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Metabolite profiles of diabetes mellitus and response to intervention in anti-hyperglycemic drugs

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Type 2 diabetes mellitus (T2DM) has become a major health problem, threatening the quality of life of nearly 500 million patients worldwide. As a typical multifactorial metabolic disease, T2DM involves the changes and interactions of various metabolic pathways such as carbohydrates, amino acid, and lipids. It has been suggested that metabolites are not only the endpoints of upstream biochemical processes, but also play a critical role as regulators of disease progression. For example, excess free fatty acids can lead to reduced glucose utilization in skeletal muscle and induce insulin resistance; metabolism disorder of branched-chain amino acids contributes to the accumulation of toxic metabolic intermediates, and promotes the dysfunction of β-cell mitochondria, stress signal transduction, and apoptosis. In this paper, we discuss the role of metabolites in the pathogenesis of T2DM and their potential as biomarkers. Finally, we list the effects of anti-hyperglycemic drugs on serum/plasma metabolic profiles.

KEYWORDS

diabetes mellitus, metabolites, amino acids, lipids, carbohydrates

1 Introduction

According to the latest statistics from the International Diabetes Federation (IDF), as of 2021, about 537 million adults worldwide had diabetes, and this number is expected to increase to 783 million by 2045, causing 6.7 million deaths and high health costs every year (1). T2DM is the most common type of diabetes mellitus, accounting for about 90% of the total number of diabetes mellitus. It is of great significance to explore the pathogenesis of T2DM and develop precise and reliable prevention and treatment strategies. T2DM is a typical metabolic disease, usually accompanied by the disorder of systemic metabolic networks including carbohydrates, lipids, and amino acids, which is very suitable for metabolomics and lipidomics studies. Metabolites are not only ending products of genome regulation and cellular energy transfer, reflecting biological situations that have occurred or are occurring in the body, but also have multiple functions such as signaling molecules,

immune regulation, and environmental sensors. Thus, the exploration of metabolite changes can reflect the metabolic phenotype of T2DM in a relatively comprehensive way. In contrast to biopsies, blood sample collection is a minimally invasive method with the advantages of rapid, economical and high availability, and is essential for facilitating mapping of disease metabolic profiles and prognostic diagnosis.

2 Metabolite profiles of T2DM

Metabolites are commonly recognized as end products of a wide range of gene transcription and biochemical reactions, and there is growing evidence that metabolites can be involved in disease development as biomarkers (Figure 1) and regulators. When acting as regulators, metabolites have an impact on the pathogenesis of T2DM in at least the following aspects: (1) Metabolites can regulate the downstream signaling pathway of insulin and directly affect insulin sensitivity; (2) Accumulation of harmful metabolic intermediates; (3) Cause organelle dysfunction; (4) Directly or indirectly mediates the inflammatory response of target tissues. A comprehensive understanding of metabolic pathways may be a novel direction for the prevention and treatment of T2DM.

2.1 Carbohydrate

Chronic hyperglycemia is not only a major feature of diabetes, but also a key factor in accelerating its progression and inducing complications. High glucose can damage the mitochondrial aerobic metabolic flux of pancreatic β-cells and reduce insulin content, which may be the pathological basis of the progressive decline of β-cell function in patients with T2DM. Excess fructose can cause a dramatic increase in hepatocyte carbohydrate response element binding protein (ChREBP) activity, which not only mediates changes in circulating triglycerides and high density lipoprotein (HDL) levels, but also is an important upstream regulator of a key enzyme in BCAA metabolism, branch chain ketoate dehydrogenase kinase (BCKDK)/metal ion-dependent protein phosphatase (PPM1K), integration affects BCAA oxidation and lipid

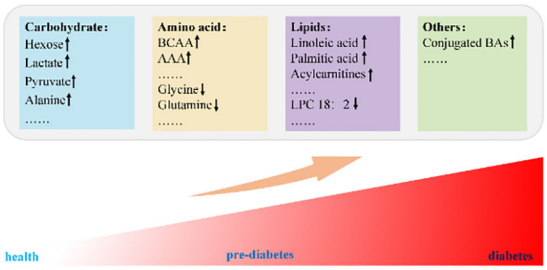

FIGURE 1
BCAA, branched-chain amino acids; AAA, aromatic amino acids; LPC, lysophosphatidylcholine; BAs, bile acids.

metabolism (2). Evidence suggests that overproduction of the subtype ChREBP-β mediates glucose toxicity and subsequent cell death in β-cells. Overexpression of the different subtypes of ChREBP-α enhances glucose-stimulated β-cell proliferation and antagonizes Chrebp-β-cell death mediated by Nuclear factor erythroid 2-related factor 2 (Nrf2) antioxidant pathway (3).

Single-cell sequencing results showed that genes related to oxidative phosphorylation and ATP synthesis were significantly downregulated in the islets of T2DM patients (4), and this phenomenon was verified in animal models. Nearly all glycolytic enzymes were significantly upregulated in the diabetic mouse islets. In contrast, genes, proteins, and BCAA metabolic pathways associated with mitochondrial oxidative phosphorylation were significantly reduced (5, 6). Haythorne et al. have found that the impairment of β-cell function by high glucose is not glucose per se, but mediated by metabolic intermediates associated with increased glycolysis flux, one or more metabolites located between phosphofructokinase (PFK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). By stimulating mammalian rapamycin complex 1 (mTORC1) and inhibiting AMPK activity, preventing pyruvate from entering the TCA cycle, and the imbalance of $NADH/NAD^+$ in mitochondria and cytoplasm exacerbates the accumulation of upstream metabolites of GAPDH, creating a vicious cycle. This may partly explain the impaired oxidative phosphorylation of mitochondria. More importantly, glucose stimulation at 8mM appears to be sufficient to initiate this cycle, suggesting that cumulative impairment of pancreatic function may have already begun in patients with early impaired glucose tolerance (7).

Impaired glucose oxidative phosphorylation metabolic pathways and increased glycolysis flux are determinants of increased lactate levels. In patients at high risk of CVD, plasma lactate, pyruvate, glycerol-3 phosphate, and isocitrate were significantly positively correlated with the risk of T2DM (23%-44% higher for every 1 SD increase) (8, 9), and was associated with various pathological phenomena of T2DM: (1) Increased blood lactate concentration reflects decreased mitochondrial oxidation capacity and is strongly positively correlated with IR index (10); (2) Blood lactate level may reflect liver dysfunction in T2DM patients (9); (3) Blood lactate levels may be indicative of susceptibility to T2DM to some extent (11). However, the effect of lactate as a signaling molecule on disease is complex and may depend on exposure duration and specificity of tissue and organ (Figure 2). Recent evidence suggests that lactate signaling is involved in inflammatory response (12–14), cells proliferation and migration (15), appetite regulation (16), redox homeostasis regulation (17), histone modification (18), and vascular cells damage (19–21). Diabetes is commonly accompanied by oxidation and systemic chronic inflammation. Elevated lactate levels can lead to an increase in $NADH/NAD^+$ ratio, and mitochondria actively oxidize lactate to produce additional ROS accordingly. When antioxidants are out of balance, oxidative damage may be caused. Lactate is one of the main fuels of TCA cycle. Acute lactate exposure can stimulate mitochondrial coupling efficiency and promote bioenergetics of mitochondria in heart, skeletal muscle, and liver (22), while chronic hyper-lactate

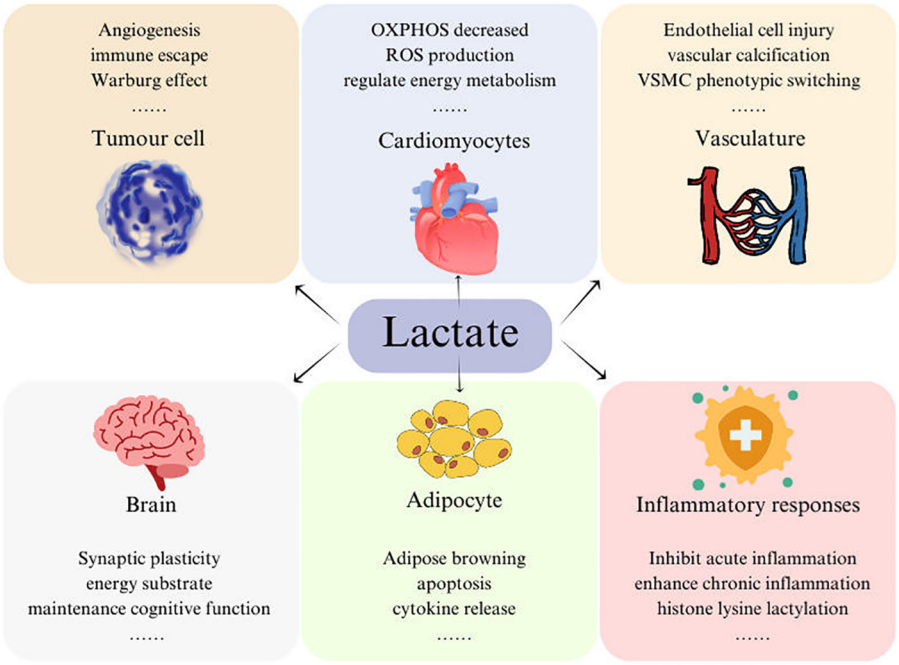

FIGURE 2
Lactate is involved in physiological and pathological processes of various tissues, including cardiovascular disease, immune response, fatty acid metabolism, cognitive function, and tumor cell angiogenesis. OXPHOS, oxidative phosphorylation; VSMC, vascular smooth muscle cell.

exposure may negatively affect mitochondrial respiration rate, reduced metabolic flexibility (23).

Chronic hyperglycemia can also trigger the activation of collateral glucose metabolic pathways, such as polyol, hexosamine, and AGE, resulting in oxidative stress, promoting the transcription of pro-inflammatory factors and apoptosis, and thus contribute to the occurrence and development of diabetes mellitus and its complications. AGE, cytokines and ROS can promote the production of triose phosphate, an intermediate of glucose metabolism, and increase *de novo* synthesis of diacylglycerol (DAG), the activator of protein kinase C (PKC) (24). In the polyol pathway, aldose reductase activated by hyperglycemia reduces glucose to sorbitol, which is further metabolized to fructose by sorbitol dehydrogenase. An increase in plasma fructose concentration is positively correlated with the development of T2DM and can lead to liver insulin resistance (IR) and non-alcoholic fatty liver disease development (25–28). In tissues such as nerves, sorbitol cannot easily cross the cell membrane, so the accumulation of sorbitol and fructose can increase the osmotic pressure in the cytoplasm and cause the leakage of myoinositol, a deficiency of which can lead to the production of DAG. On the other hand, the activation of the polyol pathway leads to the consumption of NADPH and promotes the conversion of NAD^+ to NADH, which may not only reduce the production of the antioxidant glutathione, resulting in oxidative stress (29), but also competitively inhibit GAPDH, down-regulate glycolytic flux, and promote the transfer of more glucose to non-traditional metabolic pathways (such as hexosamine), thus aggravating glucotoxicity (30, 31). As the hexosamine biosynthesis pathway is also activated in diabetes, fructose-6-phosphate is detached from glycolysis and subsequently catalyzed to glucosamine 6-phosphate by fructose 6-phosphate aminotransferase. Glucosamine 6-phosphate forms the end product uridine diphosphate-N-acetylglucosamine through acetylation and isomerization. It then serves as the basic substrates for the formation of glycosyl side chains in post-translational modifications of proteins and lipids (32). This post-translational modification can aggravate glucotoxicity by regulating target protein stability, activity and subcellular localization, which can promote liver gluconeogenesis, lead to impaired insulin signaling and pancreatic β-cell function (33), and is directly involved in the pathogenesis of several diabetic complications, especially in cardiovascular disease and kidney dysfunction (34).

2.2 Amino acid

Since Felig et al. found in the 1970s that the increased concentration of circulating amino acids in obese people is associated with decreased insulin sensitivity (35), a large number of studies have confirmed the value of amino acids in the early identification and risk stratification of diabetes and its complications (36–40). Among the known and relatively clear amino acid biomarkers, plasma branched-chain amino acids (BCAA) and aromatic amino acids (AAA) increased significantly (Table 1), while glycine and glutamine decreased in diabetes and prediabetes (25, 41–43, 52–56).

BCAA is most closely related to homeostasis model assessment for insulin resistance (HOMA-IR) and blood glucose (46, 57, 58).

TABLE 1 Association of amino acids profiles with T2DM in cohort studies.

Sample size (incident cases)	Duration of follow-up (years)	Platform	Metabolites	End point	OR/ HR (95% CI)	Ref
769	6	NMR	BCAA, phenylalanine, alanine, tyrosine↑ Glutamine↓	HOMA-IR	2.09[a] (Men)	(36)
189	12	LC-MS	BCAA, tyrosine, phenylalanine↑	Diabetes	1.70-2.42[a]	(40)
91	2	LC-MS	Glycine↓	T2DM	0.85[a]	(41)
76	5	MS-MS	BCAA, phenylalanine, alanine, glutamine and glutamate↑ Aspartate/asparagine, glycine↓	T2DM	1.56-2.22[a] 0.42-0.58[a]	(42)
340	19	NMR	BCAA, alanine, isoleucine, phenylalanine, tyrosine↑ Glycine↓	Diabetes	1.27-1.48[a] 0.77[a]	(43)
9180	5.7	LC-MS	Tryptophan↑	T2DM	—	(44)
251	3.8	LC-MS	Tryptophan↑	T2DM	1.29[b]	(45)
17	1.5	LC-MS/MS	BCAA↑	HOMA-IR	—	(46)
70	5.5	MS-MS	Aspartic acid/asparagine, phenylalanine↑ Histidine↓	Prediabetes	2.39-2.72[a] 0.89-0.90[a]	(47)
540	—	NMR	isoleucine, alanine↑	Liver/muscle HOMA-IR	—	(48)
152	1-3	LC-MS	isoleucine, alanine, proline↑ glycine, arginine↓	HOMA-IR	—	(49)
151	9.5	UHPLC-MS/MS	BCAA, alanine, glutamate, arginine↑ glycine↓	T2DM	—	(50)
16	2.3	NMR	BCAA↑	IR	—	(51)

HOMA-IR, homeostasis model assessment for insulin resistance; IR, insulin resistance; NMR, nuclear magnetic resonance; LC-MS, liquid chromatography-mass spectrometry; MS-MS, tandem mass spectrometry; GC-MS, gas chromatography-mass spectrometry; IGT, impaired glucose tolerance; BCAA, branched-chain amino acids; T2DM, type 2 diabetes mellitus; SDMA, symmetric dimethylarginine; ↑, increased; ↓, decreased; —, not available, [a], odd ratio (OR), [b], hazard ratio (HR).

The results of large sample size genome-wide association studies (GWAS) show that BCAA contributes to the increase of the incidence of IR and T2DM (59). Under physiological conditions, BCAA promotes protein synthesis or inhibits its breakdown by activating the mTOR signaling pathway, a catalytic subunit of two distinct structural and functional complexes mTORC1 and mTORC2. mTORC1 promotes protein synthesis and regulates autophagy, and mTORC2 is a classic insulin/PI3K signaling pathway effector (60). BCAA supplementation alone did not significantly affect skeletal muscle mass and glycemic control in patients with T2DM (61), nor worsen diet-induced insulin resistance and glucose intolerance in obese mice (62). Whereas HFD combined with BCAA supplementation caused chronic activation of mTORC1, p70-S6 kinase (p70S6K), and phosphorylation of insulin receptor substrate 1 (IRS1) serine, promoting the accumulation of multiple acylcarnitines in muscle, decreased insulin sensitivity (37), which can be reversed by the mTOR inhibitor rapamycin (63). Reducing dietary BCAA intake rapidly reduced diet-induced obesity, improved glucose tolerance, reversed fatty acyl-coA accumulation in skeletal muscle, normalized glycine content, and improved skeletal muscle insulin sensitivity (64, 65). In leucine-incubated skeletal muscle, AMPK activity decreased by more than 50%, phosphorylation of mTOR and p70S6K was concentration-dependent, phosphorylation of insulin-stimulated Akt was impaired, and AMPK agonist was used to inhibit these changes (66).

Paradoxically, Leucine has also been suggested to increase GLUT4-mediated glucose uptake, stimulate insulin-dependent PI3K and protein kinase C (PKC) signaling cascades, and increase mitochondrial biogenesis and substrate oxidation capacity (67, 68). Leucine supplementation has been shown to reduce body weight by 32% and improve insulin sensitivity, plasma total cholesterol, and low-density lipoprotein cholesterol (LDL) levels in mice (69). This may be related to the insulinotropic properties of BCAA (especially Leu), short-term (4 weeks) BCAA-restricted diet decreased postprandial insulin secretion, increased postprandial insulin sensitivity and mitochondrial metabolism efficiency in adipose tissue (70). On the other hand, Long-term (60 weeks) supplementation with amino acids has also been shown to improve glycemic control and insulin sensitivity in older non-

obese (BMI within 19~23) T2DM patients (71). Since protein degradation is commonly increased in populations with poor IR and T2DM control (72), and the provision of additional BCAAs in the diet can mitigate protein degradation (73), it is necessary to tailor nutritional programs to different populations. In addition, valine and isoleucine are major contributors to the production of cyclic odd-chain FA (74), among which valine not only promotes α oxidation by activating PPARα, but also promotes odd-chain FA production by providing PrCoA as a substrate (75).

Different organs also show differences in amino acid profiles, liver but not muscle IR was associated with increased levels of leucine and tyrosine, leucine deprivation enhances insulin sensitivity by increasing AMPK phosphorylation and inhibiting the mTOR/S6K pathway in the mouse liver (76), while both showed higher levels of isoleucine and alanine and lower levels of glycine (48). In the liver, BCAA supplementation activates mTORC1 and suppresses mTORC2, blocks insulin-mediated Akt2 phosphorylation, and promotes its ubiquitination and degradation, negative regulation of Akt2 increases FoxO1-mediated gluconeogenesis and inhibits liver lipogenesis mediated by the sterol-regulatory element binding protein (SREBP)1/INSIG2a signaling pathway (77). It is believed that when FA are excessive, the accumulation of metabolic intermediates of BCAA (rather than BCAA itself) can competitively "block" FA β oxidation flux, resulting in the accumulation of BCAA and incomplete oxidation products of FA, leading to a corresponding decrease in glucose utilization (37). All of these suggest that the increase of BCAA in T2DM is likely to be a downstream effect caused by obesity and IR, and then plays a further mediating role in disease development (37).

Studies have suggested that increased IR leads to increased levels of circulating fasting BCAA and inflammation (78, 79). Obesity and T2DM reduce the activity of metabolic enzymes involved in BCAA catabolism, leading to BCAA accumulation (80). BCAA catabolism involves the first transamination of BCAA aminotransferase (BCAT) to branch alpha-ketoic acid (BCKA), followed by decarboxylation of BCKA by BCKA dehydrogenase complex (BCKDC), which is activated by dephosphorylation of PPM1K phosphatase and deactivated by phosphorylation of BCKD kinase. The expression of BCAT and BCKDC is relatively low in the liver, where adipose tissue and skeletal muscle are major sites of BCAA oxidative metabolism (81, 82). In human and animal models of metabolic syndrome, hypoxia, inflammation, and ER stress in adipose tissue can lead to a significant decrease in the level of BCAA catabolic enzyme (83, 84), and the accumulation of BCAA directly inhibits the activity of pyruvate dehydrogenase (PDH) and reduces the oxidative metabolism of glucose and FA (85). Another study suggested that reduced oxygenation in adipose tissue inhibited BCAA catabolism (86). Oxygen partial pressure in subcutaneous adipose tissue was negatively correlated with the expression of markers of inflammation and fibrosis. Meanwhile, hypoxia inhibited the catabolism and oxidation of BCAA, resulting in increased plasma BCAA concentration, thus promoting IR. Surgical weight loss interventions can reverse the increase in plasma concentrations by improving BCAA metabolism in adipose tissue, suggesting that changes in plasma BCAA reflect IR or relative insulin deficiency in obesity (87). Muscle biopsies in patients with T2DM also showed decreased expression of two enzymes necessary for valine and isoleucine metabolism (88). In contrast, increased BCAA catabolism effectively reduced plasma BCAA levels in T2DM patients, significantly improved peripheral glucose utilization, and increased pyruvate mitochondrial oxidation flux by 10% in muscle (89).

In addition to BCAA and AAA, multiple additional amino acids and derivatives are associated with diabetes progression (49, 90, 91). Studies have shown that glutamate is significantly increased in people with T2DM, while glutamine and glycine are associated with a 15% and 11% reduction in diabetes risk, respectively (52). After adjusting for BMI, concentrations of aspartic acid, asparagine, and histidine were strongly correlated with the incidence of prediabetes. For every 1 standard deviation increase in baseline aspartic acid and asparagine levels, the risk of prediabetes increased by 2.72 times, while for every 1 standard deviation increase in baseline histidin level, the risk of prediabetes decreased by 10% (47). This may be related to histidine's role in regulating gluconeogenesis and anti-inflammatory (92).

Studies have shown that alanine, tryptophan, and trytophan-related metabolic intermediates are associated with a higher risk of T2DM and prediabetes (40, 43, 44, 93), and that kynurenine is the main metabolic intermediate of tryptophan, chronic inflammatory can induce activation of the tryptophan/kynurenine metabolic pathway (94), and may mediate the increased mortality associated with inflammation in T2DM (95). Circulating kynurenine levels are also affected by dietary tryptophan intake. As a result, it has been suggested that the ratio of kynurenine/tryptophan can reflect the metabolic status of tryptophan further than that of tryptophan or kynurenine concentration alone (96). This partly explains why the level of tryptophan increases at the beginning of T2DM and reverses as the disease progresses (45), and why there is no significant association between the plasma kynurenine/tryptophan ratio and T2DM risk, but the urine kynurenine/tryptophan rate is strongly associated with T2DM risk (96). In addition, indolepropionate, a tryptophan-breaking metabolite derived from the gut microbiome, was negatively associated with T2DM risk, while increased indolelactate was associated with higher T2DM risk (44, 53).

Elevated levels of lysine and its metabolic intermediate 2-aminoadipic acid were associated with an increased risk of T2DM. 2-aminoadipic acid metabolism occurs primarily in mitochondria and is broken down into acetyl-CoA before entering the (tricarboxylic acid, TCA) cycle. Plasma 2-Aminoadipic acid level increases by 47% in obesity and is positively correlated with IR (97). As a novel biomarker to predict the risk of T2DM, 2-aminoadipic acid, independent of common BCAA and AAA, has been shown to increase in concentration 12 years before the onset of diabetes symptoms (98), and as a biomarker to predict childhood obesity and related metabolic disorders 2 years later (99). In addition, the level of circulating 2-Aminoadipic acid is significantly negatively correlated with HDL, which is closely associated with cardiovascular complications such as atherosclerosis and coronary artery calcification (100, 101).

2.3 Lipids and acylcarnitines

Elevated blood levels of triacylglycerols (TAGs) are traditional risk indicators for T2DM (48, 102, 103). Free fatty acids (FFA) are non-esterified fatty acids in the serum that comes primarily from the breakdown of TAG. When caloric intake exceeds the normal storage and consumption capacity of lipids, fatty acids "spillover" will result in increased FFA (104). Elevated fasting FFA is associated with a three-fold increased risk of impaired glucose tolerance or T2DM over the next 5~8 years (105). After clinical intervention, the level of FFA can also serve as an effective prognostic evaluation index (106). FFA can be divided into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) according to the difference of hydrocarbon saturation. Serum FFA variations of different types are generally suggestive of hyperglycemia or T2DM (Table 2). Specifically, increased levels of partial n-6, n-7 and n-9 were significantly positively correlated with elevated blood glucose, while some n-3 PUFA was significantly inversely correlated with T2DM (107, 108, 113, 116). In addition, different chain lengths of SFA have particular metabolic and biological effects. Increased circulating concentrations of C15:0, C17:0 and C24:0 and very long chain of SFA are associated with lower risk of T2DM (114, 117), while C14:0, C16:0, C16:1, and C18:0 are positively correlated with T2DM risk (109, 111).

Increased FFA release and oxidation rate can antagonize glucose oxidation, resulting in the disturbance of pyruvate metabolism and impaired insulin sensitivity (118, 119), and this damage to glucose homeostasis by FFA is commonly referred to as lipotoxicity. Extensive evidence has demonstrated the role of lipotoxicity in IR and pancreatic β-cell injury (120, 121). Elevated plasma FFA levels are also known to cause TAG and DAG deposition in a variety of tissues and organs, gradually accumulating DAG enhances NADPH oxidase activity through the PKC pathway, exacerbates oxidative stress and cytokine transcription, and promotes cell differentiation, proliferation, and apoptosis. Activation of the PKC pathway is a critical mechanism leading to diabetes cardiovascular disease (122). Thus, lipid management is absolutely recommended for the prevention and treatment of vascular complications of T2DM.

The inflammatory response mediated by SFA is an essential cause of IR and β-cell damage (Figure 3). SFA and lipopolysaccharide synergistically amplifies the effects of decreased β-cell viability, increased apoptosis, and decreased basal insulin secretion, and significantly alleviates lipid-induced β-cell damage by blocking toll-like receptor 4 (TLR4) or overexpressing neutral ceramidase (NCDase) activity (123, 124). Palmitate (C16:00) is the most abundant SFA in dietary and plasma, palmitate can enhance the interaction between TLR and myeloid differentiation primary response protein MyD88, mediating β-cell death (125). On the one hand, it directly inhibits insulin signal transduction by activating the phosphorylation of Jun N-terminal kinase (JNK) and the inhibitor of nuclear factor-κB (NF-κB) kinas (IKKβ). On the other hand, degradation of inhibitor of NF-κB (IκB) leads to nuclear translocation of NF-κB, this increases cytokine transcription and exacerbates inflammation. TLR4, IKKβ, or JNK knockout inhibited the expression of inflammatory cytokines in adipocytes and macrophages and protected mice against lipid-induced IR (126–128). In contrast, some PUFA such as docosahexaenoic acid (DHA) inhibit the production of TLR4-induced inflammatory cytokines (129), improve insulin sensitivity and insulin secretion capacity to some extent, and reduce the risk of T2DM (128, 130).

In specific lipids and derivatives, baseline lysophosphatidylcholine (LPC), phosphatidylcholine (PC), sphingomyelin, and cholesterol esters were inversely associated with T2DM risk (112). LPC were strongly associated with IR and β-cell dysfunction (50). Decreased concentrations of LPC18:2 and 16:0 are associated with the onset of T2DM (41, 131), and increased levels of PC O-16:1/0:0, (O-18:1/0:0)/(P-18:0/0:0) and LPC 20:2 can increase the 10-year risk of T2DM by 29% (132). Diacylphosphatidylcholine C32:1, C36:1, C38:3 and C40:5 were positively associated with T2DM risk. PC O-20:0/O-20:0, 22:6/20:4, LPC18:0, sphingomyelin C16:1 and acyl-alkyl-phosphatidylcholine C34:3, C40:6, C42:5, C44:4 and C44:5 were negatively correlated with T2DM risk (133–135). In the early stages of dysglycemia and IR, fasting concentration of linoleoylglycerophosphocholine is decreased, independent of classical predictors, as an indicator of worsening glucose tolerance (50).

Ceramide is a relatively minor component of the total cellular lipidome with a particularly low abundance, and increased ceramide content has been shown to be positively correlated with HOMA-IR, fasting glucose, and cardiovascular diseases (136, 137). Elevated ceramides are key lipotoxic species in skeletal muscle, liver, adipose tissue, and vascular cells, and contribute to disease progression by interfering with insulin signaling, stimulating lipid uptake, and enhancing inflammatory cytokines (138–142). Of the different types of sphingolipids, C16 ceramides and C18 ceramides are more damaging to adipose and liver function. C16:0 ceramides can impair mitochondrial oxidative phosphorylation by inhibiting mitochondrial complex II and promoting mitochondrial fission, reduce mitochondrial respiration, and promote the release of cytochrome c to induce apoptosis by increasing the permeability of mitochondrial outer membrane (139, 143). Inhibition of ceramide synthesis can improve insulin sensitivity and prevent obesity-induced diabetes (144). It also increases brown adipocyte numbers, mitochondrial activity, and promotes the polarization of adipose tissue macrophages towards the M2 anti-inflammatory phenotype (145).

Acylcarnitines, metabolites of FA, play crucial roles in cellular energy metabolism and are gradually considered as influential biomarkers of metabolic disorders in metabolic syndrome, diabetes, cardiovascular diseases and other diseases. For example, C2, C3DC-CH3, C4, C5, C7 and C26 have been observed to be associated with HOMA-IR (49). In cross-sectional studies, acylcarnitines were elevated in IGT and diabetic individuals (146), reflecting incomplete fatty acid beta oxidation in the organism, but acetylcarnitine C2 did not predict IGT or T2DM years before onset, so it is more likely to be a quick-acting event (41).

Recently, FFA ligand-specific G-protein-coupled receptor (GPR) including GPR40 (also known as FFA1), GPR43 (FFA2), GPR41 (FFA3), GPR120 (FFA4) has been extensively studied. In HFD-fed mice, FFA2 function is more mediated by Gi/o and

TABLE 2 Association of lipids profiles with T2DM in cohort studies.

Sample size (incident cases)	Duration of follow-up(years)	Platform	Metabolites	End point	OR/ HR/ RR (95% CI)	Ref
91	2	LC-MS	LPC18:2↓	T2DM	0.69[a]	(41)
151	9.5	UHPLC-MS/MS	linoleoyl-glycerophosphocholine↓	T2DM	0.67[a]	(50)
540	—	NMR	TAG↑	HOMA-IR	—	(48)
152	1-3	LC-MS	Carnitine (C3, C4, C5)↑ LPC (18:1, 22:6), SM16:0, Carnitines (C9, C10:2, C18, C18:1OH, C18:2), LPE16:0, lysophosphatidylethanolamine C16:0, acetylcholine↓	HOMA-IR	—	(49)
189	12	LC-MS MS-MS	TAG (44:1, 46:1, 48:0, 48:1, 50:0, 52:1), PC (34:2, 26:2), LPE18:2↑ TAG (56:9, 58:10, 60:12), PC38:6, LPC22:6↓	T2DM	1.35-1.94[a] 0.67-0.78[a]	(102)
189/364	4.4/3.8	HPLC-MRM	LPl16:1, PC34:3, TAG50:2(16:2), TAG51:0(17:0), TAG54:7(22:6) ↑ PE38:4p(18:0p/20:4) ↓	T2DM	—	(103)
12132	16	GC	n-3 (EPA20:5), n-6 (GLA18:3, DGLA20:3, AA20:4, DTA22:4, DPA22:5)↑ n-3 (ALA18:3, DPA22:5, DHA22:6), n-6 (LA18:2)↓	T2DM	1.02-1.46[b] 0.80-0.95[b]	(107)
276	4.5	NMR	Glycerol, FFAs, total TAG, MUFAs, SFA (n-7, n-9)↑ n-6 FAs↓	T2DM	1.09-1.26[a] 0.92[a]	(108)
12132	16	GC	SFA (14:0, 16:0, 18:0)↑ SFA (15:0, 17:0, 20:0, 22:0, 23:0, 24:0)↓	T2DM	1.06-1.26[b] 0.58-0.92[b]	(109)
507	6	LC-MS	Carnitine (C0, C3DC, C8:1, C10, C14OH, C14:1OH), acylcarnitines (C16:1, C16:2, C18, C18OH, C18:1, C18:2, C20, C20:4)↑ 3-dehydroxycarnitine, 3-dehydrocarnitine, dicarboxylic (C10DC, C12DC), acylcarnitines (C12, C12OH, C12:1) ↓	T2DM	2.48-9.41[c] (models)	(110)
703	11	GC	C16:0, C16:1↑	T2DM	1.15-1.24[b]	(111)
250	3.8	LC-MS	TAG, DAG, PE↑ LP, PC-PL, SM, CE↓	T2DM	1.45-1.58[b] 0.67-0.78[b]	(112)
71	5.9	GC-MS	C20:3n-6↑	T2DM	1.53[b]	(113)
284	10	GC-MS	SFA (C20:0, 22:0, 24:0)↓	Diabetes	0.68-0.99[b]	(114)
251	3.8	LC-MS	Carnitine C4OH	T2DM	1.44[b]	(115)

T2DM, Type 2 diabetes mellitus; GC, gas chromatography; GLA, γ-linolenic acid; DGLA, dihomo-γ-linolenic acid; DTA, docosatetraenoic acid; n-6 DPA, docosapentaenoic acid; ALA, α-linolenic acid; SFA, saturated fatty acid; LC-MRM, liquid chromatography multiple reaction monitoring; HPLC, high-performance liquid chromatography; MS-MS, tandem mass spectrometry; CE, cholesteryl ester; PC, phosphatidylcholine; LPl, lyso-phosphatidylinositol; PPPE, polyunsaturated plasmalogen phosphatidylethanolamine; LPE, lysophosphatidylethanolamines; LPC, lysophosphatidylcholines; SM, sphingomyelin; TAG, triacylglycerol; DAG, diacylglycerol; PE, phosphatidylethanolamine; LP, lysophospholipid; PC-PL, phosphatidylcholine-plasmalogen; CE, cholesterol ester; ↑, increased; ↓, decreased; —, not available, [a], odd ratio (OR); [b], hazard ratio (HR); [c], relative risk (RR).

activated by short-chain FA, which inhibit insulin signaling in adipose tissue, increasing energy expenditure and improving insulin sensitivity in different tissues, including liver and muscle (147, 148). Propionic acid (C3) and valeric acid (C5) can increase basal glucose uptake in adipocytes and muscle cells by activating FFA3, while this effect is decreased after FFA3 inhibition (149).

FFA1 and FFA4, as long chain FFA receptors, are expressed in a variety of tissues and cells, such as adipocytes, macrophages, and pancreatic β-cells. FFA1 activation alone or synergistically amplifies glucose-dependent insulin secretion by affecting cellular Ca^{2+} signaling and increasing intracellular Ca^{2+} concentration (150, 151), which is critical for maintaining the homeostasis of glucose

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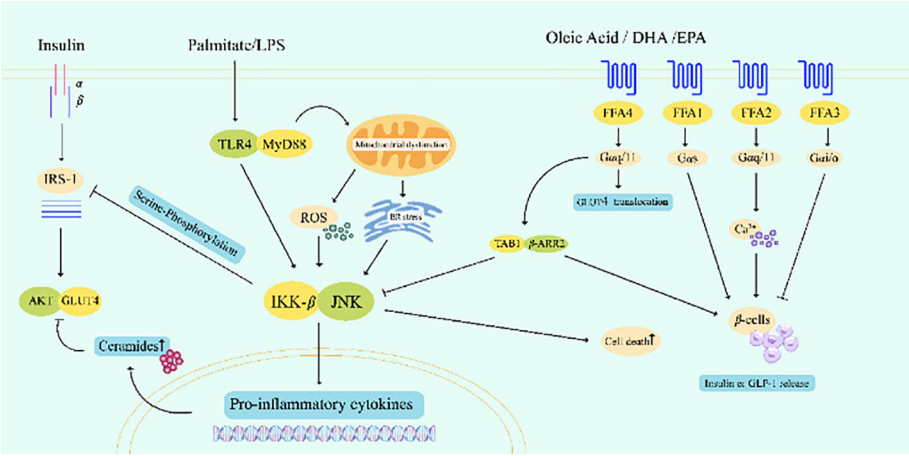

FIGURE 3
Palmitate cooperated with LPS to amplify TLR4-related signaling pathways, directly or indirectly inhibited insulin signaling, and caused β-cell apoptosis in islets. The activation of FFA4 by certain PUFAs such as oleic acid, DHA and EPA can competitively bind TAB1 through the recruitment of β-arrestin-2, inhibit the phosphorylation and activation of TAK1, inhibit the pro-inflammatory response, and promote the release of insulin and GLP-1. LPS, lipopolysaccharide; IRS-1, insulin receptor substrate 1; TLR4, toll-like receptor 4; AKT, protein kinase B; GLUT, glucose transporter; ROS, reactive oxygen species; IKK-β, inhibitor kappa B kinase-β; JNK, Jun N-terminal kinase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FFA, free fatty acid receptor; TAB1, TAK1 binding protein 1; β-ARR2, β-arrestin-2; GLP-1, glucagon-like peptide-1.

and lipid metabolism in IR individuals, making it an attractive research target for the regulation of glucose and lipid metabolism (152, 153). However, the exact mechanism of FFA1 in β-cells is still under debate. Although FFA1 mediates insulin secretion in response to acute FFA exposure, long-term activation of FFA1 is also involved in lipotoxicity to β-cells. The diversity of FFAs may partly explain the difference in efficacy, for example, palmitate increases endoplasmic reticulum stress and mitochondrial dysfunction through FFA1 activation (154–157), resulting in increased apoptosis and decreased insulin secretion. In addition, FFAR1 also responds to trans isomers of conjugated linoleic acid or arachidonic acid (158), regulating the crosstalk between Akt/mTOR and IRS-1 signaling in β-cells under lipotoxicity conditions, promoting the progression of IR and T2DM (159). Oleic acid can inhibit the activation of JNK and NF-κB, inhibit inflammatory cytokine secretion, and improve insulin sensitivity (160), while palmitoleic acid can reverse the HFD-induced proinflammatory polarization of macrophages by activating AMPK and FFA4 (161, 162), independently of the PPAR-α mechanism (163). n-3 PUFA activates FFA4, which inhibits inflammation and increases insulin sensitivity (162). This is at least in part through the regulation of NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome and macrophage conversion to the M2 anti-inflammatory standard (164), and FFA4 also mediates a variety of effects such as glucagon-like peptide-1 (GLP-1) secretion, islet function, and appetite control (165, 166).

2.4 Others

α-hydroxybutyrate (α-HB) is an organic acid derived from α-ketobutyrate, a by-product of amino acids such as methionine and threonine catabolism and glutathione synthesis (167). Increasing evidence has shown that α-HB is an early predictor of IR and impaired glucose tolerance (168, 169), the combination of α-HB and L-glycerophosphate choline showed similar accuracy to glucose in OGTT assay, and the plasma level of α-HB was negatively correlated with insulin sensitivity (Table 3). It has also been shown to be associated with β-cell dysfunction in statistical and mechanism studies (50, 51, 170). Since glutathione is a vital antioxidant, which can inhibit oxidative damage caused by an imbalance of lipid peroxides and free radicals in cells, the potential mechanism of increased α-HB may be a compensatory increase in liver glutathione synthesis flux after body REDOX dysplasia (169).

3-Hydroxyisobutyrate (3-HIB), an intermediate product of valine decomposition, is considered a higher sensitive biomarker of T2DM than valine. Impaired valine catabolism increases 3-HIB production, leading to increased lipid oxidation and acylcarnitine accumulation. Currently, 3-HIB is believed to mediate the occurrence of IR by affecting FA uptake in endothelial cells, and 3-HIB treatment has a regulatory effect on mitochondrial metabolism in white and brown adipocytes (171). Recent studies have shown that changes in the gut microbiome are involved in the metabolic disorders of T2DM, where imidazole propionate (ImP) is a product of histidine microbial metabolism. Serum ImP expression is upregulated in T2DM patients due to changes in microbial metabolism rather than histidine intake per se (172). It affects insulin receptor substrates and inflammatory signals by activating p38γ/MAPK/p62/mTORC1 signals, leading to impaired glucose metabolism (173). In addition, ImP (but not the precursor histidine) also showed a significant association with diastolic blood pressure in the overweight/obesity population, showing a possible role in CVD complications (174).

Serum concentrations of 3-carboxy-4-methyl-5-propyl-2-furan propionic acid (CMPF), the main endogenous metabolite of furan

TABLE 3 Association of metabolic intermediate profiles with T2DM in cohort studies.

Sample size (incident cases)	Duration of follow-up (years)	Platform	Metabolites	End point	OR/ HR (95% CI)	Ref
9180	5.7	LC-MS	Kynurenine, kynurenate, xanthurenate, quinolinate, indolelactate↑ indolepropionate↓	T2DM	—	(44)
251	3.8	LC-MS	Quinolinic acid↑	T2DM	1.39[b]	(45)
350	12	LC-MS/MS	2-aminoadipic acid↑	T2DM	1.59[a]	(98)
16	2	NMR	α-Hydroxybutyrate↑	IR	—	(51)
151	9.5	HPLC-MS/ MS	α-Hydroxybutyrate↑	T2DM	1.26[a]	(50)
152	1-3	LC-MS	*N*-acetyl-tryptophan, methyladenosine, N-acetyl-leucine, dimethylglycine, hypoxanthine, thiamin↑ Betaine, guanidoacetic acid, β-Amino-isobutyric acid↓	HOMA-IR	—	(49)

T2DM, type 2 diabetes mellitus; IR, insulin resistance; LC-MS, liquid chromatography-mass spectrometry; HOMA-IR, homeostasis model assessment for insulin resistance; HPLC, high-performance liquid chromatography; MS/MS, tandem mass spectrometry; NMR, nuclear magnetic resonance; ↑, increased; ↓, decreased; —, not available, [a], odd ratio (OR); [b], hazard ratio (HR).

FA, are elevated in patients with impaired glucose tolerance and T2DM, and can directly act on pancreatic β-cells to lead to impaired insulin secretion (175–177). However, one study showed the opposite result: compared with the control group, the concentration of serum CMPF in the T2DM group was lower and was negatively correlated with the changes in serum TAG (178), although this study was limited by the small sample size, it still suggested that the metabolism of CMPF might be affected by race, diet and other factors. Supplementation of n-3 FA can increase the level of CMPF, and there is a positive correlation between docosahexaenoic acid (DPA) and DHA levels and CMPF, but no significant relationship between eicosapentaenoic acid (EPA) (179). It should be noted that although exogenous n-3 FA intake (e.g. fish) can significantly increase circulating CMPF concentration, it is still far below the level of T2DM and low doses of CMPF do not have a significant effect on glucose metabolism (180).

Bile acids (BAs) can act as signaling regulators for lipids and glucose metabolism, and the concentration of BAs changed has been linked to metabolic disorders such as IR. Studies have shown that after adjusting for age, sex, BMI, waist circumference, and fasting blood glucose, increased circulating 12α-hydroxylated BAs concentration is significantly associated with increased HOMA-IR and fasting blood glucose (132, 181), but it cannot be used as an effective predictor of diabetes (182, 183). This may be because decreased insulin sensitivity and impaired glucose tolerance occur before the rise in BAs. The increase in circulating BAs is not the factor that causes the change in glucose metabolism, but its downstream effect (184). Recent study has also confirmed that the increase of circulating BAs in T2DM individuals is positively correlated with fasting blood glucose, HbA1c, and HOMA-IR, which may be due to insulin signaling dysfunction. However, insulin treatment did not significantly affect the total level of BAs. Therefore, more studies are needed on the composition of BAs and its role as a regulator in metabolic disorders (185).

3 Effects of anti-hyperglycemic medications on metabolites

Many of commonly used anti-hyperglycemic medications can also have pleiotropic effects on the metabolite profile, which may positively affect T2DM and complications. In the following, we summarize the therapeutic effects of current mainstream hypoglycemic drugs on metabolites associated with different diabetes risks.

3.1 Metformin

Metformin is a common drug for T2DM management. In randomized controlled trials, taking metformin was associated with increased levels of betaine, alanine, histidine, leucine/ isoleucine and decreased levels of carnitine, phenylalanine, tyrosine and valine (186, 187). In terms of blood lipids, metformin was associated with an increase in TAG of higher carbon numbers (188), and elevated levels of the latter seemed to predict a reduced risk of T2DM (102), and very-low-density lipoprotein (VLDL)-triglyceride levels were significantly reduced (189). In another small sample size study, T2DM patients treated with metformin had increased serum trimethylamine-N-oxide, α-HB, and tryptophan, while acetoacetic acid, phenylalanine, and LPC (16:0, 18:0, and 18:2) were decreased (190).

3.2 Thiazolidinedione

Compared with metformin, pioglitazone increased myocardial glucose uptake and decreased hepatic TAG content (191, 192), but did not show any effect on subcutaneous fat volume. Compared

with rosiglitazone, pioglitazone has a smaller increase in LDL and a larger increase in HDL, and promotes the transformation of VLDL to larger LDL by reducing asymmetric dimethylarginine levels (193, 194), which has a protective effect on cardiovascular diseases.

3.3 GLP–1 receptor agonist

Treatment with liraglutide can significantly reduce serum tyrosine, valine and isoleucine levels in obese people, but has no significant effect on T2DM patients (195, 196). After liraglutide treatment, ceramides, phospholipids, hexocyl-ceramides, LPC, sphingolipids, and TAG were significantly deregulated in T2DM patients, demonstrating the cardiovascular system benefits of liraglutide (197–200). Exenatide treatment for 6 months was effective in reducing body weight, cysteine, and FFA concentration, while levels of aminoisobutyric acid, anandamide, and sarcosine tended to increase (201, 202). The efficacy of duraglutide was also associated with a significant reduction in 2-hydroxybutyric acid and a significant upregulation of threonine compared to placebo (203). In addition, high doses of trusted downregulated BCAA, glutamate, 3-hydroxyisobutyrate, branched-chain ketoacids, and 2-hydroxybutyrate (204).

3.4 DPP-4 inhibitor

Studies have shown that 6 months of vildagliptin treatment can reduce the level of asymmetric dimethylarginine in T2DM patients (205), but has no significant effects on FFA, glycerol, lactic acid and pyruvate (206, 207).

3.5 Concomitant drugs

The present investigation shows that 3 months of metformin plus pioglitazone can significantly reduce the levels of phenylalanine/tyrosine, citrulline/arginine, and lysine/α-aminoadipic acid in T2DM and obese adults (208). Compared with treatment alone, the combination of pioglitazone and exenatide reduced hepatic fat and plasma TAG more significantly (209).

At present, the effects of hypoglycemic drugs on serum metabolites in patients with T2DM are more focused on the effects of lipids and lipoproteins, but the number of studies on amino acids and metabolic derivatives are limited. GLP-1 agonists have shown relatively better effects on lipid and amino acid metabolites, and improvements in metabolites associated with cardiovascular risk have been observed in short-term trials, but long-term follow-up evidence is still lacking. An anti-hyperglycemic drug's effect on blood metabolites needs more prospective, intervention and randomized clinical trial studies to confirm the molecular mechanism of further metabolites.

4 Perspective

As a typical metabolic disease, exploring changes in metabolites and their regulatory mechanisms is closer to the essence of T2DM. Among the promising metabolites, blood concentrations of hexose, BCAA, AAA, TAG, phospholipids and sphingomyelins were significantly and positively associated with T2DM incidence, while glycine and glutamine were negatively associated with T2DM risk. However, using only one metabolite type as a biomarker has many limitations in terms of disease duration, race, or diet, so a comprehensive judgment of multiple metabolite prediction models is necessary. Understanding the metabolism of metabolites in specific tissues and the influence of the regulation of corresponding receptors on immune response and biological efficacy, as well as verifying causality through mechanism studies, is key to metabolite research. Finally, while we have an initial understanding of the functions of metabolites as regulators, the results of dietary interventions do not completely match our expectations. How dietary nutrients cause changes in metabolic pathways and certain protein signaling pathways, as well as the role of gut flora in metabolite synthesis and downstream regulation, will be attractive topics.

Author contributions

YL mainly wrote the manuscript. DW ccollects data, corrects and provides fund support. Y-PL reviews, editing and provides fund support. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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