Tracing the Proteomic Fingerprint of the Diabetic Aorta?

Gonca Suna and Manuel Mayr

Circ Cardiovasc Genet. 2014;7:100-101
doi: 10.1161/CIRCGENETICS.114.000592
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1942-325X. Online ISSN: 1942-3268

Editorial

Tracing the Proteomic Fingerprint of the Diabetic Aorta?

Gonca Suna, MD; Manuel Mayr, MD, PhD

Diabetes mellitus is a worldwide epidemic, and the percentage of the US population diagnosed with diabetes mellitus between 1980 and 2010 increased from 2.5% to 6.9%.[1] Nearly 27% of people >65 years of age have diabetes mellitus. If current trends continue, 1 in 3 US adults will experience diabetes mellitus by 2050. This can be attributed to lifestyle choices contributing to obesity.[2] One of the main causes of death and disability in patients with diabetes mellitus are vascular complications, affecting both the macro- and the microvasculature.[3] Macrovascular manifestations include atherosclerosis leading to coronary artery disease, peripheral arterial disease, and stroke. Microvascular changes, on the other hand, encompass diabetic nephropathy, retinopathy, and neuropathy.

Article see p 161

Although there is compelling evidence for the association of diabetes mellitus and vascular disease, the underlying pathophysiological mechanisms remain to be elucidated. Advanced glycation end products, as well as reactive oxygen species, are suggested to be 2 of the major culprits leading to vascular damage.[4,5] Extracellular matrix proteins can become glycated by nonenzymatic reactions of sugar moieties, which alter protein function in target tissues.[6] Major histological changes are apparent in diabetic vessels, including increased intima–media thickness and excessive extracellular matrix deposition.[5] Yet, despite many patients with diabetes mellitus following a tight glycemic control regime, they are still experiencing vascular complications. Notably, large-scale clinical trials have failed to demonstrate that strict glycemic control leads to a reduction in cardiovascular risk.[7,8] However, although vascular complications remain as the principal causes of death and disability in diabetes mellitus, epidemiological evidence suggests an inverse association between diabetes mellitus and aortic aneurysms and dissections.[9,10] These findings demonstrate the multiple facets of diabetes mellitus on the vasculature, and a more holistic approach might advance our insights in the vascular risk associated with diabetes mellitus at a molecular level.[11]

In this issue of the journal, Husi et al[12] report the results of a proteomics analysis of the diabetic mouse aorta. Aiming at a comprehensive understanding of the molecular changes in the diabetic vasculature, they performed a comparison of 5 aortic vessels obtained from control and diabetic mice. Diabetes mellitus was induced within 2 weeks after consecutive injections of low-dose streptozotocin (STZ). STZ-induced hyperglycemia was maintained throughout the 19-week experimental period with an average blood glucose level of 395 mg/dL (21.9 mmol/L). A significant thickening of the vascular wall in diabetic mice was observed (media thickness of 28 μm in diabetic aortas versus 19 μm in control aortas). The proteomics analysis revealed 2205 nonredundant aortic proteins, of which 72 entries were selected as likely differing in both groups based on a P value of 0.05. Only limited overlap (<50%) was observed between proteins identified in the aortas from control and diabetic mice, suggesting a marked heterogeneity in cellular composition or an undersampling of the vascular proteome by mass spectrometry. More targeted proteomics approaches can overcome the dynamic range limitations because of high abundant cellular proteins.[13,14] The context of the differentially expressed proteins was then further explored by bioinformatics analyses including gene ontology, interactome mapping (by using Michigan Molecular Interactions [MiMI]), or metabolic pathway (Path Visio) analyses, as well as a literature and Online Mendelian Inheritance in Man (OMIM) database search. The bioinformatics approaches suggested that there were pronounced changes in proteins involved in myogenesis, vascularization, hypertension, hypertrophy, as well as a substantial reduction of fatty acid storage. Most notably, the identified proteins and pathways implicated decreased glycolysis and fatty acid metabolism and upregulation of an alternative ketone-body formation pathway within the vessel wall. In addition, the authors observed a pronounced increase in vascularization, oxidative stress-response proteins, as well as apoptosis-related proteins. The most prominent findings were validated using immunohistochemistry. The authors justified their selected targets for validation by the placement of the candidate proteins in molecular pathways, as well as by the availability of antibodies. For selected proteins, immunohistochemistry confirmed the changes observed in the proteomic analysis. Although the iron-dependent alcohol dehydrogenase Adhfe1, a key molecule in the postulated ketone-body formation process, could not be detected in the proteomics analysis, the authors found it upregulated in aortas of STZ-treated mice, underpinning their bioinformatics predictions.

STZ destroys insulin-producing β cells, thereby mimicking type I diabetes mellitus rather than type II diabetes mellitus in mice fed with standard diet.[15,16] Type I diabetes mellitus with its lack of insulin production, but not type II diabetes mellitus, which usually begins with insulin resistance, is frequently associated with ketoacidosis (ketone-body overproduction). The authors' hypothesis about an alternative ketone-body formation pathway may be limited to the STZ-induced diabetes mellitus

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From the King's British Heart Foundation Centre, King's College London, London, United Kingdom.

Correspondence to Manuel Mayr, MD, PhD, King's British Heart Foundation Centre, King's College London, 125 Coldharbour Lane, London SE5 9NU, United Kingdom. E-mail manuel.mayr@kcl.ac.uk

(*Circ Cardiovasc Genet*. 2014;7:100-101.)

***Circ Cardiovasc Genet* is available at http://circgenetics.ahajournals.org**

DOI: 10.1161/CIRCGENETICS.114.000592

model. Generalizing this pathway as the pathomechanism for diabetes mellitus–induced changes in the vasculature requires caution because the findings have not been replicated in a type II diabetes mellitus model. The authors ruled out the overproduction of ketone bodies as a result of the classical ketone-body formation by β-oxidation because all molecules involved in this pathway were apparently downregulated. Nonetheless, the authors' conclusion of diabetes mellitus leading to a reduction of fatty acid biosynthesis and upregulation of local ketone-body production in the vasculature cannot be substantiated by immunohistochemistry staining alone. More quantitative approaches, including real-time polymerase chain reactions, immunoblotting, and enzyme activity assays, would have been preferable as a validation method. Interpretation of the bioinformatics results without further validation at the metabolite level, therefore, remains challenging. Measurements of ketone bodies in the circulation and the vessel wall or more detailed metabolomics analysis[17] to substantiate changes in fatty acid biosynthesis could have helped in differentiating between local ketone production within the vessel wall versus general overproduction in the liver, which is known to occur under type I diabetes mellitus conditions. Another potential confounding factor for proteomics studies of the mouse aorta is the perivascular fat tissue. Unless carefully removed, enzymes of perivascular fat tissue will be detected in the proteomics analysis of murine aortas. Future studies will have to show the applicability of these results in a murine type I diabetes mellitus model to human vascular tissue.[18]

Although modern medicine has fortunately moved on from diagnosing diabetes mellitus by tasting the sugary taste of the patient's urine, we still await a cure or at least a prevention of its vascular complications.

Sources of Funding

Dr Suna is supported by a British Heart Foundation PhD Studentship. Dr Mayr is a Senior Fellow of the British Heart Foundation. The research was funded/supported by the National Institute of Health Research Biomedical Research Centre based at Guy's and St. Thomas' National Health Service (NHS) Foundation Trust and King's College London in partnership with King's College Hospital.

Disclosures

None.

References

1. Polonsky KS. The past 200 years in diabetes. *N Engl J Med*. 2012;367:1332–1340.
2. Mokdad AH, Bowman BA, Ford ES, Vinicor F, Marks JS, Koplan JP. The continuing epidemics of obesity and diabetes in the United States. *JAMA*. 2001;286:1195–1200.
3. Fisher M. Diabetes and atherogenesis. *Heart*. 2004;90:336–340.
4. Schmidt AM, Yan SD, Wautier JL, Stern D. Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. *Circ Res*. 1999;84:489–497.
5. Paneni F, Beckman JA, Creager MA, Cosentino F. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. *Eur Heart J*. 2013;34:2436–2443.
6. Brownlee M. Advanced glycosylation in diabetes and aging. *Annu Rev Med*. 1995;46:223–234.
7. Cognet I, Gimenez M. Glucose control and cardiovascular disease. Is it important? No. *Diabetes Care*. 2009;32:334–336.
8. Buse JB, Ginsberg HN, Bakris GL, Clark NG, Costa F, Eckel R, et al; American Heart Association; American Diabetes Association. Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. *Diabetes Care*. 2007;30:162–172.
9. Lederle FA. The strange relationship between diabetes and abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg*. 2012;43:254–256.
10. Prakash SK, Pedroza C, Khalil YA, Milewicz DM. Diabetes and reduced risk for thoracic aortic aneurysms and dissections: a nationwide case-control study. *J Am Heart Assoc*. 2012;1: pii: jah3-e000323.
11. Didangelos A, Stegemann C, Mayr M. The -omics era: proteomics and lipidomics in vascular research. *Atherosclerosis*. 2012;221:12–17.
12. Husi H, Van Agtmael T, Mullen W, Bahlmann FH, Schanstra JP, Vlahou A, et al. Proteome-based systems biology analysis of the diabetic mouse aorta reveals major changes in fatty acid biosynthesis as potential hallmark in diabetes associated vascular disease. *Circ Cardiovasc Genet*. 2014;7:161–170.
13. Didangelos A, Yin X, Mandal K, Saje A, Smith A, Xu Q, et al. Extracellular matrix composition and remodeling in human abdominal aortic aneurysms: a proteomics approach. *Mol Cell Proteomics*. 2011;10:M111.008128.
14. Stegemann C, Didangelos A, Barallobre-Barreiro J, Langley SR, Mandal K, Jahangiri M, et al. Proteomic identification of matrix metalloproteinase substrates in the human vasculature. *Circ Cardiovasc Genet*. 2013;6:106–117.
15. Rees DA, Alcolado JC. Animal models of diabetes mellitus. *Diabet Med*. 2005;22:359–370.
16. Leiter EH, Schile A. Genetic and pharmacologic models for type 1 diabetes. *Curr Protoc Mouse Biol*. 2013;3:9–19.
17. Stegemann C, Drozdov I, Shalhoub J, Humphries J, Ladroue C, Didangelos A, et al. Comparative lipidomics profiling of human atherosclerotic plaques. *Circ Cardiovasc Genet*. 2011;4:232–242.
18. Mayr M, Grainger D, Mayr U, Leroyer AS, Leseche G, Sidibe A, et al. Proteomics, metabolomics, and immunomics on microparticles derived from human atherosclerotic plaques. *Circ Cardiovasc Genet*. 2009;2:379–388.

KEY WORDS: Editorials ■ proteome ■ proteomics