Targeted Metabolomics Finds Its Mark in Diabetes Research

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Numerous biochemical pathways are deranged in diabetes, especially those involving metabolic fuels. Metabolomics, which seeks to simultaneously measure many small molecules, shows promise as a source of mechanistic insight into the molecular pathology of diabetes (1), but no systematic approach has yet come into wide use. Methods vary greatly from laboratory to laboratory. For most of the last decade, metabolomic studies in diabetes have been dominated by nontargeted methods in which nuclear magnetic resonance spectroscopy (NMR) or mass spectrometry (MS) are used to gather large and complex datasets from which investigators extract information on metabolites that characterize a biological condition of interest (2–8). Our own approach has centered on targeted MS methods, in which labeled internal standards are used in conjunction with narrowly focused instrumental methods to yield quantitative data on panels of chemically similar metabolites (1,9–20). Some critics do not consider such targeted studies to be "metabolomics" at all. Targeted metabolomic studies are on the rise in diabetes research (13–15,18), and an increasing number of laboratories combine both approaches or fashion hybrids of the two (16,17,19).

This month's issue of *Diabetes* includes an account of one of the largest targeted metabolomic studies of human diabetes reported to date. Floegel et al. (13) propose a model of the 7-year risk of developing type 2 diabetes (T2D) based on circulating metabolites measured at baseline in initially healthy subjects enrolled in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam (a subcohort of 2,282 individuals, compared with all 800 incident cases of T2D). This model was then partially validated in the Cooperative Health Research in the Region of Augsburg (KORA) study (n = 876; 91 with incident T2D), and related to insulin secretion and sensitivity using samples from the Tübingen Family Study (TüF; n = 76). Their results confirm and extend contemporary findings in independent studies.

Flow-injection, tandem MS (FI-MS/MS) identified 163 circulating metabolites. Elevations in phenylalanine, tyrosine, isoleucine, and valine associated with increased risk of T2D (Fig. 1). This corroborates results of recent metabolomic studies that linked increases in the three branched-chain amino acids (namely isoleucine, valine, and leucine) and the aromatic amino acids (phenylalanine, tyrosine, histidine, and tryptophan) with obesity and T2D (3–5,7,8,10–12,16,19,20). Further work is needed to determine whether these amino acids are elevated because of host genetics, diet, or the actions of gut microbes (14). Studies in laboratory animals raise suspicion that these amino acids, especially the branched-chain amino acids, might have a causal role in the development of insulin resistance (11). Glycine was an enigma—alone among the amino acids, it correlated negatively with T2D risk. We and others (3,8,11,17,20) have noted lower glycine levels in patients with obesity, impaired insulin sensitivity, or both. In examining samples from these patients, we have grown accustomed to seeing elevations of circulating metabolites that cut across a broad diversity of biochemical pathways, a global signature of disordered metabolic homeostasis—and time and again, glycine stands out as a maverick, trending in the opposite direction. Floegel et al. speculate that glycine depletion in patients at risk for T2D might result from diversion to gluconeogenesis, increased biosynthesis of glutathione to combat oxidative stress, or its use to dispose of incompletely oxidized metabolites as urinary acylglycines.

The FI-MS/MS method they used cannot distinguish glucose from its isomeric hexoses, but the positive association between the hexose feature and T2D risk remained after correction for glucose measurements made by another analytic method, suggesting that fructose and perhaps such minor hexoses as mannose and galactose might be independent risk factors.

Phosphocholine (PC)-containing lipids were also informative (Fig. 1). Risk of T2D correlated positively with four species of diacylphosphatidylcholines, especially those in which fatty acid residues were shorter and had lower degrees of saturation. Similarly, in a recent NMR metabolomic study of insulin resistance (using homeostasis model assessment of insulin resistance) in more than 7,000 human subjects, Würtz et al. (7) found a positive association between insulin resistance and an NMR feature that reports an aggregate of PC-containing metabolites. Conversely, T2D risk correlated negatively with palmitoleoyl sphingomyelin, linoleoyl lysophosphatidylcholine (15), and five PC-based plasmalogens (acyl-alkyl PCs; 18). Future studies should evaluate the potential roles of these PC lipids in such processes as hepatic lipid secretion, signal transduction, and, in the case of the plasmalogens, resistance to oxidation of unsaturated lipids (Fig. 1).

The subtle metabolic phenotype defined by these metabolites significantly improved prediction of T2D risk compared with the use of such established biomarkers as glucose and HbA_{1c}. Surprisingly, basic features of this metabolic signature of T2D risk could be discerned

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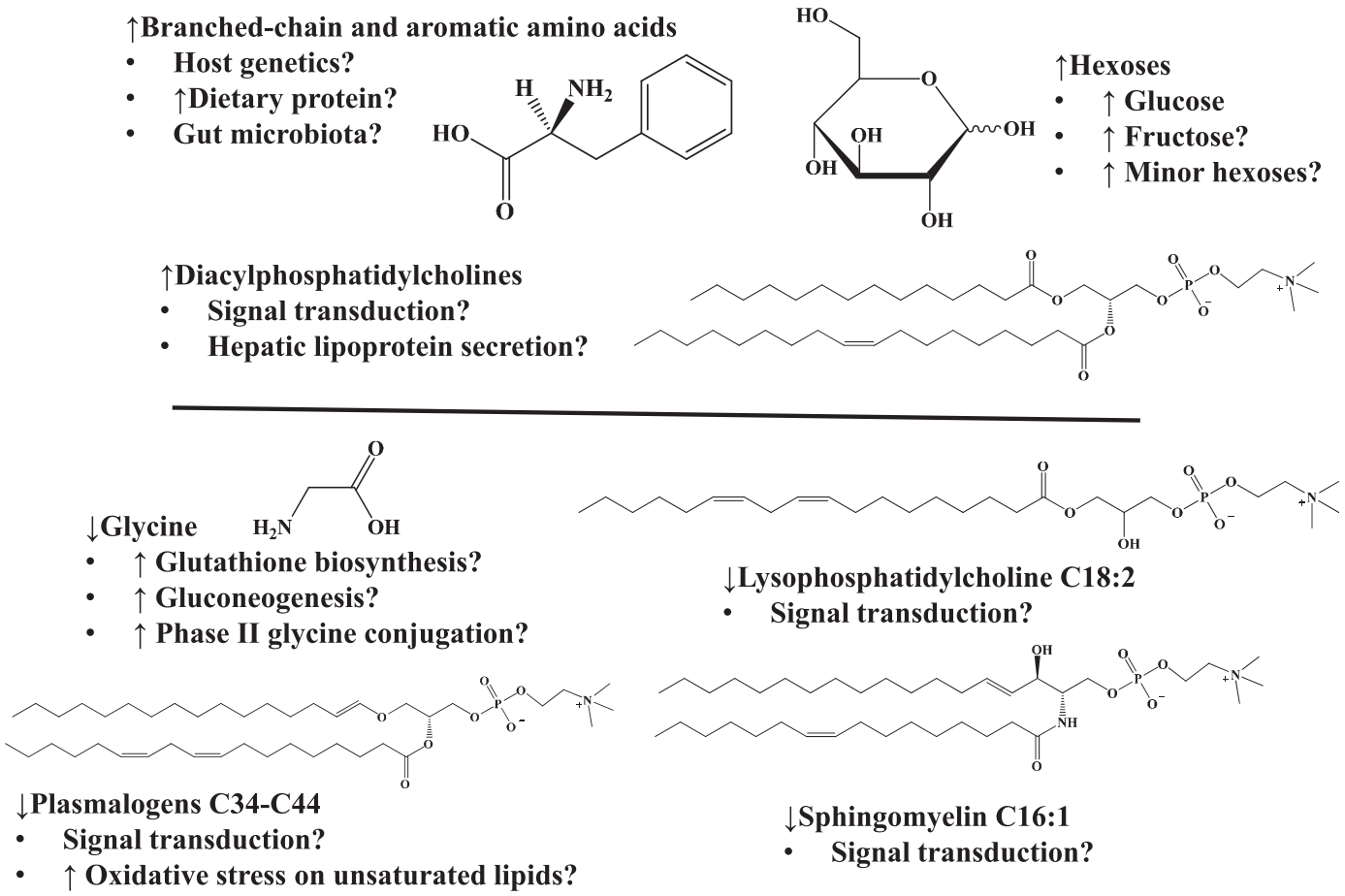

FIG. 1. Circulating metabolites associated with the 7-year risk of developing T2D (13). Growing evidence links elevated aromatic and branched-chain amino acids to the onset of insulin-resistant states, but the reasons these metabolites are enriched remain obscure. Repletion of 6-carbon sugars other than glucose might pose independent risks. Patients at risk for T2D have reduced levels of glycine, possibly from making glutathione to combat oxidative stress or the need to conjugate and excrete incompletely oxidized fuels. PC-based lipids tell a complex story that might involve signal transduction, liver function, and oxidative stress.

regardless of whether blood was drawn at random or with the donor in a fasting state. (KORA sera and TüF plasmas were from fasting subjects, whereas most EPIC-Potsdam sera were random). If this observation is verified in follow-on studies, then blood products from random phlebotomies archived in biobanks might be more useful for metabolomic studies of diabetes than one might have thought.

Multiplexed measurement of dozens to hundreds of metabolites by NMR or MS remains fraught with technical difficulties. The study by Floegel et al. (13) is significant in that it is one of the first large population studies of diabetes in which a commercial kit was used to conduct targeted, MS-based metabolomics. Encouragingly, batch effects, which can be the demons of any large metabolomic study, were controllable, with coefficients of variation between plates of less than 12% in the EPIC-Potsdam sera. These investigators are experienced with this FI-MS/MS kit (4), and they are alert to such potentially confounding effects as ion suppression and interferences (13), but suitability of such kits for use by more casual users is an open question. Their elucidation of a phenotype of T2D risk involving circulating amino acids and PC lipids shows that targeted metabolomics is coming into its own. This engenders hope that forthcoming data on metabolites, when integrated with knowledge gleaned from genomics, transcriptomics, and proteomics, will help to deliver on the promise of functional genomics and translational medicine in diabetes.

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