Metabolomics Reveals Unexpected Responses to Oral Glucose

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In this issue of *Diabetes*, Ho et al. (1) present the largest metabolomic study to date of oral glucose challenges in humans at risk for type 2 diabetes mellitus (T2DM). Their observations of unexpected metabolic responses demonstrate the value of metabolomics as a tool for discovery in diabetes research.

T2DM is widespread in developed countries, and its prevalence continues to grow. Associated morbidity and mortality are substantial, as is the economic burden imposed on society (2). Early interventions that modify diet, increase activity level, and reduce excess body weight, sometimes combined with drug therapy, are effective in preventing progression of prediabetes to T2DM (3,4). The risk of developing T2DM is currently evaluated using such traditional clinical parameters as family history and fasting plasma glucose. In the decade since the human genome was first sequenced, rapid development of the "omics" sciences and their enabling analytic technologies has fostered a search for genes, proteins, and metabolites that will strengthen existing models that predict T2DM risk (5). Diabetes is characterized by derangements in the processing of metabolic fuels. Metabolomics, in which one makes simultaneous measurements of many small metabolites, holds promise for uncovering early biomarkers of risk that can help to guide timely interventions and perhaps aid in the exploration of underlying biochemical mechanisms, as well (6).

Recent longitudinal metabolomic studies have found associations between circulating metabolites and future development of insulin resistance (IR), prediabetes, or T2DM in humans. For example, elevations in circulating branched-chain amino acids (BCAAs) and aromatic amino acids (AAAs) are biomarkers of risk (7–12). In several studies, inclusion of biomarkers identified by metabolomics improved prediction of risk models of T2DM over the use of conventional metrics alone (8,9), and changes in metabolites that predict the onset of T2DM might also correlate with positive outcomes of clinical interventions (13,14). Mechanistic links of these biomarkers to the onset of IR and T2DM are largely unknown and in need of further study (15). The aforementioned investigations of diabetic risk evaluated baseline measurements of metabolites in light of clinical outcomes months (10) to years (7–9,11,12) later.

Diabetes profoundly affects minute-to-minute processing of metabolic fuels, and investigators have begun deploying metabolomic tools to study complex, short-term responses to such challenges as feeding and exercise. The oral glucose tolerance test (OGTT) provides a dynamic view of the body's metabolic machinery in action and has long been used to detect early shifts in metabolism that mark incipient IR. Recently, metabolomic studies of the OGTT in small groups of humans have found that a glucose challenge causes a fall in free fatty acids and acylcarnitines (16,17), glycerol (18), the "ketone body" β-hydroxybutyric acid (18), a purine, hypoxanthine (18), and numerous amino acids, including the BCAAs, three of the AAAs, and three players in the urea cycle (18). In addition to glucose, an oral glucose challenge was followed by a rise in circulating lactic acid (18), free carnitine (17), hippuric acid (18), and lysophosphatidylcholines (16). Glutathione fell then rose after glucose was consumed (19). Importantly, Shaham et al. (18) presented preliminary evidence relating diminished insulin sensitivity to a blunted OGTT response in glycerol and the isomeric pair of BCAAs, leucine and isoleucine, suggesting that metabolomic analysis of samples taken before and after a glucose challenge might help in the early identification of disorders in specific biochemical pathways during the progression from early IR to the full-blown T2DM phenotype.

In the community-based study of Ho et al. (1) reported in this issue, nondiabetic men and women enrolled in the Framingham Offspring cohort (n = 377) donated blood before and 2 hours after oral glucose (75 g) after an overnight fast. Patients were middle-aged (average, 57 years), with a mean BMI of ~30 kg/m^2. Half were resistant to insulin. An impressive 91 of 110 measured metabolites changed significantly in response to the glucose challenge. Detection of these changes was no doubt favored by an inherent strength of metabolomic studies of paired samples in the OGTT: each subject serves as his or her own control. For those who wonder about the scientific value of frozen blood products stored for long periods, it is worth noting that samples for this study were collected between 1995 and 1998.

Expected responses (Fig. 1) included reductions in "ketone bodies," amino acids and their metabolites, and intermediates of the Krebs cycle, as well as an increase in glycolysis. The observed decrease in urea-cycle metabolites after oral glucose might reflect a diminished need to dispose of nitrogenous wastes from amino acid catabolism. As in their previous study (18), circulating hippuric acid increased greatly during the OGTT, ascribed to the conjugation of glycine to the benzoic acid preservative in the glucose solution consumed by the subjects.

But there were unexpected metabolic changes as well (Fig. 1), and none of these is easily explained. Numerous purines and pyrimidines fell, perhaps an effect of

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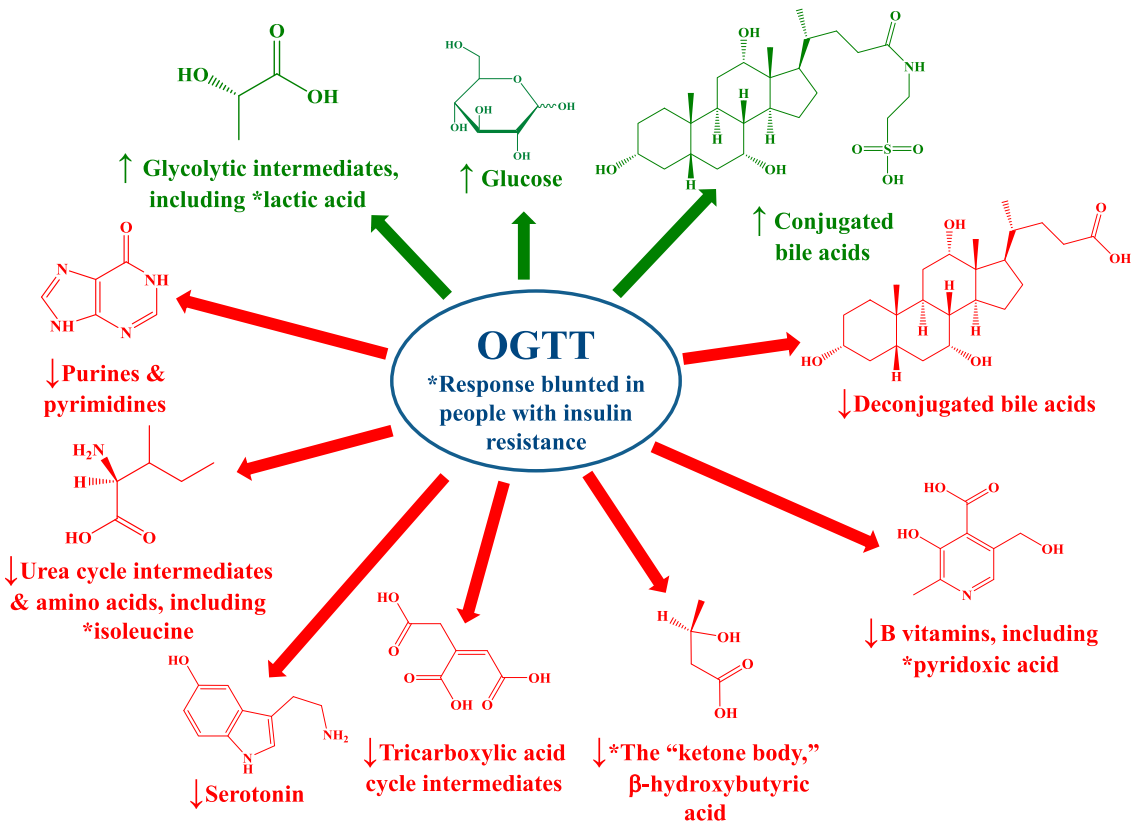

FIG. 1. Classes of circulating metabolites that changed in response to an OGTT (1). Four metabolites showed blunted responses in IR subjects (*), including a B vitamin (pyridoxic acid), a "ketone body" (β-hydroxybutyric acid), a product of glycolysis (lactic acid), and an amino acid (isoleucine).

insulin-stimulated synthesis of nucleotides and nucleic acids. Previous reports varied regarding response of conjugated bile acids to the OGTT (16,18). In the current study (1), a rise in conjugated bile acids, concomitant with a drop in unconjugated bile acids, was attributed to the action of bile acid CoA, amino acid *N*-acyltransferases, or perhaps involvement of the enteric microbiota. Five distinct B vitamins fell during the OGTT—the underlying mechanisms are unclear. Also puzzling was a more than twofold drop in circulating serotonin, accompanied by decreases in its precursor, tryptophan, and its principal catabolite, 5-hydroxyindole-3-acetic acid. Clearly, metabolomics is proving its usefulness for bringing new, functional aspects of metabolism to light.

Blunted responses to glucose in IR subjects were noted in four metabolites belonging to four different biochemical classes (Fig. 1). Further work is needed in a larger and more diverse human population to ascertain whether these findings are broadly applicable, but the study of Ho et al. (1) inspires hope that metabolomic analysis of samples from the OGTT will give sensitive and specific insight into early changes in the progression from a healthy, normoglycemic state toward T2DM.

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