REVIEW ARTICLE

The Importance of Metabolites in Modulating Insulin Sensitivity

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Abstract

BACKGROUND: Metabolism impairment in obese condition usually initially triggered by inflammation and insulin signaling impairment. The involvement of metabolites, including lipids, amino acids, and ketone bodies, in altering insulin sensitivity has been revealed after massive data sets were provided by the studies regarding metabolomics and lipidomics.

CONTENT: Metabolites were now understood to serve more than just the metabolism products, but also as active signaling molecules including in insulin and immunological actions. Different lipid metabolites can serve as signaling molecules to induce insulin resistance of sensitivity through a similar pathway, and impact on the inflammation status. Branched Chain Amino Acids (BCAA) and many amino acids have been correlated with mitochondrial dysfunction and insulin impairment. Ketogenic diet, supplementation and microbiota transplantation become the current strategies to set a preferable metabolites composition to modulate insulin sensitivity.

SUMMARY: Thousands of metabolites can now be measured using technical and bioinformatics developments. Different types of amino acids, fatty acids, and bile acids are being studied in relation to altered metabolic states, particularly obesity and type 2 diabetes mellitus. A thorough knowledge of the metabolic changes that contribute to insulin resistance might lead to the discovery of new targets for enhancing insulin sensitivity and preventing and treating many metabolic disorders.

KEYWORDS: metabolites, insulin resistance, lipids, amino acids, ketone bodies

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Introduction

Metabolism of nutrients is essential for all organisms' survival, growth, and development, with a coordinated control of several metabolic processes. The canonical insulin signaling cascade, which includes the Insulin Receptor (IR), Insulin Receptor Substrate (IRS) proteins, Phosphoinositide 3-Kinase (PI3K), and AKT, controls glucose, lipid, and protein metabolism. Insulin resistance, which is defined as poor signal transduction and physiologic responses to insulin stimulation, is a key factor in the development of Type 2 Diabetes Mellitus (T2DM). T2DM develops when insulin secretion is no longer able to compensate for insulin resistance. Integration of functional genomics, transcriptomics, proteomics, metabolomics, and lipidomics in the post-genomic age has increased our knowledge of the molecular pathways behind insulin resistance and T2DM. The technology for evaluating metabolomics and lipidomic data sets is quickly improving, allowing us to discover new metabolites that control insulin sensitivity as well as new functions for existing metabolites in this process.(1)

Human metabolic flexibility can simply defined as the capacity to respond or adapt its metabolism according to changes in metabolic or energy demand in many different levels of nutrients availability especially glucose and

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fatty acids, including nocturnal and diurnal fasted and fed conditions, exercise and environmental changes in the habitat. Thus, metabolism impairment which can be a consequence of or are interrelated with insulin resistance and obesity can be explained as metabolic inflexibility. (2) Insulin resistance is a major contributor to metabolic inflexibility in a variety of tissues and organs.(3) Impaired mitochondrial fatty acid oxidation and excess buildup of lipid metabolites such as diacylglycerol and ceramides have been highlighted as important processes driving insulin resistance in the liver and skeletal muscle.

Years before clinically manifested, usually metabolic diseases has already existed. For example, before T2DM was diagnosed as hyperglycemia, or even before insulin deficiency appears, actually pancreatic beta cell impairment has already developed.(4) Current clinical and laboratory predictors of diabetes risk, such as Body Mass Index (BMI), Waist Circumferences (WC), or fasting glucose, can be useful in predicting diabetes risk (5), but they often provide little insight into pathophysiologic mechanisms and the risk progression have been closed enough to the disease. Early identification was needed to deal with earlier prevention or delaying the disease to be started.(6-9)

Recent studies found variations in blood metabolite profiles before and after glucose loading (10-12) as well as in obese compared with lean individuals (13). What have been noticed from those studies is the differences in the quantity of some small molecules such as C3 and C5 acylcarnitines, glutamine and glutamate, and extra amino acids in both groups, suggest that individual risk and onset of T2DM can be predicted by the alteration of those plasma metabolite concentrations.(14) Metabolites can be defined as endogenous organic and inorganic compounds as results of anabolism or catabolism process. They indeed serve as signaling molecules and activate particular receptors to govern hormone production, immunological responses, insulin action, and brain function. Furthermore, determining the functional ramifications of metabolite or lipid change of protein structure and cell membrane dynamics, the sheer number of these metabolites, as well as the interaction between their metabolic pathways and their propensity to change inter-tissue communication will be critical for understanding metabolic illness and developing new treatments for T2DM.(1)

We provide this narrative review to give the current view on the importance of metabolites in insulin signaling process. We hope further studies can be made to find any regiments of diet or supplements to improve insulin sensitivity thus recover metabolism impairment.

Mechanisms of Insulin Receptor Signaling

Insulin and Insulin-like Growth Factor (IGF)-1 signaling act on two closely related tyrosine kinase receptors to maintains glucose, lipid, and energy homeostasis in the liver, skeletal muscle, and adipose tissue. Activation of receptors triggers a chain of phosphorylation events to activate enzymes that regulate many aspects of metabolism and growth. Many different points of regulation or critical nodes controls insulin/IGF-1 signaling either positively or negatively to ensure proper signal duration and intensity. Disruption in these signaling pathways can cause insulin resistance.(15)

IRS is found in almost all mammalian cells, and so most of all mammalian cells react to insulin, makes the insulin signaling become very complex. Insulin performs many roles in our body (Figure 1), such as promotes glucose uptake and protein synthesis in muscle, promotes energy storage in adipose tissue, enhance glucose utilization and triglyceride synthesis in the liver, and promotes anorexigenic and locomotor signals in the brain. Macrophages, endothelial cells, and insulin-producing pancreatic-cells are other insulin target cells having a metabolic role. Furthermore, insulin also activate some key insulin signaling mediators resulting in a cascade of interconnected signaling pathways (Figure 1) (16), including PI3K and AKT, some growth factor receptors, and some mitogenic pathways, such as Growth Factor Receptor-bound Protein 2 (GRB2) and Extracellular Signal-regulated Kinase (ERK) (17). The insulin signaling cascade is made up of reversible enzyme reactions. As a result, there are many phosphatases that stop the action of each kinase triggered by insulin. The distinct post-translational changes of the same target protein might have a variety of biological consequences. These pathways are just as crucial as signaling initiation, but due to their redundancy, they have proven more difficult to study using cellular and genetic methods.(18)

Tyrosine phosphorylation activates IR and IRS proteins, while serine/threonine phosphorylation inactivates them.(15,19) The effects are site-dependent in granular level, but serine/threonine phosphorylation is a fundamental method for fine-tuning, antagonizing, or terminating IR signaling. IRS is phosphorylated by Mammalian Target of Rapamycin (mTOR) and S6 Kinase (S6K) at several residues.(19) Knocking down S6K1 in rats decreases weight gain and improves insulin sensitivity, which is consistent with this theory and suggested that the processes

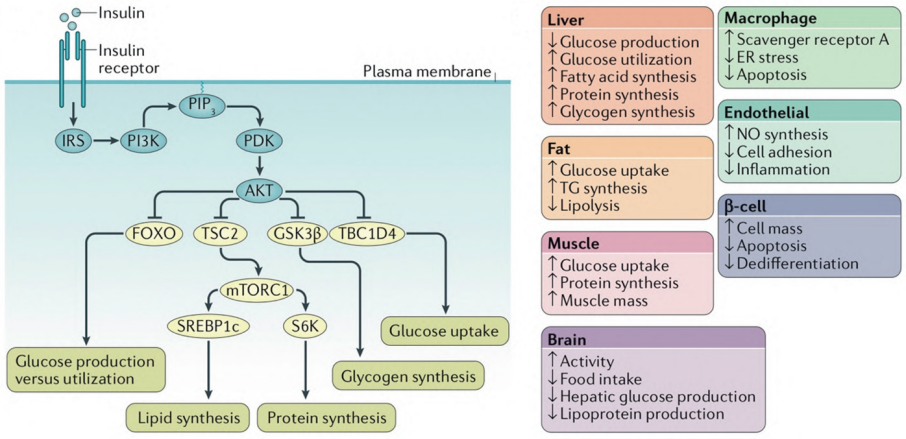

Figure 1. Insulin activates some key insulin signaling mediators. Insulin binding to its receptor activates tyrosine phosphorylation, allow the lipid kinase PI3K binding and synthesize PtdIns, also AKT phosphorylation. Activated AKT goes on to phosphorylate a number of substrates at Ser/Thr residues (FOXO, TSC2, GSK3β, and TBC1D4), which finally affect almost all cells in human body.(16) (Adapted with permission from Springer Nature). PIP_3: phosphatidylinositol (3,4,5)-trisphosphate (PtdIns(3,4,5)P3); PDK: phosphoinositide-dependent kinase; FOXO: forkhead box O; TSC2: tuberous sclerosis complex 2; GSK3β: glycogen synthase kinase-3 β; TBC1D4: TBC1 domain family member 4; mTORC1: mammalian target of rapamycin complex 1; SREBP1c: sterol regulatory element-binding protein 1.

connecting mTOR–S6K signaling to insulin resistance may be independent of IRS.(17) The significance of IRS serine/threonine phosphorylation in these phenotypes, as well as in insulin action in general, then become questioned.

Many factors can disrupt insulin signaling including hormones, inflammatory cytokines, and lipotoxicity, inducing a condition known as insulin resistance. Hormone can be the most essential negative regulator of its own signaling. As insulin levels rise, cell surface receptor downregulation reduces insulin signaling, contributing to its termination. Like other receptor tyrosine kinases, with help of a 12-amino-acid motif on the intracellular region of the IR juxta membrane domain, IR undergoes ligand-induced internalization, lysosomal destruction, or recycling back to the cell surface.(16,20) This provides a solid suggestion that insulin resistance associated with hyperinsulinemia. Because this process is solely dependent on plasma insulin concentrations, it is quickly reversed when insulin levels decrease, as in fasting.(21)

Inflammatory cytokines start a second pathway that leads to inhibitory phosphorylation (22), this can be triggered when macrophages infiltrate adipose tissue such as in obesity (23,24). Macrophages release pro-inflammatory cytokines such as Tumor Necrosis Factor (TNF)-α, Interleukin (IL)-1, and IL-6, and activate serine kinases in adipocytes such as IB Kinase (IKK), c-Jun N-terminal Kinase (JNK), S6K, and mTOR in a paracrine way.(25) These kinases phosphorylate IRS1 in an inhibitory manner, inducing insulin resistance in adipocytes. Activation of inflammatory pathways has been shown to impair glucose metabolism and create systemic insulin resistance.(25,26)

Lipotoxicity is another mechanism that causes IR antagonism. By phosphorylating Thr1160 in IR35's activation loop, diacylglycerol-dependent activation of Protein Kinase C (PKC) hinders IR autophosphorylation. Mutant animals with this phosphorylation site mutation are protected against diet-induced insulin action impairment in the liver (27) but not in the muscle, where diacylglycerol effects may instead be mediated by PKC36 (28,29). Ceramide buildup can affect AKT function by boosting the activity of PKC, which modifies AKT membrane location, and Protein Phosphatase 2A (PP2A), in addition to diacylglycerol.(30,31)

PI3K regulates the plasma membrane concentrations of the lipid second messenger PtdIns-3,4,5-P3 not just during synthesis but also during localization and degradation. Lipid phosphatases like Phosphatase and Tensin Homolog Deleted on Chromosome 10 (PTEN) and Src Homology 2-containing Inositol 5-Phosphatase 2 (SHIP2) inhibits insulin signaling by dephosphorylate PtdIns-3,4,5-P3 (32), while Protein Tyrosine Phosphatase 1B (PTP1B) dephosphorylating tyrosine residues on IR and IRS proteins. Animals ablated with PTP1B have higher insulin sensitivity.(33-35) PTP1B phosphorylation on IR and IRS seems to give major effect on the central nervous system (36-38) compare to peripheral tissue (39-41). PTP1B inhibits leptin signaling by interacting with Janus Kinase 2 (JAK2). PTP1B has the potential to be an integrative node in signal transduction underpinning common biological effects between the two hormones since the activities of leptin and insulin partially overlap.

IR/IRS, PI3K, and AKT signaling nodes meet the requirements to be referred as 'critical nodes' (Figure 2).

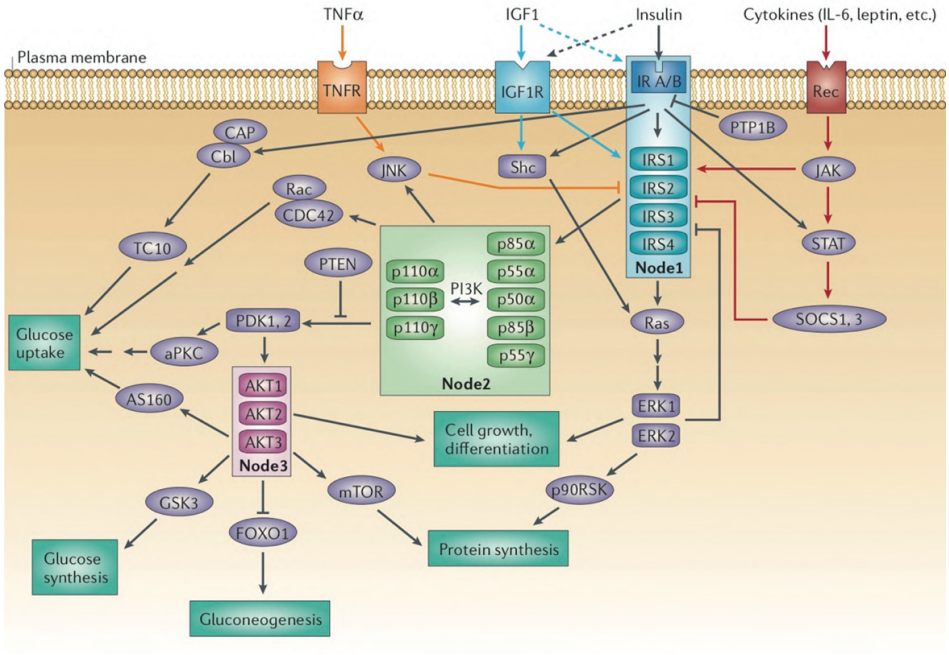

Figure 2. Critical nodes in the insulin-signaling network. (42) (Adapted with permission from Springer Nature). TNFR: tumor necrosis factor receptor; IGF1R: IGF1-receptor; CAP: c-Cbl associated protein; CDC42: cell division control protein 42 homolog; GSK3: glycogen synthase kinase-3; STAT: signal transducer and activator of transcription; SOCS1,3: suppressor of cytokine signaling 1,3.

The insulin-signaling network is a well model of important nodes, because the molecular interactions between many of its components have been verified *in vitro* and *in vivo* using genetic and biochemical techniques. Those studies show the fundamental characteristics of a critical node. Critical nodes are essential for ligand's biological activities and crosstalk sites with other signaling systems. They consist of numerous molecular isoforms that are involved in divergent signaling in a strong regulated way, either positively or negatively.(42)

Insulin Resistance as The Risk of Metabolic Disorders

A well coordination between glucose production (glycogenolysis and gluconeogenesis) in the liver and disposal (via glycogen synthesis and glucose metabolism) in skeletal muscle manage the homeostasis of glucose. Insulin resistance is defined as a reduction in a target cell's metabolic response towards insulin, or a diminished lowering impact of circulating or injected insulin on blood glucose levels at the whole-organism level.(43) It's now recognized that insulin signaling impairment is a hallmark prior to many metabolic disorders and their comorbidities include kidney failure, neuropathy, retinopathy, vascular morbidities, and ischemic heart disease.(44)

Insulin was produced by the pancreas' β-cells at feeding to reduce hepatic glucose production while boosting glucose absorption into muscle and adipose tissue. The insulin-sensitive Glucose Transporter (GLUT)4 mediates glucose absorption in muscle and fat, while the glucose transporter GLUT2 releases glucose in the liver. The important protein kinase AKT is activated by the principal canonical insulin signaling cascade, which is needed for maintaining blood glucose concentrations (15) and control glucose homeostasis such as glucose transport in adipocytes and muscle (37,38), inhibition of hepatic gluconeogenesis (45,46), and cell-autonomous activation of hepatic lipogenesis (45,47).

A coordinated tyrosine phosphorylation and dephosphorylation was activated when insulin bind with the IR in liver or muscle. In skeletal muscle, this bind causes IRS1 to be phosphorylated. IRS1 binds to and activates PI3K, which stimulates GLUT4 translocation to the plasma membrane and glucose absorption into skeletal muscle through signaling intermediates. In humans (48) and rodents (49,50), lipid infusion impairs IRS1 tyrosine phosphorylation and PI3K activation in muscle, suggest an intracellular fatty acid-derived signal (49,51) can defect insulin signaling and glucose transport. The impairment of insulin function also involved the transcription factor FOXO1 activation in the liver which increases gluconeogenesis.(52)

Obese with high levels of triglyceride (TAG) do not appear to associate with insulin resistance, but with Diacylglycerol (DAG) by activating PKC isoforms.(53,54) Traditional PKCs (cPKCs: PKC, PKCI, PKCII, and PKC), which require DAG and Ca^{2+} for activation, novel PKCs (nPKCs: PKC, PKC, PKC, and PKC), which require DAG for activation, and atypical PKCs (aPKCs: PKC and PKC), which do not require DAG or Ca^{2+} for enzymatic

activity. One study showed that transient increases in muscle DAG content can induce PKC and promotes insulin signaling inhibition via serine–threonine kinase cascade. (55,56) Furthermore, mice lacing with PKCθ (57), and mice carrying Ser→Ala mutations in key IRS1 residues prevent serine hyperphosphorylation of IRS1; both types of mice were protected from lipid-induced insulin resistance in muscle. IRS1 at Ser 1101 is a target of PKCθ, which suppresses insulin signaling, according to further *in vitro* investigations.(58)

Insulin resistance can happen in muscle and liver, even in the absence of peripheral and visceral adiposity. Ectopic intracellular lipid accumulation cause by the spillover of exceeded level of energy intake can induce insulin resistance in muscle and the liver (Figure 3). The excess of intracellular energy in the form of DAGs, the lipid-derived metabolites, causes PKCε activation in muscle and PKC activation in the liver, as well as insulin signaling suppression in both organs. Muscle cell lipid content indicates a net balance between fatty acid intake and mitochondrial fat oxidation rates. Acquired mitochondrial dysfunction become a significant predisposing factor for ectopic fat accumulation and insulin resistance in the elderly in this matter. This theory might explain the insulin resistance linked to obesity, age, lipodystrophy, prediabetes, and T2DM, as well as the reversal of insulin resistance and diabetes following weight reduction and thiazolidinedione medication.(59)

Exercise, weight reduction, and thiazolidinediones have all been shown to enhance insulin action in this paradigm. Furthermore, boosting mitochondrial uncoupling

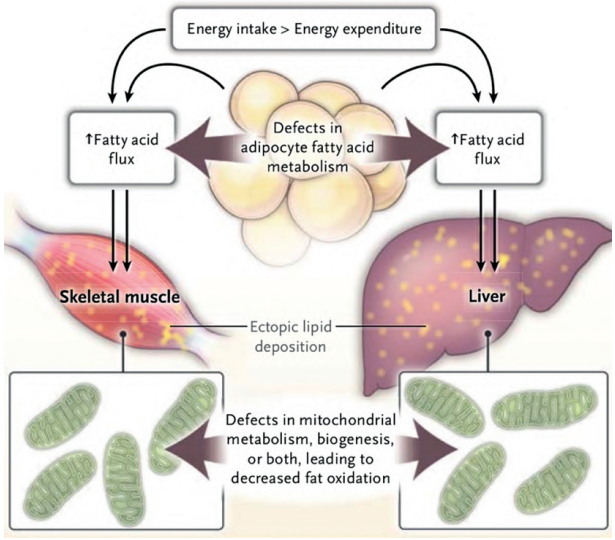

Figure 3. Mechanisms of Increased Ectopic Lipid Deposition in the Liver and Skeletal Muscle (Adapted with permission from Massachusetts Medical Society, 2014).(59)

to increase hepatic energy expenditure might be a unique method for treating the associated epidemics of nonalcoholic fatty liver disease, metabolic syndrome, and T2DM. Insulin resistance raises the risk of cancer and promotes glucose intolerance and T2DM with related comorbidities.(60) The etiology of insulin resistance, on the other hand, is complex and multidimensional, including both cell-autonomous pathways and inter-organ communication. Though the Akt pathway is often impacted, systemic insulin resistance often occur downstream or independently of insulin signaling to the protein kinase Akt, so further studies were needed to find one solid agreement between different results.(61-66)

Metabolomics and Metabolic Diseases

Metabolomics is the large-scale study focus on the quantification of thousands of different metabolites in a biological system, using advanced instrument technology such as mass spectrometres coupled with liquid or gas chromatographies. Today's most modern nontargeted metabolomics techniques (also known as "shotgun" metabolomics) can identify up to 10,000 distinct spectrum characteristics in a single biological material.(67-69) As a result of these advancements, many metabolic diseases can be “mapped”. Metabolomics have some advantages over other "-omics" technologies, since humans are currently thought to have around 6,500 distinct small molecule metabolites (70), and more sensitive new measuring methods are increasingly revealing more number of chemical species over time (67).

We understood about the cardiometabolic disorders link, where disturbed lipid homeostasis contribute the risk for cardiovascular diseases. Metabolomic testing measure specific lipid metabolites widely tested in clinical laboratories, such as triglycerides, cholesterol, and total Non-Esterified Fatty Acids (NEFAs) for more specific risk prediction. Targeted metabolomics also found that a set of metabolites in body fluids including BCAAs, aromatic amino acids (Phe and Tyr), Glu/Gln, Met, and C3 and C5 acylcarnitines have a strong correlation with insulin resistance.(13) These too contribute to cardiometabolic diseases.(71-73) Additionally, during follow-up, the cohort participants who transitioned from prediabetes to diabetes had an increase in BCAAs and a decrease in glycine.(74) Importantly, these findings have been verified using large cross-sectional populations and metabolomics.

Early detection even before the onset of disease will be very helpful for diabetic individual, since preventive

strategies have been established and the end-stage comorbidities can be avoided. Although established risk indicators like BMI and fasting glucose can help predict future diabetes risk, not all obese develop into diabetes. However, diabetes genetic polymorphism currently add a little amount to risk assessment.(75-77) A panel of more than 60 amino acids metabolites were measured once while fasting, and the results were compared to conventional risk variables (such as BMI, dietary patterns and fasting glucose). The result showed that branched-chain and aromatic amino acids appeared as predictors of future diabetes development. Further research is needed to see if plasma amino acid levels may assist select candidates for diabetes risk reduction therapies and to understand the molecular processes by which particular amino acids might cause T2DM.(14)

Lipid Metabolites in Metabolic Disorders

More than seven decades ago fatty acids were first discovered to Signal Protein-Coupled Receptors (GPCRs) (78,79), and induce various intracellular signals. Later, more lipids such as fatty acids, DAG, sphingolipids, and fatty acid esters of hydroxy fatty acids found to impact on insulin signaling thus affecting the metabolism (Figure 4).

Saturated fatty acids (SFAs) impair insulin sensitivity in animals and cell culture.(80-83) SFA-induced insulin resistance in animals and cultured cells via many pathways. SFAs drive downstream pro-inflammatory processes by activating Toll-Like Receptors (TLRs), particularly TLR4, which is found on immune cells, in White Adipose Tissue (WAT), and in the liver.(80-84) SFA-induced insulin resistance is assumed to be directly mediated through TLR4-mediated activation of the inhibitor of Nuclear Factor-kappaB (NF-κB) IKK-β and JNK pathways, most likely via MYD88.

SFA can induce insulin resistance when SFAs affect the membrane distribution of the proto-oncogene tyrosine-protein kinase SRC, resulting in its activation and stimulation of JNK signaling, which suppresses AKT in the insulin cascade.(85) The protein NLR Family Pyrin Domain Containing 3 (NLRP3) inflammasome pathway contained in NACHT, LRR, and PYD domains can be activated by palmitic acid to result in 5′-AMP-activated Protein Kinase (AMPK) inactivation and reduce insulin sensitivity, as well as impaired autophagy and the generation of mitochondrial Reactive Oxygen Species (ROS).(86) Ceramides, DAGs, and phospholipids are all substrates for SFAs, which may disrupt insulin action as well, demonstrating that SFAs can reduce insulin sensitivity in various ways.

Polyunsaturated Fatty Acids (PUFAs), particularly the important long-chain omega-3 PUFAs Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA), were known to improve insulin sensitivity in animals (87-89), but still debatable in human (87,90). Plant-based oil consumption, particularly those found in olive oil, has been linked to lower cardiovascular mortality and increased insulin sensitivity because they contains Monounsaturated Fatty Acids (MUFAs).(91-94) High Fat Diet (HFD)-fed mice with oleic acid showed insulin sensitivity improvement by increasing IL-1, suppressing JNK and NF-κB.(95) This is because oleic acid act as an endogenous ligand of the Peroxisome Proliferator-Activated Receptor-α (PPAR-α).(96,97)

Another lipid metabolite is palmitoleate, can be found from animal fat or plant seed oils, but it may also be made from scratch using *de novo* lipogenesis. Palmitoleate activates AMPK and G-Protein Coupled Receptor 120 (GPR120) but not PPAR-α (98-100), reverse macrophage polarization into M2, although these results were still contradictive in different studies.(101-103) Fatty Acyl Esters of Hydroxy Fatty Acids (FAHFAs) are structurally unique lipids produced by humans, animals, and plants that have favorable metabolic and anti-inflammatory properties. (104) More than 1,000 FAHFAs and more than 20 FAHFA families are estimated to exist in nature.(104-106) Each family can be differed by its ester link position between acyl chains.(106)

Palmitic Acid Esters of Hydroxy Stearic Acids (PAHSAs) are found in the greatest concentrations in white and brown adipose tissue in mice, compared to other tissues; adipose tissue has eight PAHSA isomers, whereas other tissues include at least three, and mouse and human serum contain six.(104) Insulin-resistant mice and humans have lower amounts of PAHSA in their serum and subcutaneous WAT, and serum PAHSA levels correspond with insulin sensitivity in.(104) In chow-fed mice that are glucose intolerant due to age or insulin-resistant mice on an HFD, a single oral dosage of 5-PAHSA or 9-PAHSA improves glucose tolerance. PAHSAs increase glucose-stimulated insulin and Glucagon-Like Peptide 1 (GLP1) secretion in elderly mice on a chow diet, but same secretory effects are not detected in HFD-fed animals. GLP1 increases insulin production, which lowers blood sugar levels.

Ceramides are precursors of the cell's most important sphingolipids, such as sphingomyelin and gangliosides. Some studies showed inflammation-induced insulin resistance by ceramide via NLRP3 inflammasome

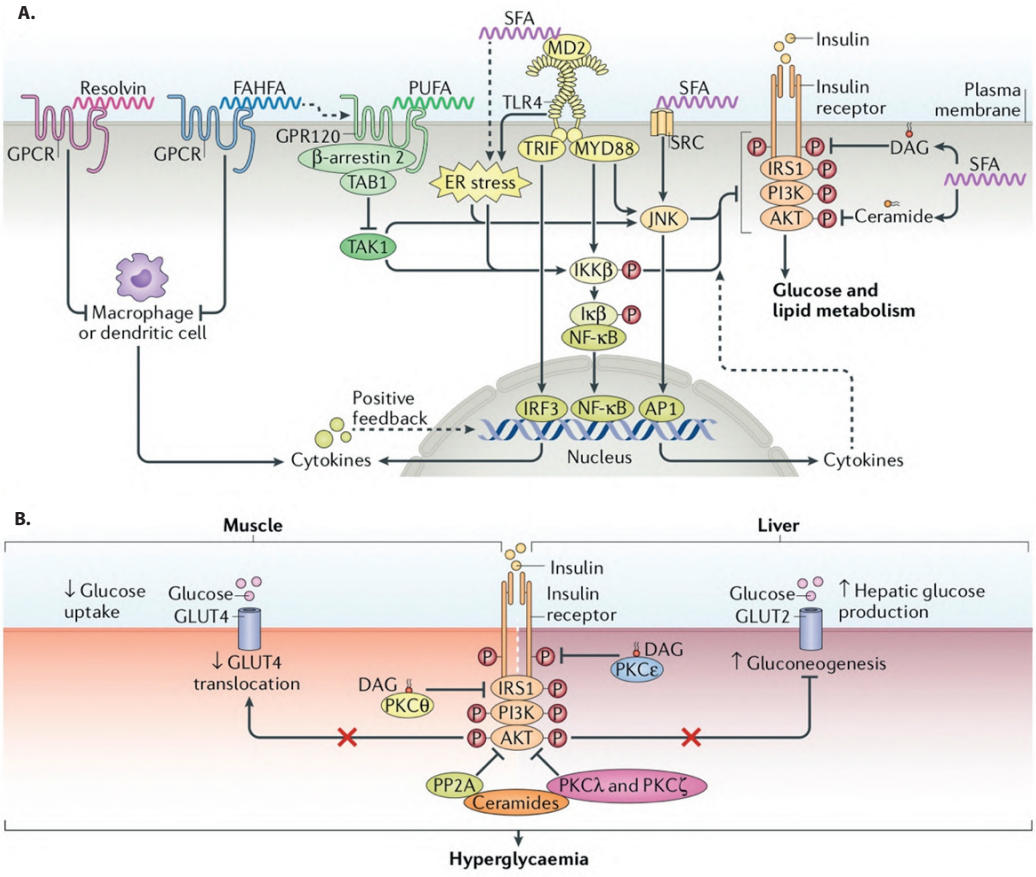

Figure 4. Lipid metabolites as signaling molecules that regulate metabolism, induce insulin sensitivity or insulin resistance. A: Insulin sensitivity induced by lipid metabolites; B: Insulin resistanceinduced by lipid metabolites.(1) (Adapted with permission from Springer Nature).

activation, and can also lead to pancreatic injury.(107-109) Fibroblast Growth Factor (FGF)21, like adiponectin, causes a slew of hypermetabolic reactions that boost glucose and fat consumption.(110) The Scherer group has postulated that this favorable metabolic state is driven by a FGF21–adiponectin–ceramidase axis.(111) FGF21 was discovered to reduce ceramide levels while concurrently promoting adiponectin expression. Adiponectin deficiency rendered mice resistant to FGF21-mediated metabolic stimulation and ceramide reduction.(30,112)

The link of dyslipidaemia to obesity that leads to poor clinical outcomes is complicated and involves a vast number of lipid species, whose homeostasis is regulated by a variety of metabolic pathways. Many sphingolipid and phospholipid metabolites have been identified as important factors in the development of insulin resistance, type 2 diabetes, and cardiovascular disease. Lipidomics, a relatively recent area, has aided in the definition of these interactions and has revealed new research and treatment options.(113)

Amino Acid Metabolites and Insulin Resistance

More than four decades ago, a link between high blood amino acid levels and obesity and insulin resistance was observed.(114) The development of metabolomics analysis and mass spectrometry-based isotope labeling techniques has reignited interest in this field. A panel of lipid metabolites and amino acids can be useful in predicting insulin resistance earlier. BCAAs (leucine, isoleucine, and valine) is the most studied amino acids metabolites associated with insulin resistance.(13,14,115)

BCAA actually have benefit in nutrient signaling effects, but since many studies found the increase level of BCAAs in obesity and metabolic syndrome, then some paradox opinions raised if BCAA roles as the cause, or just the marker of this impairment.(116) Under normal conditions of energy homeostasis, BCAAs may promote improved glucose uptake/insulin sensitivity. On the other side, when

there is excess energy and abundance metabolites including BCAAs, cells lose the optimum ability to degrade those metabolites, and those metabolites became accumulated (Figure 5).(117) Thus, higher level of BCAAs might be a biomarker for the onset of metabolic syndrome.(118)

Alternatively, some theories also raised that persistent high level of BCAAs become toxic and contribute impairing insulin signaling. Leucine, isoleucine, and valine are involved to stimulate mTOR leading to enhanced protein synthesis, and increased levels of BCAAs results in uncoupling of insulin signaling at an early stage. BCAAs are closely connected to lipid metabolism, and the accumulation of mitotoxic metabolites causes an increase in acylcarnitine buildup in muscle (13), potentially exacerbating mitochondrial dysfunction (119).

Another plasma amino acids associated with obesity and insulin resistance were Aromatic Amino Acids (AAAs), methionine, carnitine, choline and betaine.(14,118) Subjects who performed gastric bypass surgery showed better metabolic features when they have lower AAAs.(120) However, further studies were needed to confirm if AAA levels connected to insulin resistance. Methionine serves as a methyl donor for DNA, histones, and other proteins.(121-124). Methionine restriction in mice decreases adiposity and improves insulin sensitivity and fatty liver, while in humans, restricting methionine consumption results in weight loss despite increasing calorie intake, with a 58 percent drop in serum methionine.(125) Within the first 12 hours of methionine restriction, FGF21 levels in plasma and liver increase tenfold, and lasts for several weeks. (122) However, FGF21 induction may be required for the effects of methionine restriction on energy expenditure but not on weight loss.(123) Methionine restriction can induce adiponectin secretion and improved insulin sensitivity indirectly.(126)

Carnitine is a tiny water-soluble vitamin-like molecule that plays a variety of important roles in intermediate metabolism. The principal physiological role is associated to cellular energy production activities via Long Chain Fatty Acids (LCFA) transfer from the cytosol into the mitochondria, where they are degraded by β-oxidation. This function is important since neither free LCFA nor their Coenzyme-A esters can traverse the inner mitochondrial membrane on their own, but only can be transported in carnitine ester form. (127) L-carnitine have anti-inflammatory and antioxidant properties (128-130), improving insulin sensitivity, protein nutrition, dyslipidemia, and membrane integrity (131). L-carnitine level in plasma and tissue are relatively low, and tightly regulated by carrier-mediated gastrointestinal absorption, endogenous biosynthesis, extensive renal tubular reabsorption, and compartmentalization via carrier-mediated transport between plasma and tissue.(132)

Choline and betaine are quaternary ammonium compounds which synthesized *de novo* in our body or obtained from food like eggs, beef, pig, liver, soybean, and wheat germ for choline, because they provide the most methyl groups in the diet, whereas wheat bran, wheat germ, and spinach have the most betaine.(133-134) A S-adenosylmethionine-dependent methylation process catalyzed by phosphatidylethanolamine N-methyltransferase forms phosphatidylcholine (lecithin), the most common choline molecule, endogenously from phosphatidylethanolamine. This is a significant source of choline when compared to food consumption, particularly in premenopausal women.(135) Choline is an epigenetic regulator of gene expression, vital for lipid metabolism,

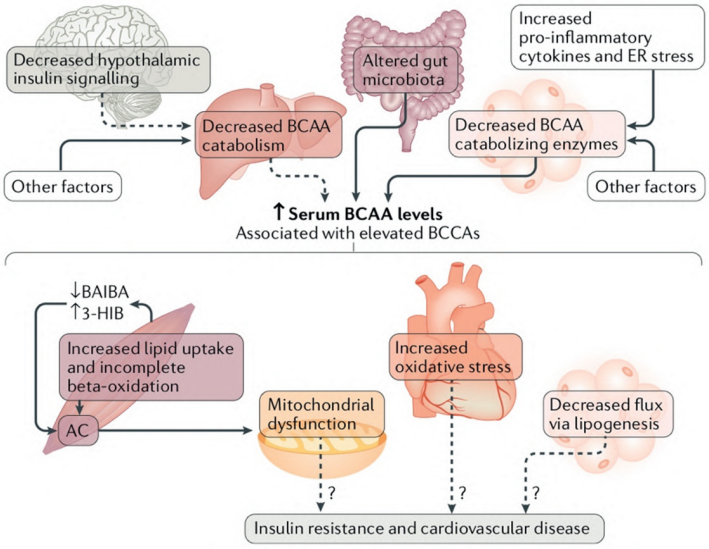

Figure 5. Increased levels of branched-chain amino acids are associated with insulin resistance.(1) (Adapted with permission from Springer Nature).

cell membrane integrity, and nerve function (136) and a precursor of lipoproteins, membrane phospholipids, and the neurotransmitter acetylcholine (133).

Choline oxidation, performed by the mitochondrial enzyme choline dehydrogenase, produces betaine in the kidney and liver.(133,137,138) In humans, betaine serves two purposes. It's an organic osmolyte that builds up in a number of cells, including renal medullary cells, in of hypertonicity body.(139) Betaine acts as a methyl donor for homocysteine remethylation into methionine in the Betaine-Homocysteine Methyltransferase (BHMT)7 reaction.(140) This explains why betaine supplementation reduces plasma Total Homocysteine (tHcy) levels (141) and is inversely linked to plasma betaine levels (142,143). Choline and betaine were shown to have opposite associations with essential metabolic syndrome components, indicating a disturbance of the mitochondrial choline dehydrogenase pathway.(144)

Keton Bodies, A Ketogenic Diet and Insulin Sensitivity

Knowing the roles of metabolites in modulating insulin sensitivity, some strategies have been developed to optimize the metabolites composition in human bodies, including ketogenic diet, supplementation rich in intermediate metabolites and cofactors, and probiotics.

Some condition including starvation, CR, high-intensity exercise, and the low-carbohydrate ketogenic diet can raise the ketone bodies β-hydroxybutyrate (BHB) levels. (145,146) Ketones are produced in the liver during periods of nutritional scarcity and replace the energy for important organs such as brain and heart, and TCA cycle intermediates are prioritized utilized for gluconeogenesis.(46) When liver 3-Hydroxy-3-Methylglutaryl-CoA Synthase 2 (HMGCS2) was knocked down, ketogenesis didn't happen and acetyl-CoA was accumulated, leads to mitochondrial pyruvate carboxylase and gluconeogenesis. In mice with HMGCS2 knockout, HFD result in severe Non-Alcoholic Fatty Liver Disease (NAFLD) and Non-Alcoholic Steatohepatitis (NASH).(147)

Metabolomics profiling, both targeted and nontargeted, has improved our understanding of the metabolic alterations that occur during the transition from Normal Glucose Tolerance (NGT) to T2DM.(148). A variety of metabolite biomarkers have been discovered that potentially predict the onset of diabetes. BCAAs (149), aromatic amino acids (14,150), glycine (151), α-hydroxybutyric acid (α-HB) (152), and Linoleoyl-Hlycerophosphocholine (L-GPC) (150,153) are only a few of them. Ketogenic diets, which contain more than 70% fat calories and less than 10% carbohydrate calories, boost energy expenditure, decrease obesity, and improve insulin sensitivity in mice and humans. (64,154) Although ketogenic diets are helpful for weight reduction, some may find it difficult to stick to them. As a result, unraveling the processes underlying ketogenic diet-induced weight reduction, including metabolite changes, might lead to new targets for obesity therapy.

Microbiota-generated Metabolites

Dietary fiber is categorized into soluble and insoluble. Gut microbiota digest soluble fiber into short-chain fatty acids (SCFAs) acetate, propionate, and butyrate.(155) In both lean (156,157) and obese diabetic individuals (158,159), fiber-rich diets increase insulin sensitivity and glucose tolerance. SCFAs are thought to be involved in the positive benefits of soluble fiber via regulating whole-body energy balance. (160) SCFAs are also signaling molecules that operate as endogenous ligands for the G-protein-coupled receptors Free Fatty Acid Receptor (FFAR)3 and FFAR2, as well as modulators of the epigenome through histone acetylation. (161) Numerous effects are mediated by signaling through these receptors, including the creation of glucagon-like peptide 1 in enteroendocrine cells (162), control of adiposity, and alterations in intestinal transit time (163).

The recently discovered link between gut microbiota makeup and obesity and related illnesses has reignited interest in SCFAs.(155) Obese people's microbiota may have a larger capacity to convert food into energy, than lean people's microbiota (164,165), and many studies have found that HFD have a significant impact on the gut microbiota composition in rats (165-168). Furthermore, a recent study in obese people found that certain microbiome compositions are linked to impaired glucose control.(169,170)

In humans and animals, obesity and insulin resistance are linked to shifting in gut microbial composition. The Firmicutes/Bacteroidetes ratio is commonly used as a marker for obesity dysbiosis. Bacteroidetes mainly produce acetate and propionate, while Firmicutes produce more butyrate, the health-promoting molecule that increase insulin sensitivity, exert anti-inflammatory activities, regulate energy metabolism, and improve leptin gene expression.(171)

Fecal transplanting the microbiota from obese mice or people into germ-free animals changes the amounts of

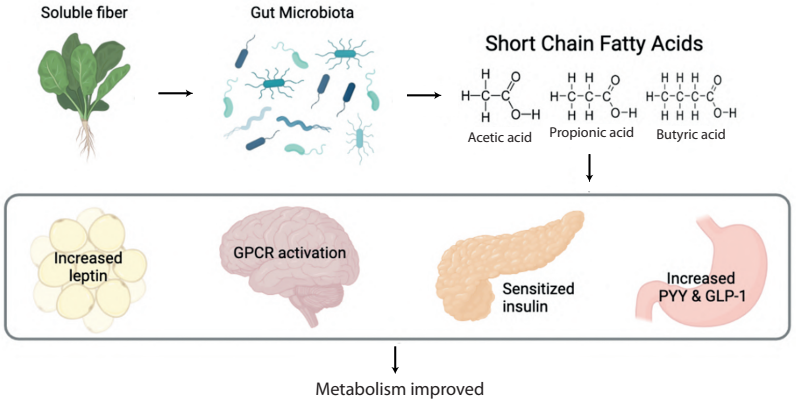

Figure 6. SCFAs improved metabolism via insulin sensitization and hunger inhibition. (created with Biorender.com).

SCFAs and promotes obesity.(172,173) SCFAs (especially propionate) have also been shown to reduce glucose levels because SCFA can activate intestinal gluconeogenesis and sensitizing systemic insulin.(170) While acetate, may pass the blood–brain barrier in both rats and humans (174,175), and send their anorexigenic effects by brain GPCR activation via the periportal neural system. SCFAs also inhibit hunger by increasing the production of leptin from WAT (176,177) and peptide YY (PYY) and GLP1 from the stomach (162,178-180). Therefore, lean, healthy human donors transplantation enhance insulin sensitivity and increase the number of butyrate-producing bacteria in obese people. (181) Figure 6 shows how SCFA can improved metabolism via insulin sensitization and hunger inhibition.

Altered gut microbiota in rodents can increase acetate production and stimulate parasympathetic activity which increase glucose-stimulated insulin secretion, ghrelin secretion, hyperphagia, thus promotes obesity. Hence, increased acetate synthesis as a result of a nutrient–gut microbiota interaction can be a potential treatment for obesity.(182)

In mice models, dietary supplementation of butyrate can prevent and cure diet-induced obesity and insulin resistance in similar mechanism to CR (183,184), which is linked to increased energy expenditure and mitochondrial function induction. Butyrate activity may be mediated by stimulation of PPAR-γ Coactivator (PGC)-1α activity to activate AMPK and suppress the histone deacetylases. Butyrate and its derivatives might be useful in the prevention and treatment of metabolic syndrome in people.(185)

Protein Acetylation in Metabolism

Post-translational modification of proteins is used by organisms ranging from yeast to humans to change the structure and function of proteins, result in metabolites level changes. These metabolites, in turn, serve as direct substrates for post-translational modifications such as phosphorylation, ADP-ribosylation, and acetylation of proteins.(186-188) The protein function and cellular metabolism can be synchronized due to the direct relationship between metabolites and post-translational modification, and this leads to a better understanding of how they affect protein activity and metabolic signaling pathways with the identification of novel signaling roles for intermediary metabolites and cofactors formerly thought to be only co-enzymes, such as NAD^+ and acetyl-CoA.(189) The dynamic function of NAD^+ in sirtuin activity regulation propose a hypothesis about NAD regulation on protein deacetylation.(190)

Acetylation is a reversible process mediated by lysine acetyltransferases (KATs) and deacetylases (DACs) that affects protein activity.(189,191) KATs and DACs may modulate the acetylation of proteins in the insulin signaling cascade as well as other proteins involved in glucose and fat metabolism to affect insulin sensitivity. General Control Non-depressible 5 (GCN5) is a histone acetyltransferase that may regulate hepatic gluconeogenesis in two ways. GCN5 acetylates PGC-1 to suppress its activity in the fed state, resulting in decreased expression of gluconeogenic enzymes and decreased glucose synthesis. (192,193) A comprehensive overview of the role of protein O-GlcNAcylation in insulin signaling, fat and glucose metabolism due to glucosamine-6-phosphate acetylation have been published.(194) Overall, protein acetylation is a key regulator of insulin sensitivity and metabolism, and studies with sirtuins showed prospective results.

Sirtuins (SIRT), particularly SIRT1, can reverse p300-mediated protein acetylation, which is linked to enhanced insulin sensitivity.(195) Mice with SIRT1 heterozygous knockout exhibit hepatic steatosis and impaired energy balance, while overexpression of SIRT1 improve insulin sensitivity and hepatic steatosis.(196-200) SIRT1 in WAT

and liver, but not muscle, modulates insulin sensitivity and glucose metabolism.(196-200) SIRT1 deacetylating PPAR-γ in WAT and turn it into BAT for higher thermogenesis, insulin sensitivity, and glucose metabolism.(201) It also deacetylates PGC-1α and activate FOXO1 to promote hepatic gluconeogenesis.(193,202-204) Under some circumstances, the effect of SIRT1-mediated deacetylation on Farnesoid X Receptor (FXR) and CREB-Regulated Transcription Coactivator 2 (CRTC2) counteract its effect on PGC-1 and FOXO1.(205-307)

Metabolites like zinc and β-hydroxybutyrate, which control acetylation and/or deacetylation directly or indirectly, can also regulate metabolism. A meta-analysis found that zinc supplementation improved glycemic control and lipid metabolism in individuals with T1 or 2 diabetes (208), indicating that there is a relationship between zinc availability and cellular metabolism. Similarly, diets that cause the liver to produce ketones, which give β-hydroxybutyrate, have a variety of positive effects on metabolic and degenerative processes, some of which may be linked to β-hydroxybutyrate's capacity to suppress class I KDACs (209) and activate SIRT1 expression (210). The 'omics research has clearly opened the door to the acetylome, and makes acetylation in regards of insulin signaling and glucose metabolism issue grows. It's tempting to deduce functional results (in this case, insulin action) from changes in protein and enzyme acetylation, and we indeed still need further studies.

Conclusion

Identification of novel molecules that both inducing and interfering insulin signaling will be useful for preventing and treating many metabolic diseases and related disorders. Metabolites can serve as signaling molecules and activate particular receptors to govern hormone production, immunological responses, insulin action, and brain function. Metabolites panel, as well as the interaction between their metabolic pathways and their propensity to change inter-tissue communication, make this a fascinating area for understanding metabolic illness and developing new treatments.

Authors Contribution

AM drafted and wrote the manuscript, NMD edited the manuscript, AW proposed the manuscript topic, supervised, and edited the manuscript.

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