Diabetologia (2012) 55:1559–1563
DOI 10.1007/s00125-011-2445-5

THEN AND NOW

Endothelial dysfunction in type 2 diabetes

A. Natali · E. Ferrannini

Received: 1 June 2011 / Accepted: 6 September 2011 / Published online: 20 January 2012

Abstract The mechanisms responsible for the accelerated atherosclerosis observed in type 2 diabetes are not fully understood. One of the earliest events in the development of atherosclerosis is endothelial dysfunction, namely, a reduction in nitric oxide (NO) synthesis or its bioavailability within the peri-endothelial environment, where it is responsible for maintenance of vascular tissue integrity. The clinical evaluation of this pathway is hampered by the fact that in vivo NO cannot be directly measured; however, exploiting a novel, complex and elegant experimental setup, McVeigh and co-workers (*Diabetologia* 1992;35:771–776) were the first to document that NO bioavailability in type 2 diabetic patients is indeed reduced. In this edition of 'Then and now' that paper is reappraised not only for its originality, but also for the broad and extensive evaluation of the vascular functions explored, the complete clinical characterisation of patients enrolled and for the fact that all the major findings were subsequently replicated.

Keywords Endothelium · Type 2 diabetes

Abbreviations

EMP	Endothelial microparticle
EPC	Endothelial progenitor cell
L-NMMA	N^G-Monomethyl-L-arginine
PET	Positron-emitting tomography

A. Natali (✉) · E. Ferrannini
Department of Internal Medicine, University of Pisa,
Via Roma 67,
56100 Pisa, Italy
e-mail: anatali@ifc.cnr.it

Background

The endothelium plays a central role in maintaining vascular homeostasis through the release of vasodilating and vasoconstricting substances. Seminal work by Furchgott and Zawadzki in 1980 [1] revealed that the endothelium is responsible for vascular relaxation induced by acetylcholine, a muscarinic receptor agonist. The clinical relevance of this pathway in human disease, including hypertension and hypercholesterolaemia, was reported in 1990 [2, 3]. Endothelial biology took centre stage in 1998 when the Nobel Prize in Physiology or Medicine was awarded to Robert F. Furchgott, Louis J. Ignarro and Ferid Murad for their discoveries concerning the role of NO as a signalling molecule controlling vasodilation. The translational impact of this key discovery has been suggested by in vitro studies that have established a role of the endothelium—and NO—in protecting vessels from atherosclerosis [4]. With regard to diabetes, early after the seminal paper by Furchgott and Zawadzki [1], endothelial dysfunction was demonstrated in experimental animal models of diabetes [5], while its mechanisms were described by Bucala et al. [6].

The *Diabetologia* paper

The work by McVeigh and co-workers [7] was the first in vivo demonstration of the presence of endothelial dysfunction in type 2 diabetic patients. Specifically, they demonstrated that, in type 2 diabetic patients, the ability of resistance vessels to vasodilate in response to endothelium- or smooth muscle cell-dependent stimuli was impaired (Fig. 1c, d, respectively), while neither the vascular structure (Fig. 1a) nor unstimulated (basal; Fig. 1b) endothelium-dependent blood flow appeared

to be compromised (as illustrated in the figures from the original paper reproduced in Fig. 1). The authors also showed that this vascular dysfunction is caused by reduced NO release. To accomplish this, they used a technique that relies upon the measurement of perfused forearm blood flow by strain-gauge plethysmography in response to an intra-arterial infusion of either acetylcholine (an endothelium-dependent dilator) or nitroglycerine (a smooth-muscle-dependent dilator). This method is still considered state-of-the art for the study of endothelial function in resistance arteries. The number of vascular tests, combined with the thorough clinical characterisation of the patients, makes this study a very rich source of information. In addition to the 'standard' responses to acetylcholine and nitrates, the investigators also evaluated the response to ischaemia and the effect of blocking NO synthesis with N^G-monomethyl-L-arginine (L-NMMA)—a competitive inhibitor of NO-synthase—on both acetylcholine-stimulated and basal blood flow.

The main findings by McVeigh et al [7] have since been replicated and have stood the test of time. In particular, the impaired vasodilatory response to nitrates, a somewhat neglected aspect of vascular dysfunction in diabetes, has been confirmed by other investigators, notably in the elegant study by Creager and co-workers [8]. Similarly, the lack of significant associations between the vascular dysfunction and the presence of vascular complications or degree of metabolic control, although quite surprising, has been reported by subsequent studies [9, 10]

Limitations

The finding that vascular dysfunction in type 2 diabetes is caused by reduced NO release is highly original. Nevertheless, the study by McVeigh et al [7] was subject to limitations that warrant some discussion. For example, the post-ischaemic vasodilatory response following only 5 min of ischaemia does not accurately explore structural changes of the resistance vessels, as claimed in the paper; rather, it provides another index of endothelial function, since the vasodilation is largely sustained by the blood flow acceleration caused by the drastic reduction in peripheral resistance. The mean absolute blood flow values achieved with the 5 min ischaemia (15 ml min^{-1} dl^{-1}) are far below what is now known to be the maximal blood flow of the forearm (~30 ml min^{-1} dl^{-1}). Reflecting minimal resistance, maximal blood flow makes it possible to estimate vascular structural changes; maximal flow rates are usually obtained after 10 min of ischaemia combined with 1 min of forearm exercise [11].

Another limitation of the study by McVeigh et al is related to the authors' suggestion that the endothelial dysfunction in type 2 diabetes was caused by reduced NO synthesis. The L-NMMA infusion rate used was low (500 μg/min) and the infusion occurred only after interrupting the maximal acetylcholine infusion (to describe the kinetics of blood flow recovery). To determine the extent to which an impaired response to acetylcholine is caused by reduced NO synthesis, a more correct approach is to repeat the stepwise acetylcholine infusion on top of a constant infusion

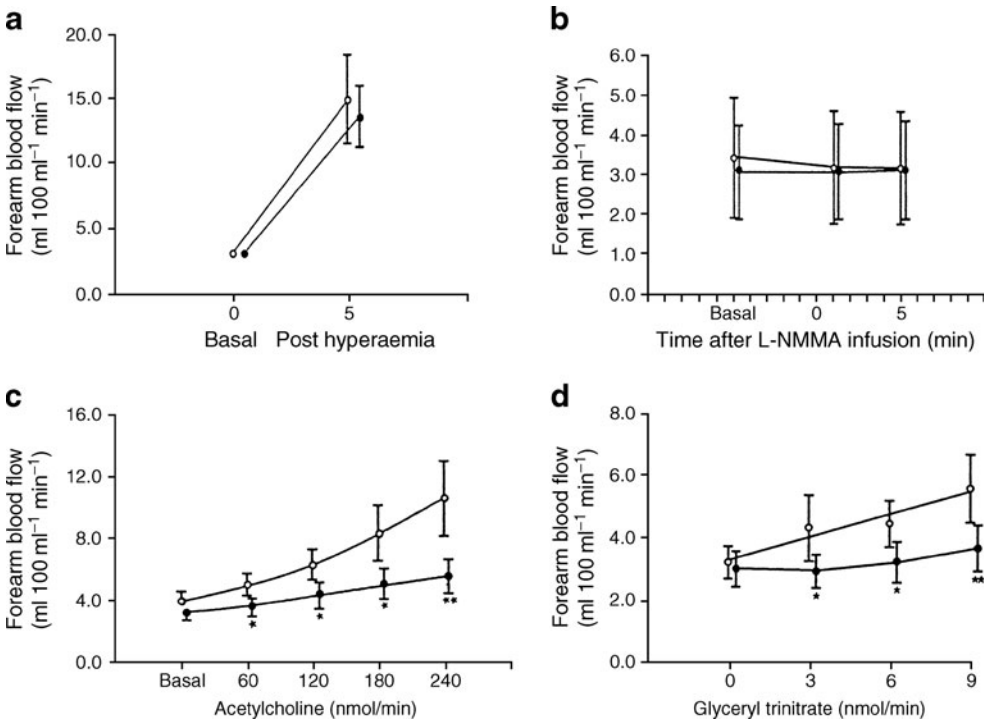

Fig. 1 **a** Blood flow before and after 5 min of ischaemia in 21 type 2 diabetic patients (black symbols) and 13 nondiabetic controls (white symbols). Blood flow responses to intraarterial infusions of (**b**) L-NMMA, at the rate of 2 μmol/min, (**c**) acetylcholine and (**d**) and glyceryl trinitrate. The error bars represent the 95% CIs of the mean values. *$p<0.01$, ** $p<0.001$. Reproduced from [7] with permission of Springer Science + Business Media

of L-NMMA (at rates ranging from 900 to 1,300 μg/min) to fully block NO synthesis. This procedure allows the career estimation of the contribution of NO to the full vascular response to the muscarinic receptor agonist, which is known to also activate non-NO-mediated vasodilatory pathways.

Additionally, the coexistence of a profound impairment in the response to nitrates raises the possibility that the primary defect in type 2 diabetes occurs in smooth muscle, as discussed as an alternative mechanism by McVeigh et al. Nevertheless, although not fully supported by the data, the authors' suggestion of a reduced NO bioavailability in type 2 diabetes was later demonstrated to be essentially correct [10].

What came afterwards

Endothelial dysfunction has been detected in several other vascular districts: in epicardial vessels and resistance vessels of the coronary circulation (assessed by measuring lumen diameter or blood flow changes in response to acetylcholine); in leg and arm conductance vessels (evaluated with the use of the flow-mediated dilatation technique); and in the skin microcirculation (by laser-Doppler iontophoresis). Notably, endothelial dysfunction of both coronary and forearm resistance arteries has been shown to predict coronary events. Patients with type 2 diabetes not only show an impaired vasodilation but also a basal (unstimulated) enhanced release of endothelin-1, a potent vasoconstrictor [12]. The endothelium produces other vasoconstrictors, the prostanoids, but they do not seem to contribute to the vascular dysfunction in diabetes [8].

A correlation analysis performed on a cohort of 95 patients with type 2 diabetes (Fig. 2) highlights that factors responsible for the reduced response to acetylcholine appear to be more closely related to the triad of inflammation–insulin resistance–obesity than to diabetes directly. This interpretation is based on cross-sectional association studies [9, 10], as well as prospective randomised clinical trials [13] and clinical investigations, that provide evidence for a marginal contribution of both chronic [14] and acute [15] hyperglycaemia, at least within the concentration range commonly seen in type 2 diabetes.

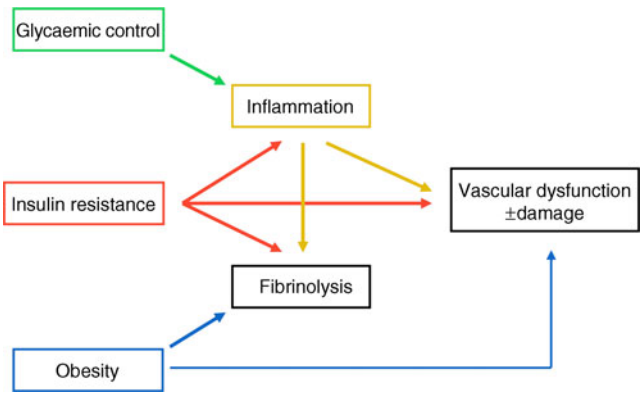

Fig. 2 Synthesis of correlation analysis in 95 patients with type 2 diabetes undergoing a forearm vascular study and extensive clinical characterisation. Copyright 2006 American Diabetes Association. From [9]. Reprinted with permission from The American Diabetes Association

Since insulin induces NO release in vitro and vasodilation in vivo [16], theoretically, defective insulin-induced vasodilation might impair insulin action on glucose uptake in target tissues by curbing the supply of both substrate and stimuli. Detailed studies in experimental animal models of diabetes support this mechanism [17], whereas the evidence in humans is indirect and probably relevant to pharmacological insulin concentrations. Nevertheless, the hypothesis that endothelial dysfunction contributes to the cellular insulin resistance of obesity or hypertension, when directly tested in vivo in man [18, 19], has not been confirmed.

The mechanisms underlying endothelial dysfunction have been partly clarified such that impaired vasodilation is caused by a reduced NO bioavailability, which in turn results from enhanced oxidative stress. In addition to hyperglycaemia-induced mitochondrial dysfunction, which is probably relevant at extreme glucose concentrations, the enzymes NAD(P)H-oxidase and xanthine oxidase seem to play key roles in superoxide production by endothelial cells in response to inflammation [20]. From a therapeutic perspective, both enzymes are activated by angiotensin II, and xanthine oxidase is inhibited by allopurinol, the antihyperuricaemic drug. Indeed, endothelial dysfunction can be reversed, as supported by evidence from randomised double-blind, placebo-controlled studies showing that enalapril [21], allopurinol [22], metformin [23] and thiazolidinediones [13] significantly improve endothelial function in patients with type 2 diabetes.

What is to come

Novel methods to assess endothelial function will allow a more thorough assessment of the relevance of, and the mechanisms behind, endothelial dysfunction in the pathogenesis of type 2 diabetes. The perfused forearm technique is invasive and requires sophisticated technology and expertise, and is only a correlate of endothelial function in the coronary vasculature. Pulse wave analysis with arterial tonometry is a validated alternative technique that is being used on an epidemiological scale. This method gives us the opportunity to evaluate whether information on endothelial function has clinical utility [24]. Conversely, positron-emitting tomography (PET) allows the non-invasive measurement of the changes in myocardial perfusion induced by pharmacological (dipyridamole) or physiological (cold) stimuli that reflects endothelial function of the coronary micro- and macrocirculation [25]. PET offers the unique

opportunity of evaluating the integrity of the endothelium directly in the heart in clinical studies focused on both the pathogenesis [26] and treatment [27] of endothelial dysfunction.

The availability of novel compounds that act as NO-donors will enable assessment of the relevance of NO in protecting vessels from atherosclerosis. Particularly relevant will be the use of hybrid drugs, such as the modified non-steroidal anti-inflammatory drugs (NSAIDs), currently under development [28].

The contribution of the endothelium to the maintenance of vascular integrity is not limited to NO release. Two more recently characterised endothelial functions appear potentially relevant to atherosclerosis. They involve endothelial cell turnover, at least the component that is sustained by the endothelial progenitor cells (EPCs), and the shedding of cell microparticles (EMPs) into the circulation.

The bone marrow continuously produces EPCs, which sustain reparative processes of the endothelium. Circulating EPC levels reflect the efficiency of this process. Clinical studies provide evidence that EPC levels are reduced in diabetic patients and increased in response to measures that control oxidative stress, inflammation and traditional diabetes risk factors. Cell-based therapy to mobilise or expand the EPC pool are under investigation [29]. EMP is an emerging marker of endothelial dysfunction and apoptosis and levels are elevated in a number of pathological states, including cardiovascular disease [30]. EMP contains membrane, cytoplasmic and nuclear constituents that are characteristic of their precursor cells (such as adhesion molecules and microRNAs) that may influence vascular homeostasis [31]. EMP levels in type 2 diabetic patients correlate with indices of endothelial dysfunction, the presence of coronary artery disease and late-stage complications of the disease [32].

In conclusion, the study by McVeigh and colleagues rightly deserves to be considered a pioneering investigation. These workers were the first to translate landmark discoveries in endothelial biology into a physiological context of clinical relevance. Their finding that impaired endothelium-dependent and independent vasodilation is implicated in the pathogenesis in type 2 diabetes paved the road for many subsequent investigations into the causes and treatment of atherogenesis in humans.

Contribution statement Both authors were responsible for the conception of the manuscript and drafted the article and gave approval for publication of the final version.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

References

1. Furchgott RV, Zawadzki JV (1980) The obligatory role of endothelial cells in the relaxation of smooth muscle by acetylcholine. Nature 288:373–376
2. Panza JA, Quyyumi AA, Brush JE, Epstein SE (1990) Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. N Engl J Med 323:22–27
3. Creager MA, Cooke JP, Mendelsohn ME et al (1990) Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. J Clin Invest 86:228–234
4. Bruckdorfer KR, Jacobs M, Rice-Evans C (1990) Endothelium-derived relaxing factor (nitric oxide), lipoprotein oxidation and atherosclerosis. Biochem Soc Trans 18:1061–1063
5. Fortes ZB, Garcia Leme J, Scivoletto R (1983) Vascular reactivity in diabetes mellitus: role of the endothelial cell. Br J Pharmacol 79:771–781
6. Bucala R, Tracey KJ, Cerami A (1991) Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. J Clin Invest 87:432–438
7. McVeigh G, Brennan GM, Johnston GD et al (1992) Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non insuline-dependent) diabetes mellitus. Diabetologia 35:771–776
8. Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA (1996) Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. J Am Coll Cardiol 27:567–574
9. Natali A, Toschi E, Baldeweg S et al (2006) Clustering of insulin resistance with vascular dysfunction and low-grade inflammation in type 2 diabetes. Diabetes 55:1133–1140
10. Makimattila S, Liu M-L, Vakkilainen J et al (1999) Impaired endothelium-dependent vasodilation in type 2 diabetes. Relation to LDL size, oxidized LDL, and antioxidants. Diabetes Care 22:973–981
11. Natali A, Taddei S, Quinones-Galvan A et al (1997) Insulin sensitivity, vascular reactivity, and clamp-induced vasodilatation in essential hypertension. Circulation 96:849–855
12. Cardillo C, Campia U, Bryant MB, Panza JA (2002) Increased activity of endogenous endothelin in patients with type II diabetes mellitus. Circulation 106:1783–1787
13. Natali A, Baldeweg S, Toschi E et al (2004) Vascular effects of improving metabolic control with metformin or rosiglitazone in type 2 diabetes. Diabetes Care 27:1349–1357
14. Beckman JA, Goldfine AB, Gordon MB, Garrett LA, Creager MA (2002) Inhibition of protein kinase C beta prevents impaired endothelium-dependent vasodilation caused by hyperglycemia in humans. Circ Res 90:107–111
15. Beckman J, Goldfine A, Gordon M, Creager M (2001) Ascorbate restores endothelium-dependent vasodilatation impaired by acute hyperglycemia in humans. Circulation 103:1618–1623
16. Baron A (1994) Hemodynamic actions of insulin. Am J Physiol 267:E187–E202
17. Clark MG (2008) Impaired microvascular perfusion: a consequence of vascular dysfunction and a potential cause of insulin resistance in muscle. Am J Physiol Endocrinol Metab 295:E732–E750
18. Laine H, Yki-Jarvinen H, Kirvela O et al (1998) Insulin resistance of glucose uptake in skeletal muscle cannot be ameliorated by enhancing endothelium-dependent blood flow in obesity. J Clin Invest 101:1156–1162
19. Natali A, Quiñones-Galvan A, Pecori N, Sanna G, Toschi E, Ferrannini E (1998) Vasodilatation with sodium nitroprusside does

not improve insulin action in essential hypertension. Hypertension 31:632–636
20. Gao X, Picchi A, Zhang C (2010) Upregulation of TNF-α and receptors contribute to endothelial dysfunction in Zucker diabetic rats. Am J Biomed Sci 2:1–12
21. O'Driscoll G, Green D, Rankin J, Stanton K, Taylor R (1997) Improvement in endothelial function by angiotensin converting enzyme inhibition in insulin-dependent diabetes mellitus. J Clin Invest 100:678–684
22. Butler R, Morris AD, Belch JF, Hill A, Struthers AD (2000) Allopurinol normalizes endothelial dysfunction in type 2 diabetes with mild hypertension. Hypertension 35:746–751
23. Mather K, Verma S, Anderson T (2001) Improved endothelial function with metformin in type 2 diabetes mellitus. J Am Coll Cardiol 37:134450
24. Rubinshtein R, Kuvin JT, Soffler M et al (2010) Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. Eur Heart J 31:1142–1148
25. Schindler TH, Schelbert HR, Quercioli A, Dilsizian V (2010) Cardiac PET imaging for the detection and monitoring of coronary artery disease and microvascular health. JACC Cardiovasc Imaging 3:623–640
26. Prior JO, Quinones MJ, Hernandez-Pampaloni M et al (2005) Coronary circulatory dysfunction in insulin resistance, impaired glucose tolerance, and type 2 diabetes mellitus. Circulation 111:2291–2298
27. Schindler TH, Facta AD, Prior JO et al (2007) Improvement in coronary vascular dysfunction produced with euglycaemic control in patients with type 2 diabetes. Heart 93:345–349
28. Lazzarato L, Donnola M, Rolando B et al (2008) Searching for new NO-donor aspirin-like molecules: a new class of nitrooxy-acyl derivatives of salicylic acid. J Med Chem 51:1894–1903
29. Fadini GP, Avogaro A (2010) Potential manipulation of endothelial progenitor cells in diabetes and its complications. Diabetes Obes Metab 12:570–583
30. Mallat Z, Benamer H, Hugel B et al (2000) Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. Circulation 101:841–843
31. Hunter MP, Ismail N, Zhang X et al (2008) Detection of microRNA expression in human peripheral blood microvesicles. PLoS One 3:e3694
32. Tushuizen ME, Diamant M, Sturk A, Nieuwland R (2011) Cell-derived microparticles in the pathogenesis of cardiovascular disease: friend or foe? Arterioscler Thromb Vasc Biol 31:4–9