Editorials

Association of Haptoglobin Genotype in Adolescents with Type 1 Diabetes Mellitus and Endothelial Function: An Unresolved Issue

Michael Shechter MD MA

Heart Institute, Sheba Medical Center, Tel Hashomer, Israel
Affiliated to Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

The endothelium comprises the innermost layer of blood vessels and is thus the largest organ of the body. This endothelial surface of blood vessels functions as a regulatory organ maintaining vasomotor tone, while the non-thrombogenic nature of its surface regulates platelet activity and blood fluidity by the secretion of anticoagulant and fibrinolytic factors [1].

Endothelial dysfunction is a major factor contributing to the atherogenic process [2,3]. Abnormal function of the endothelium is detectable prior to observable intimal lesions in patients with risk factors for atherosclerosis [4-6]. Endothelial dysfunction is a systemic disorder and a key variable in the pathogenesis of atherosclerosis and its complications [3]. Current evidence suggests that endothelial status is not determined solely by the individual risk factor, but rather may be regarded as an integrated index of all atherogenic and atheroprotective factors present in an individual, including both known and as yet unknown variables and genetic predispositions. Endothelial dysfunction reflects a vascular phenotype prone to atherogenesis and may therefore serve as a marker of inherent atherosclerotic risk. In line with this hypothesis, dysfunction of either the coronary or peripheral vascular endothelium was shown to constitute an independent predictor of cardiovascular events, providing valuable prognostic information in addition to that derived from conventional risk factor assessment [7]. Interventions, such as risk factor modification and treatment with various drugs, including statins [8,9], angiotensin-converting enzyme inhibitors [10], sibutramine [11], in addition to adjunct therapy, such as food supplements [12] and enhanced external counterpulsation therapy [13], may improve endothelial function, potentially leading to improved prognosis [3]. Therefore, given its reversibility and suitability as a diagnostic tool in identifying patients at risk, together with its control over efficacy of therapy in clinical practice, endothelial dysfunction may be an attractive primary target in the effort to optimize individualized therapeutic strategies to reduce cardiovascular morbidity and mortality [14,15].

While numerous important functions of the endothelium have been recognized in the past, many recent experimental and clinical studies have focused on endothelium-derived nitric oxide, probably due to the fact that one of its main functions, vascular relaxation, can be easily assessed in humans *in vivo* [16]. Nitric oxide, a potent endogenous vasodilator that appears to be responsible for the maintenance of basal vascular tone, is also thought to exert other important effects, such as inhibition/modulation of platelet aggregation, leukocyte adhesion, cell respiration, and apoptosis [17].

Over the past decade a non-invasive technique has been developed to evaluate endothelium-dependent, brachial artery flow-mediated vasodilation [16-22]. This stimulus provokes the endothelium to release nitric oxide with subsequent vasodilation that can be imaged and quantitated as an index of vasomotor function. The advantages of this high frequency ultrasonographic imaging of the brachial artery are twofold: it is non-invasive and also facilitates repeated measurements [16].

This raises the question of whether brachial artery flow-mediated vasodilation could be a useful marker of preclinical vascular disease in the pediatric population. Celermajer et al. [23] note that the risk factors that predispose to atherosclerosis are associated with endothelial dysfunction throughout life, and suggest that the procedure could have value for risk modification during the preclinical phase of vascular disease. Influencing factors, such as vessel diameter, age, and therapeutic interventions, may be of less significance in children and adolescents than in adults. Furthermore, ultrasound testing is reproducible, non-invasive, low risk, and non-painful, making it an ideal procedure for the young [24].

Type I diabetes mellitus is a well-established risk factor for premature cardiovascular disease [25], while micro- and macrovascular complications are related to long-term glycemic control [26] and other risk factors such as blood pressure and lipids. Since children rarely experience cardiovascular events, endothelial function is a surrogate marker of CVD and can be monitored non-invasively using either the traditional reactive hyperemia measurement by brachial artery reactivity high resolution ultrasound testing [16], or by a more novel technique, reactive hyperemia-peripheral artery tonometry [27]. Both these

---

CVD = cardiovascular disease

techniques are non-invasive, bedside available, non-painful, relatively safe and relatively inexpensive tests of short duration (< 30 minutes). The BRT technique, although operator dependent, is highly reproducible, with widespread international use, and uniformed by the International Brachial Artery Reactivity Task Force published in 2002 [16]. The PAT technique, on the other hand, is a more recent innovation, independently operated, with much less worldwide experience. While the BRT technique explores vascular smooth muscle cells as the cause of non-dilation of the blood vessels instead of pure endothelial dysfunction, the PAT technique does not provide such data. Finally, while BRT can predict cardiovascular events, PAT score has not yet been used to evaluate the risk of future such events in either adult or pediatric populations. Further studies are therefore needed to validate the relevance of low PAT scores in detecting CVD and predicting future cardiovascular events [27].

Although the incidence of cardiovascular disease is higher in diabetic compared to non-diabetic populations, there are geographic and ethnic differences in the risk of CVD among diabetic patients that cannot be fully explained by differences in conventional CVD risk factors between these groups. These studies suggest that genetic factors could contribute to differences in susceptibility to CVD among individuals with diabetes mellitus. In humans, there are two general classes of alleles for the haptoglobin gene, designated 1 and 2 [28]. Haptoglobin alleles differ significantly in their relative frequency among different ethnic groups. The protein product of the 1 allele is significantly more antioxidant than that produced by the 2 allele [29]. Levy and colleagues [30] recently demonstrated that haptoglobin phenotype is predictive of microvascular (those who are homozygous for haptoglobin 1 allele are at decreased risk for developing retinopathy and nephropathy) and macrovascular (those with the 1-1 haptoglobin phenotype are the least likely to develop post-percutaneous coronary angioplasty restenosis) [31] complications in adult patients with diabetes mellitus. Levy et al. [32] also demonstrated in a case-controlled sample from the Strong Heart Study, in a population-based longitudinal study of CVD in American Indians, that haptoglobin phenotype is an independent risk factor for CVD in diabetic individuals.

Since the haptoglobin phenotype is a risk for micro- and macrovascular complications in diabetic patients, the question is whether diabetic children with the 1-2 and 2-2 haptoglobin phenotype carry the potential for worse endothelial function than those with the haptoglobin phenotyope 1-1. Furthermore, if the haptoglobin phenotype 1-1 is associated with better endothelial function, this non-invasive test could be used to monitor those diabetic children who are at risk for future cardiovascular complications.

Since children with type I diabetes rarely experience cardiovascular events, Shachor-Meyouhas and co-workers [33], in this issue of IMAJ, examined the association of a diabetic profile, haptoglobin genotype and peripheral endothelial function using

PAT in 15 type 1 diabetic patients aged 14–20 years, without obvious microvascular/neuropathy complications. The authors divided the 15 patients into two groups: normal (n=8) and impaired (n=7) endothelial function. Although the two groups were comparable regarding age, body mass index, systolic and diastolic blood pressure, lipoproteins and fasting glucose, patients with impaired endothelial function had a significantly higher level of HbA1C compared to those with normal endothelial function. Furthermore, there was a significant negative association between HbA1C levels and endothelial function. Nevertheless, the authors did not find any correlation between the haptoglobin genotype and endothelial function.

The article by Shachor-Meyouhas et al. [33] carries some major limitations which could lead to the following negative results. First, the sample size is relatively small and therefore a power calculation is crucial for estimating the number of patients needed for the study. Second, most studies looking at endothelial function as a surrogate end-point demand fasting for at least 10–12 hours [16]. Vogel and team [34] demonstrated that endothelial function is transiently impaired over a 6 hour period following a single high fat meal [34]. However, in the current study participants were asked to fast for at least 3 hours before endothelial function testing. Thus, the endothelial function results could be influenced by various types of food intake. Third, since blood tests were not performed on the same day as endothelial function assessment, factors influencing endothelial function, such as fasting blood glucose, HbA1c or lipid profile cannot be correlated to the endothelial function results. For example, it could be that on the day of endothelial function assessment the children had ingested high fasting blood glucose or high triglycerides, thereby impairing endothelial function. Fourth, since, as previously described, the PAT technique, in contrast to the BRT technique, does not explore the vascular smooth muscle cells as a potential cause of non-dilation of the blood vessels, the low score provided by PAT in the current study could result from smooth muscle dysfunction rather than endothelial dysfunction per se.

Thus, although the questions raised by Shachor-Meyouhas and associates [33] in the current study are extremely important, we are still left with unanswered questions. Is haptoglobin phenotype 1-2 or 2-2 associated with endothelial dysfunction in diabetic children without micro- or macrovascular complications? Can endothelial dysfunction in diabetic children without micro- or macrovascular complications predict long-term cardiovascular outcome? A prospective, carefully designed, long-term follow-up study in children is required to answer these important questions.

BRT = brachial artery reactivity test
PAT = peripheral artery tonometry

References

1. Biegelsen ES, Loscalzo J. Endothelial function and atherosclerosis. *Coron Artery Dis* 1999;10:241–56.
2. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1900s. *Nature* 1993;362:801–9.
3. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction. A marker of atherosclerotic risk. *Arterioscer Thromb Vasc Biol* 2003;23:168–75.

4. Zeiher AM, Drexler H, Wollschlager H, Just H. Modulation of coronary vasomotor tone in humans: progressive endothelial dysfunction with different early stages of coronary atherosclerosis. *Circulation* 1991;83:391–401.

5. Celermajer DS, Sorensen KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992;340:1111–15.

6. Reddy KG, Nair R, Sheehan HM, Hodgson JM. Evidence that selective endothelial dysfunction may occur in the absence of angiographic or ultrasound atherosclerosis in patients with risk factors for atherosclerosis. *J Am Coll Cardiol* 1994;23:833–43.

7. Halcox JPJ, Schenke WH, Zalos G, et al. Prognostic value of coronary vascular endothelial dysfunction. *Circulation* 2002;106:653–8.

8. Vogel R. Cholesterol lowering and endothelial function. *Am J Med* 1999;107:479–87.

9. Shechter M, Sharir M, Labrador MJ, Forrester J, Merz CN. Improvement in endothelium-dependent brachial artery flow-mediated vasodilation with low-density lipoprotein cholesterol levels below 100 mg/dl. *Am J Cardiol* 2000;86:1256–9.

10. Anderson TJ, Elstein E, Haber H, Charbonneau E. Comparative study of ACE-inhibition, angiotensin II antagonism, and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF Study). *J Am Coll Cardiol* 2000;35:60–6.

11. Shechter M, Beigel R, Freimark D, Matezky S, Feinberg MS. Short-term sibutramine therapy is associated with weight loss and improved endothelial function in obese patients with coronary artery disease. *Am J Cardiol* 2006;97:1650–3.

12. Shechter M, Sharir M, Labrador MJ, Forrester J, Silver B, Beirey Merz CN. Oral magnesium therapy improves endothelial function in patients with coronary artery disease. *Circulation* 2000;102:2353–8.

13. Shechter M, Matetzky S, Feinberg MS, Chouraqui P, Rotstein Z, Hod H. External counterpulsation therapy improves endothelial function in patients with refractory angina pectoris. *J Am Coll Cardiol* 2003;42:2090–5.

14. Drexler H. Endothelium as a therapeutic target in heart failure. *Circulation* 1998;98:2652–5.

15. Shechter M, Marai I, Marai S, et al. The association of endothelial dysfunction and cardiovascular events in healthy subjects and patients with cardiovascular disease. *IMAJ* 2007;9:271–9.

16. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. A report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002;39:257–65.

17. Verma S, Anderson TJ. Fundamentals of endothelial function for the clinical cardiologist. *Circulation* 2002;105:546–9.

18. Laurent S, Lacolley P, Brunel P, Laloux B, Pannier B, Safar M. Flow-mediated vasodilation of brachial artery in essential hypertension. *Am J Physiol* 1990;258:H1004–11.

19. Arbel Y, Dvir.D, Feinberg MS, Beigel R, Shechter M. The association between right coronary artery morphology and vascular endothelial function. *Int J Cardiol* 2007;115:19–23.

20. Freimark D, Feinberg M, Matetzky S, Shechter M. The impact of intermittent intravenous dobutamine therapy on endothelial function in patients with severe chronic heart failure. *Am Heart J* 2004;148:878–82.

21. Shechter M, Ginzberg S, Scheinowitz M, Feinberg MS, Laron Z. Obese adults with primary growth hormone resistance (Laron syndrome) have normal endothelial function. *Growth Horm IGF Res* 2007;17:165–70.

22. Shechter M, Sherer Y. Endothelial dysfunction: a crystal ball prediction for enhanced cardiovascular risk? *IMAJ* 2003;5:736–8.

23. Celermajer DS, Sorensen KE, Bull C, Robinson J, Dearfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol* 1994;24:1468–74.

24. Slyper AH. What vascular ultrasound testing has revealed about pediatric atherogenesis, and a potential clinical role for ultrasound in pediatric risk assessment. *J Clinc Endocrinol Metab* 2004;89:3089–95.

25. Orchard TJ, Costacou T, Kretowski A, Nesto RW. Type 1 diabetes and coronary artery disease. *Diabetes Care* 2006;29:2528–38.

26. Nathan DM, Cleary PA, Backlund JE, et al., for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* 2005;353:2643–53.

27. Haller MJ, Stein J, Shuster J, et al. Peripheral artery tonometry demonstrates altered endothelial function in children with type 1 diabetes. *Pediatric Diabetes* 2007;8:193–8.

28. Langlois MR, Delanghe JR. Biological and clinical significance of haptoglobin in humans. *Clin Chem* 1996;42:1589–600.

29. Melamed-Frank M, Lache O, Enav BI, et al. Structure/function analysis of the anti-oxidant properties of haptoglobin. *Blood* 2001;98:3693–8.

30. Nakhoul F, Zoabi R, Kantor Y, et al. Haptoglobin phenotype and diabetic nephropathy. *Diabetologia* 2001;44:602–4.

31. Roguin A, Hochberg I, Nikolsky E, et al. Haptoglobin phenotype as a predictor of restenosis after percutaneous transluminal coronary angioplasty. *Am J Cardiol* 2001;87:330–2.

32. Levy AP, Hochberg I, Jablonski K, et al. Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: The Strong Heart Study. *J Am Coll Cardiol* 2002;40:1984–90.

33. Shachor-Meyouhas Y, Pillar G, Shehadeh N. Uncontrolled type 1 diabetes mellitus and endothelial dysfunction in adolescents. *IMAJ* 2007;9:637–40.

34. Vogel RA, Corretti MC, Plotnick GD. Effect of a single high-fat meal on endothelial function in healthy subjects. *Am J Cardiol* 1997;79:350–4.

Correspondence: Dr. M. Shechter, Heart Institute, Sheba Medical Center, Tel Hashomer 52621, Israel.
Phone: (972-3) 530-2645; Fax: (972-3) 534-3882
email: shechtes@netvision.net.il

The innocent and the beautiful have no enemy but time

William Butler Yeats (1865-1939), Irish poet and dramatist and one of the foremost figures of 20th century literature. He was awarded the Nobel Prize for Literature in 1923, yet his best work was done in the years after he received the prize.