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Metabolic Profiling of Diabetes: From Black-Box Epidemiology to Systems Epidemiology

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Type 2 diabetes (T2D)[2] is a heterogeneous condition that is characterized by increased insulin resistance and impaired insulin secretion. A progressive disorder with an insidious onset, T2D typically progresses from an early asymptomatic insulin resistance state to mild glucose intolerance and eventually to frank T2D that requires pharmacologic interventions. Whether insulin resistance or impaired insulin secretion is the primary defect in the pathogenesis of T2D remains a matter of debate. Interestingly, most genetic variants identified from recent genome-wide association studies are related to decreased β-cell function or impaired insulin secretion, which implicates a key role for β-cell dysfunction in the development of T2D. Still, obesity, with its fundamental influence on insulin resistance, is the single most important risk factor for T2D.

Although T2D is largely predictable through anthropometric, lifestyle, and clinical factors and is preventable through diet and exercise, the metabolic pathways underlying the progression from normal glycemia to a prediabetes state and later to T2D are not completely understood. Classic epidemiology typically relates lifestyle and environmental exposures to chronic disease end points, such as T2D. This approach (sometimes referred to as "black-box epidemiology") has identified many important lifestyle and environmental risk factors for chronic diseases, but it often does not illuminate biological mechanisms that underlie observed associations. Recent advances in "omics" technology, however, have enabled epidemiologists to incorporate novel biomarkers at multiple levels into human observational studies, with the potential to shift the research paradigm from the traditional black-box strategy to a systems approach *(1, 2)*.

This new model integrates a wide range of information—genetic predisposition (genome), epigenetic changes (epigenome), the expression of genes (transcriptome), proteins (proteome), metabolites (metabolome), and gut microbiota (microbiome)—into population-based studies to improve our understanding of the biological mechanisms that underlie disease pathophysiology in humans. Systems epidemiology is at the intersection of human observational or interventional studies and the concept of systems biology, which uses a holistic and integrated approach to understand complex phenotypes. It couples traditional epidemiologic methods with modern high-throughput technologies to enhance biological understanding of metabolic pathways in humans.

Recently, Wang and colleagues *(3)* used liquid chromatography in combination with tandem mass spectrometry to identify metabolites in plasma that robustly predict the future risk of T2D among apparently healthy normoglycemic individuals. This investigation was conducted as a case–control study nested within the Framingham Offspring Study. Among 2422 nondiabetic participants at baseline, 201 developed new-onset diabetes during 12 years of follow-up. The authors used fasting blood samples collected at baseline to screen a panel of 61 metabolites and found that 5 branched-chain and aromatic amino acids (i.e., isoleucine, leucine, valine, tyrosine, and phenylalanine) had highly significant associations with future risk of developing diabetes. A combination of 3 amino acids (isoleucine, tyrosine, and phenylalanine) was associated with an even greater risk, >5-fold higher for individuals in the top quartile vs the bottom quartile. These results were replicated in an independent European cohort (the Malmö Diet and Cancer study).

This groundbreaking work suggests that amino acid metabolism may play a role in the pathogenesis of T2D and that amino acid profiling could help predict future risk long before any clinical manifestations. Although prior cross-sectional studies using mass spectrometry have linked branched-chain amino acids (BCAAs) with insulin resistance, the work of Wang et al. was the first prospective study to show that amino acid profiles at baseline were significant and independent predictors of incident diabetes. The prospective design allowed investigators to demonstrate the changes in amino acid metabolism

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[2] Nonstandard abbreviations: T2D, type 2 diabetes; BCAA, branched-chain amino acid.

that occur well before the clinical diagnosis of diabetes.

Prior clinical and animal experimental studies have shown that increased concentrations of BCAAs may contribute to the development of insulin resistance and that intakes of these amino acids may also modulate insulin secretion. Several decades ago, Felig et al. observed that BCAAs were increased in obese individuals, compared with normal weight-, age-, and sex-matched controls and that the increase was directly correlated with the fasting insulin concentration, a marker of insulin resistance *(4)*. The causal relationship between the increase in BCAAs and insulin resistance remains to be determined, however. Although BCAAs can promote insulin resistance by impairing insulin-signaling pathways, the catabolism of amino acids is also enhanced in insulin-resistant and obese individuals, leading to a secondary increase in BCAAs in the circulation. Therefore, it is possible that BCAAs and insulin resistance have bidirectional effects leading to a vicious cycle that eventually causes hyperglycemia and T2D.

Laferrère et al. *(5)* recently showed that weight loss induced by gastric bypass surgery—but not dietary intervention—was associated with significantly reduced circulating total amino acids, especially BCAAs. This finding suggests that reduction in BCAAs, rather than simply weight loss, may contribute to the rapid improvement in glucose homeostasis and the resolution of T2D seen with gastric bypass surgery. It remains to be determined, however, whether the improved insulin sensitivity observed after the surgery is responsible for the reduction in BCAAs or vice versa.

The study by Wang and colleagues underscores the importance of using well-phenotyped prospective cohorts in metabolic profiling. The Framingham Offspring Study has collected detailed information on diabetes risk factors, with long-term follow-up and high rates of follow-up. These factors allow for detailed adjustment for potential confounders and minimize possible biases due to reverse causation and selection, which can often occur in cross-sectional or retrospective case–control studies. In addition, the prospective nested case–control design improves the efficiency of the study. Over the past several decades, many large prospective studies of lifestyle and chronic diseases have been established worldwide. Contemporary cohort studies have the advantages of very large size, long-term follow-up, high rates of follow-up, availability of archived biological samples, and repeated measures of diet and lifestyle. Future research will need to harness the resources from large, well-powered population-based studies for initial discovery and validation of novel biomarkers by means of the "systems epidemiology" approach.

Although omics-based biomarkers and signatures identified from population-based studies can potentially be used to identify high-risk groups for targeted prevention and treatment, the translation of research findings into practice is not a straightforward process. For example, the recent advent of genome-wide association studies has led to major advances in the identification of common genetic variants that contribute to diabetes susceptibility. To date, at least 40 genetic loci have been convincingly associated with T2D, but these loci confer effects of only modest size and do not add to the clinical prediction of diabetes beyond that of traditional risk factors, such as obesity, physical inactivity, family history of diabetes, and certain clinical parameters. Likewise, despite the strong association between BCAAs and T2D risk seen in the Framingham study, the addition of BCAA concentrations yielded only modest gains in the ability to predict the disease, compared with traditional risk factors. Previous studies have shown that body mass index combined with a family history of diabetes and clinical parameters (e.g., blood glucose, increased blood pressure, low HDL, and increased triglycerides), reliably discriminates groups at relatively high risk of developing T2D within 5–10 years from groups at lower risk. Therefore, measuring the plasma concentrations of BCAAs is unlikely to lead to substantial gains in our ability to predict diabetes onset beyond that of the traditional risk factors and routinely measured clinical parameters. Further investigation is needed to determine whether amino acid profiling can aid in the early detection of prediabetes and subtle metabolic abnormalities long before the emergence of traditional markers, such as impaired fasting glucose or glucose intolerance.

Despite the lack of immediate clinical utility of most of the novel biomarkers identified to date, the integration of high-throughput technologies (e.g., metabolic profiling of small molecules such as lipids, sugars, and amino acids involved in cellular function) has the potential to unlock the "black box" in chronic disease epidemiology. The technologies are still in the early stages of development, however, and their application to large-scale cohort studies has just started. Future technological advances in automation and high-throughput methods with improved analytical sensitivity and reproducibility, enhanced bioinformatics and analytical tools, and reduced costs will enable more widespread use of these techniques in human observational and interventional studies. Although the systems epidemiology approach can offer deeper understanding of molecular pathways underlying epidemiologic observations, whether it can improve early disease detection, clinical diagnosis, and prognosis,

and contribute to personalized prevention and treatment remains to be seen.

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