Identifying Biomarkers of Subclinical Diabetes

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The epidemic of type 2 diabetes and the ever-increasing economic burden on society is a constant reminder of the continuing need for better therapeutic approaches to delay and/or prevent the onset of overt diabetes. Although tremendous scientific advances in our understanding of the signaling pathways and metabolism of individual tissues that are considered central to the pathophysiology of diabetes have occurred over the last several decades, efforts to reliably detect the subclinical stages early in the pathological process have remained elusive (1).

Studies aimed at detecting early stages in any disease are key for defining successful therapy, and in the case of type 2 diabetes, it is especially critical given the morbidity and mortality arising from uncontrolled hyperglycemia and multiorgan complications associated with this disease. The initial promise of the "omics" approach that evolved rapidly over the last decade has yet to deliver a cheap, widely-used biomarker that can reliably predict the development of overt diabetes. This may be related in part to the polygenic nature of the disease itself, the variable time of onset, and the progressive and unpredictable multiorgan pathophysiology that continues to challenge scientists. Given these variables, investigators have focused on finding a marker that can be linked to a consistent feature that characterizes overt diabetes such as hyperinsulinemia (e.g., proinsulin) to predict the ensuing uncontrolled hyperglycemia. However, few studies have been successful in identifying a marker of β-cell failure.

Renner and colleagues have taken advantage of the finding that patients with type 2 diabetes manifest defects in glucose-dependent insulinotropic polypeptide (GIP) activity (2). To explore whether this observation can be harnessed to unmask subtle changes in circulating metabolites, they first "created" transgenic pigs by expressing a dominant-negative GIP receptor ($GIPR^{dn}$) restricted to the pancreatic β-cells (3). In a follow-up study reported in this issue of *Diabetes*, Renner et al. (4) observed that the progressive deterioration of glucose homeostasis due to the defect in incretin signaling in the β-cells, coupled with the ability to monitor β-cell mass, makes the model especially suitable for identifying potential biomarkers. The authors subjected plasma samples, collected during intravenous glucose tolerance tests in 2.5- and 5-month-old pigs, to targeted mass spectrometry (5) and report an elevation in several plasma metabolites in young animals when β-cell mass is still normal. In the older animals, these metabolites declined, whereas other metabolites were found to correlate with the reduced β-cell mass, implying a direct or indirect link with β-cell function. Intriguingly, mice expressing a $GIPR^{dn}$ manifest a more severe phenotype (6) compared with GIP receptor knockout mice, suggesting either activation of compensatory pathways in the latter and/or species-specific differences (7).

The elevation in several amino acids (leucine, valine, tyrosine, phenylalanine, ornithine, histidine, arginine) at the earlier time point in the transgenic pigs is interesting for several reasons. First, detecting biomarkers very early in the disease process is desirable for quicker intervention. Second, elevated amino acids, when the measured β-cell mass is still intact, indicate global metabolic alterations that are linked to defective insulin secretion well before overt secondary effects of reduced mass become apparent. Third, the report by Renner et al. comes on the heels of another study on the follow-up of humans over a 12-year period showing a rise in some of the same amino acids (leucine/isoleucine, valine, tyrosine, phenylalanine) that were associated with an increased risk of developing diabetes (8). One difference that makes the Renner study significant is that the report by Wang et al. (8) included individuals who also manifested higher BMIs and homeostasis model assessment of insulin resistance, indicating potential involvement of obesity and/or progression of disease at the time of the study. This raises questions about the specificity of the changes in the amino acids that were observed in the latter report. Notwithstanding, the similarity in the changes in the amino acids in the two species is worth further consideration.

In addition to amino acids, several other metabolites detected in the pig model have also been reported in humans. For example, short-chain acylcarnitine levels are generally increased in insulin-resistant obese individuals and correlate with HbA_{1c} (9). Their elevation in 2.5-month-old pigs suggests that it is a sensitive parameter that is altered very early in the pathological process of poor incretin signaling in β-cells. Since ~50% of these changes in metabolites were evident even in fasting samples in the young pigs, they likely reflect altered metabolic status due to long-term defects and underscore their significance as potential candidate biomarkers.

The decline in the metabolites in 5-month-old transgenic pigs, when β-cell mass is clearly reduced, suggests the markers may predict disease progression. It would be important to clarify whether the changes in circulatory concentrations observed in these specific amino acids occur consistently and independently of the normal aging process. If the changes continue to be significant in a larger number of both male and female experimental pigs, it would provide further evidence that the changes are independent of sex hormones, which are known to contribute to altered glucose homeostasis (10). Further, since the changes in some of these metabolites have been reported in proatherogenic and proinflammatory states, it is not clear whether the specificity of

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using them to predict type 2 diabetes is confounded by other complications that are usually associated with the disease.

HbA_{1c} continues to be a standard measure that reflects recently elevated blood glucose levels and is used as a diagnostic test for evaluating progression of disease. It is also considered a risk factor for complications (11). It would have been useful if a correlation between the metabolites and HbA_{1c} had been available at both ages but especially in the young transgenic pigs. Several additional questions are worth pursuing: *1*) Are these biomarkers valid even when the animals manifest insulin resistance or become obese? Such studies would, in part, address the critique that the study by Renner et al. is unifactorial and does not directly represent the multifactorial nature of type 2 diabetes. *2*) Do the metabolites change earlier than 2.5 months, and if so, will early therapeutic intervention in the young susceptible pigs reverse or delay progression of the disease? *3*) Do changes in the candidate metabolites correlate with previously reported biomarkers such as Tmem27, a protein that is shed from β-cells (12)? *4*) Because the $GIPR^{dn}$ is restricted to the β-cell, could it be used as a model to examine the suggested role of osteopontin in the modulation of islet function (13), and islet vascular tissue (L. Groop, personal communication)? Although some of the findings by Renner et al. are already in agreement with a previous report in humans (8), it would be important to reproduce the findings in a second cohort, preferably one that is neither insulin resistant nor obese. This reproducibility would meet the definition of a biomarker (1) and provide evidence that the biomarkers are not only for pigs. Successful identification of a reliable biomarker of subclinical diabetes would directly influence the management of the disease, potentially reduce morbidity and mortality associated with chronic hyperglycemia, and have a major impact on health care costs.

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