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Metabolites as regulators of insulin sensitivity and metabolism

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Abstract

The cause of insulin resistance in obesity and type 2 diabetes mellitus (T2DM) is not limited to impaired insulin signalling but also involves the complex interplay of multiple metabolic pathways. The analysis of large data sets generated by metabolomics and lipidomics has shed new light on the roles of metabolites such as lipids, amino acids and bile acids in modulating insulin sensitivity. Metabolites can regulate insulin sensitivity directly by modulating components of the insulin signalling pathway, such as insulin receptor substrates (IRSs) and AKT, and indirectly by altering the flux of substrates through multiple metabolic pathways, including lipogenesis, lipid oxidation, protein synthesis and degradation and hepatic gluconeogenesis. Moreover, the post-translational modification of proteins by metabolites and lipids, including acetylation and palmitoylation, can alter protein function. Furthermore, the role of the microbiota in regulating substrate metabolism and insulin sensitivity is unfolding. In this Review, we discuss the emerging roles of metabolites in the pathogenesis of insulin resistance and T2DM. A comprehensive understanding of the metabolic adaptations involved in insulin resistance may enable the identification of novel targets for improving insulin sensitivity and preventing, and treating, T2DM.

Nutrient metabolism is fundamental for the survival, growth and development of all organisms and it requires the coordinated regulation of many metabolic pathways. Insulin regulates the metabolism of carbohydrates, lipids and proteins through the canonical insulin signalling cascade, which involves the insulin receptor (IR), insulin receptor substrate (IRS) proteins, PI3K and AKT[1,2]. Insulin resistance — defined as impaired signal transduction and biological actions in response to insulin stimulation — is a fundamental mechanism causing type 2 diabetes mellitus (T2DM). When insulin secretion can no longer compensate for

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insulin resistance, T2DM occurs. Despite increasing knowledge of the insulin signalling cascade, few insulin-sensitizing agents are available to treat T2DM in the clinic. Directly targeting the insulin signalling pathway to improve insulin sensitivity in patients with T2DM is difficult, and there are concerns that activating PI3K and AKT may increase the risk of, or exacerbate, cancer[3].

In the post-genomic era, the integration of functional genomics, transcriptomics, proteomics, metabolomics and lipidomics has broadened our understanding of the molecular mechanisms underlying insulin resistance and T2DM. The technology for analysing metabolomics and lipidomic data sets is rapidly evolving, and it has enabled us to identify novel metabolites that regulate insulin sensitivity and/or novel roles for known metabolites in this process.

In this Review, we provide an overview of the current landscape of metabolite families, in particular, of lipids and related metabolites, which have emerged from omics studies as being linked to insulin sensitivity. Lipids have diverse roles as signalling molecules, metabolic substrates and cellular membrane components. Lipids can also modify proteins that influence insulin sensitivity. Chain length and the degree of desaturation of the fatty acid moieties in lipid molecules increase the complexity of assigning biological roles to lipid classes. Moreover, whether lipids are synthesized endogenously or obtained through diet influences their accumulation and/or metabolism and subsequent biological roles. Thus, it is not surprising that controversy exists regarding the causative role of, and mechanisms for the effects of, specific lipid classes on the development of insulin resistance. However, new high-resolution metabolomic techniques enable the identification of lipid subclasses and novel families of lipids that regulate insulin sensitivity.

In addition to lipids, we also provide updates on amino acids and other metabolites such as ketones. We primarily focus on data from preclinical studies in animal models in which the mechanisms of insulin sensitivity have been investigated. However, we relate these data to human studies and highlight discrepancies between preclinical and human data. It is still unknown whether many of the metabolite alterations discussed play a causative role in, or are markers of, insulin resistance. Also, when such alterations are known to be causative, the underlying mechanism is often unknown. Finally, we discuss how targeting the pathways that regulate metabolites involved in insulin resistance may lead to novel strategies for treating T2DM.

Lipids signal to regulate metabolism

Our understanding of lipids as signalling molecules can be traced back more than seven decades, when fatty acid-derived eicosanoids such as prostaglandins, thromboxanes and leukotrienes were found to signal through G protein-coupled receptors (GPCRs) to exert a broad spectrum of biological functions[4,5]. Since then, other signalling lipids (such as fatty acids, diacylglycerol (DAG), sphingolipids and fatty acid esters of hydroxy fatty acids) that regulate intracellular pathways to impact insulin action and metabolism have been identified (FIG. 1).

Saturated fatty acids.

Saturated fatty acids (SFAs) (see Supplementary Box 1) are detrimental to insulin sensitivity in animals and cell culture[6–9]; SFAs impair insulin signalling by activating pro-inflammatory signalling pathways and/or by providing substrates for the synthesis of potentially detrimental lipids such as DAG and ceramides (FIG. 1a) (see below). However, human studies evaluating the role of SFAs on insulin resistance and diabetes present conflicting data. Intralipid infusion or a high-SFA diet (80% calories from fat with ~63% from SFAs) for 24 hours induces insulin resistance[10–12]. By contrast, chronic consumption of dietary SFAs is not associated with incident diabetes. However, replacing SFAs with unsaturated fats in diets may have some beneficial effects for insulin sensitivity and is likely to reduce the risk of T2DM[13,14].

Multiple mechanisms contribute to SFA-induced insulin resistance in animals and cultured cells. SFAs activate Toll-like receptors (TLRs), especially TLR4, which is expressed on immune cells, in white adipose tissue (WAT) and in liver, to stimulate the downstream pro-inflammatory processes[6–9,15]. Pharmacological inhibition, or whole-body or WAT-specific deletion, of TLR4 in mice prevents insulin resistance caused by lipid infusion[9,15,16]. Moreover, mice with a liver-specific knockout of the gene encoding TLR4 are protected from high-fat diet (HFD)-induced glucose intolerance, insulin resistance and hepatic steatosis[17]. Therefore, TLR4 is necessary for SFA-induced insulin resistance even though structure studies and binding assays indicate that SFAs are not direct ligands of TLR4 (REFS[18,19]). One study suggested that palmitic acid, referred to as C16:0 (where C16 indicates the number of carbon atoms and 0 indicates the number of double bonds), directly binds to the TLR4 co-receptor myeloid differentiation protein 2 (MD2; also known as Ly96))[20] and that the knockdown or inhibition of MD2 prevented the pro-inflammatory response to palmitic acid[20]. Further studies are needed to determine whether the SFA–MD2 interaction is important for SFA-induced insulin resistance.

TLR4 activation initiates intracellular signalling pathways involving the adaptor proteins myeloid differentiation primary response protein MYD88 and TIR-domain-containing adaptor-inducing interferon-β (TRIF; also known as TICAM1), which activate pro-inflammatory pathways to increase the production of pro-inflammatory cytokines; these pro-inflammatory cytokines induce feedforward signalling cascades to exacerbate pro-inflammatory effects (FIG. 1a). TLR4-mediated activation of inhibitor of nuclear factor-κB (NF-κB) kinase subunit-β (IKKβ) and Jun N-terminal kinase (JNK) pathways, likely via MYD88, is thought to directly mediate SFA-induced insulin resistance. Indeed, IKKβ and JNK knockout mice are protected from HFD-induced insulin resistance[21,22], but the mechanism remains to be elucidated. Although IKKβ-mediated and JNK-mediated phosphorylation of IRS1 on Ser307 in mice (Ser312 in humans) impairs insulin signalling in vitro[23,24], the relevance of this phosphorylation in SFA-induced insulin resistance is questionable, as IRS1-S307A knock-in mice (that is, mice expressing IRS1 that cannot be phosphorylated at Ser307) are insulin resistant and glucose intolerant[25,26].

SFAs induce endoplasmic reticulum (ER) stress in metabolic organs and immune cells, which can activate NF-κB signalling and the inflammatory cascade to exacerbate insulin resistance[27,28]. Whole-body knockout of TLR4 in mice attenuates HFD-induced ER stress in

muscle, liver and adipose tissue, indicating that TLR4 is necessary for SFA-induced ER stress[29]. However, in cultured myotubes, the TLR4-specific inhibitor TAK-242 attenuated palmitate-induced inflammatory cytokine expression but not ER stress, suggesting that TLR4 signalling is not the predominant mediator of SFA-induced ER stress under some conditions[30]. SFAs may also induce ER stress by altering the composition of intracellular phospholipids. For example, phosphatidylcholines (PCs) with incorporated SFAs integrate into the ER membrane, impair its fluidity and trigger ER stress in multiple cell types, including macrophages[31,32]. Furthermore, thioesterase superfamily member 2 (THEM2; also known as ACOT13), a long-chain fatty acyl-CoA thioesterase that incorporates SFAs into PCs, was found to reduce the fluidity of the ER membrane, resulting in increased hepatic ER stress, insulin resistance and gluconeogenesis[33].

SFA-induced insulin resistance may also occur when SFAs alter the membrane distribution of proto-oncogene tyrosine-protein kinase SRC, leading to its activation and the induction of JNK signalling; JNK signalling inhibits AKT in the insulin cascade[34]. In addition, palmitic acid may trigger the NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome pathway to reduce insulin sensitivity by inactivating 5′-AMP-activated protein kinase (AMPK), which results in defective autophagy and the generation of mitochondrial reactive oxygen species (ROS)[35]. SFAs are also substrates for ceramides, DAGs and phospholipids, which also may impair insulin action (see below), further highlighting that SFAs can impair insulin sensitivity via multiple pathways.

Although animal and cell culture studies have provided important insights into the mechanisms of SFA-induced insulin resistance, how these mechanisms translate to dietary SFA intake in humans, which is not associated with insulin resistance, is unclear[13,14]. The composition of the SFAs and of other nutrients in diets may mitigate the association between SFA consumption and insulin resistance. Indeed, multiple studies show that even-chain SFAs, especially palmitic acid (16:0) and stearic acid (18:0), are associated with insulin resistance, whereas odd-chain SFAs may be associated with a lower incidence of T2DM[36–40]. Future studies focusing on the roles of different species of SFAs in insulin resistance may solve some controversies in human studies and provide information for dietary guidelines.

Polyunsaturated fatty acids.

In contrast to SFAs, polyunsaturated fatty acids (PUFAs), in particular, the essential long-chain omega-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Supplementary Box 1), are anti-inflammatory and have been associated with improved insulin sensitivity in animal studies[41–43]. However, the effects of PUFAs in humans are controversial, and most studies suggest that omega-3 PUFAs have no beneficial effects on cardiovascular outcomes or glucose levels[41,44].

Omega-3 PUFAs exert anti-inflammatory effects by activating the GPCR GPR120 (also known as FFAR4), which recruits the adaptor protein β-arrestin 2; β-arrestin 2 sequesters TAK1-binding protein 1 (TAB1), resulting in the inhibition of TGFβ-activated kinase 1 (TAK1; also known as MAP3K7) to prevent JNK and IKKβ activity (FIG. 1a). DHA-enriched diets decrease inflammatory macrophage infiltration and inflammation in the WAT

of wild-type, but not GPR120-knockout, mice[42]. Several cell-based studies also suggest that GPR120 mediates the anti-inflammatory effects of PUFAs[45,46]. However, whether GPR120 promotes the insulin-sensitizing effects of omega-3 PUFAs is less clear[47,48].

Using untargeted lipidomic approaches, the omega-3 PUFA metabolites specialized pro-resolving mediators (SPMs) were found to be present during the resolution phase of acute inflammation[49]. SPMs are structurally classified as resolvins, protectins and maresins, and resolvins are further classified as E series (RvE1–RvE3) or D series (RvD1–RvD5) depending on whether they are generated from EPA or DHA, respectively. DHA is also a precursor for protectins (such as protectin D1 (PD1)) and their isomers, protectin DX (PDX), maresin 1 (MaR1) and MaR2. The production of some SPMs, and particularly of RvD1, RvD2 and PD1, is impaired in the WAT of obese mice and humans[50,51]. Treating obese mice with RvD1 and RvE1 improves insulin sensitivity and attenuates WAT and hepatic inflammation[52,53]. The reduced inflammation is at least partially the result of macrophage polarization towards an M2 (that is, alternatively activated) and anti-inflammatory phenotype[54]. Several GPCRs mediate the anti-inflammatory effects of SPMs, although the signalling pathways have not been fully elucidated[55–57] (FIG. 1a). For example, the GPCRs *N*-formyl peptide receptor 2 (FPR2) and GPR32 are necessary for the RvD1-mediated inhibition of TAK1 (REF.[56]). RvE1 interacts with the GPCR CHEMR23 (also known as CMKLR1) to enhance macrophage phagocytosis through the PI3K–AKT–ribosomal protein S6 kinase pathway[58]. RvE1 treatment also induces the expression of adiponectin, glucose transporter 4 (GLUT4; also known as SLC2A4) and IRS1 in WAT and improves insulin sensitivity in *ob*/*ob* mice[52], but the role of CHEMR23 in causing these effects is unknown. Further studies are needed to determine the physiological roles of these SPM receptors in regulating insulin sensitivity and glucose homeostasis.

Monounsaturated fatty acids.

Monounsaturated fatty acids (MUFAs) are enriched in plant-based oils including avocado, olive and peanut oils. The most common dietary MUFA is oleic acid (C18:1 n-9; where n-9 indicates that the single double bond is between carbons 9 and 10), followed by palmitoleic acid (POA, also known as palmitoleate, C16:1 n-7) and vaccenic acid (C18:1 n-6) (see Supplementary Box 1). The intake of MUFAs, especially those in olive oil, may be associated with reduced cardiovascular mortality and improved insulin sensitivity[13,59,60]. Untargeted plasma metabolomics data obtained using liquid chromatography followed by mass spectrometry and combined with Mendelian randomization analysis identified a causative role for reduced serum levels of oleic acid and POA in insulin resistance[61]. Oleic acid reverses palmitate and tumour necrosis factor (TNF)-induced impairments in AKT phosphorylation by inhibiting JNK and NF-κB activation in cells[62]. Furthermore, feeding mice an oleic acid-rich HFD reduced the secretion of interleukin-1β (IL-1β) from adipose tissue and improved insulin sensitivity[63]. Oleic acid is also a substrate of oleoylethanolamide, which is reported to act as an endogenous ligand of peroxisome proliferator-activated receptor-α (PPARα) to regulate food intake and body weight[64,65].

Palmitoleate is a common constituent of animal fats and plant seed oils, but it can also be synthesized through de novo lipogenesis. Palmitoleate produced from adipocytes may

regulate systemic insulin sensitivity[66]. Indeed, palmitoleate treatment reverses HFD-induced pro-inflammatory macrophage polarization, potentially by activating AMPK and GPR120 but not PPARα in mice[42,67,68]. However, human studies of the relationship between palmitoleate and metabolic syndrome are conflicting[61,69,70]. Thus, most PUFAs and MUFAs have anti-inflammatory effects in cells and mice, but dietary PUFAs and MUFAs have limited or no beneficial effects on insulin sensitivity in humans. The PUFA and MUFA doses used in mice are too high and not suitable for human use. Nevertheless, understanding which signalling pathways these lipids regulate may yield novel anti-inflammatory and insulin-sensitizing targets.

Branched fatty acid esters of hydroxy fatty acids.

Branched fatty acid esters of hydroxy fatty acids (FAHFAs) are structurally novel lipids that are synthesized in humans, animals and plants and that have beneficial metabolic and anti-inflammatory effects[71]. An in silico analysis predicted that >1,000 FAHFAs exist in nature[72], and >20 FAHFA families have been identified[71–73]. These families are differentiated by their acyl-chain composition; each family contains isomers that differ in the position of the ester bond between acyl chains. FAHFAs containing DHA are present at low levels in serum and WAT in mice and humans, and these levels are increased by long-term treatment with omega-3 fatty acid[73].

Palmitic acid esters of hydroxy stearic acid (PAHSAs) have been studied most extensively. PAHSAs are present at highest levels in white and brown adipose tissue compared with other tissues in mice; adipose tissue has eight PAHSA isomers, other tissues have at least three PAHSA isomers, and mouse and human serum have six[71]. PAHSA levels are reduced in the serum and subcutaneous WAT of insulin-resistant mice and humans, and serum PAHSA levels correlate with insulin sensitivity in humans[71]. A single oral dose of 5-PAHSA or 9-PAHSA in mice fed chow that are glucose intolerant from ageing or in insulin-resistant mice on a HFD improves glucose tolerance. In aged mice on a chow diet, PAHSAs augment glucose-stimulated insulin and glucagon-like peptide 1 (GLP1) secretion in vivo but these secretory effects are not seen in HFD-fed mice. GLP1 can decrease blood sugar levels by enhancing insulin secretion. It also reduces appetite, resulting in weight loss in some people. GLP1 analogues are currently being used to treat T2DM and have been shown to have cardioprotective effects. Chronic PAHSA treatment improves insulin sensitivity and glucose tolerance in mice on both diets[74]. In vitro, PAHSAs directly enhance the secretion of GLP1 from entero-endocrine cells and glucose-stimulated insulin secretion (GSIS) from human pancreatic islets. They also enhance insulin-stimulated glucose transport and GLUT4 translocation in adipocytes. PAHSAs are anti-inflammatory. They decrease HFD-induced adipose inflammation in obese, insulin-resistant mice and attenuate lipopolysaccharide (LPS)-induced dendritic cell activation and cytokine production in vitro[71] (FIG. 1a). The anti-inflammatory effects are further demonstrated by their ability to reduce the severity of colitis in a mouse model[75].

PAHSAs activate the long form of GPR120 albeit at high concentrations. GPR120 is necessary for PAHSAs to enhance insulin-stimulated glucose transport and GLUT4 translocation to the plasma membrane in adipocytes[71]. PAHSAs are also selective agonists

for GPCR 40 (GPR40; also known as FFAR1), as demonstrated by the fact that they increase Ca^{2+} flux through this receptor but not cAMP generation. PAHSA-mediated GPR40 activation promotes glucose homeostasis, as blocking GPR40 reverses the improvements in glucose tolerance and insulin sensitivity observed in PAHSA-treated chow- and HFD-fed mice and directly inhibits PAHSA-mediated augmentation of GSIS in human islets[74]. Although PAHSAs do not activate the GLP1 receptor, it is necessary for them to enhance glucose tolerance[74], perhaps because PAHSAs stimulate the secretion of GLP1 from entero-endocrine cells. As GPR120 and GPR40 do not mediate all PAHSA effects, PAHSAs are likely to activate other GPCRs, the identification of which could drive the discovery of new treatments for T2DM and immune-mediated diseases. The naturally evolved relative affinities that PAHSAs have for multiple GPCRs may make them more effective at treating these disorders than synthetic agonists.

FAHFA biosynthesis is stimulated by increased glucose uptake into adipocytes, which activates the lipogenic and glycolytic transcription factor carbohydrate-responsive element-binding protein (ChREBP)[71]. Full-length FAHFAs are synthesized from labelled FAHFA precursors in mice[71], but the biosynthetic enzymes responsible for this are not yet known. However, several FAHFA-degrading enzymes have been identified[76]. A gain-of-function mutation in carboxyl ester lipase (CEL), a major FAHFA hydrolase in pancreas[77], is thought to cause type 8 maturity-onset diabetes of the young[78]. As this mutant has greater hydrolytic activity against FAHFAs than wild-type CEL[77], patients expressing it may undergo increased FAHFA hydrolysis in the pancreas. This could result in lower FAHFA levels, which might impair GSIS and thereby result in diabetes.

Interestingly, R-form stereoisomers of 9-PAHSA are more abundant than S-form stereoisomers in WAT[79]. Cells favour the production of R-9-PAHSA, and CEL selectively hydrolyses S-9-PAHSA[79]. These observations highlight the role of stereochemistry in the production and degradation of PAHSAs and may facilitate the identification of the enzymes responsible for PAHSA biosynthesis[79]. In summary, FAHFAs are anti-diabetic and anti-inflammatory lipids that are synthesized in mammalian tissues. Levels of at least one family member of FAHFAs, PAHSAs, are reduced in insulin-resistant people. Understanding the pathways that regulate PAHSA biosynthesis and degradation could inform approaches to restore PAHSA levels and prevent and treat T2DM and inflammatory diseases.

Diacylglycerol.

Ectopic lipid accumulation in muscle and liver is associated with insulin resistance in animals and humans[80]. However, whether lipid deposition causes insulin resistance, or vice versa, and which lipids in muscle and liver might cause insulin resistance, are unclear. The increased triglyceride (TAG) levels that are associated with obesity do not appear to cause insulin resistance[81,82]. DAG, an immediate precursor of TAG (BOX 1), is elevated in muscle and liver in obesity and T2DM and has been linked to insulin resistance in some but not all studies, by activating protein kinase C (PKC) isoforms, which modify the phosphorylation of insulin signalling molecules (see below) (FIG. 1b). The PKC family of serine/threonine-protein kinases contains conventional PKCs (cPKCs: PKCα, PKCβI, PKCβII and PKCγ), which require DAG and Ca^{2+} for activation, novel PKCs (nPKCs: PKCδ, PKCε, PKCη and

PKCθ), which require DAG for activation and atypical PKCs (aPKCs: PKCζ and PKCλ), which do not require DAG or Ca^{2+} for enzymatic activity.

An increase in muscle and hepatic DAGs in obesity and T2DM leads to the activation of the nPKCs PKCθ and PKCε[81,82] (FIG. 1b). In both mice and humans, lipid infusion increases the accumulation of DAG in muscle, enhances the activity of PKCθ and causes insulin resistance[83,84]. Mice lacking PKCθ are protected from insulin resistance in muscle during lipid infusion[85]. Mechanistically, PKCθ promotes the phosphorylation of IRS1 at Ser1101, which prevents it from activating AKT[83,86]. In liver, increased DAG enhances the activity of PKCε, which leads to the phosphorylation of IR at Thr1160 to impair insulin signalling[87,88]. Mice that express a version of IR that cannot be phosphorylated at Thr1160 (Thr1160Ala) are protected from HFD-induced hepatic insulin resistance[87]. Knocking down PKCε in the liver of rats fed a HFD reverses lipid-induced defects in insulin signalling and hepatic insulin resistance[89]. Therefore, the ectopic accumulation of DAG in muscle and liver may modulate insulin sensitivity by activating nPKCs.

In insulin-resistant states such as obesity and T2DM, insulin fails to suppress hepatic gluconeogenesis, while insulin-stimulated lipogenesis remains intact[88,90]. The ‘selective’ hepatic insulin resistance occurs downstream of IR activation. It is unclear how DAG-mediated PKCε activation and impairment of insulin signalling at the IR level fit with the mechanism of selective hepatic insulin resistance. In addition, animal models lacking or overexpressing genes that regulate DAG metabolism do not always support a causative role of DAG in insulin resistance[88] (BOX 1). Indeed, the genetic deletion of several lipolysis-related genes causes hepatic steatosis and elevated DAG levels but not insulin resistance[91,92]. Total levels of DAG are also higher in muscles of endurance-trained athletes, and these athletes have a higher sensitivity to insulin[93]. The accumulation of DAG in membranes versus lipid droplets has different effects on PKC activation[80], and DAG species with different acyl chains activate PKC with varying potencies[94], further complicating the role of DAG in insulin resistance. Future studies should investigate specific DAG species and their activation in individual cellular compartments to determine their role in insulin sensitivity.

Sphingolipids and ceramides.

Sphingolipids are structurally similar to glycerolipids but have a serine (rather than glycerol) backbone to which acyl chains are attached. Sphingolipids may act as structural components of membranes and as signalling molecules[95]. Ceramides, which contain a long-chain amino alcohol sphingosine and a fatty acid, are the best-studied sphingolipids in relation to insulin resistance (BOX 2). Although elevated ceramide levels are usually associated with insulin resistance, not all ceramide species are metabolically detrimental, and the chain length and saturation of fatty acids in ceramides affect their biological functions[96].

Insulin resistance may lead to elevated ceramide levels by causing inflammation, reducing adiponectin levels and increasing the availability of ceramide precursors[97,98]. Elevated ceramides may in turn further impair insulin sensitivity[99]. Inhibition of de novo ceramide synthesis decreases intramyocellular ceramide levels and improves insulin sensitivity in obese mice[100]. Furthermore, the genetic deletion or pharmacological inhibition of

sphingomyelinase, which generates ceramides from sphingomyelin, protects against diet-induced hyperglycaemia and insulin resistance[101]. Mechanistically, ceramides interfere with insulin signalling (FIG. 1b) by allosterically activating protein phosphatase 2A (PP2A), which dephosphorylates AKT at Thr308 and Ser473, the phosphorylation of which is critical for insulin-stimulated AKT activation[102,103]. Ceramides also directly activate the aPKCs PKCλ and PKCζ, which prevents AKT from binding to phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) to block insulin signalling[104–106].

The association between ceramides and insulin resistance has not been observed in all studies[107]. Mass spectrometry has enabled the detection of ceramide species with different length fatty acid chains and different degrees of saturation[108], and data suggest that specific ceramide species rather than the total ceramide levels influence insulin resistance[96,108]. In humans, the levels of muscle C18:0, C16:0 and C18:1 ceramide species, but not total ceramides, inversely correlated with insulin sensitivity[109,110]. Ceramide species containing C16–C22 long-chain fatty acids (LCFAs) and >C22 very-long-chain fatty acids (VLCFAs) were increased and decreased, respectively, in the liver of mice on a HFD[111]. Consistent with this, overexpression in hepatocytes of ceramide synthase 2 (CERS2), which catalyses the synthesis of VLCFA ceramides, enhanced insulin signalling despite elevating the total level of ceramide[111]. CERS2 haploinsufficiency was compensated for by an increase in CERS6 expression, which resulted in the increased production of C16:0 ceramide in liver and predisposed mice to steatohepatitis and insulin resistance[112]. Furthermore, hepatic overexpression of CERS6 impaired insulin-stimulated AKT phosphorylation[112]. Conversely, CERS6 knockout reduced C16:0 ceramide in WAT and liver and ameliorated HFD-induced obesity and insulin resistance[113]. Therefore, specific ceramide species may play different roles in regulating insulin sensitivity.

Lipids as metabolic substrates

Over half a century ago, Randle and colleagues recognized that fatty acids and glucose compete for oxidation; in isolated heart and skeletal muscle, utilization of one of these nutrients directly inhibited the use of the other without hormonal mediation[114]. This substrate competition forms the basis of the concept of ‘metabolic flexibility’, which refers to metabolic substrate shift during physiological changes such as fasting and feeding, although hormones are involved at the whole-body level[115]. Kelley and colleagues used the term ‘metabolic inflexibility’ to describe the impairment in substrate shift between fatty acid and carbohydrate utilization in obesity and T2DM[116,117].

Lipids play a central role in metabolic inflexibility and the associated insulin resistance. For example, excessive dietary lipid intake and enhanced lipogenesis may cause ectopic lipids to accumulate in metabolic organs. Furthermore, adipose lipolysis is increased in obesity and T2DM, increasing the release of glycerol and fatty acids[118–120] (FIG. 2). Inflammatory cytokines such as TNF and IL-6 enhance lipolysis by increasing the expression of lipolytic enzymes[121]. IL-6-neutralizing antibodies reduce lipolysis and improve insulin sensitivity in HFD-fed rats[119]. Adipocyte-secreted retinol-binding protein 4 (RBP4) increases the production of pro-inflammatory cytokines from macrophages, which promotes adipocyte lipolysis[122,123]. Furthermore, insulin suppresses adipose lipolysis by inhibiting the rate-

limiting enzyme hormone-sensitive lipase (HSL)[124]. Therefore, insulin resistance itself enhances lipolysis[118]. All these events increase the uptake and deposition of lipids and alter metabolic flux in muscle and liver.

Increased fatty acid influx can impair mitochondrial function in muscle. Under physiological conditions, fatty acids are activated to acyl-CoAs, which are converted into acylcarnitines so that acyl-CoAs can be transported across the mitochondrial membrane for oxidation. Acylcarnitines are sensitive biomarkers of shifts in nutrient delivery to, and flux through, mitochondrial metabolic pathways. In obesity and T2DM, impaired mitochondrial function in muscle and increased fatty acid influx causes incomplete β-oxidation, leading to the accumulation of acyl-CoAs and their acylcarnitine counterparts[125,126] (FIG. 2). In cultured muscle cells, acylcarnitines impair insulin signalling and/or glucose uptake and induce oxidative stress and mitochondrial dysfunction[125,127]. In mice, inhibition of muscle acetylcarnitine export causes the accumulation of C6–C20-chain acylcarnitines and glucose intolerance[128]. Pharmacological inhibition of L-carnitine transport decreases the level of acylcarnitines in plasma and tissue and improves insulin sensitivity in obese mice[129]. These results from cell culture and mouse studies suggest that acylcarnitine accumulation from increased lipid influx and deposition in muscle mechanistically link metabolic inflexibility to insulin resistance. However, human studies on the roles of acylcarnitines and L-carnitine supplementation are lacking[130]. Note that it is possible that acylcarnitines with different carbon lengths may have different effects on insulin resistance.

Lipids as metabolic substrates may also regulate insulin sensitivity by modulating hepatic glucose production (FIG. 2). The glycerol released from lipolysis can serve as a direct substrate for hepatic gluconeogenesis[131]. Fatty acids released by lipolysis are delivered to liver and metabolized to acetyl-CoA[119], which enhances the activity of pyruvate carboxylase to push pyruvate into the gluconeogenic pathway[119]. Therefore, by acting as metabolic substrates, lipids may influence glucose homeostasis by regulating muscle insulin signalling and hepatic gluconeogenesis.

Phospholipids and insulin sensitivity

Phospholipids are major components of cellular membranes, and they comprise two fatty acids at stereospecific numbering (sn)-1 and sn-2 positions and an alcohol-modified phosphate group at the sn-3 position. Phosphatidylcholine (PC) is the most abundant phospholipid, accounting for 40–50% of total cellular phospholipids; phosphatidylethanolamine (PE), which is localized in mitochondrial inner membranes, is the second most abundant phospholipid. PC and PE synthesis is controlled by different enzymes, but PE can be enzymatically converted into PC (BOX 3). Specific PC species rather than total PC concentrations regulate insulin sensitivity.

In the 1930s, Charles Best, who co-discovered insulin, reported that the PC precursor choline reduces fat deposition in rat liver[132], providing evidence that choline and PC may regulate lipid metabolism. Choline-deficient diets are now commonly used to induce hepatic steatosis in mice[133,134]. Furthermore, decreasing the levels of hepatic PC and PE by knocking down enzymes involved in their synthesis (BOX 3) exacerbates HFD-induced non-

alcoholic steatohepatitis (NASH) without, surprisingly, causing insulin resistance[135,136]. These data suggest that total levels of PC and PE do not regulate insulin sensitivity.

Instead, specific phospholipids may regulate insulin sensitivity. Indeed, PC(C12:0/C12:0) (with C12:0 fatty acids at n-1 and n-2 positions) activates the orphan nuclear receptor liver receptor homologue 1 (LRH1; also known as NR5A2) to increase bile acid synthesis, resulting in improved hepatic steatosis, insulin sensitivity and glucose homeostasis in mice[137]. PPARδ-activated hepatic PC(18:0/18:1) promotes fatty acid uptake and metabolism in muscle in a PPARα-dependent manner, and treating obese mice with PC(18:0/18:1) improves glucose tolerance and insulin sensitivity[138]. Phospholipids can be regulated by 'remodelling' (also called the Lands cycle), which changes the fatty acids at positions sn-1 and sn-2. Lysophosphatidylcholine acyltransferase-3 (LPCAT3) preferentially synthesizes unsaturated fatty acid-containing PCs and is induced by liver X receptors (LXRs) and PPARδ in liver[139,140]. Hepatic knockdown of LPCAT3 induces ER stress and inflammation[139], while its overexpression improves insulin sensitivity and glucose tolerance in wild-type and *ob*/*ob* mice[139,141]. Future studies identifying the intracellular signals generated by specific phospholipid species will help clarify their role in insulin sensitivity.

Lipids and metabolites modify proteins

Post-translational modifications (PTMs) of proteins increase functional diversity of the proteome without altering amino acid sequence. PTMs regulate protein activity, localization and interaction with other cellular components. Although protein phosphorylation and protein dephosphorylation are common PTMs that extensively regulate protein function, lipids and other non-lipid metabolites may serve as substrates for additional PTMs, among which acetyl-CoA-mediated protein acetylation has been extensively studied.

Acetyl-CoA and protein acetylation.

Acetyl-CoA links nutrient metabolism to many biological functions, including the PTM of proteins at lysine residues by acetylation. Acetylation, which regulates protein function, is a reversible process that is mediated by lysine acetyltransferases (KATs) and deacetylases (DACs)[142,143] (Supplementary Box 2). In yeast, stem cells and cancer cells, the activity of KATs and DACs is regulated by the availability of acetyl-CoA, with high acetyl-CoA levels increasing protein acetylation. However, acetyl-CoA does not modify proteins 'nonspecifically' because the KATs have varying K_d for acetyl-CoA[144–148]. Unfortunately, elucidating whether acetyl-CoA levels regulate protein acetylation in metabolic tissues and organs is challenging. In vivo intracellular acetyl-CoA levels are regulated by the availability of multiple substrates (glucose, lipids and amino acids), metabolic flux (as determined by the tricarboxylic acid (TCA) cycle, de novo lipogenesis, ketogenesis and steroid synthesis)[149] and inter-organ crosstalk (for example, the elevation of acetyl-CoA in liver mediated by adipose lipolysis)[119]. Acetyl-CoA dynamics may explain the discrepancies in tissue acetyl-CoA levels recorded in obese mice on a HFD with studies reporting elevated[119] or reduced[149] hepatic acetyl-CoA levels. Measuring subcellular acetyl-CoA levels is also technically challenging. Therefore, a major focus has been on studying the regulation of

KATs and DACs by hormones and environmental cues and how this affects the function of target proteins.

KATs and DACs may regulate insulin sensitivity by modulating the acetylation of proteins in the insulin signalling cascade (BOX 4; FIG. 3a) and of other proteins involved in glucose and lipid metabolism (FIG. 3b). The histone acetyltransferase GCN5 (also known as KAT2A) may play a dual role in regulating hepatic gluconeogenesis. In the fed state, GCN5 acetylates PPARγ co-activator-1α (PGC-1α) to inhibit its activity, resulting in the reduced expression of gluconeogenic enzymes and reduced glucose production[150,151]. In fasted and obese diabetic states, GCN5 is phosphorylated by protein kinase A (PKA), which increases its acetyltransferase activity for histones and attenuates that for PGC-1α. The increased histone acetylation recruits transcription factors such as forkhead box protein O1 (FOXO1) and hepatocyte nuclear factor 4α (HNF-4α)) to gluconeogenic genes to promote gluconeogenesis[152]. Hepatic gluconeogenesis is also regulated by p300, which acetylates and stabilizes CREB-regulated transcription co-activator 2 (CRTC2), a transcriptional cofactor of cAMP-responsive element-binding protein (CREB)[153]. This mechanism appears to be important for the activation of early gluconeogenic genes because CRTC2 is deacetylated by sirtuin 1 (SIRT1) and degraded later during fasting[153]. p300 also acetylates glucokinase (GCK) regulatory protein (GKRP), which increases its stability, sequesters GCK in the nucleus and inhibits the participation of GCK in glycolysis[154]. Finally, in the liver of obese mice, p300 promotes hyper-acetylation of the bile acid receptor farnesoid X-activated receptor (FXR) to impair its activity[155,156]. FXR deficiency causes ectopic lipid deposition in liver and muscle, leading to insulin resistance[157]. Thus, protein acetylation regulates glucose and lipid metabolism.

p300-mediated protein acetylation can be reversed by sirtuins, especially SIRT1, which is associated with increased insulin sensitivity[158]. Global SIRT1 overexpression improves insulin sensitivity, glucose tolerance and hepatic steatosis[159,160], and SIRT1 heterozygous knockout mice have hepatic steatosis and disrupted energy homeostasis[161,162]. Studies from global and tissue-specific SIRT1 transgenic and knockout mice indicate that SIRT1 in WAT and liver, but not muscle, regulates insulin sensitivity and glucose metabolism[161–166]. SIRT1 may promote WAT remodelling by deacetylating PPARγ to attain a more thermogenic brown adipose tissue (BAT)-like phenotype, thereby improving insulin sensitivity and glucose metabolism[167]. Although multiple studies indicate that SIRT1 activation in liver increases insulin sensitivity[161,168,169], understanding the molecular mechanisms involved is complicated by the fact that SIRT1 deacetylates and activates PGC-1α and FOXO1, which promote hepatic gluconeogenesis[151,170–172]. SIRT1-mediated deacetylation of FXR and CRTC2 may also offset its effects on PGC-1α and FOXO1 under certain conditions[155–157].

SIRT3, SIRT4 and SIRT5, which are localized in mitochondria, also regulate lipid and glucose metabolism. SIRT3 is dominant, and it regulates global mitochondrial protein acetylation[173]. Mitochondrial proteins are hyperacetylated in SIRT3 knockout mice and, although this is associated with reduced ATP production in metabolic organs such as liver, heart and kidney, it does not produce a metabolic phenotype in mice on a chow diet[174,175]. However, on a HFD, SIRT3 knockout mice are prone to obesity, insulin resistance and hepatic steatosis[173,176]. Interestingly, muscle and liver-specific SIRT3 knockout mice show

no metabolic abnormalities, even on a HFD[177], which is consistent with growing evidence showing that impaired mitochondrial function is associated with, but may not cause, insulin resistance[178–180]. SIRT7 deletion has been reported to both prevent and promote hepatic steatosis[181–183]. These often contrasting metabolic effects seen in different sirtuin studies reflect the complexity of studying how protein acetylation regulates glucose and lipid metabolism; different diets may alter the tissue levels of acetyl-CoA and NAD^+, which are critical for p300 and sirtuin activity[184]. In addition, protein acetylation is temporally and spatially regulated, as observed in GCN5, p300 and SIRT1-modulated gluconeogenesis[152,153].

Histone deacetylase 3 (HDAC3) regulates hepatic lipid metabolism via its interaction with Rev-erbAα (also known as NR1D1), a nuclear receptor that regulates circadian rhythm (FIG. 3b). During the fed period, when the animal is active, low Rev-erbAα levels reduce the association of HDAC3 with the liver genome, resulting in lipid synthesis and accumulation. During the inactive and fasting period, increased Rev-erbAα levels and HDAC3 activity suppress lipid accumulation[185]. Liver-specific HDAC3 deletion causes continuous lipid synthesis, leading to severe hepatosteatosis[186], yet these mice are more sensitive to insulin, perhaps owing to the limited availability of gluconeogenic substrates[186]. By contrast, mice with a muscle-specific deletion of HDAC3 are insulin resistant[187] yet have greater endurance and resistance to muscle fatigue than wild-type mice, as they activate anaplerotic pathways that are driven by AMP deaminase 3 and the catabolism of branched-chain amino acids (BCAAs)[187]. Furthermore, loss of HDAC3 in heart and/or skeletal muscle results in lipid accumulation and cardiac hypertrophy[188,189]. Insulin also promotes the HDAC3-mediated deacetylation and activation of phosphoglycerate kinase 1 to increase glycolytic ATP production and regulate redox state[190]. The relevance of this pathway in insulin resistance remains to be investigated.

Acetyl-CoA can also be used to acetylate glucosamine-6-phosphate to synthesize uridine-diphosphate-GlcNAc, which is the substrate for protein *O*-GlcNAcylation — that is, the attachment of *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) moieties to proteins. The role of protein *O*-GlcNAcylation in regulating insulin signalling and lipid and glucose metabolism was recently reviewed[191]. Overall, protein acetylation is a critical regulator of insulin sensitivity and metabolism, and this is a complex and evolving field of research.

In addition to acetylation, lysine residues in proteins can be post-translationally modified by succinylation, malonylation and glutarylation, all of which involve the addition of acyl moieties[192,193]. Proteomics has identified thousands of proteins that are modified by these metabolites, although the functional consequences of these modifications are largely unknown. The substrates for these modifications, such as succinyl-CoA, malonyl-CoA and glutaryl-CoA, are intermediates of multiple metabolic pathways, including the TCA cycle, lipogenesis, fatty acid oxidation and amino acid metabolism. Future studies will clarify whether these PTMs regulate or reflect insulin sensitivity.

Palmitate and protein palmitoylation.

LCFAs such as palmitic acid or myristic acid can also modify proteins. Palmitoylation of proteins at cysteine residues is established by a family of 24 Asp–His–His–Cys motif-

containing acyltransferases (DHHCs)[194] and removed by palmitoyl protein thioesterases[27,195]. *N*-myristoylation is mediated by peptide *N*-myristoyltransferase 1, which irreversibly attaches myristate to a conserved acceptor glycine either co-translationally or post-translationally[196]. Hundreds of proteins can be acylated, but few acylated proteins are known to be involved in insulin signalling and/or action. A recent non-targeted proteomic analysis revealed that GLUT4, proteins in the GLUT4 storage vesicle (including vesicle-associated membrane protein 2 (VAMP2) and insulin-responsive aminopeptidase (IRAP)) and other proteins involved in GLUT4 trafficking (including synaptosomal-associated protein 23 (SNAP23), syntaxin 4 and AKT substrate of 160 kDa (AS160; also known as TBC1D4)) are palmitoylated in adipocytes[197] (FIG. 3c). Palmitoylation of GLUT4 at Cys223, which is mediated by zinc-finger DHHC domain-containing protein 7 (DHHC7; also known as ZDHHC7), is critical for insulin-stimulated GLUT4 translocation[198,199]. GLUT4 translocation does not occur in the adipocytes of DHHC7 knockout mice, which develop insulin resistance and glucose intolerance[198]. Although DHHC7 has substrates in addition to GLUT4, this study demonstrates that protein palmitoylation has metabolic consequences. Interestingly, the induction of de novo lipogenesis by insulin in endothelial cells seems to be critical for the palmitoylation of endothelial nitric oxide synthase (eNOS; also known as NOS3), and disruption of this pathway, as seen in insulin resistance, leads to endothelial dysfunction and inflammation[200]. Furthermore, HFD-induced insulin resistance and the accumulation of palmitic acid in the hippocampus increase the expression of DHHC3 (also known as ZDHHC3) in a FOXO3-dependent manner, leading to palmitoylation of the AMPA glutamate receptor subunit GluA1, which reduces its activity and impairs cognitive function[201]. Additional studies are required to characterize the mechanisms by which protein palmitoylation influences insulin action, metabolic homeostasis and cognitive function.

Short-chain fatty acids in metabolism

Acetate, propionate and butyrate represent 90% of short-chain fatty acids (SCFAs), which are fermentation products of dietary complex carbohydrates such as fibres that are released by the gut microbiota[202,216]. Obesity and insulin resistance in humans and rodents are associated with alterations in gut microbial composition, with the enrichment of certain species of bacteria such as the butyrate-producing Firmicutes. and propionate-producing Bacteroidetes being different between healthy, lean and obese, insulin-resistant states[202,203]. Microbiota transplantation from obese mice or humans into lean, germ-free mice alters the levels of SCFAs and increases adiposity[202,204]. Conversely, faecal transplants from lean, healthy human donors into obese individuals improve insulin sensitivity and increase the abundance of certain butyrate-producing bacteria[205]. Studies in small cohorts of humans also suggest that SCFAs (particularly propionate) lower glucose levels[206,207]. Thus, an altered microbiota and consequently altered SCFA production may affect insulin sensitivity and energy metabolism in patients with obesity and T2DM.

Most but not all studies show that SCFA administration reduces body weight and/or adiposity in rodents and humans by suppressing appetite and increasing energy expenditure and oxidative metabolism[208–213]. SCFAs act through GPR41, GPR43 and GPR109 (REFS[214–218]). SCFAs, particularly acetate, can cross the blood–brain barrier in rodents and

humans[211,219], so their anorexigenic effects may be caused by GPCR activation in the brain. SCFAs also suppress appetite by stimulating the secretion of leptin from WAT[220,221] and of peptide YY (PYY) and GLP1 from the gut[208,222–224]. In addition, SCFAs activate intestinal gluconeogenesis, which has systemic insulin-sensitizing effects and promotes satiety and increased energy expenditure via the periportal neural system[209].

SCFAs alter peripheral metabolism by suppressing lipolysis and increasing oxidative metabolism in WAT, liver and skeletal muscle in an AMPK-dependent and/or PPARα-dependent manner[225,226]. Furthermore, SCFAs increase the number, differentiation and activity of colonic regulatory T cells[227,228] and suppress adipose inflammation in mice[220,229]. In humans, SCFAs suppress the pro-inflammatory effects of LPS in several immune cell types[230,231], and acetate reduces the levels of circulating TNF in obese women[222]. However, the metabolic effects of SCFAs may be species-specific, as SCFAs promote adipogenesis in murine and porcine, but not human, adipocytes in a GPR43-dependent manner[232–234]. Furthermore, acetate increases GSIS from mouse, but not human, islets, which may also be a result of species-specific differences in GPR43 signalling[214,235]. In conclusion, SCFAs have promising metabolic effects in rodents. However, long-term SCFA intervention studies have marginal benefits in humans[216], so their relevance to insulin sensitivity in humans is unclear.

Amino acids regulate insulin resistance

The association of elevated serum amino acid levels with obesity and insulin resistance was observed more than four decades ago[236]. The advancement in metabolomics analysis and mass spectrometry-based isotope labelling techniques has renewed interest in this area, especially towards understanding the relationship between BCAAs and insulin resistance.

Branched-chain amino acids.

Elevated circulating levels of the BCAAs leucine, isoleucine and valine has been associated with obesity, insulin resistance, T2DM, fatty liver, coronary artery disease and other complications of metabolic syndrome[237–241]. Elevated BCAA levels may also serve as a biomarker for the development of metabolic syndrome[242]. Bariatric surgery and pharmacological or dietary interventions causing weight loss and metabolic improvements lower the level of circulating BCAAs[237,243–247]. Several mechanisms have been implicated in raising BCAA levels in insulin resistance and/or obesity (FIG. 4). The expression level of BCAA catabolic enzymes in WAT is reduced in animal models and humans with obesity and/or insulin resistance[246,248,249]. This reduction in BCAA catabolism in WAT may be due to increased WAT inflammation and ER stress[250]. Transplanting WAT from normal mice reduces the elevated BCAA levels in mice with systemic genetic deletion of a rate-controlling BCAA catabolic enzyme[249,251]. How liver and muscle contribute to elevated BCAA levels in the states of insulin resistance and obesity is less clear as the expression of BCAA catabolic enzymes has been reported to be increased, decreased or unchanged under these conditions[246,249,252–254]. Increased enrichment of BCAA-producing bacteria in the gut microbiota[255] and disruption of hypothalamic insulin signalling that results in decreased

hepatic BCAA catabolism[252] has been reported to also contribute to elevated BCAAs in obesity and insulin resistance.

It is unclear whether the elevated levels of BCAAs associated with obesity and/or insulin resistance cause insulin resistance. Mendelian randomization in a large human genetic study suggests that elevated levels of BCAAs cause insulin resistance and T2DM[256]. However, animal and human studies supplementing with or restricting the levels of BCAAs report conflicting effe cts[241,254,257–260]. The rodent studies are confounded by several variables including species (mouse versus rat), the type of BCAA supplemented (leucine seems to be permissive for insulin signalling and induces satiety[257,258]) and the route of BCAA administration. Data suggesting that BCAAs promote mitochondrial dysfunction are stronger. BCAAs are tightly linked to lipid metabolism (FIG. 4), and BCAA overload increases acylcarnitine accumulation in muscle[241], which may exacerbate mitochondrial dysfunction[126]. The conjugation of acyl-CoA to glycine and their export from skeletal muscle can limit acylcarnitine accumulation in the muscle. Indeed, obese and insulin-resistant Zucker fatty rats have lower muscle glycine and urine acetyl-glycine levels than their lean counterparts, and BCAA restriction restores these parameters and is associated with improved metabolic homeostasis[254]. Serum 3-hydroxyisobutyrate (3-HIB), a valine metabolite secreted by muscle, is elevated in obesity and may stimulate fatty acid uptake and promote lipid accumulation in muscle[261]. By contrast, the valine metabolite β-aminoisobutyric acid (BAIBA), which is also secreted by muscle (in a PGC-1α-dependent manner), increases thermogenesis and the oxidative capacity of liver and adipose tissue via PPARα. Unlike 3-HIB levels, BAIBA levels are associated with reduced cardiometabolic risks and are increased by exercise in humans[262]. In addition, BCAA catabolism is impaired in heart failure, resulting in the accumulation of BCAA metabolites that may cause oxidative stress and impair mitochondrial function[263,264]. BCAAs also contribute to lipogenic substrates acetyl-CoA and propionyl-CoA in adipocytes. The impaired catabolism of BCAAs in adipocytes in obesity may result in less substrate and therefore reduced de novo lipogenesis, which is associated with insulin resistance[265]. Furthermore, monomethyl branched-chain fatty acids (mmBCFAs), which are synthesized from BCAAs, are decreased in WAT of individuals with obesity and insulin resistance and increased after gastric bypass surgery[266]. However, the biological functions of mmBCFAs are unknown.

In short, BCAA catabolism activates pathways that cause mitochondrial dysfunction, but whether mitochondrial dysfunction causes insulin resistance is unclear. Therefore, further studies are needed to verify whether elevated levels of circulating BCAAs in obesity, insulin resistance and T2DM cause insulin resistance.

Methionine and circulating aromatic amino acids.

Methionine is an essential amino acid in humans; the methionine derivative *S*-adenosyl-methionine is a methyl donor for the methylation of DNA, histones and other proteins. Methionine restriction rapidly reduces adiposity and improves insulin sensitivity and fatty liver in mice[267–270]. In humans, restriction of methionine intake with a 58% decline in serum methionine causes weight loss despite being coupled with increased energy intake[271]. It is not clear how methionine restriction increases energy expenditure while also increasing

food intake[267–270]. Levels of fibroblast growth factor 21 (FGF21) are increased tenfold in plasma and liver within the first 12 hours of methionine restriction, and this increase is sustained over several weeks[268]. However, the induction of FGF21 may be necessary for the effects of methionine restriction on energy expenditure but not weight loss, suggesting that other factors are involved in regulating methionine restriction-induced leanness[270]. Methionine restriction also induces adiponectin secretion, although adiponectin is dispensable for methionine restriction-mediated insulin sensitivity[272]. Methionine restriction-mediated insulin sensitivity is partially cell autonomous, as methionine depletion from cell culture media increases insulin-stimulated AKT phosphorylation in HepG2 cells[268]. Methionine restriction also alters histone and DNA methylation in liver and WAT[273], but whether these epigenetic modifications influence methionine restriction-mediated energy expenditure and insulin sensitivity is unknown.

Metabolomics studies show that the circulating aromatic amino acids (AAAs) phenylalanine and tryptophan are elevated in individuals with obesity and insulin resistance[242,247]. Importantly, AAAs may predict whether individuals will develop T2DM[274,275]. Metabolic improvements as a result of gastric bypass surgery are associated with lower AAAs[276]. To date, the relationship between AAAs and insulin resistance is only correlational. Further studies are needed to determine whether elevated AAAs cause insulin resistance and/or obesity.

Other metabolites

In addition to lipids and amino acids, other metabolites, including ketones, bile acids and nucleotides, may also be involved in regulating insulin sensitivity.

Ketone bodies, a ketogenic diet and insulin sensitivity.

Ketone bodies (that is, acetone, acetoacetate and β-hydroxybutyrate (β-OHB)) are alternative metabolic substrates during periods of nutrient deprivation. Ketones are both derived from acetyl-CoA and metabolized to acetyl-CoA, which interfaces with metabolic pathways such as fatty acid oxidation, BCAA metabolism and glycolysis. During periods of nutrient deprivation, ketones are generated in the liver and delivered to metabolically active organs such as the brain and heart for energy production. Ketogenesis is enhanced during fasting when the expression and activity of the rate-limiting ketogenic enzyme 3-hydroxy-3-methylglutaryl CoA synthase (HMGCS2) are induced and TCA cycle intermediates are preferentially used for gluconeogenesis. HMGCS2 knockdown in liver blocks ketogenesis, resulting in the accumulation of acetyl-CoA; the accumulation of acetyl-CoA activates mitochondrial pyruvate carboxylase to drive pyruvate towards gluconeogenesis[119]. Therefore, mice with hepatic HMGCS2 knockdown are hyperglycaemic on a chow diet, and they develop severe fatty liver and NASH owing to the increased flux of acetyl-CoA into de novo lipogenesis on a HFD[277]. HMGCS2 is also expressed in extrahepatic organs such as kidney, muscle, heart and adipose tissue[278], but the physiological relevance is unclear.

Ketone bodies are also signalling molecules. β-OHB binds to GPR109A and inhibits WAT lipolysis[279], which may serve as a negative feedback mechanism given that lipolysis provides ketogenic substrates. β-OHB also antagonizes GPR41 signalling in the sympathetic

nervous system, which lowers sympathetic tone[280] and may be a physiological adaptation to starvation. In addition, β-OHB inhibits HDAC1 and HDAC2 activity, which increases histone acetylation at promoters of FOXO3 and metallothionein 2 (REF.[281]) — factors that help protect against oxidative stress. Finally, β-OHB may inhibit the NLRP3 inflammasome[282,283]. Although WAT lipolysis, the oxidative stress response and inflammation contribute to insulin sensitivity[284,285], it is unclear whether ketone bodies such as β-OHB influence lipid and glucose metabolism through these pathways.

Ketogenic diets, which generally have >70% calories from fats and <10% calories from carbohydrates, increase energy expenditure, reduce obesity and enhance insulin sensitivity in rodents and humans[286,287]. Although the beneficial effects of ketogenic diets in mice require an increase in FGF21 levels, ketogenic diets reduce circulating levels of FGF21 in humans[288,289], indicating that the FGF21 pathway is not important in humans on a ketogenic diet. Beta-adrenergic receptors are required for a ketogenic diet-induced increase in energy expenditure in mice because mice lacking all three beta-adrenergic receptors do not increase energy expenditure and gain weight on a ketogenic diet, despite elevated FGF21 levels[290]. Although ketogenic diets are effective for weight loss, it may be hard for individuals to follow these diets.

Therefore, elucidating the mechanisms for ketogenic diet-induced weight loss, including alterations of metabolites, may provide novel targets for obesity treatment.

Bile acids.

Bile acids (Supplementary Box 3) are signalling molecules that regulate glucose and lipid metabolism through FXR and the Takeda G-protein coupled receptor 5 (TGR5; also known as GPBAR1). Bile acid-activated FXR reduces the activity of transcription factors hepatocyte nuclear factor 4α (HNF4α) and LRH1 to inhibit the synthesis of liver bile acid, forming a negative feedback loop. Furthermore, FXR activation in intestine induces the expression of the intestinal hormone FGF15 in mice (FGF19 in humans), which inhibits cholesterol 7α-hydroxylase (CYP7A1; the rate-limiting enzyme for bile acid synthesis) in liver by activating FGF receptor 4 (FGFR4)–β-klotho signalling[291]. In addition to negatively modulating bile acid production, FXR activation regulates lipid and glucose metabolism and therefore insulin sensitivity. Specifically, FXR knockout mice are insulin resistant and glucose intolerant owing to the deposition of ectopic lipids in liver and muscle[157]. FXR also inhibits the hepatic lipogenic factor sterol regulatory element-binding protein 1 (SREBP1) and promotes PPARα-induced β-oxidation[292].

TGR5, which is expressed ubiquitously, increases intracellular cAMP signalling[293,294]. Dietary supplementation of cholic acid in mice prevents and reverses HFD-induced weight gain, potentially by increasing TGR5-dependent energy expenditure in muscle and BAT[295,296]. This could result from the conversion of tetraiodothyronine (T_4) into the more potent triiodothyronine (T_3) (REFS[295,296]), which activates genes via thyroid hormone receptors to promote energy metabolism. The cholic acid-derived secondary bile acid deoxycholic acid (DCA) is more potent in activating, and has a higher specificity for, TGR5 than cholic acid. Disruption of FGFR4–β-klotho signalling by knocking out β-klotho results in higher levels of DCA, which activates TGR5 and stimulates BAT thermogenesis[297]. The

β-klotho knockout mice are therefore resistant to diet-induced obesity[298]. TGR5 is also expressed on certain intestinal entero-endocrine cells (L cells) and epithelial cells, from which it facilitates GLP1 release. Interestingly, TGR5 also increases GLP1 production from pancreatic β-cells by increasing the activity of prohormone convertase 1 (PCSK1), which cleaves proglucagon to generate GLP1 (REF.[299]). Bile acids or selective agonists of TGR5 improve liver and pancreatic function and enhance glucose tolerance in obese mice[300]. The biological functions of TGR5 may also explain many of the beneficial metabolic effects of gastric bypass surgery, as weight loss, improved fatty liver and reduced insulin resistance are lost in TGR5 knockout mice subjected to vertical sleeve gastrectomy[301]. In short, bile acid-mediated FXR and TGR5 pathways appear to be attractive targets for treating obesity and diabetes.

Nucleotides.

More than 30 years ago, Weber and colleagues described the role of insulin in regulating the synthesis of nucleotides[302]. Although the roles of purines in cellular energetics and signalling are well defined[303], an interest in the pyrimidine nucleoside uridine in metabolism has emerged. Fasting increases the levels of circulating uridine by inducing the production of uridine from adipose tissue, which lowers core body temperature and metabolic rate; refeeding reduces plasma uridine levels, as uridine synthesis in adipose tissue is decreased and biliary clearance of uridine is increased[304]. In humans, plasma uridine levels are increased in individuals with T2DM[305] and hypertension[306] and correlate with insulin resistance. In agreement, short-term or chronic uridine supplementation in mice results in glucose intolerance and reduced insulin signalling[307]. However, uridine supplementation also ameliorates hepatic steatosis and has cardioprotective effects[308,309]. Thus, the full spectrum of effects of uridine in cardiometabolic disease needs further study.

Perspective

Recent technological and bioinformatics advances have enabled the measurement of thousands of metabolites in biological samples. Signatures of amino acid, fatty acid and bile acid subtypes are being identified in association with altered metabolic states, especially obesity and T2DM. This presents several challenges, including that of determining which metabolite signatures cause the metabolic aberrations in question and which are consequences of them. Also, how these changes in metabolite levels are initiated, and in which tissues, needs to be determined. Studies also need to address how dietary nutrients alter cellular metabolites and the cellular pathways that these regulate, whether the effects of gut-derived metabolites are local or peripheral and the mechanisms involved. Additionally, it will be key to determine which observations in animal models are relevant to humans. Deepening our mechanistic understanding of how metabolites act as signalling molecules, and engage specific receptors to regulate hormone secretion, immune responses, insulin action and neuronal function, will be important in addressing these issues. In addition, it will be important to identify the functional consequences of the metabolite or lipid modification of protein structure and cell membrane dynamics. The sheer number of these metabolites and the interplay between their metabolic pathways, coupled with their ability to alter inter-

tissue communication, make this a compelling frontier for understanding metabolic disease and inventing novel approaches to treat T2DM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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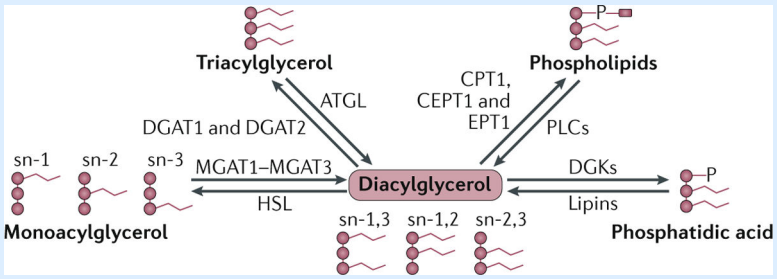
Box 1 |

Diacylglycerol synthesis

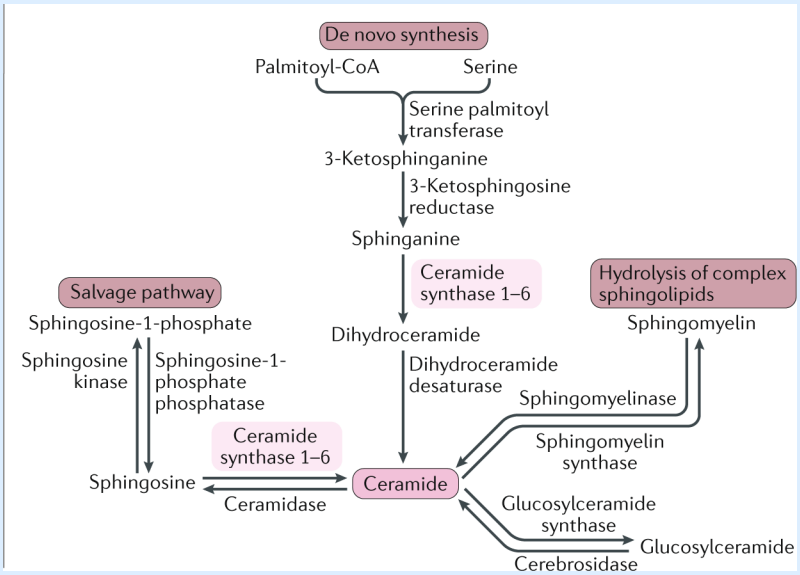
Diacylglycerol (DAG) levels are dynamically regulated by multiple pathways (see the figure). First, DAG can be synthesized from monoacylglycerol catalysed by monoacylglycerol acyltransferases (MGAT1–MGAT3), which esterify monoacylglycerol at stereospecific numbering 1 (sn)-1, sn-2 or sn-3 of glycerol to form sn-1,2, sn-1,3 or sn-2,3 DAG. second, DAG can be generated by the hydrolysis of triacylglycerol (triglycerides) and membrane phospholipids by adipose triglyceride lipase (ATGL) and phospholipases (PLCs), respectively. third, dephosphorylation mediated by lipins (lipin 1 (LPIN1) and LPIN3) of phosphatidic acid (PA) that is embedded in the endoplasmic reticulum membrane also generates DAGs. Each of these reactions can be reversed to reduce DAG levels. specifically, hormone-sensitive lipase (HSL) hydrolyses DAGs to generate monoacylglycerols. Diacylglycerol acyltransferase 1 (DGAT1) and DGAT2 esterify DAG to generate TAG. DAGs can be substrates for the synthesis of phospholipids, which is catalysed by cholinephosphotransferase 1 (CPT1), choline/ethanolaminephosphotransferase 1 (CEPT1) and ethanolaminephosphotransferase 1 (EPT1). Finally, DAGs can be phosphorylated by DAG kinase (DGK) to generate PA (see the figure). All these pathways have been studied in the context of insulin resistance, with inconclusive results. Knockdown of MGAT1 in liver improves glucose tolerance and hepatic insulin signalling in high-fat-diet-fed and *ob/ob* mice but also unexpectedly increases DAG levels[310]. However, the systemic deletion of *Mgat1* in *ob/ob* mice does not alter insulin sensitivity[311]. The adenovirus-mediated knockdown of LPIN1 and LPIN2 reduces hepatic DAGs and the activation of protein kinase Cε (PKCε) and improves insulin sensitivity[312,313]. However, liver-specific knockout of LPIN1 does not affect insulin sensitivity[88,314]. DGKδ haploinsufficiency increases the level of DAG in muscles and causes insulin resistance[315], but DGKθ overexpression in primary hepatocytes causes insulin resistance despite decreased levels of DAG[316]. Finally, and paradoxically, the overexpression of DGAT2 in liver increases the level of DAGs, whereas DGAT2 knockdown reduces the accumulation of hepatic lipids and improves insulin sensitivity[317–319]. The discrepancies in data derived from these models highlight the complex regulation of cellular DAG levels; altering one regulatory pathway may trigger compensatory changes in other pathways. In addition, different DAG species may have different effects on PKC activity[94]. However, as most studies reporting that an increase in DAG-induced PKC activation causes insulin resistance do not identify the DAG species involved, it is unclear whether specific DAG species or DAG levels are important.



Box 2 |

Sphingolipid metabolism

Ceramides can be generated through de novo synthesis, the salvage pathway or the hydrolysis of complex sphingolipids[320] (see the figure). De novo synthesis occurs in the endoplasmic reticulum, where palmitoyl-Coa and serine are condensed by serine palmitoyltransferase to form 3-ketosphinganine. 3-Ketosphinganine is then reduced to sphinganine by 3-ketosphingosine reductase, which is further acylated through a family of six ceramide synthase (CERS) enzymes (CERS1–CERS6) and desaturated by dihydroceramide desaturase to form ceramides. Each CERS isoform preferentially generates ceramides with specific acyl-chain lengths. For instance, CERS1, CERS5 and CERS6 generate C18 ceramides and C16 ceramides, respectively, and CERS2 selectively produces ceramides containing very-long-chain fatty acids (C22–C26 ceramides). In the salvage pathway, the sphingosine backbone of ceramides is broken down and reused for ceramide synthesis. Ceramides are degraded by ceramidases, which deacylate ceramides to generate sphingosine. Sphingosine is then phosphorylated by sphingosine kinase 1 (SPK1) or SPK2 to sphingosine-1-phosphate (S1P). S1P binds to S1P receptors (S1PR1–S1PR5), which are G protein-coupled receptors. S1P may induce insulin resistance in liver and muscle[321,322], although adiponectin-stimulated S1P is associated with improved hepatic insulin sensitivity[97]. Finally, ceramides may be released from the hydrolysis of complex sphingolipids such as sphingomyelin (via the action of sphingomyelinase) and glycosphingolipids such as glucosylceramide (via the action of glucosylceramide synthase (GCS).

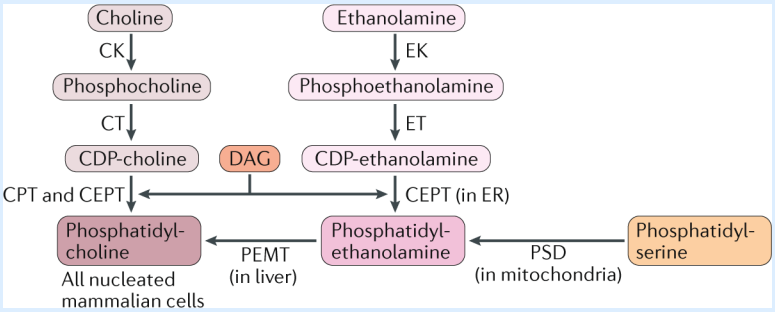


Box 3 |

Synthesis of phosphatidylcholine and phosphatidylethanolamine

In the majority of cells, phosphatidylcholine (PC) is synthesized via the cytidine 5-diphosphate (CDP)-choline pathway, in which choline is phosphorylated to phosphocholine by choline kinase (CK) and then converted into CDP-choline by CTP:phosphocholine cytidylyltransferase (CT; encoded by PCYT1) (see the figure). subsequently, CDP-choline is combined with diacylglycerol (DAG) by two enzymes that are integrated into the endoplasmic reticulum (ER): CDP-choline:1,2-diacylglycerol cholinephosphotransferase (CPT) and CDP-choline:1,2-diacylglycerol choline/ethanolamine phosphotransferase (CEPT). The CDP-choline pathway is present in all nucleated mammalian cells. However, in the liver, up to 30% of PC is generated by the conversion of phosphatidylethanolamine (PE) into PC by phosphatidylethanolamine *N*-methyltransferase (PEMT) (see the figure).

PE can be synthesized by two major pathways: the CDP-ethanolamine pathway in the ER and the phosphatidylserine decarboxylase (PSD) pathway in mitochondria. the CDP-ethanolamine pathway is similar to that of PC synthesis. Phosphoethanolamine is converted into CDP-ethanolamine by CTP:phosphoethanolamine cytidylyltransferase (encoded by PCYT2) and then added to DAG by CEPT to form PE. the PSD pathway occurs exclusively in mitochondria, where phosphatidylserine (PS) is decarboxylated by PSD to form PE. The synthesis of PS, which is controlled by two PS synthases, is the rate-limiting step for PE synthesis in the PSD pathway. EK, ethanolamine kinase.



Box 4 |

Acetylation of insulin signalling proteins

Acetylation of insulin receptor substrate (IRS) proteins by the histone acetyltransferase p300 and their deacetylation by histone deacetylase 2 (HDAC2) are both associated with impaired insulin signalling[323,324]. Mass spectrometry identified 15 acetylated lysine residues in IRS1 and IRS2 in mouse liver[324]. Serial mutagenesis revealed that acetylation of Lys1017, Lys1080 and Lys1131 in IRS1 and of Lys1173 and Lys1264 in IRS2 may be critical for insulin signalling[324]. Additional acetylation sites (Lys52 and Lys61) in human IRS1 were identified by non-targeted proteomics, but their relevance to insulin signalling is unclear[325,326]. p300 and the p300/CBP-associated factor (P/CAF) also acetylate AKT in the pleckstrin homology (PH) domain at Lys14 and Lys20, thereby blocking AKT binding to phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) at the plasma membrane[327]. Conversely, deacetylation of these lysine residues by sirtuin 1 (SIRT1, which is NaD^+ dependent), promotes AKT–PIP_3 binding to activate aKt[327]. In addition, p300 and SIRT1 regulate AKT activation by modulating the acetylation of rapamycin-insensitive companion of mtOR (RICTOR), an essential scaffold protein within mTOR complex 2 (MTORC2). RICTOR acetylation increases mTORC2 activity and promotes AKT phosphorylation at Ser473, which is critical for insulin-stimulated AKT activation[328]. Finally, SIRT7 suppresses AKT phosphorylation at Ser473 via deacetylating and activating FK506-binding protein, a scaffold protein for the AKT-specific phosphatase PH domain leucine-rich repeat-containing protein phosphatase (PHLPP), in cancer cells[329]. However, the significance of acetylation of proteins involved in insulin signalling in systemic insulin resistance is unclear. For instance, although SIRT1 deacetylates and activates AKT in cultured cells[327], transgenic SIRT1 overexpression in skeletal muscle does not increase AKT phosphorylation or insulin sensitivity[330].

Toll-like receptors

(TLRs). A family of pattern-recognition receptors with a role in innate immunity. For example, TLR4 is activated upon dimerization with the co-receptor myeloid differentiation protein 2 (MD2); signalling via TLR4–MD2 results in the transcription of pro-inflammatory genes.

Mendelian randomization

A novel epidemiological study design that uses genetic variants to investigate the causal relationship of a biomarker to the risk of having a phenotype or disease.

Stereoisomers

Two molecules that have the same molecular formula but differ in the orientation of atoms. Enantiomers are stereoisomers that are mirror images of each other; an enantiomer is labelled ‘R’ in its right-handed configuration and ‘S’ in its left-handed configuration.

Stereospecific numbering (sn).

The sn position is often used to define the configuration of glycerol-containing metabolites. When the OH group on the second carbon (sn-2) of glycerol is oriented to the left, the top (first) carbon is at the sn-1 position and the bottom (third) carbon is at the sn-3 position.

Non-alcoholic steatohepatitis (NASH).

Hepatitis caused by excessive fat deposition in the liver that is not related to heavy alcohol use.

Lands cycle

Cycles to remodel phospholipids by first de-acylating and then re-acylating them, thereby altering the fatty acid moiety to generate mature phospholipids.

Anaplerotic pathways

Pathways that replenish tricarboxylic acid cycle intermediates, which can then be used for energy production or for gluconeogenesis in the liver.

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Periportal neural system

The nervous system that innervates the liver.

L cells

A subset of entero-endocrine cells that secrete gut peptides such as glucagon-like peptide 1 (GLP1), incretins, etc.

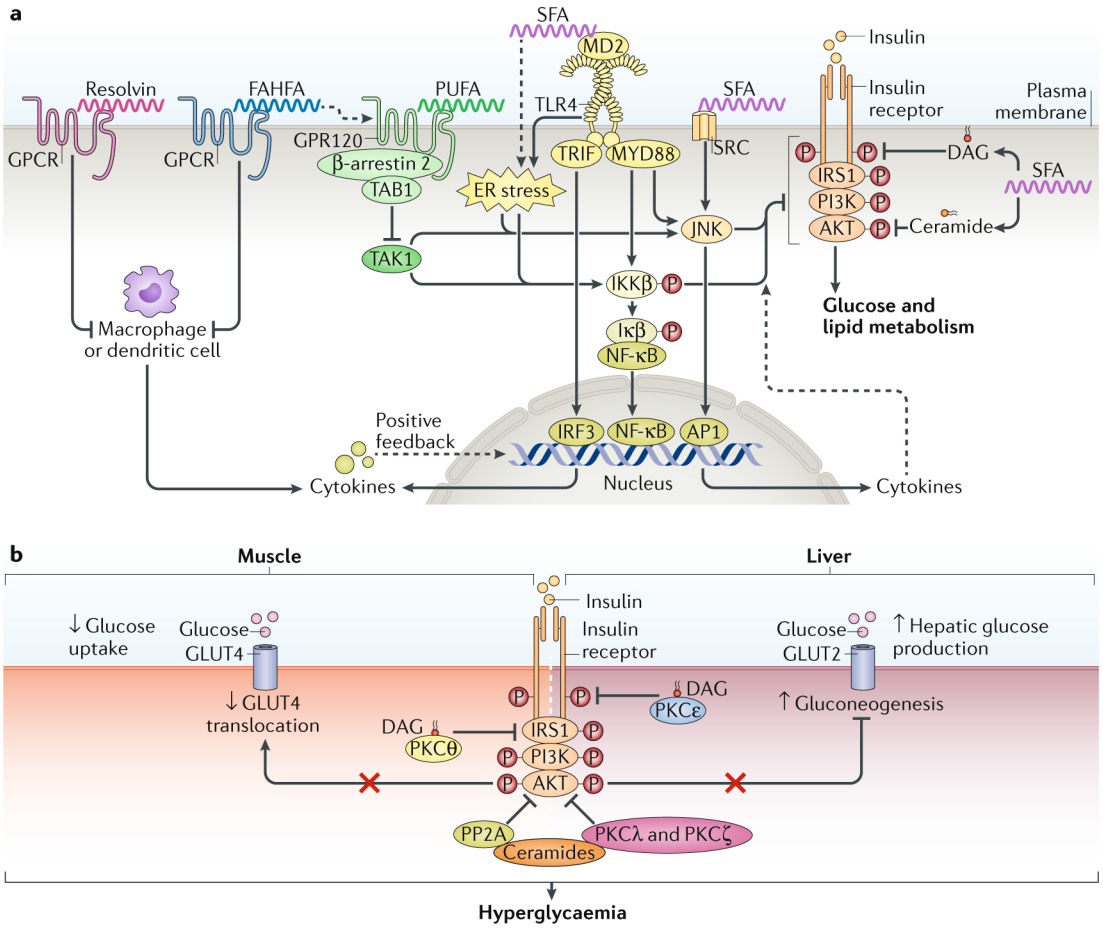

Fig. 1 |. Lipids as signalling molecules that regulate metabolism.

Several signalling lipids, including fatty acids, fatty acid esters of hydroxy fatty acids (FAHFAs), diacylglycerol (DAG) and ceramides, regulate intracellular pathways to influence insulin resistance. **a** | Fatty acids regulate insulin sensitivity and inflammation. Saturated fatty acids (SFAs) activate Toll-like receptor 4 (TLR4), possibly via its co-receptor, myeloid differentiation protein 2 (MD2), which, through the adaptor proteins TIR-domain-containing adaptor-inducing interferon-β (TRIF) and myeloid differentiation primary response protein MYD88, increases the activity of pro-inflammatory transcription factors. TRIF activation promotes the nuclear translocation of interferon regulatory factor 3 (IRF3) to increase the expression of cytokines. MYD88 activation increases phosphorylation of inhibitor of nuclear factor-κB (NF-κB) kinase (IKKβ), which further phosphorylates inhibitor of NF-κB (IκB), leading to nuclear translocation of NF-κB to increase pro-inflammatory cytokine expression. MYD88 also activates Jun N-terminal kinase (JNK) to increase the activity of transcription factor activator protein 1 (AP1), thereby altering the expression of cytokines. Pro-inflammatory cytokines, through their receptors (not shown), further activate these pro-inflammatory transcription factors to establish a positive feedback loop for sustained inflammation. SFA-activated TLR4 and cytokine production impair insulin signalling through IKKβ and JNK activation, but the direct targets of IKKβ and JNK in the insulin signalling pathway remain to be identified. SFA-mediated activation of endoplasmic reticulum (ER) stress, the SRC–JNK pathway and the incorporation of SFAs into

diacylglycerol (DAG) and ceramides also impair insulin signalling by inhibiting phosphorylation of the insulin receptor, insulin receptor substrate 1 (IRS1) or AKT. Polyunsaturated fatty acids (PUFAs) exert anti-inflammatory effects by activating G protein-coupled receptor 120 (GPR120), which recruits β-arrestin 2 and sequesters TAK1 binding protein 1 (TAB1) to inhibit the TAK1-mediated activation of JNK and IKKβ. PUFA, FAHFAs and resolvins may exert anti-inflammatory effects by activating G protein-coupled receptors (GPCRs) in antigen-presenting cells such as macrophages to inhibit cytokine production. The dashed lines indicate indirect effects. **b** | DAG and ceramide may induce insulin resistance. DAG accumulates ectopically in insulin-resistant muscle and liver. In muscle, DAG-activated protein kinase Cθ (PKCθ) promotes the phosphorylation of IRS1 on Ser1101 in mice, impairing IRS1 phosphorylation on tyrosine and attenuating insulin signalling. In liver, DAG-activated PKCε promotes phosphorylation of IR on Thr1160 to suppress insulin signalling. As a result of DAG increase, glucose uptake via insulin responsive glucose transporter 4 (GLUT4) in muscle is reduced, and glucose output via GLUT2 from liver is increased; these changes induce hyperglycaemia. Ceramides contribute to hyperglycaemia by activating protein phosphatase 2 A (PP2A), which dephosphorylates AKT, and stimulating PKCλ and PKCζ, which prevent AKT from associating with the membrane, thereby inhibiting AKT activity.

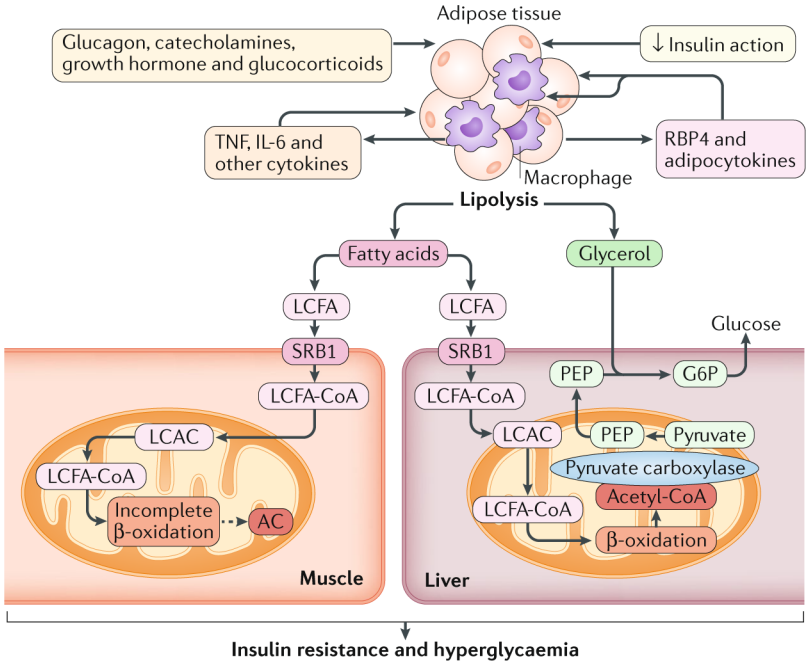

Fig. 2 |. Alterations in lipid metabolism are associated with insulin-resistant states.

Obesity and type 2 diabetes mellitus are associated with increased lipolysis in adipose tissue owing to the action of, or resistance to, multiple hormones and to the increased production of cytokines (such as tumour necrosis factor (TNF) and interleukin-6 (IL-6)) by adipose tissue macrophages. The release of TNF and IL-6 from macrophages is potentiated by the secretion, from adipocytes, of adipocytokines such as retinol-binding protein 4 (RBP4). Fatty acids including long-chain fatty acids (LCFAs) that are released by lipolysis are taken up by muscle and liver via the fatty acid transporter scavenger receptor class B member 1 (SRB1). In muscle, LCFA thioesters (LCFA-CoAs) are imported into mitochondria for β-oxidation via the carnitine shuttle, in which LCFA-CoAs are converted into long-chain acylcarnitines (LCACs). Incomplete β-oxidation in insulin-resistant states causes accumulation of acylcarnitines (ACs) (shown as a dashed line) of varying lengths, which are associated with insulin resistance and hyperglycaemia. In the liver, LCFAs are imported into the mitochondria and are oxidized to generate acetyl-CoA, which activates pyruvate carboxylase, leading to increased production of phosphoenolpyruvate (PEP) from pyruvate. Glycerol generated from lipolysis, in addition to PEP, is converted into glucose-6-phosphate (G6P), resulting in increased glucose production. Overall, the increased flux of metabolic substrates into liver causes insulin resistance and hyperglycaemia. Although an increase in AC muscle content correlates with insulin resistance, a causative effect has not been established in vivo.

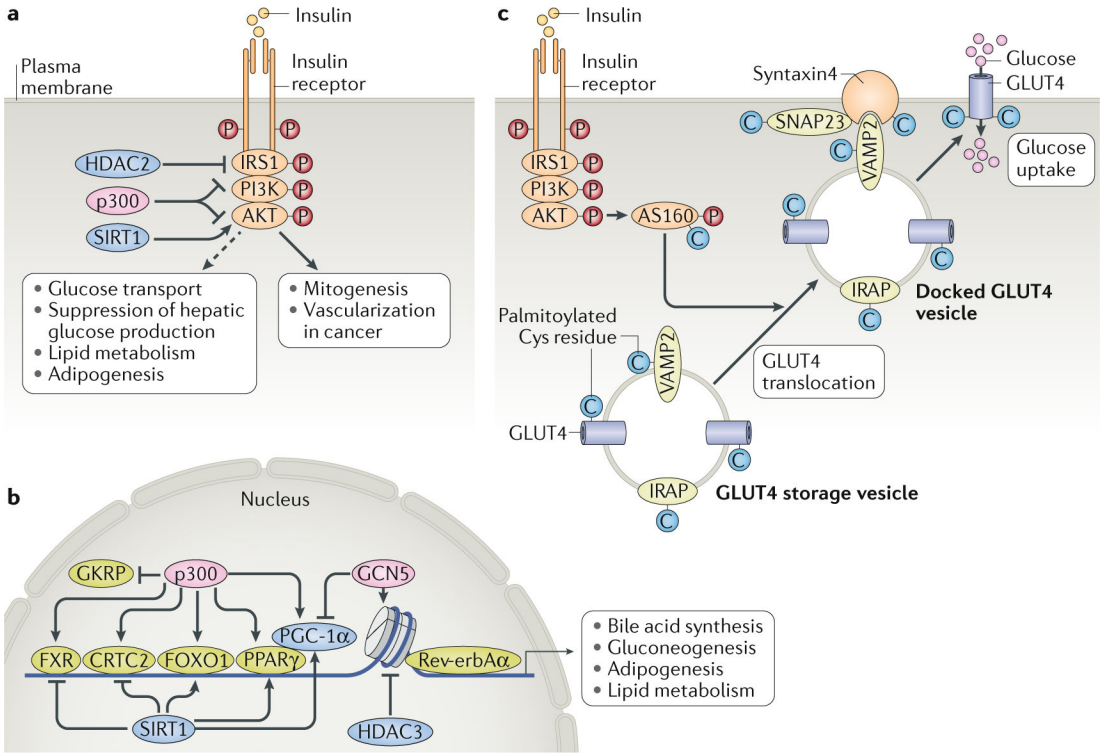

Fig. 3 |. Lipids and metabolites modify proteins to regulate metabolism.

Protein acetylation regulates insulin signalling and glucose metabolism. **a** | Histone acetyltransferase p300, histone deacetylase 2 (HDAC2) and sirtuin 1 (SIRT1) modulate acetylation of insulin receptor substrate 1 (IRS1) and/or AKT in cancers, causing mitogenesis and vascularization, but their effects on activation of insulin-stimulated metabolic pathways remain to be determined (shown as a dashed line). **b** | In the nucleus, these acetyltransferases and deacetylases also modulate the activity of transcription factors, including farnesoid X-activated receptor (FXR), CREB-regulated transcription co-activator 2 (CRTC2), forkhead box protein O1 (FOXO1) and peroxisome proliferator-activated receptor-γ (PPARγ), and of PPARγ co-activator 1α (PGC-1α)) to regulate bile acid synthesis, gluconeogenesis, adipogenesis and lipid metabolism. Moreover, the modulation of histone acetylation by histone deacetylase 3 (HDAC3), which interacts with Rev-erbAα, regulates the circadian rhythm of lipid and glucose metabolism. p300 also acetylates glucokinase (GCK) regulatory protein (GKRP), which sequesters GCK in the nucleus to inhibit its participation in glycolysis. **c** | Protein palmitoylation regulates glucose transport in adipocytes. In adipocytes, glucose transporter 4 (GLUT4), components of the GLUT4 storage vesicle (namely, vesicle-associated membrane protein 2 (VAMP2) and insulin-responsive aminopeptidase (IRAP)) and GLUT4 trafficking proteins (including synaptosomal-associated protein 23 (SNAP23), syntaxin 4 and AKT substrate of 160 kDa (AS160)) are palmitoylated at cysteine residues, which regulates their activity. Decreased palmitoylation is associated with impaired insulin-stimulated GLUT4 translocation.

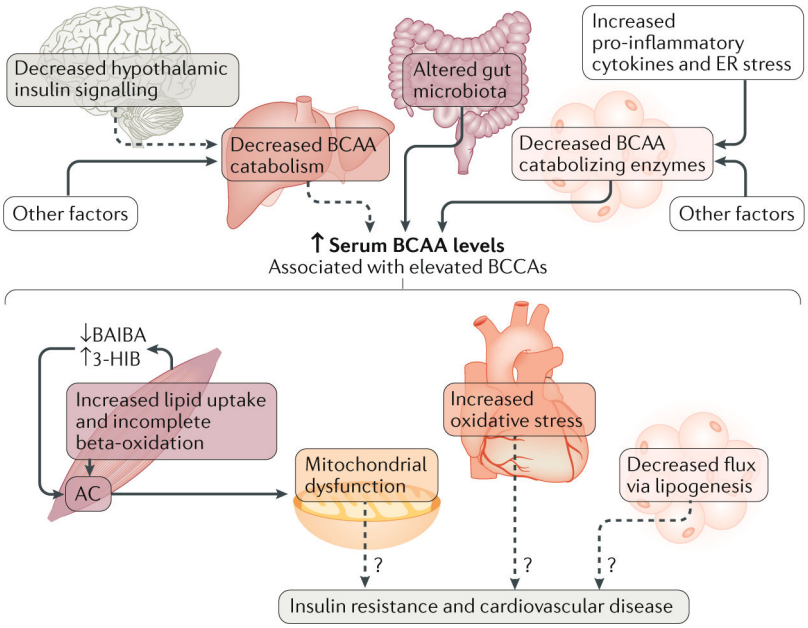

Fig. 4 |. Increased levels of branched-chain amino acids are associated with insulin resistance. Several factors may increase serum levels of branched-chain amino acids (BCAAs) in states of insulin resistance and obesity, including increased BCAA production by gut microbiota, decreased expression of BCAA catabolizing enzymes in white adipose tissue (WAT) resulting from increased WAT inflammation and endoplasmic reticulum (ER) stress, and possibly decreased catabolism of hepatic BCAAs, which is linked to impaired hypothalamic insulin signalling. Although elevated serum levels of BCAAs are associated with insulin resistance and cardiovascular disease, whether this is causative needs further investigation. BCAAs increase incomplete lipid oxidation in muscle, resulting in the accumulation of acylcarnitines and mitochondrial dysfunction. Altered valine catabolism in muscle increases the production of 3-hydroxyisobutyrate (3-HIB) and decreases the secretion of β-aminoisobutyric acid (BAIBA), which results in increased lipid oxidation and the accumulation of acylcarnitines. Elevated cardiac BCAAs may cause oxidative stress in heart. In adipose tissue, impaired BCAA catabolism may reduce substrate flux into lipogenesis. All these factors may contribute to metabolic dysfunction in insulin resistance, type 2 diabetes mellitus and cardiovascular disease. Dashed lines indicate possible effects that have not been conclusively validated.