THE ROLE OF DIGLYCERIDES IN LIPID-INDUCED INSULIN RESISTANCE

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Abstract

The cause of lipid-induced insulin resistance has been an intensively-discussed question since the last century. Many experiments have proven the positive correlation between insulin resistance and diglyceride (DAG), which is a bioactive lipid, and an essential intermediate in TAG synthesis and lipolysis. Among the three structural and stereoisomers, sn-1,2 DAG can activate protein kinase C enzymes, mainly PKCε in the liver and PKCθ in the skeletal muscle, and thereby inhibit insulin signaling pathways. While sn-1,2 DAG is primarily formed during TAG synthesis, multiple mechanisms are available for its metabolism. The Bradford Hill criteria are applied and discussed to better evaluate the role of DAG in the development of lipid-induced insulin resistance. To help understand how DAG affects insulin signaling and type 2 diabetes, a network containing glucose metabolism, lipid metabolism, the effect of exercise, and insulin signaling is generated for the liver, muscle, and adipose tissue, and how each PKC isoenzymes influence the network are discussed. Beyond its direct inhibition of hepatic insulin signaling, PKCε might alter hepatic metabolism through indirect effect from adipose tissue. The crosstalk between the liver and adipose tissue has been further demonstrated through adipose lipolysis, secretion of signaling molecules, inflammation, and how these mechanisms deteriorate hepatic insulin sensitivity.

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Introduction

Type 2 diabetes has been a threatening health problem since the 21st century. In 2019, there are around 463 million diabetic patients globally, which accounts for 8.8% of the adult population (Magkos et al.). Type 2 diabetes is marked by insufficient insulin production and insulin resistance. There are multiple hypotheses on the problem that causes insulin resistance, and the DAG/nPKC hypothesis is one of them. It states that the elevated intracellular DAG content, due to overnutrition, will activate PKCs and inhibit normal insulin signaling (Magkos et al.).

Association between DAG and IR

Multiple experiments have proven the positive correlation between DAG and insulin resistance in both liver and skeletal muscle. Two methods that quantify the level of insulin resistance are widely used: homeostatic model assessment of insulin sensitivity (HOMA-IR) and hyperinsulinemic-euglycemic clamp (HEC). Kumashiro et al. demonstrated that hepatic DAG content is more correlated with hepatic insulin sensitivity than other lipids and markers of inflammation (Magkos et al.). DAG accounts for 64% of the variability in insulin resistance, based on HOMA-IR data obtained from human liver biopsies. A similar result was obtained from animal models, using HEC to access hepatic insulin sensitivity (Samar I. Itani et al.). During an HEC experiment of six-hour lipid infusion to human muscle, PKC activity, and DAG mass was increased to 400%, accompanied with a 43% decrease in insulin activity, while there is no change on ceramide mass and little change in IκB-α (Samar I. Itani et al.). The vital role of DAG played in mediating insulin resistance also has been observed in experiments modulating enzymes or pathways involved in DAG formation and

metabolism. Mice without the function of glycerol-3-phosphate O-acyltransferase (GPAT), which plays an essential role in the TAG synthesis pathway, were protected from hepatic insulin resistance under a high-fat diet, due to decreased DAG and TAG content (Neschen et al.). Another enzyme involved in TAG synthesis, phosphatidate phosphatase (PAP), upon inhibition of its coding gene lipin-1 or lipin-2, decreases hepatic DAG and TAG levels and improves glucose tolerance. Knockdown of Acc1 and Acc2 to promote beta-oxidation led to reduced DAG content and PKC activity, and protection from hepatic insulin resistance. The reversal of the above experiments all resulted in hepatic insulin sensitivity deterioration as expected (Erion and Shulman; Petersen and Shulman). Similar enzyme modifications also were performed in skeletal muscle. Overexpression of lipin-1 in mice skeletal muscle resulted in obesity and insulin resistance (Phan and Reue). In rodent models, downregulation of DAGKδ, a DAG kinase that converts DAG to phosphatidic acid, raised myocellular DAG level and negatively influenced insulin signaling and glucose uptake (Chibalin et al.).

The role of DGAT, which is in charge of the final step of TAG synthesis in hepatic and muscular insulin resistance, is sometimes controversial. The two isoenzymes, DGAT1 and DGAT2, have distinct properties (More details in the section on DAG metabolism), functions, and the influence of their deficiency. The knockdown of DGAT1 in rodent livers did not affect TAG levels (Cheol et al.). However, while in skeletal muscle, such knockdown resulted in a 30~40% decrease of TAG, no change or even decreased level of DAG, and improved insulin sensitivity (Chen et al.). Interestingly, the overexpression of DGAT1 in mice skeletal muscle also decreased DAG content, increased TAG, and protected muscle from insulin resistance (Liu et al.). Considering the role DAG played in PKC activation and downstream insulin signaling disruption, the latter experiment's result is expected. The reduced DAG level in DGAT1 knockout might be due to the MGAT activity via DGAT1, which

means DAG formation also gets hindered. Such conflicts continue with DGAT2. Inhibition of hepatic DGAT2 resulted in decreased TAG and DAG levels and improved insulin sensitivity, as overexpression led to increased TAG and DAG levels (Jornayvaz et al.)(Cheol et al.). The impaired suppression of hepatic glucose production during HEC might be the reason for the increased DAG content. Nonetheless, muscular DGAT2 overexpression produced decreased DAG level, increased ceramide level, and modest glucose intolerance (Levin et al.). This might be caused by the ceramide effect on insulin signaling that will be explained later. The complex role DGAT plays in raising DAG/TAG content needs further experiments to elucidate all the factors that affect insulin resistance. Nonetheless, the existing experimental data definitely demonstrates the strong association between DAG and insulin resistance.

DAG stereoisomers

DAG is a glyceride composed of a glycerol backbone and two fatty acid chains. Depending on the position of fatty acid chains on the backbone, DAG has three structural isomers, 1,2 DAG, 2,3 DAG, and 1,3 DAG, according to IUPAC nomenclature (See Figure 1). 1,3 DAG can be either chiral if the two fatty acid ester chains are identical, or achiral if they are not. Due to this uncertainty and the lack of experimental evidence on species of the fatty acid esters, racemic (rac)-1,3 DAG will be used in the following content. 1,2 and 2,3 DAGs have the same linkage between atoms but the different geometric positions in space. The stereospecific numbering (sn) is added as a prefix to emphasize the stereoisomerism. Even though they all have the same chemical structure, these DAG isomers come from different sources, participate in different signaling pathways, and get converted into different substrates (Eichmann and Lass).

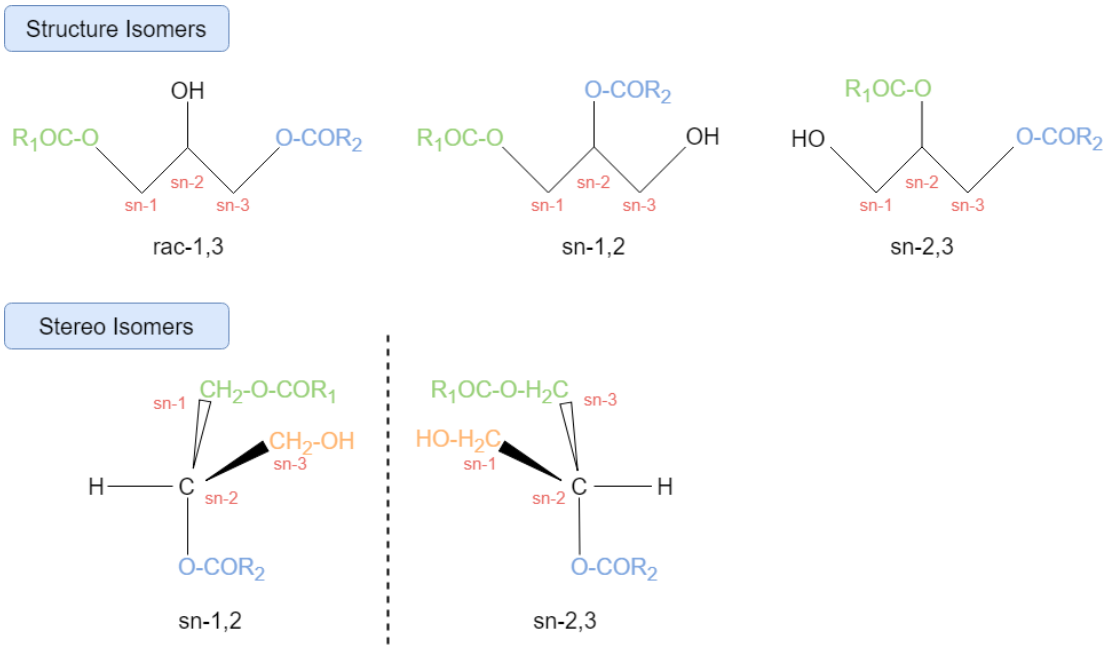

Figure 1: Depiction of the structural and stereoisomers of diglycerides. Adapted from figure 1 in Ref.34. sn-1,2 DAG and sn-2,3 DAG have the same molecular formula and constitution but biochemical properties. Only sn-1,2 DAG can activate PKC signaling pathways.

Extracellular DAG formation

Diet is the most accessible answer when people think about where does DAG in our body come from. DAGs are widely used in processed food like bread, ice cream, and peanut butter, as the emulsifier, because it has both a hydrophilic and hydrophobic end. It also is present in some seed oils in a portion of around 5%. Nevertheless, according to a WHO (World Health Organization) evaluation, monoglyceride (MAG) and DAG only attribute to one percent of daily glyceride intake, and almost all the dietary glycerides are in the form of triglyceride (*Monoglycerides: What Are They and Are They Safe?*).

After being emulsified by bile acids, triglyceride is further broken down into MAG, DAG, and free fatty acids by the pancreas lipase. Pancreas lipase and other TAG lipases involved in lipid digestion,

like gastric lipase and lingual lipase, preferably hydrolyze TAG at sn-1 and sn-3 positions (Carrière et al.). However, these enzymes will also act on DAG to release two fatty acids leaving a MAG as the result of the hydrolysis process. The successive two-step hydrolysis indicates that the DAG produced is unlikely to participate in any intracellular signaling pathways due to its short lifetime (Eichmann and Lass).

Next, fatty acids and MAGs will group with bile acids to form small micelles and enter the epithelial cells of the small intestine. In the endoplasmic reticulum (ER), they get recombined into triglycerides and transported to Golgi to form chylomicrons, accompanying lipoproteins, cholesterols, and other lipids. The final step of lipid absorption is the exocytosis of chylomicrons and transport through the lymphatic system and then into the general circulation system (*Absorption of Lipids*). Chylomicrons have three time-dependent stages: nascent chylomicrons, mature chylomicrons, and chylomicron remnants. As nascent chylomicrons circulate with blood, they exchange apolipoproteins with high-density lipoproteins (HDL). Apolipoprotein E (ApoE) and Apolipoprotein C-II (ApoC2), a vital coenzyme in the activation of lipoprotein lipase (LPL), are given to chylomicrons in this process and transform them into mature chylomicrons (Sareen S. Gropper and Smith). LPL is found primarily on the surface of the endothelial cells in the capillary of adipose, heart, and muscle tissues. Responsible for TAG hydrolysis, LPL prefers the sn-1 position of TAG and sn-2, sn-3 position for DAG, and therefore generates two fatty acids and one sn-3 or sn-2 MAGs. When most TAGs get hydrolyzed, and ApoC2 is returned to HDL, chylomicrons will enter the third stage: chylomicron remnants. In this stage, they have an average size of 30~80 nm and are ready to be removed from the circulation with the help of ApoC2 and LDL receptors in the liver (Feingold and Grunfeld)(*Hepatic Uptake of Chylomicron Remnants - PubMed*). Through phospho lipolysis, hepatic

lipase (HL) can also help generate remnants that can be rapidly cleared out by the liver, and it has the same stereo preference with LPL in TAG hydrolysis (Crawford and Borensztajn). A significant number of fatty acids are generated during the three stages and participate in many lipid metabolism processes as they cross the cell membrane by either simple diffusion or fatty acid transporters like CD36 and FATPs, which will be discussed later in this thesis.

Intracellular DAG formation

Unlike extracellular DAG formation, intracellular formation gives DAG the potential to join vital signaling pathways. In this thesis, only DAG in the liver, muscle, and adipose tissue will be considered and discussed because of their relative importance. There are three primary mechanisms for catabolic DAG formation: by phospholipase C (PLC), by sphingomyelin synthase (SMS), and by TAG lipases.

Catabolic DAG formation

DAG can come from the catabolism process of phospholipid. Phospholipase is capable of hydrolyzing phospholipids at the membrane. Among the four major classes (PLA, PLB, PLC, PLD), PLA and PLB cleave the sn-1 and sn-2 acyl chain, while PLC cuts before the phosphate and releases sn-1,2 DAG and PLD cuts after the phosphate (Eichmann and Lass). Sphingomyelin synthase (SMSs) can also generate sn-1,2 DAG by transferring phosphorylcholine residue to a ceramide backbone to produce sphingomyelin and DAG (Voelker and Kennedy). Adipose triglyceride lipase (ATGL) plays a significant role in TAG turnover, and its mRNA is expressed in almost all tissues but most highly in adipose tissues. It specifically hydrolyzes TAG at the sn-2 position and generates rac-1,3 DAG. In the presence

of comparative gene identification-58 (CGI-58), a post-transcriptional regulator for ATGL, however, it can also attack the sn-1 position and result in sn-2,3 DAG (Eichmann et al.). ATGL displays no activity on DAG and MAG. The downstream metabolism for DAG is mainly regulated by hormone-sensitive lipase (HSL), which exhibits higher activity on DAG than TAG. These two enzymes contribute to 90% of TAG catabolism in adipose tissue and display their full activity at lipid droplets' surface (Schweiger et al.). A third TAG lipase, triacylglycerol hydrolase/carboxylesterase-3 (TGH/Ces3), targets predominantly short-chain TAG and hydrolyzes them in the ER with an unknown stereo preference (Lehner and Verger).

Anabolic DAG formation

The primary intracellular anabolic DAG production is through the glycerol 3-phosphate (G3P) pathway. Free fatty acids cross the membrane, enter the cell, and are oxidized by FA-CoA ligase. The product, fatty acid CoA (FA-CoA), will work with enzymes glycerol-3-phosphate O-acyltransferase (GPAT) and 1-acylglycerol-3-phosphate-O-acyltransferase (AGPAT) to acylate G3P and generate phosphatidic acids (PA). Phosphatidic acid phosphatase (PAP) is the regulatory enzyme for the conversion from PA to DAG and gives exclusively sn-1,2 stereoisomers. While the G3P pathway mainly happens in the liver and adipose tissue, MGAT is responsible for another intracellular DAG synthesis pathway, primarily in intestines. As mentioned above, fatty acids and MAG will reform TAG after they get into the epithelial cells of the small intestine. The stereoselectivity of MGAT largely depends on its isoforms. MGAT3 is considered as the main enzyme for the reesterification process and generate sn-1,2 or sn-2,3 DAG (Eichmann and Lass). Nevertheless, MGAT can esterify DAG into

TAG further and leads to the minimal possibility for this DAG to affect downstream signaling pathways.

Summary

In summary, the three mechanisms of intracellular sn-1,2 DAGs formation are: SMS transfers phosphorylcholine residue to ceramide backbone to generate DAG; phospholipid gets hydrolyzed by PLC and results in DAG; PAP converts PA to DAG, which is the second last step for de novo lipogenesis. SMS is expressed ubiquitously in the body, and at the subcellular level, its distribution depends on subtypes. SMS1 localizes only to the luminal side of the trans-Golgi; SMS2 is on trans-Golgi and the extracellular leaflet of the plasma membrane; SMSr localizes on the ER. Such localization influences the functions of DAG produced by SMS subtypes. DAG produced by SMS1 and SMS2 on the trans-Golgi can activate PKD and further secretory vesicle formation. DAG generated by SMS2 on the plasma membrane has access to PKCs, but it is also mostly consumed by SMS2 to maintain sphingomyelin levels decreased by sphingomyelinases (Eichmann and Lass). At the plasma membrane, PLC controls the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2), generating DAG and inositol 1,4,5-trisphosphate (IP3), which can open Ca2+ channel on the ER (Czech). Together with DAG, the increased Ca2+ level will result in short-lived PKC activation, contrasting to the chronic activation by long-termed elevated DAG level produced by PAP on the ER during de novo lipogenesis because of lipid oversupply (Schmitz‐Peiffer). Overall, the sn-1,2 DAG capable of activating PKCs and responsible for long-term activation is mainly produced during de novo lipogenesis.

DAG metabolism

To investigate the quantitative influence of DAG, the metabolism of intracellular sn-1,2 DAG is as crucial as the formation. Acting as an intermediate element in de novo lipogenesis, one way of DAG consumption is acylation by diglyceride acyltransferase (DGAT). The two types of DGAT, DGAT1, and DGAT2 exhibit different localization and efficacy. DGAT1 is mainly localized to the ER membrane, and DGAT2 is both on the ER membrane and the lipid droplets (LDs). Therefore, the DAG produced at the LDs will only be available for DGAT2, but those produced at the ER membrane can be utilized by DGAT1 and DGAT2. DGAT1 is more efficient in the consumption of sn-DAGs, while DGAT2 prefers rac-DAGs (Eichmann et al.). DGAT2 seems to play a more vital role in TAG synthesis: it is more potent and has a higher affinity to its substrate; the deficiency of DGAT2 can be lethal in mice while the deficiency of DGAT1 only leads to moderate reduction of TAG (Yen et al.). As the deficiency of DGAT1 is not accompanied by the increase of DGAT2 mRNA expression, DGAT1 might be a potential target for diabetes treatment (Chen et al.).

While DGAT activity is dependent on the substrate availability, it can be modified at the mRNA level by multiple factors. In adipocytes, mRNA levels of DGAT1 and DGAT2 are increased by glucose and insulin (DGAT2 only) (Yen et al.). DGAT is also upregulated during adipogenesis, indicating that modifications on transcription factors like C/EBPα or PPARγ will affect DGAT expression (Payne et al.). In hepatocytes, the inhibition of the MEK-ERK signaling pathway increases DGAT mRNA level and enhances VLDL secretion (Tsai et al.). Leptin is another factor contributing to decreased DGAT mRNA expression. Mice with deficient leptin receptor or leptin resistance show enhanced DGAT2 mRNA expression in WAT, skeletal muscle, and small intestine (Wakimoto et al.). Alteration on nutrient

states, like administration of glucagon, epinephrine, or long-chain fatty acids, decreases DGAT activity (Yen et al.).

Another mechanism is DAG hydrolysis by HSL, as mentioned above, as the second step in TAG hydrolysis. Nonetheless, HSL targets sn-3 positions specifically, therefore, cannot consume sn-1,2 DAG. Instead, DAGLα and β are two sn-1 specific DAG lipase. DAGLα is mainly expressed in the brain and pancreas, while DAGLβ is in bone marrow, lymph nodes, spleen, and liver. DAGLα localizes to the plasma membrane, whereas DAGLβ also localizes to the LDs. Due to their subcellular localization, DAGLα and β both have access to DAG produced by hydrolysis of PL (Eichmann and Lass).

In addition to DAG acyltransferases and lipases, sn-1,2 DAG can be consumed through the phosphorylation by diacylglycerol kinase (DGK). DGK phosphorylates the hydroxyl group of DAG to form PA. Therefore, DGK is instrumental in balancing the intracellular DAG and PA levels. Ten DGK isozymes are widely spread in almost all organs and organelles, and they are all sn-1,2 DAG specific kinases. The last mechanism is the Kennedy pathway (CDP-choline pathway), which converts DAGs to either phosphatidylcholine or phosphatidylethanolamine (PE), catalyzed by CDP-choline:1,2-diacylglycerol choline phosphotransferases (CPT) or ethanolamine phosphotransferase (EPT), respectively(Eichmann and Lass).

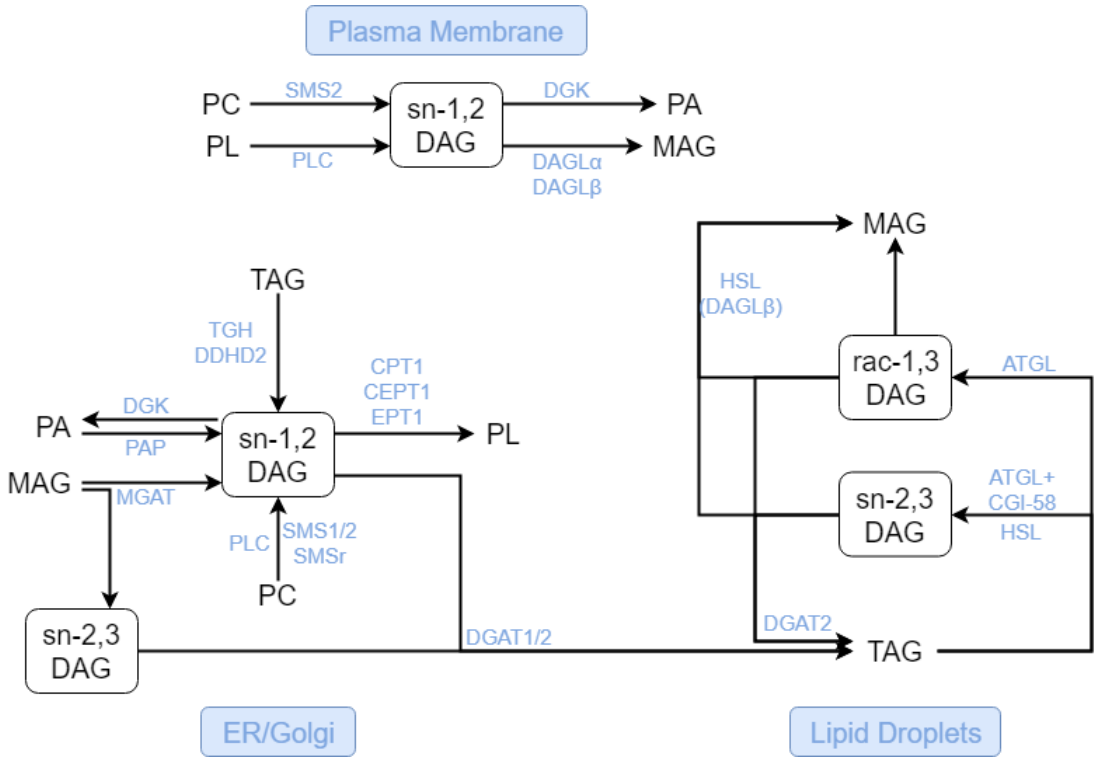

Figure 2: Mechanisms of DAG formation and metabolism in three subcellular compartments. Adapted from figure 6 in Ref.34. Only sn-1,2 DAG at plasma membranes or ER/Golgi can reach and activate PKCs. Descriptions and abbreviations are included in the above section.

Bradford Hill criteria to justify DAG and IR relation

To better evaluate DAG's role in the development of lipid-induced insulin resistance, the Bradford Hill criteria, a group of nine principles to establish the cause-effect relationship among public health researches, can be applied (Petersen and Shulman, "Roles of Diacylglycerols and Ceramides in Hepatic Insulin Resistance").

The first principle is strength. How well does DAG positively correlate with lipid-induced insulin resistance? Kumashiro et al. proved that DAG content in hepatic lipid droplets is responsible for 64% of the variability of hepatic insulin resistance (Kumashiro et al.). In vivo experiments done by Samar et al. show that a 300% increase in DAG mass in human muscle resulted in a 43% decrease in insulin

activity (Samar I. Itani et al.). Both experiments highlighted the considerable influence of DAG in insulin function.

The second principle is consistency. Is this positive correlation between DAG and insulin resistance consistent among different researches? In addition to the above two experiments, Faidon et al. also demonstrated a negative correlation between DAG level and insulin-suppressed hepatic glucose production (Magkos et al.). An experiment done by Szendroedia et al. found that there is a transient increase in cytosolic DAG content during the acute induction of muscle insulin resistance (Szendroedi et al.).

The third principle is specificity. Is DAG accumulation associated specifically with insulin resistance? By activating PKC isomers, DAG is capable of inducing inflammatory signaling pathways (Petersen and Shulman, “Roles of Diacylglycerols and Ceramides in Hepatic Insulin Resistance”). Also, DAG metabolism can interact with TOR and S6K signaling and therefore influence oxidative stress resistance (Lin et al.). However, traces of insulin resistance appear only after three days of a high-fat diet in mice, and without significant inflammation, implying non-inflammatory responses are the ones initiating insulin resistance (Petersen and Shulman, “Mechanisms of Insulin Action and Insulin Resistance”).

The fourth principle is temporality. Does DAG accumulate before the onset of insulin resistance? Elevated DAG level usually comes from overnutrition. An excess fatty acid supply to the liver and muscle pushes the TAG synthesis and produces more DAG. Insulin resistance is a time-taken process, usually takes at least days to develop, but the accumulation of DAG can simply happen after a meal. However, to formally fit this criterion, a time point with an increased DAG but unchanged insulin

sensitivity will be necessary (Petersen and Shulman, “Roles of Diacylglycerols and Ceramides in Hepatic Insulin Resistance”).

The fifth principle is biological gradient. Is there a dose-response data available? HOMA-IR and HEC are used by researchers to quantify insulin resistance, so the correlation between DAG and insulin resistance always covers a wide range. In an experiment done by Kumashiro et al., for example, a linear relationship is presented between total DAG content and HOMA-IR (Kumashiro et al.).

The sixth principle is plausibility. Is there a plausible mechanism between DAG and insulin resistance? An elevated sn-1,2 DAG content can activate PKCs isomers (mainly PKCε in the liver and PKCθ in skeletal muscle). Activated PKCs thereby can inhibit insulin signaling pathways by either phosphorylating IRS1 or insulin receptors. More detailed mechanisms are explained in the *Lipid-Induced Insulin Resistance* section.

The seventh principle is coherence. Does all data fit the model? While the majority of researches affirmed the DAG-PKC-IR hypothesis, some experiments do show opposite results. Takase et al. injected a dose of DAG into non-diabetic men with or without insulin resistance and resulted in decreased plasma TAG level (Takase et al.). And genetic modulation on DGAT shows unexpected results in TAG content and insulin sensitivity (Cheol et al.; Liu et al.). These situations might be explained by the specificity of DAG stereoisomers and subcellular locations, plus the complexity of DGAT1/2 regulating DAG metabolism. Both are explained in previous sections.

The eighth principle is experiment. Do experiments fit the model? Various experiments modulating enzymes involved in DAG formation and metabolism can justify the role of DAG in insulin resistance. Mice with inhibited GPAT or PAP activity, both are engaged in DAG formation, are protected from

hepatic insulin resistance under the high-fat diet (Neschen et al.). Downregulation of DAGKδ raised the DAG level in skeletal muscle and negatively influenced insulin signaling (Chibalin et al.). More examples are discussed in the *Association between DAG and IR* section.

The last principle is analog. Is there any similar cause-effect pair? The correlations between DAG and hepatic insulin resistance/muscular insulin resistance are very similar cause-effect pairs, and both are well-studied hypotheses. The main difference between the two is the type of PKCs activated upon DAG elevation. DAG in the liver mainly activates PKCε, and in the skeletal muscle, it stimulates PKCθ. Despite these two major PKC isomers, other PKCs are distributed differently among tissues, resulting in the activation of various pathways.

Why not ceramide?

Similar to DAG, ceramide is another bioactive lipid, composed of sphingosine and a fatty acid. It can be produced by de novo synthesis and sphingomyelin hydrolysis. It is observed that during excess fatty acids supply, there is elevated ceramide content in the muscle, accompany with the development of insulin resistance (de la Maza et al.). Ceramide functions as a part of the microdomain on the cell membrane to help stabilize the structure and modulate receptors distribution on the membrane (Sokolowska and Blachnio-Zabielska). Despite that, it also activates many signaling pathways and regulates glucose and lipid metabolic pathways. Therefore ceramide has been considered as a potent contributor to lipid-induced insulin resistance. Ceramide can inhibit PI3K activity and thereby block the stimulation of insulin on Akt signaling pathways (Powell et al.). Indirect inhibition of Akt by ceramide is performed by the activation of protein phosphatase 2A (PP2A) or activation of atypical PKC isoforms, PKCζ/λ in skeletal muscle (Sokolowska and Blachnio-

Zabielska). In addition to interfering glucose metabolism, ceramide stimulates fatty acids uptake and suppresses fatty acids oxidation, especially in the liver (Chaurasia and Summers). Lastly, ceramide participates in inflammatory responses by activating pro-inflammatory adipocytokines and stimulating cell death (Galadari et al.). However, many recent studies questioned the positive correlation between ceramide and insulin resistance (Samuel and Shulman). Compared with DAG, ceramide lacks specificity and necessity to induce insulin resistance. Experiments showed increased ceramide level, in obesity or type 2 diabetes, always accompanies an increased level of overall lipids, so it's hard to specify the role of ceramide in such a situation. And many experiments with lipid-induced insulin resistance show no change on ceramide level, indicating that ceramide is not necessary for the development of insulin resistance (Magkos et al.; Samar I. Itani et al.).

PKC isoenzymes

Protein kinase C is a family of protein kinase enzymes. By phosphorylating the serine or threonine amino acid residues on proteins, it's capable of manipulating many critical signaling pathways in the human body. PKC can be activated by the concentration of DAG or calcium ions. Categorized by activation conditions, its fifteen isozymes are divided into three groups: conventional PKCs (cPKCs, including PKCα, PKCβI, PKCβII, and PKCγ), novel PKCs (nPKCs, including PKCδ, PKCε, PKCη, and PKCθ), and atypical PKCs (aPKCs, including PKCζ and PKCλ). cPKCs need Ca2+, DAG, and phospholipids for activation, while nPKCs require DAG only, and aPKC solely depends on phospholipid (*Protein Kinase C - Wikipedia*). The auto-inhibited inactive PKCs move freely in the cytosol. Once they hit DAG or other activation factors, conformational changes will enable their phosphorylation ability, increase the hydrophobicity, and promote the binding of PKCs to membrane

lipids (Igumenova; Khalil). Upon activation, PKCs can help regulate a vast range of cellular functions, including cell growth, immune responses, receptor inhibition, etc. In the case of insulin resistance, the primary role of PKC is the inhibition effect on insulin receptor (INSR) and insulin signaling mediators like GIV/Girdin, while the exact mechanisms will depend on individual PKC isozymes.

PKCs localization

The isozymes species presented are tissue-specific. Past experiments have proven the connection between individual PKC isozymes and insulin resistance in different tissues, especially the liver and skeletal muscle. In an in-vitro experiment, muscle fiber strips from obese patients were under insulin stimulation. Increased translocation of PKCβ, PKCθ, and PKCδ to the membrane fraction was observed, while such an increase was not seen for PKCζ, PKCε, and PKCμ (S. I. Itani et al.). In rat skeletal muscle, activation and translocation of PKCθ and PKCε were found. By comparison, PKCθ was not detected in human liver, as PKCε took the major role instead, together with PKCα, PKCβ, PKCζ, PKCι, and PKCδ (Kumashiro et al.). PKCε is also the dominant PKC isoform in the liver of fat-fed rat (Samuel and Shulman). For subcellular localization, PKCs are mainly bounded to the plasma and endomembrane, while nPKCs significantly localize to the Golgi, possibly due to the localization of their adapter protein RACKs (Petersen and Shulman, “Mechanisms of Insulin Action and Insulin Resistance”). Organ and cellular level localization of DAG, PKC, and enzymes involved in DAG formation and consumption is essential to clarify the interaction further.

Connection to IR

Among the fifteen isoenzymes, PKCθ, PKCε, PKCδ, PKCζ, and PKCβ are well studied in recent years for their tight connections to insulin resistance. During a five-hour acute lipid infusion done by Jason Kim et al. in 2004, PKCθ knockout mice showed a 40~50% decrease in insulin receptor substrates 1 (IRS1) phosphorylation and associated PI3K activity in skeletal muscle (J. K. Kim et al.). However, such decline was not observed for PKCθ knockout mice after 14 weeks of high-fat feeding, possibly due to the effect of hepatic insulin resistance or the activation of other PKC isoenzymes (Gao et al.). For PKCε, a decrease in its expression can enhance the hepatic insulin response, and complete knockout can protect the liver from diet-induced insulin resistance after one week of high-fat diet (Petersen and Shulman). While PKCθ and PKCε disturb normal insulin function via their inhibition effect on IRS1, PKCδ, PKCζ, and PKCβ kick in through diverse mechanisms. An increase of PKCδ in the liver or whole body will exacerbate glucose intolerance. The corresponding IKK-β activation by PKCδ during a lipid infusion indicates that PKCδ might regulate glucose homeostasis through an inflammatory pathway (Petersen and Shulman). The activation of PKCζ, while closely related to the hepatic DAG level, has less effect on insulin signaling than PKCε. Ceramides play a more vital role for PKCζ as ceramides can impair the interaction between PKCζ and Akt2. Therefore, Akt2 cannot be activated in response to insulin (Petersen and Shulman). The translocation of PKC-βII to the membrane was increased with DAG mass during euglycemic-hyperinsulinemic clamping. All the evidence has proven the notable role PKC isoenzymes played in the development of insulin resistance.

Nutrient flux and the role of insulin

To fully understand the exact role DAG and PKCs played in the scenario of insulin resistance, they need to be put into the broader background. Glucose and lipid flux compose the basic structure of

this network, and they are influenced and connected by the insulin signaling like flesh on bones. The comprehensive network of the liver, muscle, and adipose tissue, and their interventions are depicted in Figure 5.

Glucose metabolism

Dietary glucose distribution to tissue cells is gated by glucose transporter type 2 (in the liver) and type 4 (in adipose tissue and skeletal muscle). At the same time, the rate of entry is proportional to the blood glucose level. After the entrance, glucose has two destinations, converted to glycogen (Glycogenesis) to store energy or broken down (Glycolysis) to provide energy to the body. Glycogenesis is catalyzed by glycogen synthase (GS), and the reverse reaction, glycogenolysis, is controlled by glycogen phosphorylase (GP). In the postprandial state, with the presence of a large amount of glucose, glycogenolysis is inhibited in the liver, and glycogenesis is activated by insulin (Kasvinsky et al.). As the glucose level falls, the glycogen stored in the liver will be broken down into glucose and become the primary source of blood glucose to fuel the whole body (*Glycogen - Wikipedia*). Glycogen formed in the muscle, by contrast, can only be used internally (*Glycogen Biosynthesis; Glycogen Breakdown*). Although murine studies have shown that adipose tissue can store glycogen, it only exists in a minimal amount under normal state, and therefore not included in Figure 5.

While glycogenesis functions as energy storage, glycolysis is the first step to extract energy from glucose. The intermediates of glycolysis, dihydroxyacetone phosphate (DHAP) can be transformed into glycerol-3-phosphate (G3P) and participate in triglyceride synthesis. And the final products of glycolysis are two ATP, two pyruvates, and two NADH molecules (*Glycolysis | Cellular Respiration |*

Biology (Article) | Khan Academy). Under hypoglycemia, pyruvate will be transformed back to glucose by pyruvate carboxylase, and this process is inhibited by insulin through FoxO1 (*Gluconeogenesis - Wikipedia*). With normal insulin function and glucose level, pyruvate instead will be transported into the mitochondria by pyruvate translocase and decarboxylated into acetyl-CoA by pyruvate dehydrogenase complex, thereby join the citric acid cycle (TCA cycle). During the TCA cycle, acetyl-CoA will be converted to citrate and undergo a series of reactions to generate energy and regenerate citrate (*Citric Acid Cycle - Wikipedia*). Under a high insulin level, instead of continuing to the next cycle, citrate will be removed from the mitochondria to the cytoplasm, and turned back into acetyl-CoA (Stryer). Acetyl-CoA in the cytoplasm is the starting molecule in de novo lipogenesis (DNL), a vital source of intracellular non-esterified (free) fatty acids. Because of the high similarity of the energy production process among different tissues, Figure 5 is simplified in the muscle and adipose tissue part.

Fatty acid metabolism

Despite DNL, three other mechanisms contribute to the intracellular free fatty acids pool in the liver. The first source is the exogenous FFAs in the circulation, from spillover or lipolysis from adipose tissues. Exogenous FFAs are demonstrated to be the largest source of FAs in hepatic triglycerides through isotope studies, and such ratio increases under fasting and NAFLD (Barrows and Parks). The metabolism of circulating chylomicron is the second source for hepatic FFAs. With the help of LPL and HL, a large amount of FFAs is released during the three stages of chylomicron, as explained in the Extracellular DAG Formation section. Glycerol is another product in this process, and after phosphorylation by glycerol kinase to G3P, it also joins TAG synthesis (Venugopal et al.). These

exogenous FFAs will enter the hepatocyte through fatty acid translocase (CD36) and fatty acid transport protein (FATP). Unexpectedly, CD36 is not required for FAs uptake under normal conditions, suggesting FATP might be in charge of a larger portion of the task (Coburn et al.). The last part of the hepatic FFA pool is from lipolysis, which releases three FAs and one glycerol from one TAG. The detailed pathway of TAG synthesis and lipolysis has been described in the previous section, and the steps are simplified for clarity in the muscle and adipose part of Figure 5.

The FFA pools in the skeletal muscle and adipose tissue share similarities in endogenous sources but are different in exogenous sources. In skeletal muscle, FFAs from adipose tissue supply for the first part. The second part comes from LPL hydrolyzation of triglycerides in very-low-density lipoproteins (VLDLs), which is exported from the liver. In adipose tissue, VLDLs and chylomicrons together contribute to the exogenous FAs. Adipose LPL activity can be influenced by modulation in apolipoproteins (Apo). ApoC3 has an inhibitory effect on LPL activity, while ApoAV and ApoC2 stimulate its activity (Schaap et al.; Samuel and Shulman). FFAs in these organs will then be converted to fatty acyl-CoA by fatty acyl-CoA synthetase (FACS) and utilized by either joining TAG synthesis or entering the mitochondria beta-oxidation. During beta-oxidation, FA-CoAs are broken down into acetyl-CoA and thereby participate in the TCA cycle for energy production. And an increased acetyl-CoA content will activate pyruvate carboxylase (PC) and, as a result, enhance gluconeogenesis (Samuel and Shulman).

Exercise

Exercise has been considered as a reliable way to prevent the onset of type 2 diabetes and improve insulin sensitivity. Random trails have shown that a proper diet and 150 minutes of physical exercise

per week can reduce 58% potential risk for type 2 diabetes among 522 middle-aged, overweight subjects(Tuomilehto et al.). Another study has proven that long-term exercise is more effective than diet in reducing visceral fat and hepatic TAG content (Koh et al.). These studies justified the critical role exercise could play in preventing type 2 diabetes. There are multiple mechanisms involved to make such an improvement to happen. Muscle contraction leads to increases in AMP/ATP ratio, calcium ions concentration, reactive oxygen species (ROS), and PKC. These changes stimulate many signaling cascades in myocytes. Among them, the major pathway is initiated by AMPK activation.

Independent of insulin action, exercise, or muscle contraction can activate AMP-activated protein kinase (AMPK) directly and indirectly through its upstream enzymes LKB1 and CaMKK, and thereby regulate muscular metabolism (Koh et al.). Activating by an increased AMP/ATP ratio, AMPK targets to restore the ATP level by inhibiting anabolic processes in order to minimize ATP consumption, and activating catabolic processes to maximize ATP production (See Figure 3).

In anabolic pathways, AMPK phosphorylates and inhibits ACC1 and ACC2 (which are responsible for the last step of DNL), and the key enzyme in cholesterol synthesis, HMG-CoA reductase (HMGCR) (Carling et al.; MUNDAY et al.). AMPK can also phosphorylate ChREBP and SREBP, and both are important enzymes in lipogenesis. These together result in an inhibition effect on fatty acid and sterol synthesis. AMPK is also capable of reducing gluconeogenesis through inhibiting the activity of cAMP response element-binding protein (CREB) and forkhead box protein O (FOXO) pathways. Glycogen synthase GYS1 and GYS2 are phosphorylated by AMPK, and glycogen storage is reduced (Herzig and Shaw).

In catabolic pathways, the goal of AMPK is to break down glucose and lipids to generate energy. The inhibition effect of AMPK on ACC2 decreases the malonyl-CoA content and increases beta-oxidation (Abu-Elheiga et al.). Similar to ChREBP and SREBP, AMPK regulates histone acetyltransferase p300 and hepatocyte nuclear factor 4 alpha (HNF4α) at the transcription level (W. Yang et al.; Stapleton et al.). The phosphorylation of thioredoxin-interacting protein (TXNIP) and TBC1D1 by AMPK increase the membrane localization of GLUT1 and GLUT4, and glucose uptake and utilization by the cells (Wu et al.; Chavez et al.). PLD1 phosphorylation also shows an indirect positive influence on glucose uptake (J. H. Kim et al.). Lastly, PFK1, a rate-limiting enzyme in glycolysis, is upregulated by AMPK (Bando et al.). AMPK also promotes lipolysis by activating ATGL. AMPK has an inhibitory effect on HSL activity, but HSL activity during exercise was elevated by 80% compared to the rest state, according to the human experiment done by Matthew et.al. The controversial results are due to PKA phosphorylation and upregulation of HSL during exercise, which compensates or even overcome the effect of AMPK in prolonged exercise (Herzig and Shaw; Watt et al.). The inhibitory and activating phosphorylation of AMPK on lipid and glucose metabolism enzymes are shown in Figure 5.

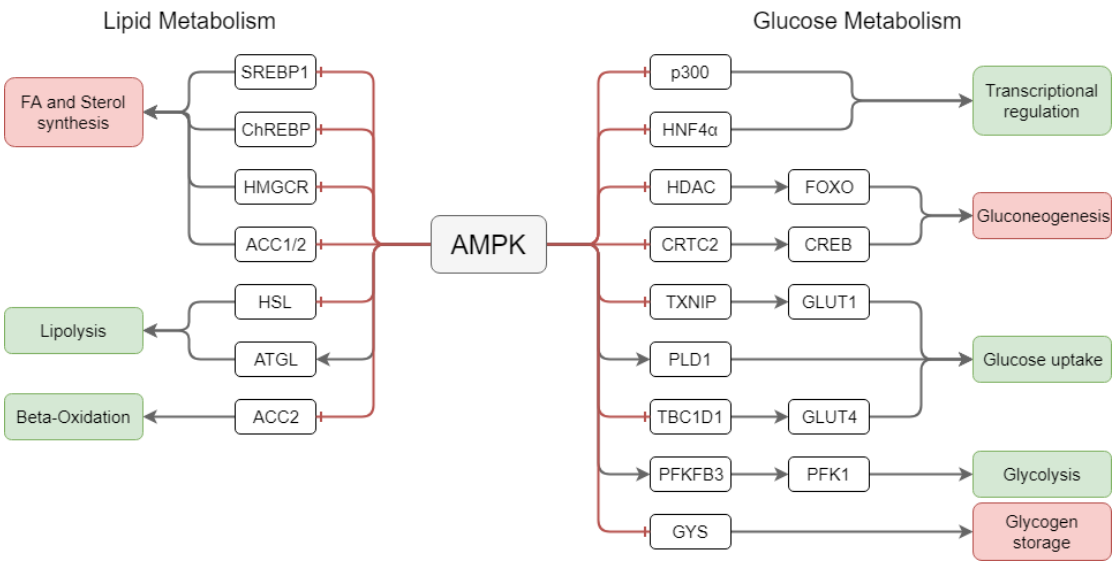

Figure 3: AMPK regulation on lipid and glucose metabolism. Adapted from figure 2 in Ref.51. The red box means that the process has been inhibited and the green box means activation. The red arrows represent the inhibitory phosphorylation by AMPK, and black arrows are the activatory phosphorylation. To restore ATP levels, AMPK will inhibit anabolic processes (FA and sterol synthesis, gluconeogenesis, glycogen storage) and activate catabolic processes (lipolysis, beta-oxidation, glucose uptake, glycolysis, transcriptional regulation).

In addition to metabolic regulation, AMPK has been proven to be closely related to muscle mitochondria biology. Mitochondrial dysfunction and reduced biogenesis are found in people with type 2 diabetes. As stated in a study in 2010, after 12 weeks of exercise, the damaged mitochondrial function has been restored, accompanied by increased insulin sensitivity (Meex et al.). The improvement in mitochondrial function is mainly achieved by various regulation mechanisms by AMPK. First, AMPK activates peroxisome proliferator-activated receptor-γ (PPARγ) co-activator 1α (PGC1α) and increases mitochondrial biogenesis gene expression. Second, AMPK modulates mitochondria dynamics through phosphorylates ULK1, therefore urges the degradation of damaged mitochondria by autophagy. Third, AMPK phosphorylates mitochondrial fission factor (MFF) on Ser155 and Ser172. MKK is the main receptor for dynamin-like protein (DRP1), which is fundamental

in mitochondria fission and facilitates mitophagy (Herzig and Shaw). Overall, AMPK improves muscle mitochondria function by increasing biogenesis, mitophagy, and autophagy.

Insulin Signaling

Besides AMPK, another key regulator of this complex nutrient flux network is insulin and its downstream signaling pathway. Binding of insulin to the insulin receptor leads to its autophosphorylation and tyrosine phosphorylation of IRS1, therefore starts the insulin signaling cascade (Schmelzle et al.). Phosphorylation of IRS1 allows it to bind with the regulatory subunit (P85) of phosphoinositide 3-kinases (PI3K), and release the inhibition effect of P85 on the catalytic subunit (P110) (Taniguchi et al.). Activated PI3K phosphorylates phosphatidylinositol (4,5)-bisphosphate (PIP2) into phosphatidylinositol (3,4,5)-trisphosphate (PIP3) (*Phosphatidylinositol (3,4,5)-Trisphosphate - Wikipedia*). After being recruited and binding to PIP3, protein kinase B (Akt2 specifically) can be activated by either phosphoinositide-dependent kinase 1 (PDK1) at Thr-308 or the mammalian target of rapamycin complex 2 (mTORC2) at Ser-473 (Sarbassov et al.; Betz et al.). As mTORC2, upon PI3K activation, can trigger Akt2 signaling cascade, mTORC1 activity, by contrast, is inhibited by Akt2, which stops mTORC1 from activating PI3K, and form a positive feedback loop (G. Yang et al.). Playing a crucial role in summarizing multiple inputs that modulate glucose and lipid metabolism, and passing the signals down to target proteins, Akt2 regulates glycogenesis, gluconeogenesis, GLUT4 translocation, and lipolysis. One of the target proteins is glycogen synthase kinase 3 (GSK-3), which can phosphorylate GS and therefore upregulates glycogenesis (EMBI et al.). Akt2 is also able to phosphorylate FoxO1 on Thr-24, Ser-256, and Ser-319 irreversibly and exclude it from the nucleus (Rena et al.). Since FoxO1 can increase the transcription of glucose 6-phosphatase

(G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK), which are both required for gluconeogenesis, the inactivation of Akt2 will eventually decrease gluconeogenesis and reduce glucose level (Hasselgren; Nakae et al.). Mice with knockdown of FoxO1 showed a decreased ATGL expression, indicates that FoxO1 also has a positive correlation with lipolysis (Chakrabarti and Kandror). Additionally, by binding to the promoter sites and stopping transcription of PPAR gamma, FoxO1 negatively regulates adipogenesis.

Another primary function of Akt2 is to stimulate glucose transporter type 4 (GLUT4) translocation to the cell membrane and facilitate glucose uptake into the cell. Unlike GLUT4, GLUT2, mainly expressed in the liver, does not rely on insulin activity for uptake (*GLUT2 - Wikipedia*). Distinct from above, Akt2 regulates lipolysis through an indirect mechanism by activating phosphodiesterase 3B (PDE-3B), lowering cAMP levels, inhibiting cAMP-dependent protein kinase (PKA) activity, and reducing lipolysis, in order (Czech et al.; *Protein Kinase A - Wikipedia*). PKA at the same time can be upregulated by β-adrenergic receptor (βAR), the target receptor for many neurotransmitters like norepinephrine and epinephrine produced by the body (*Adrenergic Receptor - Wikipedia*).

Other than Akt2, insulin can participate in glucose and lipid metabolism through many other pathways. In adipose tissue, besides the indirect inhibitory effect of Akt2 on lipolysis (HSL specifically), phosphorylation of IRS1 can directly inhibit ATGL, the enzyme responsible for the conversion from TAG to DAG (Zhao et al.). The activation of mTORC by insulin can increase sterol regulatory element-binding transcription factor 1 (SREBP-1) gene expression, thus promote lipogenesis through upregulation of acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and Stearoyl-CoA desaturase-1 (SCD1) (Ru et al.; S. Li et al.). Surprisingly, the expression of SREBP-1c can

be induced by feeding only, independent of insulin. Knockdown of the insulin receptor in ob/ob mice, nonetheless, showed zero induction of SREBP, which means under the circumstances of obesity or type 2 diabetes, the compensation effect from other signaling pathways is no longer sufficient, and the SREBP expression solely depends on insulin action (Haas et al.). Another important enzyme that is closely related to SREBP and lipogenesis regulation is ChREBP. ChREBP is activated by glucose and fructose levels and is independent of insulin. In hepatocytes, ChREBP controls several regulatory enzymes of glycolysis and lipogenesis, like liver-type pyruvate kinase, the last step of glycolysis, and all the enzymes in lipogenesis (ATP citrate lyase (ACLY), ACC, FAS, and SCD1) (Uyeda and Repa). Liver X receptor is the shared regulator for both SREBP and ChREBP by activating their transcription, and it activates lipogenic enzymes like ACC and FAS directly, too. ChREBP also regulates SREBP-1c expression, while SREBP, other than producing LXR ligands, also supports ChREBP expression under certain dietary conditions. The interplay between LXR, SREBP, and ChREBP is necessary for lipogenic mRNA inductions (Linden et al.) (See Figure 4).

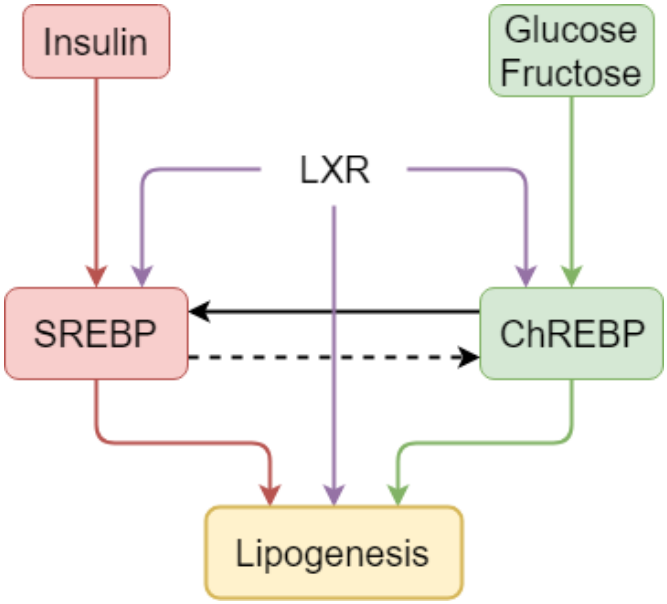

Figure 4: Interplay between SREBP, ChREBP, and LXR. Adapted from figure 8 in Ref.71. A Detailed description is in the above section.

In skeletal muscle, the translocation of glucose transporter type 4–containing (GLUT4-containing) storage vesicles (GSVs) is influenced by signals more than just Akt2. Akt2 activation leads to the phosphorylation and inactivation of TBC1D1 and Akt substrates of 160 kDa (AS160) at Ser-318, Ser-570, Ser-588, Thr-642, and Thr-751 residues. AS160 inhibits GLUT4 translocation in an unphosphorylated state, so its phosphorylation increases the GSVs translocation to the cell membrane (Taylor et al.). Experiments show that in patients with obesity or type 2 diabetes, such phosphorylation has been impaired in skeletal muscle. Yet, a 90% reduction on GLUT4 translocation only corresponds to 39% less AS160 phosphorylation, indicating some other mechanisms are also damaged in patients (Huang and Czech). One possible mechanism is that insulin promotes the binding of vesicular protein VAMP2 to syntaxin-4 (as vesicle SNAP receptors and target membrane, respectively), and increases GSVs docking to the membrane by 40-fold according to experimental data (Duffield Brewer et al.; Brady and Saltiel). Additionally, exercise as an insulin-independent

pathway improves GLUT4 translocation. Muscle contraction increases the AMP/ATP ratio and calcium content in the skeletal muscle, thus activates AMPK and Ca2+/calmodulin-dependent protein kinase II (CamKII), respectively (Stanford and Goodyear). AMPK and Akt2 both inhibit AS160, but similar to the insulin signaling branch, disruption of AMPK and Akt2 cannot completely inhibit the exercise branch of AS160 phosphorylation, meaning additional mechanisms are also available.

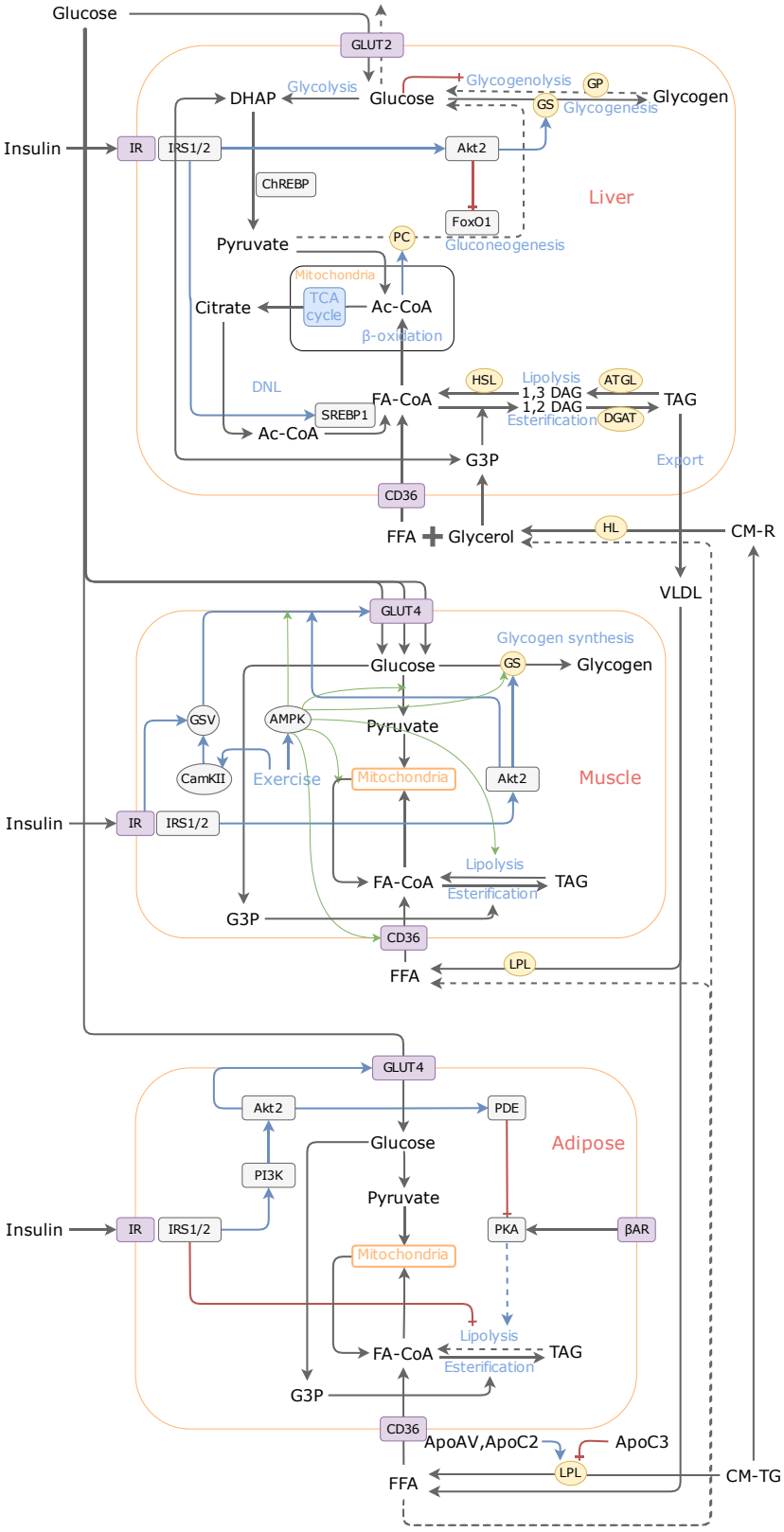

Figure 5A: Normal Insulin Signaling and its effect on lipid and glucose metabolism. Adapted from figure 1 in Ref.106. The blue, red, black arrows represent signaling cascade, inhibitory cascade, and substrate conversion, respectively. Green arrows are used to distinguish the effect of exercise.

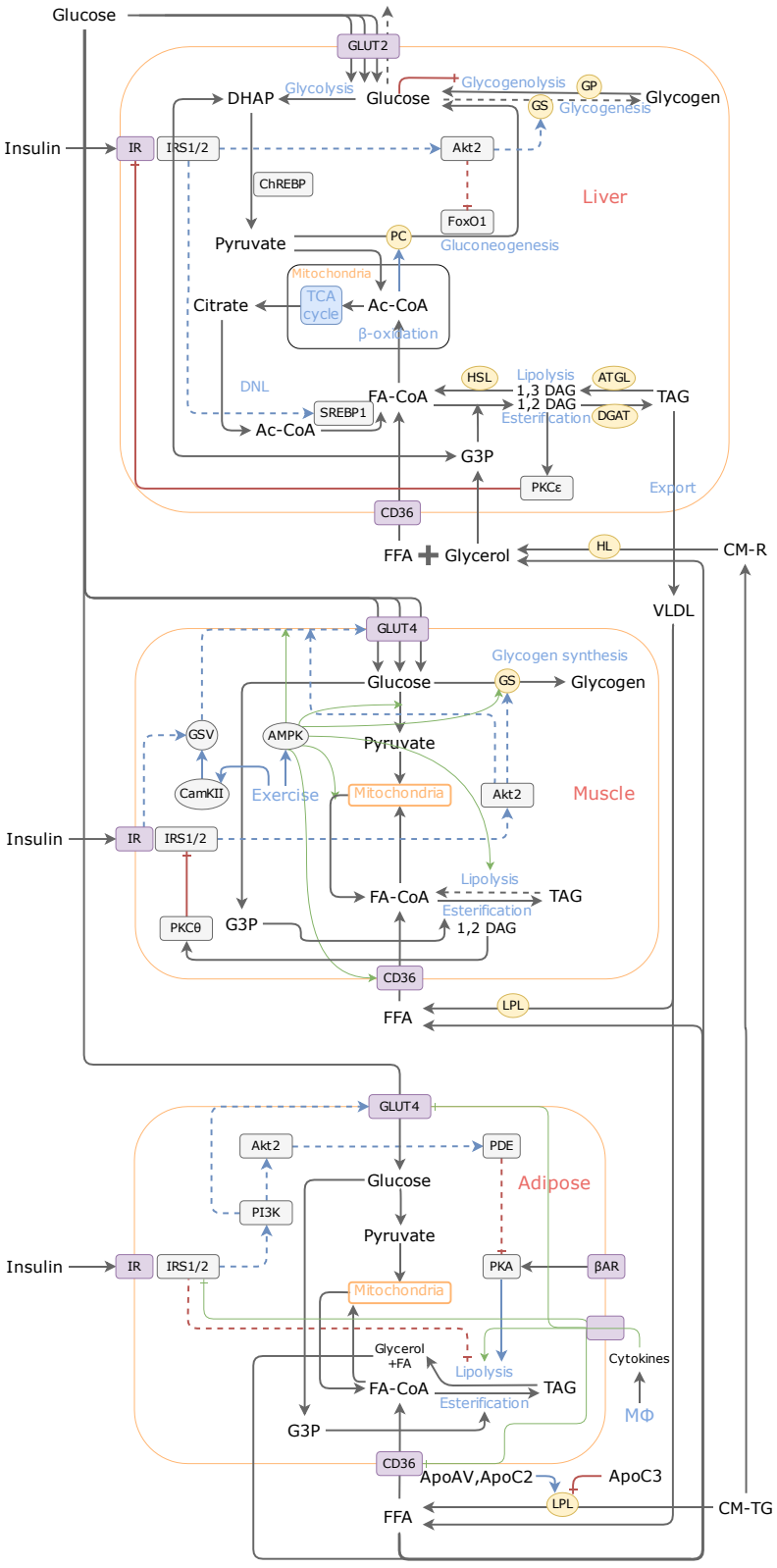

Figure 5B: Lipid and glucose metabolism under insulin resistance. Adapted from figure 1 in Ref.106. The blue, red, black arrows represent signaling cascade, inhibitory cascade, and substrate conversion, respectively. Green arrows are used to distinguish the effect of exercise and inflammation.

Pathogenesis of Lipid-induced Insulin Resistance

Under the circumstances of insulin resistance, the primary changes in the nutrient flux are elevated DAG and PKC activity, inactivated insulin receptors, and inflammation. Multiple pathological reasons can cause an increase in intracellular DAG content. Lipodystrophy, which may lead to insufficient healthy fat tissue, will redistribute fat into the liver and muscle (Luzi et al.). For offsprings of type 2 diabetic patients, a low rate of muscular fatty acid oxidation might occur, and induce increased fat content (Morino et al.). Lastly, overnutrition will boost the amount of fatty acid distributed to the liver and muscle. As the amount goes over the capability of beta-oxidation, TAG synthesis, and storage, it will lead to increased lipid content and insulin resistance in the liver and muscle (Weiss et al.).

Mechanism of PKC inhibition of insulin signaling

Increased distribution of fatty acids into the liver and the skeletal muscle will enlarge the intracellular free fatty acids pool and enhance the esterification of fatty acids into TAG. A chronic sn-1,2 DAG elevation is present under this condition. In the skeletal muscle, sn-1,2 DAG activates PKCβ, PKCθ, and PKCδ, while in the liver, mainly PKCε is activated, accompanying with PKCα, PKCβ, PKCζ, PKCι, and PKCδ (Kumashiro et al.; S. I. Itani et al.). As mentioned in the *PKC isoenzymes* section, these PKC isomers kick in the insulin signaling pathway distinctly and cause different levels of effect. For simplification and clarity, the two major PKC isomers were chosen in most related studies: PKCε for the liver and PKCθ for the skeletal muscle.

The inhibitory effect of PKC on insulin signaling is diverse. The direct serine/threonine phosphorylation is the most apparent mechanism and has been well-studied for a long time. Nonetheless, the specific correlation between one PKC isomer and one phosphorylation site has not been revealed until the 21st century. The phosphorylation of IRS1 serine/threonine sites blocks IRS1 tyrosine phosphorylation and downstream activation of the Akt pathway. However, the fact that IRS1 can be phosphorylated at more than 50 serine/threonine sites and one site can be phosphorylated by multiple stimuli complexes the question. In 2004, PKCθ was proven to phosphorylate IRS1 on Ser1101 by Yu Li et al. and a mutation on Ser1101 protected IRS1 from PKCθ and restored insulin signaling (Y. Li et al.). In acute muscular insulin resistance, an increase in PKCθ translocation and IRS1 Ser1101 phosphorylation was observed (Szendroedi et al.). Ser1101 can also be phosphorylated by TNF alpha, fatty acids, amino acid-activated kinase S6 kinase 1 (S6K1), and phorbol esters (Tremblay et al.; Y. Li et al.). However, the modulations on other IRS1 phosphorylation sites Ser307 and Ser302 didn't result in the expected effect on insulin signaling, showing that the phosphorylation regulation of IRS1 might be a combination of multiple stimuli (Copps, Hançer, et al.; Copps, Hancer, et al.). Other than IRS1, PKCθ phosphorylates GIV on the Ser1689 site and decreases PI3K-Akt signaling (López-Sánchez et al.). A mutant in the GIV Ser1689 site can completely inhibit glucose uptake in the muscle (Ma et al.). By phosphorylating PDK1 Ser504 and Ser532 in myotubes, PKCθ can stop further PDK1 phosphorylation and activation on Akt (Wang et al.). PKCε displays a more direct and one-way mechanism: phosphorylation on insulin receptor Thr1160 in the kinase activation loop, therefore destabilizing it and inhibit insulin receptor kinase activity (Petersen et al.).

Question and conflicts on PKCε

However, the direct inhibition effect of PKCε on the liver insulin signaling has been challenged by experimental results of liver-specific deletion of PKCε. Previous experiments of whole-body PKCε knockout in mice exhibited protection from lipid-induced insulin resistance and impaired glucose-stimulated insulin secretion in the pancreas (Schmitz-Peiffer). Nevertheless, controversial observations keep questioning the importance of PKCε in hepatic insulin signaling. It is observed that in a high-fat diet, hepatic insulin resistance and glucose intolerance occur before the defect in hepatic insulin signaling (Raddatz et al.). Also, the deletion of PKCε does not affect tyrosine phosphorylation of insulin receptor and Akt activation in hepatocyte (Schmitz-Peiffer et al.). Experiments showed that Akt could be fully activated under a 40% reduction in insulin receptor protein levels, or deficient in PI3K, and 85–90% reduction in Akt still allowed normal downstream signaling process. The spare space of Akt activation, further, raised questions on the hypothesis that PKCε regulates glucose homeostasis through hepatic insulin signaling (Schmitz-Peiffer).

Recently, tissue-specific deletion of PKCε was experimented by Amanda and his colleagues. Unexpectedly, mice with liver-specific deletion of PKCε was not protected from high-fat-diet (HFD) induced hepatic insulin resistance or glucose intolerance, but deletion in adipose tissue improved glucose intolerance with altered adipocyte protein phosphorylation, hepatic gene expression, and TAG storage (Amanda Brandon et al.). Such divergence illustrates the fact that hepatic deletion results in minimal effect in hepatic insulin resistance compared to whole-body deletion, suggesting the mechanism of PKCε on glucose metabolism actually happens somewhere other than the liver. PKCε might contribute to the crosstalk between the adipose tissue and the liver. The exact

mechanism of how PKCε influences adipose tissue and thereby regulates hepatic glucose metabolism and insulin signaling still needs more research, but some possible mechanisms have been proposed. First, PKCε can increase the phosphorylation of proteins involved in cell adhesion and endosome functions, resulting in the accumulation of larger adipocytes. Second, PKCε will increase secreted adipokines from adipose tissue, affecting glucose production in the liver. Lastly, PKCε also enhances lipolysis and provide more fatty acids and glycerols to the liver, promoting gluconeogenesis (Schmitz-Peiffer).

Adipose tissue regulates hepatic insulin sensitivity

The negative effect of adipose PKCε activation on whole-body insulin sensitivity is only a minor part of the crosstalk between adipose tissue and whole-body insulin resistance. Like hepatic and muscular insulin resistance, adipose insulin resistance is revealed by suppressing insulin's ability to activate glucose uptake, lipid uptake, and suppress lipolysis (Samuel and Shulman). The glucose uptake of white adipose tissue (WAT) only contributes to 5~10% of whole-body glucose uptake, but the GLUT4 inhibition in mice adipose tissue can lead to hepatic and muscular insulin resistance with unchanged adiposity or BMI, indicating an indirect systemic effect of adipose tissue on whole-body insulin signaling (Samuel and Shulman). Such influence is exerted mainly via the release of substrates during adipose lipolysis, secretion of signaling molecules, and inflammation.

Adipose lipolysis plays a vital role in whole-body insulin sensitivity. As the major sources of NEFAs in the circulation, adipose lipolysis generates fatty acids and glycerols, promoting hepatic gluconeogenesis and driving hyperglycemia. In models of lipase deficiencies, hepatic and muscle lipid content was reduced, and insulin sensitivity was improved (Morigny et al.). The inhibition of

adipose lipolysis normalizes hepatic glucose metabolism (Perry et al.). Compared with other tissues, adipose tissue is extremely sensitive to insulin, and modest insulin resistance will result in a great increase in lipolysis and worsen whole-body insulin sensitivity, according to the dose-response curve for lipolytic suppression by insulin (Petersen and Shulman, "Mechanisms of Insulin Action and Insulin Resistance"). Other than lipolysis, the inhibition of insulin activity in adipose tissue also downregulates glucose intake and ChREBP activation. Similar to in the liver and muscle, ChREBP in adipose tissue stimulates lipogenic gene expression and reduces lipid delivery to other tissues. Many beneficial substrates for insulin sensitivity and glucose tolerance are released during the de novo lipogenesis, like monomethyl branched-chain fatty acids. Glucose uptake in adipose tissue also produces G3P, which facilitates lipogenesis. Another mechanism of adipose regulation on whole-body insulin resistance is through the release of adipocytokines and lipokines. Two major lipokines are palmitoleic acid, which can enhance hepatic and muscular insulin sensitivity and block fat accumulation in the liver, and fatty acid esters of hydroxy fatty acids (FAHFAs), which improve glucose tolerance and reduce adipose inflammation (Cao et al.; Yore et al.). Adipocytokine, by contrast, is a vast family, including leptin, adiponectin, interleukin-6 (IL-6), retinol-binding protein 4 (RBP4), tumor necrosis factor-alpha (TNFα), etc. Adiponectins, as they bind to receptors in the skeletal muscle and the liver, will reduce gluconeogenesis and enhance fatty acid oxidation (Díez and Iglesias). Other adipocytokines will be discussed in the next section.

The role of leptin in lipid-induced insulin resistance

Among all the hormones produced by adipose tissue, leptin is the most abundant. It has a multidimensional influence in insulin resistance, which can be either central, meaning effect on the

hypothalamus, or peripheral, on sites other than hypothalamus (Paz-Filho et al.). By activating receptors on the hypothalamus, leptin suppresses appetite and increases energy expenditure, in the form of metabolism, physical activity, and thermogenesis, by decreasing expression of neuropeptide Y and agouti-related peptide (AgRP), and increasing expression of anorexigenic peptide α-MSH (Su et al.; Pan et al.). Leptin receptors are also expressed in peripheral tissues, including the liver, muscle, adipocytes, pancreas, kidneys, gut, etc. In human and mice islets, leptin has been shown to have an inhibitory effect on insulin secretion (Ceddia et al.). In adipose tissue, leptin stimulates lipolysis and inhibits insulin action on glucose uptake (Frühbeck et al.). Hepatic insulin action is also modulated by leptin in controversial ways that leptin attenuates tyrosine phosphorylation of IRS1 but increases PI3K activity (Cohen et al.). Leptin may increase muscular glucose uptake independent of insulin action, but multiple experiments show that such an effect is mediated by the hypothalamus, possibly through the activation of AMPK (Marino et al.).

On the other hand, leptin can directly stimulate fatty acid oxidation in the skeletal muscle, independent of the central nervous system (Margetic et al.). Under insulin resistance and hyperinsulinemia, there is an increased level of leptin in the plasma and gene expression in the adipose tissue, but still, the leptin failed to control obesity even with the treatment of leptin (Pan et al.). Such ineffectiveness of leptin is called leptin resistance and is found to be proportional to an individual's adipose mass and BMI. Mechanisms that might explain leptin resistance include damaged transport of leptin through the blood-brain barrier, weakened leptin activity, ER stress, inflammation, etc (Pan et al.). Leptin's loss of function will deteriorate insulin sensitivity and result in metabolic dysfunction.

Adipose inflammation

Aside from energy storage, adipose tissue acts as an active regulator in immunity and inflammation by secretion of pro-inflammatory and anti-inflammatory cytokines. Obesity, marked by an increased amount of adipose tissue, is always accompanied by chronic low-level inflammation and elevated pro-inflammatory cytokine secretion (Lee et al.). Adipocyte hyperplasia and hypertrophy both contribute to adipose tissue expansion, and such expansion results in various changes that might induce inflammation, including adipose cell death, hypoxia, increased ER stress, more chemokines, and altered fatty acid flux (Sun et al.). First, the cell death rate rises dramatically in obesity due to the cytotoxic effects of adipocyte hypertrophy. Obesity-associated adipose cell death, unlike normal apoptosis, exhibits a combination property of necrosis and apoptosis. Those necrotic adipocytes will attract macrophages to form crown-like syncytia (multinucleated cell) around them, a hallmark for chronic inflammation (Cinti et al.). Macrophages in adipose tissue were estimated to have a 40% increase in obese mice compared with lean mice (Weisberg et al.). Second, clinical observations implied that in obesity, adipose tissue is poorly oxygenated (Kabon et al.). Hypoxia can upregulate many pro-inflammatory adipokines like macrophage migration inhibitory factor (MIF), IL-6, and vascular endothelial growth factor (VEGF) (Sun et al.). Third, the excess nutrient drives the accumulation of misfolded/unfolded protein, increases ER stress, and therefore activates the Unfolded Protein Response (UPR). UPR can upregulate JNK and enhance inflammation (Fu et al.). Forth, macrophages and hypertrophic adipocytes will induce chemotactic MCP-1/CCR2 pathways and promote the recruitment of monocytes and differentiation in adipose tissue (Kanda et al.). Lastly, in hypertrophic adipocytes, an elevated FFA content is released from lipolysis and activates

the TLR4 signaling pathway, which will stimulate the inflammatory response and macrophage accumulation (Shi et al.).

Among all the pro-inflammatory adipocytokines, TNFα is the dominant mediator in the systemic inflammatory response and a bridge between obesity, inflammation, and insulin resistance development. TNFα is mainly produced by macrophages in adipocytes. The local accumulation of macrophages in obesity, as discussed above, results in overexpression of TNFα. Inhibition of TNFα or TNFα receptors in obese mice protected them from developing insulin resistance (Nieto-Vazquez et al.). After TNFα being produced by macrophages, it will be cleaved by metalloprotease TNF alpha converting enzyme (TACE) localized on the Golgi, and separated into transmembrane TNFα and soluble TNFα, recognized primarily by TNF receptor 2 (TNFR2) and TNF receptor 1 (TNFR1), respectively (Wajant and Siegmund). TNFR1 is expressed in most tissues, and TNFR2 typically presents in immune cells.

The binding of TNFα to TNFRs initiates a series of inflammatory responses. The activation of TNFR leads to conformational changes, dissociates the silencer of the death domain (SODD) protein, and allows the binding of adaptor protein (TRADD) to the death domain. Three pathways are triggered upon TRADD binding, resulting in activation of NF-κB, activation of JNK and MAPK, and cell death (Wajant et al.). TRADD will recruit receptor interacting protein-1 (RIP-1), a serine/threonine kinase, and TNFR-associated factor 2 (TRAF2), an E3 ubiquitin ligase to form a complex and get released from the TNFR. RIP-1 will further attract MEKK-3 and transform growth factor-beta (TGFβ)-activated kinase (TAK1) to activate IκB kinase (IKK). Such complex then phosphorylates IκBα, which normally bind to and inhibit NF-κB (Parameswaran and Patial). NF-κB is released and translocated to the

nucleus to mediate transcription of proteins involved in cell growth and death, development, oncogenesis, inflammatory, and stress responses (Chen and Goeddel). Alternative activation of NF-κB is through NF-kappa-B-inducing kinase (NIK), binding to TRAF2. The stimulation of TNFR also activates apoptosis-signaling kinase-1 (ASK-1), which binds to the TRAF complex. Activated ASK-1 can stimulate MAP2Ks, MEK-4, and MEK-6, and further activate c-Jun N-terminal kinases (JNKs) and p38 MAPK (Parameswaran and Patial). They will then initiate transcription factor AP-1 and therefore controls several cellular processes, including differentiation, proliferation, and apoptosis (Ameyar et al.). Multiple other mechanisms are involved in the activation of JNK upstream kinases by TRAF, like MEKK1 and TAK1 (Wu and Zhou). The last TNFR pathway is to trigger cell death. Upon activation, TRADD binds to FADD and recruits cysteine protease caspase-8, which induces cell death (Wajant et al.).

Despite the whole-body effect on cell survival, apoptosis, and inflammation, TNFα is also a decisive role in tissue-specific inflammation, contributing to the development of insulin resistance. According to a patient study in 2008, the TNFα level significantly correlates with BMI, beta-cell function, and insulin resistance (Swaroop et al.). In insulin-sensitive organs like the liver and muscle, TNFα directly inhibits insulin signaling pathways by reducing GLUT4 expression (Olson). Through activation of JNK, TNFα also induces serine phosphorylation of IRS-1 and inhibits downstream signaling of PI3K (Fasshauer and Paschke; Akash et al.). In addition to the ubiquitous regulation on insulin signaling, TNFα modulates lipid metabolism and related protein in adipose tissues, by decreasing fatty acid oxidation in muscle, inhibiting LPL activity, enhancing lipolysis, and decreasing FA uptake to the adipose tissue (Ruan and Lodish; Kern et al.). All these result in an elevated level of free fatty acids in

the plasma and contribute to the development of insulin resistance. Plus, TNFα also suppresses insulin secretion in by TNFα-induced inflammation results in apoptosis of β-cells (Akash et al.).

However, traces of insulin resistance, such as incapable of suppressing adipose lipolysis, appear after only three days of high-fat diet in mice without significant inflammation, implying non-inflammatory responses are the one that initiates adipose insulin resistance (Petersen and Shulman, “Mechanisms of Insulin Action and Insulin Resistance”). Moreover, pharmacologic or genetic suppression of inflammation didn’t result in improved insulin sensitivity (Morigny et al.). These evidences suggest that adipocyte dysfunction might play a more critical role in the early stages of the development of insulin resistance compared with inflammation.

The interplay of hepatic, muscular, adipose insulin resistance

The interplay among hepatic, muscular, adipose insulin resistance on glucose and lipid metabolism is vital for a comprehensive understanding of lipid-induced insulin resistance. Obesity is often the precursor of the onset of insulin resistance. Adipocyte hyperplasia and hypertrophy, and failure of insulin suppression, lead to enhanced lipolysis and release increased free fatty acids and glycerols in the circulation. These free fatty acids and glycerols are distributed to the liver and the skeletal muscle as substrates for TAG synthesis and beta-oxidation. In the lipid metabolism branch, the increased free fatty acids influx in the liver and muscle pushes the esterification of fatty acids into TAG in a substrate-dependent manner, independent of insulin signaling. Glycerols get converted to G3P, one of the starting materials for TAG synthesis, and further increase TAG production. The elevated DAG level activates PKCs and therefore inhibits normal insulin signaling and decreases insulin sensitivity. Then the insulin-stimulated glucose uptake in the muscle was reduced, together

with insulin-induced glycogen synthesis. These two factors weaken the muscle's ability to consume and store glucose in the form of glycogen, thus diverting more glucose into the liver. In the hepatic insulin resistance, hyperglycemia and hyperinsulinemia enhance lipogenesis and VLDL secretion out of the liver.

In the glucose metabolism branch, the free fatty acids are converted to FA-CoA and enter the mitochondria for beta-oxidation, which generate an elevated level of acetyl-CoA inside the mitochondria. Acetyl-CoA, in turn, activates pyruvate carboxylase to enhance gluconeogenesis, which leads to increased glucose production. Glycerols, in addition to TAG synthesis, also contribute to gluconeogenesis by a substrate-push mechanism. Under insulin resistance, glycogen synthesis is suppressed by decreased activation of GS and decreased inhibition of FoxO1, which originally inhibits gluconeogenesis. The direct and indirect mechanisms together lead to expanded glucose production(Samuel and Shulman).

Conclusion

Upon fatty acid surplus, lipid accumulation in the liver and skeletal muscle will increase the DAG content and thereby enhance the activation of PKC isomers. Such activation can inhibit normal insulin function and increase insulin resistance. On the other hand, overnutrition leads to the expansion of adipose tissue and upregulates adipose lipolysis and inflammation. The fatty acids and glycerols generated from adipose lipolysis further deteriorate lipid and glucose metabolism in the liver and the skeletal muscle. The two-sided effect of excess fatty acids and their interplay demonstrate that the cause of insulin resistance is multidimensional and controlled by various factors.

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Education

Chemical Engineering M.S. /June 2020

John Hopkins University

Relevant courses: Thermodynamics, Transport Phenomena, Interfacial Science, Lab Safety, Metabolism, Software Carpentry, Pharmacokinetics

Biochemistry/Chemistry B.S. /June 2018

University of California, San Diego Overall GPA: 3.43

Relevant courses: Introduction to Medicinal Chemistry, Human Nutrition, Drug Synthesis and Design, Biochemical Structure and Function/Energetics and Mechanism, Pharmacology and Toxicology, Introduction to Computational Chemistry, Public Speaking, Analytical Chemistry, Organic Chemistry, Inorganic Chemistry, Physical Chemistry

Laboratory Experience

Analytical Chemistry Lab (Fall 2016)

- determine the concentration of various elements in seawater by using Argentometric Titrations, ISE electrode, conductivity meter, and atomic absorption;

- use excel to manage a large amount of data and get wanted information.

Organic Chemistry Lab (Summer 2016 to Fall 2017)

- design and run multiple steps synthesis of an organic compound

- use TLC analysis, IR spectrum, GC analysis, and melting&boiling test to identify an unknown

- apply proper purification process to obtain pure compound

Working Experience

Changzhou Siyao Pharmaceutical Co. (Summer 2017)

- as a lab assistant in the drug development department

- get familiar with drug manufacture process and related machines, participate in quality inspection and transcribe contracts.

Neuroscience Lab (Winter 2017 and Spring 2018)

- research on a new drug for Rett Syndrome;

- record neuron activities through calcium imaging and distinguish between neuron and astrocytes.

Autism Research Lab (Winter 2017)

- use Matlab programming to analyze open field test data

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