Metabolomic Prediction of Diabetes and Cardiovascular Risk

Samuel Dagogo-Jack

Division of Endocrinology, Diabetes and Metabolism, University of Tennessee Health Science Center, Memphis, Tenn., USA

Metabolomics

Type 2 diabetes mellitus (T2DM), currently an epidemic, is expected to disproportionately affect developing countries in the coming decades [1]. Thus, primary prevention of T2DM is a global imperative. However, a thorough understanding of its pathophysiology is a prerequisite to the development of comprehensive strategies for diabetes prevention. One emerging area in the field of metabolism is the discovery of the role of small molecules and metabolites in the regulation of carbohydrate and lipid metabolism, and their association with metabolic disorders. The small molecules and metabolites under evaluation include branched chain amino acids (BCAAs, isoleucine, leucine, and valine), aromatic amino acids (AAAs, phenylalanine, tyrosine), methionine, glutamine, acylcarnitines, urea cycle intermediates, purine degradation products, bile acids, ketone bodies and lipid moieties [2]. The circulating levels of these metabolites are typically measured using liquid chromatography/mass spectrometry, and analysis of their expression profiles in relation to integrated metabolic pathophysiology constitutes the tenets of the emerging science of metabolomics.

Association with Insulin Resistance

Insulin resistance is an underlying defect in patients with T2DM; it precedes the clinical diagnosis of diabetes by several years, and is demonstrable in people with pre-

diabetes (impaired fasting glucose, impaired glucose tolerance) and even those with high-normal glucose levels [3]. Numerous studies have demonstrated greater insulin resistance (i.e., lower insulin sensitivity) in certain demographic groups: older subjects vs. younger subjects; African Americans vs. Caucasians; sedentary persons vs. active persons, among other examples. However, these differences in insulin sensitivity have not been fully explained at the biological or molecular level. Recently, studies utilizing metabolite profiling have found different metabolomic signatures in insulin-sensitive vs. insulin-resistant subjects, under both fasting and nonfasting conditions [2, 4, 5].

Under fasting conditions, plasma levels of BCAAs, AAAs, proline and methionine are correlated with measures of insulin sensitivity [2, 4, 5]. When the internal milieu is challenged with exogenous glucose (as occurs during oral glucose tolerance test), discernible excursions occur in the plasma levels of amino acids and other metabolites in healthy subjects. Interestingly, the expected excursions in BCAAs, methionine, and bile acids are markedly attenuated in prediabetic individuals with insulin resistance [6]. Based on regression analysis, excursions in leucine, isoleucine and glycerol levels predicted fasting insulin (a surrogate of insulin resistance) more robustly than any individual metabolite excursion. Thus, fasting and glucose-stimulated levels of BCAAs and other metabolites predict insulin resistance, and by extension the risk of T2DM [2, 4, 5].

Predicting Diabetes

The Framingham Heart Study is a long-term, ongoing cardiovascular observational study of residents of the town of Framingham, Mass., USA that was initiated in 1948. Case-control analyses of archived specimens collected at baseline from the Framingham cohort uncovered amino acid patterns that significantly predicted the development of T2DM 12 years later [6, 7]. Specifically, elevated concentrations of 3 BCAAs (isoleucine, leucine, valine) and 2 AAAs (tyrosine and phenylalanine) in baseline plasma specimens strongly predicted incident diabetes 12 years later [6, 7]. It was observed that combination of any 3 of these 5 amino acids improved the prediction of future diabetes, compared with individual amino acids. The three amino acids with the strongest predictive power for future T2DM were isoleucine, phenylalanine and tyrosine. Individuals at the top quartile of this trio of amino acids had >5-fold increased risk of future diabetes, compared to those in the lowest quartile [6, 7].

The Framingham study cohort is predominantly Caucasian; thus, future studies need to determine whether ethnic and racial differences exist in the expression of metabolites vis-à-vis insulin resistance and diabetes risk. As already noted, African Americans, Hispanics and other US ethnic minority groups tend to have greater insulin resistance (and increased risk of T2DM) compared to Caucasians. The plausibility of differential metabolite signatures as a potential underlying basis of interethnic differences in insulin resistance and T2DM risk is a tantalizing notion. Furthermore, it would be important to determine whether metabolomic expression mirrors the known sequence of transition from normoglycemia → prediabetes → T2DM. The demonstration of such a sequence would mean that intermediate metabolite alterations could emerge as a tool for prediction of prediabetes, thus allowing early interventions to prevent progression to diabetes. The post-hoc data analyzed in the Framingham cohort [6, 7] do not permit such an approach, as the endpoint of the metabolomic analysis was established diabetes.

Association with Cardiometabolic Risk

The clustering of certain traits (obesity, hypertension, dyslipidemia, insulin resistance, dysglycemia), collectively known as cardiometabolic risk factors, strongly predicts susceptibility to T2DM and cardiovascular disease. To assess whether metabolomic profiles are related to the

aggregation of cardiometabolic risk factors, Cheng et al. [8] recently analyzed 45 metabolites in plasma specimens obtained from participants in the Framingham Heart Study (n = 1,015) and the Malmö Diet and Cancer Study (n = 746). Remarkably, the cardiometabolic risk factors (obesity, hypertension, insulin resistance, dyslipidemia) were associated with expression patterns of BCAAs, other hydrophobic amino acids, tryptophan breakdown products, and nucleotide metabolites. Specifically, glutamine levels ($p < 0.001$) and glutamine-glutamate ratio ($p < 0.001$) were significantly associated with measures of insulin resistance in the Framingham cohort, and the findings were replicated in the Malmö cohort [8]. A high glutamine-glutamate ratio predicted a 21% lower risk of incident diabetes among the Framingham subjects [8]. These data indicate that the metabolomic landscape is fluid, and predict that future studies would identify even more metabolites of significance to human metabolic pathophysiology.

Mechanisms

The temporal sequence in the Framingham and other studies suggest that BCAAs, AAAs and other metabolites might have direct roles in the pathogenesis of insulin resistance and T2DM. If so, the fact that the predictive effect of these small molecules was evident 12 years before the occurrence of diabetes is truly remarkable. However, current data do not exclude the alternative concept that these metabolites might simply be early markers of insulin resistance or nutrient excess, rather than causal entities. Intervention studies are needed to the relationship between metabolomic profile and the risk of obesity and diabetes. One such study [9] compared the profiles of circulating amino acids and acylcarnitines in individuals who lost 10 kg of body weight either through gastric bypass surgery or dietary restriction. As reported, total amino acids, BCAAs, and their oxidative metabolites decreased after gastric bypass surgery, but not after diet-induced weight loss [9]. The authors concluded that gastric bypass surgery (independently of weight loss), leads to alterations in metabolomic expression that might explain the superior improvement in glucose homeostasis, compared to dietary weight loss. However, it must be noted that the macro- and micronutrient composition of dietary prescriptions could have effects on metabolomic expression. For example, activation of the glutamine pathway through exogenous administration of glutamine improves glucose tolerance and blood pressure in mice

[8]. Thus, meticulous dietetic research is needed to discover the appropriate dietary regimens that would have the most beneficial impact on metabolomic markers/mediators of disease.

In summary, emerging data from the field of diabetes and obesity research indicate that analysis of the patterns of expression of circulating metabolites and small molecules (metabolomics) is a valuable tool for predicting future risk of diabetes and cardiometabolic disorders. Interventions aimed at reducing weight (and, thus, diabetes risk), trigger robust alterations in metabolomic profiles in a manner that suggests that these metabolites play a role in modulating the metabolic benefits of such interventions. Despite these fascinating developments, it

remains for future studies to dissect the exact mechanisms linking metabolomic profiles to the risk of T2DM. Once the exact mechanisms are unraveled, the next step would be to determine whether diabetes and cardiometabolic risks can be averted by altering metabolomic signatures through selective lifestyle and other interventions [10].

Acknowledgments

The author is supported in part by grants from the National Institutes of Health R01 DK067269, DK62203 and DK 48411.

References

- 1 Echouffo-Tcheugui JB, Dagogo-Jack S: Preventing diabetes mellitus in developing countries. *Nat Rev Endocrinol* 2012, E-pub ahead of print.
- 2 Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq AM, Shah SH, Arlotto M, Slentz CA, Rochon J, Gallup D, Ilkayeva O, Wenner BR, Yancy WS, Jr., Eissensohn H, Musante G, Surwit RS, Millington DS, Butler MD, Svetkey LP: A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab* 2009;9:311–326.
- 3 Dagogo-Jack S, Askari H, Tykodi G: Glucoregulatory physiology in subjects with low-normal, high-normal, or impaired fasting glucose. *J Clin Endocrinol Metab* 2009;94:2031–2036.
- 4 Zhao X, Peter A, Fritsche J, Elcnerova M, Fritsche A, Haring HU, Schleicher ED, Xu G, Lehmann R: Changes of the plasma metabolome during an oral glucose tolerance test: is there more than glucose to look at? *Am J Physiol Endocrinol Metab* 2009;296:E384–E393.
- 5 Shaham O, Wei R, Wang TJ, Ricciardi C, Lewis GD, Vasan RS, Carr SA, Thadhani R, Gerszten RE, Mootha VK: Metabolic profiling of the human response to a glucose challenge reveals distinct axes of insulin sensitivity. *Mol Syst Biol* 2008;4:214.
- 6 Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, O'Donnell CJ, Carr SA, Mootha VK, Florez JC, Souza A, Melander O, Clish CB, Gerszten RE: Metabolite profiles and the risk of developing diabetes. *Nat Med* 2011;17:448–453.
- 7 Rhee EP, Cheng S, Larson MG, Walford GA, Lewis GD, McCabe E, Yang E, Farrell L, Fox CS, O'Donnell CJ, Carr SA, Vasan RS, Florez JC, Clish CB, Wang TJ, Gerszten RE: Lipid profiling identifies a triacylglycerol signature of insulin resistance and improves diabetes prediction in humans. *J Clin Invest* 2011;121:1402–1411.
- 8 Cheng S, Rhee EP, Larson MG, Lewis GD, McCabe EL, Shen D, Palma MJ, Roberts LD, Dejam A, Souza AL, Deik AA, Magnusson M, Fox CS, O'Donnell CJ, Vasan RS, Melander O, Clish CB, Gerszten RE, Wang TJ: Metabolite profiling identifies pathways associated with metabolic risk in humans. *Circulation* 2012;125:2222–2231.
- 9 Laferrère B, Reilly D, Arias S, Swerdlow N, Gorroochurn P, Bawa B, Bose M, Teixeira J, Stevens RD, Wenner BR, Bain JR, Muehlbauer MJ, Haqq A, Lien L, Shah SH, Svetkey LP, Newgard CB: Differential metabolic impact of gastric bypass surgery versus dietary intervention in obese diabetic subjects despite identical weight loss. *Sci Transl Med* 2011;3:80re2.
- 10 Dagogo-Jack S, Egbuonu N, Edeoga C: Principles and practice of nonpharmacological interventions to reduce cardiometabolic risk. *Med Princ Pract* 2010;19:167–175.