Lipidomics in diabetes

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Purpose of review
Multiple studies have shown a strong association between lipids and diabetes. These are usually described through the effects of cholesterol content of lipid particles and in particular low-density lipoprotein. However, lipoprotein particles contain other components, such as phospholipids and more complex lipid species, such as ceramides and sphingolipids. Ceramides, such as sphingolipids are also produced intracellularly and have signalling actions in regulating cell metabolism including effects on inflammation, and potentially have a mechanistic role in the development of insulin resistance.

Recent findings
Recently, techniques have been developed to analyse detailed molecular profiles of lipid particles – lipidomics. Proteomics has confirmed the different proteins associated with different particles but far less is known about the relationship of individual lipid species with diabetes and cardiovascular risk. A number of studies have now shown that the plasma lipidome, and in particular, ceramides and sphingolipids may predict the development of diabetes.

Summary
Lipidomics had identified ceramides and sphingolipids as potential mediators of cellular dysfunction in diabetes. Further work is required to ascertain whether they have clinical utility.

Keywords
ceramide, diabetes, lipidomics, sphingolipid

INTRODUCTION

There is a long-standing association between lipids and diabetes. Lipid particles contain a combination of different phospholipids, triglycerides and cholesterol as well as proteins involved in control of lipid-modifying enzymes, transfer factors and receptor ligands. Epidemiological studies show a strong association of both low-density lipoprotein cholesterol (LDL-cholesterol) and high-density lipoprotein cholesterol (HDL-cholesterol) content with type 2 diabetes (T2DM) [1] whereas glycaemia and blood pressure show a stronger association with type 1 diabetes (T1DM) [2]. However, these associations underestimate the complexity of these particles as amounts and species of proteins, lipids and phospholipids vary with the major underlying lipid particle species and with disease states. The new techniques associated with quantifying these relationships form part of the new 'omics', which include proteomics, lipidomics [3▪▪] and their relationship to metabolism – metabolomics [4]. Proteome studies have identified that HDL is associated with proteins associated with inflammation, complement and coagulation while LDL particles have a far simpler proteome [5]. However, fewer studies have investigated lipid subspecies and the lipidome in diabetes, its complications and cardiovascular disease (CVD).

INFLAMMATION-ASSOCIATED LIPIDS AND DIABETES

The two main types of diabetes are associated with different mechanisms of causation. In T1DM, an autoimmune reaction to pancreatic beta cells is key whereas T2DM is linked to insulin resistance and its secondary consequences including adiposopathy. Autoimmune disease is directly related to increased levels of inflammation including activation of key signalling lipids, such as lyso-lecithins, prostanoids and other arachidonic acid derivatives and some sphingolipids.

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Curr Opin Endocrinol Diabetes Obes 2022, 29:124–130
DOI:10.1097/MED.0000000000000704

KEY POINTS

- Lipidomics can identify subspecies within lipid fractions that may be associated with chronic disease.
- Ceramides and sphingolipids are lipid intermediates with signalling properties.
- Elevated ceramide levels are associated with autoimmune disease and risk of type 2 diabetes in epidemiological studies.

With insulin resistance, the changes are more complex and may differ between tissues. Hepatic insulin resistance is linked to alterations in ceramides [6,7] and in function of the innate immune system receptors, such as toll receptors [8]. The adipose tissue is not only engaged in management of stored lipids but also has a paracrine function, secreting hormones and complement proteins, and interacts with tissue macrophages [9]. Recent analysis of adipocytes from Indian Asian patients with T2DM has suggested that alteration in signalling RNAs may play a role in these changes including regulation of peroxisomal proliferator activating receptor (PPAR) pathways [10].

PHOSPHOLIPIDS AND DIABETES

The surface of lipoprotein particles is composed of phospholipids as are cell membranes. Numerous studies have profiled the response of these to dietary modification [11▪▪]. Measurement of plasma and erythrocyte lipid and phospholipid concentrations in epidemiological and intervention studies has often been performed. In the Kuopio Ischaemic Heart Disease Risk Factor Study ($n = 2332$) over a 19-year follow-up, the risk of new type 2 diabetes showed a 25% [95% confidence interval (CI) 2–43%] lower relative risk (RR) ($P_{quartile} = 0.02$) with higher choline intake driven by 41(22–55)% lower RR ($P_{quartile} < 0.001$) attributable to phosphatidylcholine intake [12▪]. More detailed lipid subspecies analysis of odd chain fatty acids (OCFA)-containing lipids included triacylglycerols (TAGs), free FAs (FFA), cholesteryl esters, phosphatidylcholines, phosphatidyl-ethanolamines, lysophosphatidylcholines (LPCs), lysophosphatidylethanolamines (LPEs), monoacylglycerols (MAGs) and diacylglycerols (DAGs) has been conducted in 820 patients with T2DM and 1248 controls from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study ($n = 27\,548$). These OCFAs were enriched in MAGs, and the contribution of C15:0 versus C17:0 differed across lipid classes. In women, several OCFA-containing phospholipids were inversely associated with T2D risk with PC (C15:0), $HR_{quintile}$ 0.56(0.32–0.97); PC (C17:0), hazard ratio per standard deviation (SD): 0.59 (0.48–0.71); LPC (C17:0), $HR_{quintile}$ 0.42 (0.23–0.76). In men, LPC (C15:0) levels were associated with T2D risk ($HR_{quintile}$ 1.96 (1.06–3.63)) whereas cholesteryl ester (C17:0), MAG (C15:0), and DAG (C15:0) were inversely associated with T2D risk. A positive correlation of fat-rich dairy and fibre-rich foods with OCFA-lipid levels was found while red meat intake showed an inverse correlation [13].

DIETS, LIPIDOMICS AND GENETICS

Attempts have been made to study the interaction between diet, lipid species and high-risk genotypes for T2DM. In 21 men homozygous for high-risk TCF7L2 challenged with two isocaloric (450 kcal) liquid meals were conducted with high-carbohydrate (89% of energy) or normocarbohydrate (45% of energy) meals. Lower postprandial glucose curves seen were correlated with lower phospholipids (−37 to −53%), lysoPL (−29 to −86%), sphingolipids (−32 to −47%), arachidonic (−36%) and oleic (−63%) acids, and leukotrienes (−65% to −83%). Sphingosine concentrations were higher (125–832%) after normal carbohydrate meals, whereas acylcarnitines were lower (−21 to −61%) [14]. This suggests that risk genotypes interact with carbohydrate content in meals to influence lipid subspecies.

LIPIDOMICS OF PHOSPHOLIPIDS IN EPIDEMIOLOGICAL STUDIES OF NEW DIABETES

Detailed quantitative techniques involving lipidomic analysis have been applied to such studies in populations either with diabetes or at risk of developing it. A study of three Swedish cohorts ($N = 3638$) including twins and including validation in the Cooperative Health Research Augsburg (KORA) S4 cohort ($n = 855$) investigated 5961 metabolic features and found 1120 were associated with T2DM and IFG of which 70 were metabolites replicated in three cohorts. Including the validation cohort, 15 metabolites were associated with new T2DM after adjustment for age, sex, BMI, waist circumference and fasting glucose. These included the bile acid deoxycholic acid and MAG (18:2) added little to standard diabetes mellitus risk scores [15].

A study of 107 men with T2DM and 216 controls from the longitudinal METSIM study assessed lipid markers at baseline and after 5-years follow-up. Validation was performed in 631 men from the general population. Lipidomic analysis was performed on a panel of 277 plasma lipid species. Higher levels of TAGs and di-acyl-phospholipids

and lower levels of alkyl-acyl phosphatidylcholines was seen in incident T2DM. LysoPC (18:2), phosphatidylcholine (32:1), phosphatidylcholine (34:2e) and phosphatidylcholine (36:1) and TAG (17:1/18:1/18:2) added to a model that included metabolic risk factors and FINDRISC variables. After adjusting for age and BMI, these lipids had respective odds ratios of 0.32, 2.4, 0.50, 2.2 and 0.31 for progression to T2DM [16].

A lipidomic analysis in China of 667 patients with new T2DM and 200 propensity-matched controls was performed. The validation cohort constituted 250 patients with new T2DM and 724 matched control individuals with normal glucose regulation. Differential correlation network analyses showed 38 lipid species, including TAGs, lysophosphatidylinositols (LPIs), phosphatidylcholines, polyunsaturated fatty acid (PUFA)-plasmalogen phosphatidyl-ethanolamines (PUFA-PEps) and cholesteryl esters, were consistently associated with risk of new T2DM. The lipids identified as significant included LPI16:1, PC34:3, PE38:4p (18:0p/20:4), TAG50:2 (16:2), TAG51:0 (17:0) and TAG54:7 (22:6), and in this study improved prediction of incident diabetes [17].

Attempts have been made to identify specific lipid subspecies as biomarkers of insulin resistance. A study was performed in 62 subjects characterized using a 5-point oral glucose tolerance test (OGTT) of whom 15 underwent hyperinsulinemic-euglycemic clamp. Linoleoylglycerophosphocholine (LGPC) were negatively correlated with glucose disposal in the clamp ($r = -0.56$, $P = 0.03$) but not with OGTT results. Participants without T2DM with higher LGPC levels had higher glucose excursions in the OGTT (P trend $= 0.02$) [18].

SPHINGOLIPIDS AND DIABETES

Ceramides and sphingolipids are a complex series of mediators derived from serine and the saturated fatty acid palmitic acid (C16:0) [19▪▪]. Ceramide is necessary for synthesis of complex glycosphingolipids and pathways exist for tis catabolism and salvage (Fig. 1). Both ceramides and sphingolipids are known to be intracellular signalling molecules

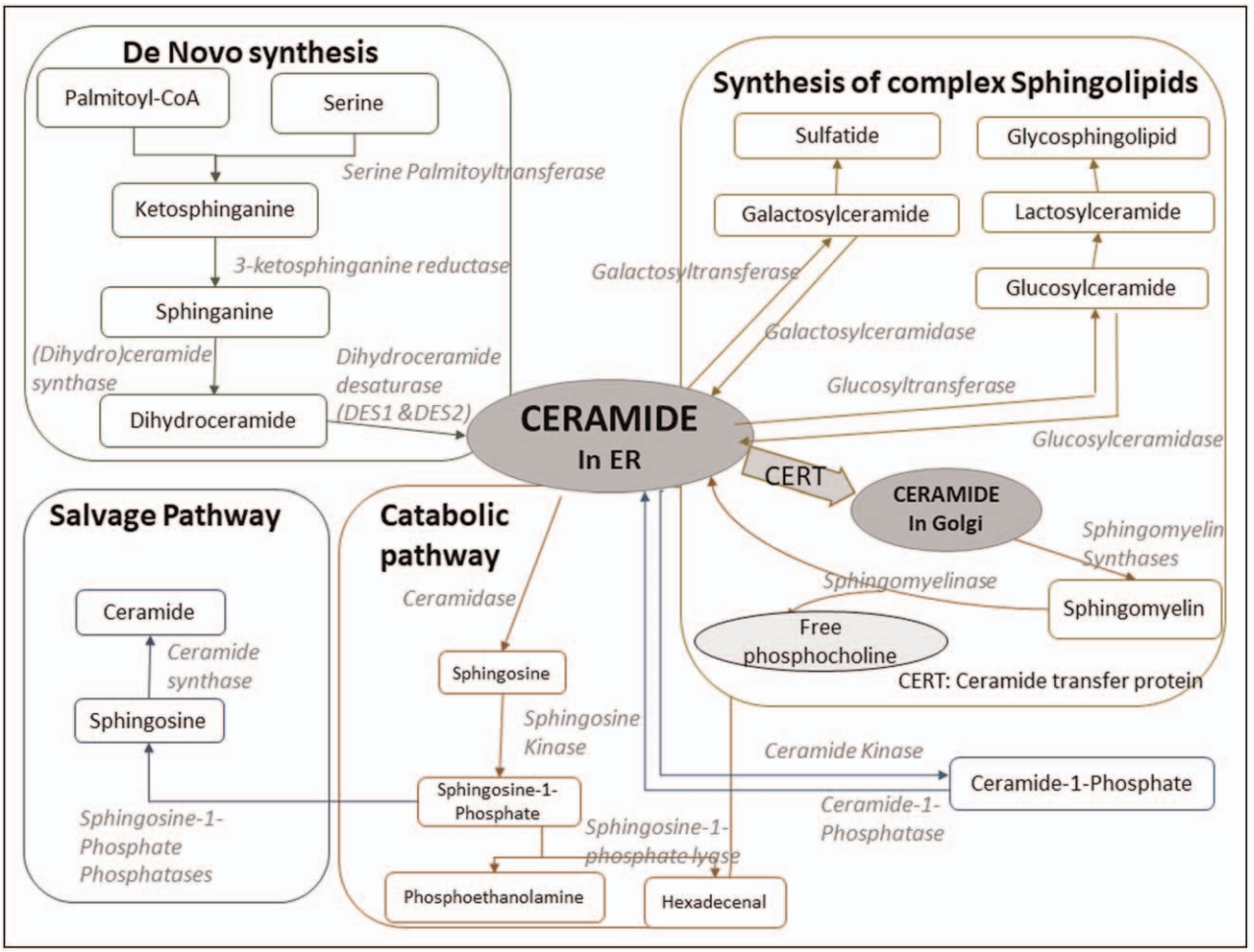

FIGURE 1. Pathways involved in synthesis, metabolism and salvage of ceramides and sphingolipids and their derivatives.

[19▪▪]. Genetic mutations in sphingolipids are associated with a variety of monogenic diseases including renal disease (e.g. Fabry's disease).

There is increasing interest in subtler perturbations in sphingolipid metabolism found in common diseases [19▪▪,20▪▪,21] including T1DM andT2DM as these are associated with processes involved in control of inflammation [22] and beta-cell function [20▪▪,23▪,24,25]. Sphingolipids are known to be associated with alterations in immune system function. Correlations have been found between plasma and hepatocyte sphingolipid concentrations and incidence of islet cell antibodies in type 1 diabetes [26]. In nonobese diabetic mice, this is mediated through activation of Ca^{2+}-independent phospholipase A2β (iPLA2β) via macrophages. Hydrolysis of the sn-2 glycerophospholipids (see below) by iPLA2β leads to the generation of eicosanoids, which can trigger immune responses leading to β cell death [27]. Similar eicosanoid signatures have been found in children with autoantibodies or recent-onset T1DM [27].

Ceramides are involved in insulin secretion. Sulphated galactosyl-ceramide increases exocytosis of secretory granules and activates K(ATP)-channels increasing insulin secretion while sulphated lactosylceramide (s-lacCer) is present in pancreatic islets [28]. In a case-control study of 569 individuals, low serum sulfatide concentrations were associated with Type 2 diabetes, independent of traditional risk factors for diabetes (tertile odds ratio (OR) 2.1 (1.1, 3.9) in men, and 2.3 (1.2–4.3) in women). Patients with T2DM had detectable s-lacCer (OR 1.7 (0.9, 3.4) in men, and 7.6 (3.8, 15.2) in women). Sulphatides were negatively correlated with insulin resistance (HOMA-IR) [29].

LIPIDOMICS OF SPHINGOLIPIDS IN EPIDEMIOLOGICAL STUDIES OF NEW-ONSET TYPE 2 DIABETES

Lipidomic analyses have been conducted in epidemiological cohort studies of high-risk groups for T2DM. Metabolomic profiling of 43 sphingolipids classified into six scores was performed in fasting serum in a Hispanic cohort. After adjustment for risk factors, a ceramide score [quartile RR 2.40 (1.24–4.65); $P = 0.003$] and sphingomyelins with fully saturated sphingoid–fatty acid pairs [RR 3.15 (1.75–5.67); $P < 0.001$] were associated with new T2DM. The associations were attenuated after adjustment for other traditional risk factors (e.g. triglycerides) but the saturated sphingomyelin score remained significant [RR 1.98 (1.09–3.59); $P = 0.03$] [30].

In the Strong Heart Study ($n = 2145$), plasma ceramide, sphingomyelin and glucosylceramide species levels were higher in older participants and lacCer levels were higher in participants with lower BMIs. Most ceramides and lower lacCer(16:0) were associated with higher glucose levels. No association of sphingomyelin species or glucosyl-ceramide species was found with glucose [31]. A further analysis of sphingolipids in 435 American-Indian participants in the Strong Heart Study and 1902 participants from the associated family study was performed. Higher levels of ceramides containing stearic acid [Cer-18; 1.22 (1.09–1.37) per SD], arachidic acid [Cer-20; 1.18 (1.06–1.31)] and behenic acid [Cer-22; 1.20 (1.08–1.32)] were associated with T2DM [32▪].

In a prospective study of 2174 participants from China, 76 sphingolipids were quantified using lipidomics. Eleven novel and three reported sphingolipids, namely ceramides (18:1/18:1, 18:1/20:0, 18:1/20:1, 18:1/22:1), saturated sphingomyelins (C34:0, C36:0, C38:0, C40:0), unsaturated sphingomyelins (C34:1, C36:1, C42:3), hydroxyl-sphingomyelins (C34:1, C38:3) and a hexosylceramide (d18:1/20:1), were associated with new T2DM after adjustment for standard risk factors (RRs 1.14–1.21; all $P < 0.001$). Network analysis showed identified five lipid modules were associated with T2DM, of which two containing saturated sphingomyelins showed the strongest association (quartile RRs 1.59 and 1.43; $P < 0.001$). Mediation analysis suggested that the associations of 13 sphingolipids were largely mediated through β-cell dysfunction (HOMA-B mediation proportion: 11–42%; $P < 0.001$). Mendelian randomization in the cohort for ceramide (d18:1/20:1) also showed an association with T2DM [OR 1.15 (1.05–1.26); $P = 0.002$] [33].

LACTOSYLCERAMIDE, INFLAMMATION AND DIABETES

More specifically among lipid-derived factors, lactosylceramide (LacCer) (CD17) is a glycosphingolipid component of lipid rafts involved in inflammation [34]. A number of pathways activate LacCer synthesis by phosphorylating LacCer synthase (β-1,4 galactosyltransferase) including those involving platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α) and oxidized low-density lipoprotein (ox-LDL) This pathway activates nicotinamide adenine dihydrogen phosphate (NADPH) oxidase to generate reactive oxygen species (ROS), other signalling pathways involved in inflammation, such as cytosolic phospholipase A2 (cPLA2) [28]. LacCer may also have direct actions on mitochondria and be implicated in the pathogenesis of diabetes-related cardiac dysfunction. In mice with streptozocin-induced T1DM ceramide synthesis by desaturase 1, (dihydro)ceramide synthase (CerS)2, serine palmitoyl transferase 1 and mitochondrial CerSs was increased

but lack of changes in mitochondrial ceramide profiles suggest that this was only a turnover effect. Neutral ceramidase (NCDase) knockdown increased LacCer suggesting crosstalk between glucosylceramide synthase-mediated and NCDase-mediated ceramide pathways. Elevated LacCer in mitochondria was associated with decreased respiration and calcium retention capacity (CRC) indicating mitochondrial dysfunction [35]. Plasma hexosylceramides (H) and lacer were measured in a cohort of 432 patients from the DCCT/Epidemiology of Diabetes Interventions and Complications cohort with T1DM. The association of ceramides with the risk of developing macroalbuminuria (albumin excretion rate >300 mg/24 h) or chronic kidney disease (eGFR < 60 ml/min) over a period of 21–28 years was investigated. Decreases in long and very long chain LacCers were associated with macroalbuminuria [36].

LACTOSYLCERAMIDE AND NONALCOHOLIC FATTY LIVER DISEASE

Hepatic dysfunction, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH) and cirrhosis are complications associated with diabetes and are associated with altered ceramide metabolism [37,38]. A study using euglycaemic clamp was conducted in 21 insulin-resistant obese patients with or without NAFLD and/or NASH and 7 healthy lean individuals using tissue biopsies during bariatric or elective surgery. In patients, hepatic total ceramides were higher by 50% in NASH and 33% in NAFLD Hepatic dihydroceramides (16:0, 22:0 and 24:1) and lacCer were increased in patients with NASH. Serum ceramides and dihydroceramides (hepatic dihydroceramides 22:0 and 24:1) correlated negatively with whole-body but not with hepatic insulin sensitivity. Hepatic oxygen use correlated positively with serum lacCer, hepatic sphinganine and lacCer(14:0). Liver lipid peroxides correlated with the respective hepatic sphingolipids [39].

INTERVENTION STUDIES

Epidemiological studies can only establish correlations as opposed to causation. Intervention studies are required to demonstrate that changing ceramides may be related to clinically relevant outcomes. A relationship exists between adipocyte sphingolipid and PPARs. In a randomized 6-month trial in 37 insulin-resistant subjects, the effects of pioglitazone on plasma ceramide concentrations were investigated. Pioglitazone therapy reduced plasma ceramide concentrations: C18:0 ($P = 0.001$), C20:0 ($P = 0.0004$), C24:1 ($P = 0.009$), dihydroceramide C18:0 ($P = 0.005$), dihydroceramide C24:1 ($P = 0.004$), lacCer C16:0 ($P = 0.02$) and HC C16:0 ($P = 0.0003$), C18:0 ($P < 0.0001$), C22:0 ($P < 0.0001$) and C24:1 ($P = 0.0006$). Overall reductions were found in grouped ceramide species: ceramides ($P = 0.03$), dihydroceramides ($P = 0.02$), HC ($P = 0.00001$) and LacCers ($P = 0.02$) and in total ceramides ($P = 0.001$). Some ceramide species correlated negatively with the change induced in insulin sensitivity (DHC C16:0; $r = -0.54$; $P = 0.02$) and others positively including lacCer C24:0 ($r = 0.53$; $P = 0.02$) and lacCer C24:0 ($r = 0.48$; $P = 0.05$) [40]. In mouse nonobese diabetic (NOD) models of diabetes fenofibrate (a PPAR-alpha agonist) increases very-long-chain C22:1 and C24:0 sphingomyelin and C24:0 ceramide and decreases C16:0, C16:1 and C18:0 improving the pancreatic lipidome [41].

Statins are first-line therapy for atherosclerosis in diabetes but are associated with increased dysglycaemia [42]. Ceramides were analysed from a lipid turnover study of 12 patients on placebo, low-dose (10 mg) or high-dose (40 mg) rosuvastatin. Baseline plasma ceramides were associated with very LDL (VLDL) apolipoprotein (apo)-B_{100} concentrations ($r = 0.58$, $P < 0.05$) and inversely with fractional catabolic rate (FCR; $r = -0.67$, $P = 0.02$). Changes in ceramides after treatment with high-dose statin were inversely associated with FCR ($r = -0.62$, $P = 0.03$) independent of changes in lipids. Plasma sphingomyelin levels were unaffected [43].

A number of novel agents are being investigated that affect HDL metabolism. A study of apabetalone (RVX-208) a bromodomain (BRET) inhibitor with effects on inflammation and HDL in 20 male patients measured ceramide levels over 4 weeks showed increased six sphingolipid and four phospholipid classes in the HDL lipidome ($P < 0.05$), but did not change standard lipids [44]. Medium-sized HDL particles increased by 11% ($P = 0.01$) whereas small HDL particles decreased by 10% ($P = 0.04$). Postchallenge plasma glucose profiles changed to delayed (30 min) and sustained ($P = 0.003$) profiles with suppression of endogenous glucose production ($P = 0.014$). The rate of glucose disappearance was lower but no effects were seen on glucose oxidation or total glucose disposal. However, cardiovascular outcome studies have shown no effect of apabetalone in reducing cardiovascular events or new diabetes in man so its development has been discontinued [45].

CONCLUSION

There is substantial evidence from cell and animal studies that changes in ceramide metabolism are associated and potentially may be causative in altering glucose and lipid metabolism. However, data in man is far more limited and mostly derived from associations found in prospective cohort

epidemiological studies. Few studies have been conducted in populations with diabetes and those mostly in epidemiological cohorts to ascertain progression to T2DM. Modern analytical techniques, such as lipidomics measure multiple species, where the degree of intercorrelation between various species is unclear [4]. Metabolomic pathway analysis is in its infancy. Metabolomics and its lipid subsection are likely to identify pathogenic mechanisms contributing to development or progression of diabetes and its complications and provide validation of genetic studies by identifying the pathways affected, and thus potential sites for drug targeting. The complexities induced by this mean that large and very well designed studies are required to avoid artefactual findings induced by statistical errors. It will probably require analysis of cohorts of the size of UK Biobank to identify the best statistical analytical techniques for epidemiological datasets (possibly including machine-learning approaches) to give reliable findings on the relationship of ceramides to new-onset diabetes. Studies of phospholipids and their derivatives suggest that many changes may be secondary to diet rather than because of alterations in intrinsic metabolism. In contrast, lipidomics of sphingolipids show associations with metabolic derangements with a strong link to pathways involving inflammation. To date, only data on lacCer seems to give any consistent association with type 2 diabetes but this association requires further validation. In intervention studies, it will require analysis of biobanked samples from long-term intervention trials to identify whether established therapies derive some of their benefits through actions on ceramide pathways. These may suggest whether ceramide-focused interventions have a role to play in the management of diabetes or its complications.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Turner RC, Millns H, Neil HA, *et al.* Risk factors for coronary artery disease in noninsulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23). BMJ 1998; 316:823–828.
2. Nathan DM, Lachin J, Cleary P, *et al.* Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. N Engl J Med 2003; 348:2294–2303.
3. ■■ Meikle TG, Huynh K, Giles C, *et al.* Clinical lipidomics: realizing the potential of lipid profiling. J Lipid Res 2021; 62:100127.
Review of lipidomics and how it can be used in research.
4. Tolani P, Gupta S, Yadav K, *et al.* Big data, integrative omics and network biology. Adv Protein Chem Struct Biol 2021; 127:127–160.
5. Bourgeois R, Girard A, Perrot N, *et al.* A comparative analysis of the lipoprotein(a) and low-density lipoprotein proteomic profiles combining mass spectrometry and Mendelian randomization. CJC Open 2021; 3:450–459.
6. Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. Cell 2012; 148:852–871.
7. Chavez JA, Summers SA. A ceramide-centric view of insulin resistance. Cell Metab 2012; 15:585–594.
8. Field BC, Gordillo R, Scherer PE. The role of ceramides in diabetes and cardiovascular disease regulation of ceramides by adipokines. Front Endocrinol (Lausanne) 2020; 11:. 569250.
9. James DE, Stockli J, Birnbaum MJ. The aetiology and molecular landscape of insulin resistance. Nat Rev Mol Cell Biol 2021; 22:751–771.
10. Saxena A, Mathur N, Tiwari P, Mathur SK. Whole transcriptome RNA-seq reveals key regulatory factors involved in type 2 diabetes pathology in peripheral fat of Asian Indians. Sci Rep 2021; 11:10632.
11. ■■ Wali JA, Jarzebska N, Raubenheimer D, *et al.* Cardio-metabolic effects of high-fat diets and their underlying mechanisms-a narrative review. Nutrients 2020; 12:1505.
Mechanisms involved in insulin resistance including ceramides.
12. ■ Virtanen JK, Tuomainen TP, Voutilainen S. Dietary intake of choline and phosphatidylcholine and risk of type 2 diabetes in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. Eur J Nutr 2020; 59:3857–3861.
Role of ceramides in peripheral fat tissue metabolism in high-risk subjects for diabetes.
13. Prada M, Wittenbecher C, Eichelmann F, *et al.* Association of the odd-chain fatty acid content in lipid groups with type 2 diabetes risk: a targeted analysis of lipidomics data in the EPIC-Potsdam cohort. Clin Nutr 2021; 40:4988–4999.
14. Adamska-Patruno E, Samczuk P, Ciborowski M, *et al.* Metabolomics reveal altered postprandial lipid metabolism after a high-carbohydrate meal in men at high genetic risk of diabetes. J Nutr 2019; 149:915–922.
15. Fall T, Salihovic S, Brandmaier S, *et al.* Nontargeted metabolomics combined with genetic analyses identifies bile acid synthesis and phospholipid metabolism as being associated with incident type 2 diabetes. Diabetologia 2016; 59:2114–2124.
16. Suvitaival T, Bondia-Pons I, Yetukuri L, *et al.* Lipidome as a predictive tool in progression to type 2 diabetes in Finnish men. Metabolism 2018; 78:1–12.
17. Lu J, Lam SM, Wan Q, *et al.* High-coverage targeted lipidomics reveals novel serum lipid predictors and lipid pathway dysregulation antecedent to type 2 diabetes onset in normoglycemic chinese adults. Diabetes Care 2019; 42:2117–2126.
18. Perez-Matos MC, Morales-Alvarez MC, Toloza FJK, *et al.* The phospholipid linoleoylglycerophosphocholine as a biomarker of directly measured insulin resistance. Diabetes Metab J 2017; 41:466–473.
19. ■■ Turpin-Nolan SM, Bruning JC. The role of ceramides in metabolic disorders: when size and localization matters. Nat Rev Endocrinol 2020; 16:224–233.
Actions of ceramides are dependent on cell types, tissues and subcellular compartments.
20. ■■ Choi RH, Tatum SM, Symons JD, *et al.* Ceramides and other sphingolipids as drivers of cardiovascular disease. Nat Rev Cardiol 2021; 18:701–711.
Link of ceramide metabolism to mechanisms of cardiovascular disease.
21. Chaurasia B, Summers SA. Ceramides in metabolism: key lipotoxic players. Annu Rev Physiol 2021; 83:303–330.
22. Albeituni S, Stiban J. Roles of ceramides and other sphingolipids in immune cell function and inflammation. Adv Exp Med Biol 2019; 1161:169–191.
23. ■ Guitton J, Bandet CL, Mariko ML, *et al.* Sphingosine-1-phosphate metabolism in the regulation of obesity/type 2 diabetes. Cells 2020; 9:1682.
Role of sphingosine derivatives in type 2 diabetes.
24. Aji G, Huang Y, Ng ML, *et al.* Regulation of hepatic insulin signaling and glucose homeostasis by sphingosine kinase 2. Proc Natl Acad Sci U S A 2020; 117:24434–24442.
25. Jessup CF, Bonder CS, Pitson SM, Coates PT. The sphingolipid rheostat: a potential target for improving pancreatic islet survival and function. Endocr Metab Immune Disord Drug Targets 2011; 11:262–272.
26. Webb-Robertson BM, Bramer LM, Stanfill BA, *et al.* Prediction of the development of islet autoantibodies through integration of environmental, genetic, and metabolic markers. J Diabetes 2021; 13:143–153.
27. Nelson AJ, Stephenson DJ, Bone RN, *et al.* Lipid mediators and biomarkers associated with type 1 diabetes development. JCI Insight 2020; 5:e138034.
28. Chatterjee S, Balram A, Li W. Convergence: lactosylceramide-centric signaling pathways induce inflammation, oxidative stress, and other phenotypic outcomes. Int J Mol Sci 2021; 22:1816.
29. Buschard K, Fredman P, Bog-Hansen E, *et al.* Low serum concentration of sulfatide and presence of sulfated lactosylceramid are associated with type 2 diabetes. The Skaraborg Project. Diabet Med 2005; 22:1190–1198.
30. Chen GC, Chai JC, Yu B, *et al.* Serum sphingolipids and incident diabetes in a US population with high diabetes burden: the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). Am J Clin Nutr 2020; 112:57–65.

31. Jensen PN, Fretts AM, Yu C, *et al.* Circulating sphingolipids, fasting glucose, and impaired fasting glucose: the Strong Heart Family Study. EBioMedicine 2019; 41:44–49.
32. Fretts AM, Jensen PN, Hoofnagle AN, *et al.* Plasma ceramides containing ■ saturated fatty acids are associated with risk of type 2 diabetes. J Lipid Res 2021; 62:. 100119.

Association of sphingolipids containing saturated fatty acids with onset of type 2 diabetes.

33. Yun H, Sun L, Wu Q, *et al.* Associations among circulating sphingolipids, beta-cell function, and risk of developing type 2 diabetes: a population-based cohort study in China. PLoS Med 2020; 17:e1003451.
34. Messner MC, Cabot MC. Glucosylceramide in humans. Adv Exp Med Biol 2010; 688:156–164.
35. Novgorodov SA, Riley CL, Yu J, *et al.* Lactosylceramide contributes to mitochondrial dysfunction in diabetes. J Lipid Res 2016; 57:546–562.
36. Lopes-Virella MF, Baker NL, Hunt KJ, *et al.* Glycosylated sphingolipids and progression to kidney dysfunction in type 1 diabetes. J Clin Lipidol 2019; 13:481.e1–491.e1.
37. Bikman BT, Summers SA. Sphingolipids and hepatic steatosis. Adv Exp Med Biol 2011; 721:87–97.
38. Summers SA. Ceramides: nutrient signals that drive hepatosteatosis. J Lipid Atheroscler 2020; 9:50–65.
39. Apostolopoulou M, Gordillo R, Koliaki C, *et al.* Specific hepatic sphingolipids relate to insulin resistance, oxidative stress, and inflammation in nonalcoholic steatohepatitis. Diabetes Care 2018; 41:1235–1243.
40. Warshauer JT, Lopez X, Gordillo R, *et al.* Effect of pioglitazone on plasma ceramides in adults with metabolic syndrome. Diabetes Metab Res Rev 2015; 31:734–744.
41. Holm LJ, Haupt-Jorgensen M, Giacobini JD, *et al.* Fenofibrate increases very-long-chain sphingolipids and improves blood glucose homeostasis in NOD mice. Diabetologia 2019; 62:2262–2272.
42. Preiss D, Seshasai SR, Welsh P, *et al.* Risk of incident diabetes with intensive-dose compared with moderate-dose statin therapy: a meta-analysis. JAMA 2011; 305:2556–2564.
43. Ng TW, Ooi EM, Watts GF, *et al.* Association of plasma ceramides and sphingomyelin with VLDL apoB-100 fractional catabolic rate before and after rosuvastatin treatment. J Clin Endocrinol Metab 2015; 100:2497–2501.
44. Siebel AL, Trinh SK, Formosa MF, *et al.* Effects of the BET-inhibitor, RVX-208 on the HDL lipidome and glucose metabolism in individuals with prediabetes: a randomized controlled trial. Metabolism 2016; 65:904–914.
45. Ray KK, Nicholls SJ, Buhr KA, *et al.,* BETonMACE Investigators and Committees. Effect of apabetalone added to standard therapy on major adverse cardiovascular events in patients with recent acute coronary syndrome and type 2 diabetes: a randomized clinical trial. JAMA 2020; 323:1565–1573.