Perspectives in Diabetes

Microvascular Function in Human Diabetes

A Physiological Perspective

John E. Tooke

The late complications of diabetes represent in large part microvascular dysfunction. The development of techniques to measure microvascular function has resulted in a clearer picture of the stages of development of microangiopathy and the key pathophysiological processes involved. Considerable evidence supports the hemodynamic hypothesis of pathogenesis, which argues that early insulin-dependent diabetes is characterized by increased microvascular pressure and flow. Resultant injury to the microvascular endothelium causes adaptive microvascular sclerosis contributing to a loss of vasodilatory reserve and autoregulatory capacity with increasing disease duration. High susceptibility to microangiopathy appears to be characterized by both high capillary pressure and increased permeability, although the interrelationship between these variables needs to be better defined. In normotensive non-insulin-dependent diabetes subjects, a different pattern of microvascular functional abnormalities is apparent; it is hypothesized that these differences represent the impact of a prediabetic insulin-resistant phase on microvascular behavior and may in part explain the differential expression of vascular pathology in the two major types of diabetes. The physiological framework that has been defined reveals those pivotal processes upon which scientific attention should be centered and facilitates the generation of plausible molecular and cellular mechanisms that fit the physiological facts. *Diabetes* 44:721–726, 1995

Despite the importance of diabetic microangiopathy in health terms, the pathogenesis of small vessel damage remains elusive, an ignorance which in all probability attests to the complexity of the processes involved rather than a lack of candidate mechanisms. The generation of plausible candidate mechanisms, however, has arguably been hampered by the difficulties involved in studying human microvascular function in an unambiguous manner without disturbing the very quantity that is being measured. In recent years a variety of techniques have become available for the study of human microvascular function, which have been successfully applied to the study of hypertension (1), edema states (2), cardiac failure (3), shock (4), peripheral arterial (5), and venous (6) insufficiency, hemorheological disorders (7) as well as diabetes (8). The application of these techniques has allowed the building of a physiological framework to define the cascade of functional events that characterizes the microangiopathic process; against this blueprint, cellular and molecular mechanisms that fit the physiological facts can be scrutinized.

The term "diabetic microangiopathy" has undoubtedly been a valuable one in concentrating attention on the culpable section of the circulation in our search for an understanding of the basis of most of the so-called late complications of diabetes. Retinopathy and nephropathy undeniably involve microvessels; considerable evidence suggests that microvascular involvement contributes to foot pathology in diabetes (9) as well as to diabetic cardiopathy (10), and more recently, the role of microvascular ischemia in the etiology of diabetic neuropathy has been re-emphasized (11). However, like all generic terms, diabetic microangiopathy implies uniformity and commonality whereas physiological studies point to a diversity of processes in time (diabetes duration), place (organ bed), and more controversially, type of diabetes. As regards the temporal evolution of diabetic microangiopathy, it is helpful to consider various stages of development (12), at the same time recognizing that cross-sectional studies can produce confusing results in view of the fact that different individuals and organ beds progress through the various stages at different rates due to differing levels of prevailing glycemia and the variable expression of myriad intrinsic factors loosely lumped together as susceptibility. The various stages may be defined as an initial functional stage that is largely reversible with normalization of blood glucose levels; there follows a period of structural adaptation and remodeling of the microvasculature leading ultimately to microvascular failure, often precipitated by some terminal event in much the same way as myocardial infarction reveals the existence of coronary atherosclerosis. In certain organ beds, (misplaced) reparative or reactive mechanisms occur, which compound the pathological consequences and clinical presentation (e.g., retinal neovascularization).

Although it has been customary to think in terms of two major types of diabetes, insulin-dependent (IDDM) and non-insulin-dependent (NIDDM), the heterogeneity of NIDDM is increasingly recognized, fueled by the discovery of the

From the Department of Vascular Medicine, Postgraduate Medical School, University of Exeter, Devon, U.K.

Address correspondence and reprint requests to Professor John E. Tooke, The Department of Vascular Medicine, Postgraduate Medical School, Barrack Road, Exeter EX2 5DW, Devon, U.K.

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EDRF, endothelium-derived relaxing factor; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus.

underlying enzymatic lesion (13) in an extreme minority of cases. Considerable evidence now exists for important differences in the pathophysiology of angiopathy in the two main types of diabetes (14), which in this brief review will therefore be considered separately.

MICROVASCULAR FUNCTION AND PHYSIOLOGICAL THEORIES OF THE PATHOGENESIS OF MICROANGIOPATHY

The microcirculation is concerned with the transport and exchange of nutrients and waste products of metabolism, tissue defense and repair, and the often conflicting demand of maintenance of tissue fluid economy. All of these processes may be affected by diabetes, but concentration has centered on transport and exchange, functions determined by flow, pressure, and their regulation and the intrinsic permeability of the capillary wall.

Early measurements of flow in whole organs or at the tissue level pointed to a sequence of changes in the relevant vascular beds, which formed the basis of a hemodynamic hypothesis of diabetic microangiopathy. First advanced by Parving et al. (15) and elaborated by others (16,17), the hypothesis states that early diabetes is characterized by increased microvascular flow (and by implication pressure) in the relevant tissues. This luxury perfusion results in increased shear stress and tangential pressure on the microvascular endothelium, which elaborates more extravascular matrix proteins as an injury response. In time, the consequent microvascular sclerosis, involving the ubiquitous basement membrane thickening as well as arteriolar hyalinosis, limits the capacity of the microvasculature to dilate at times of increased flow demand and interferes with the process of autoregulation.

Considerable evidence exists for this sequence of events as regards blood flow for the retinal, renal, and peripheral circulations (17), and the concept receives compelling support from the clinical observations that carotid and renal artery stenosis may protect against the development of ipsilateral retinal (18) and renal microangiopathy (19), respectively.

There has often been the tacit assumption that an early increase in flow equates with an increase in microvascular pressure (20). At both a theoretical and practical level this is not so, for capillary pressure is a function of the ratio of pre- and postcapillary resistance. Accordingly, a proportionate reduction in both pre- and postcapillary resistance will result in increased flow but unchanged mean capillary pressure. A concomitant increase in capillary pressure implies a relative increase in postcapillary resistance, a concept that is commonplace as far as the renal microcirculation is concerned. Although glomerular hypertension has been demonstrated in animal models of diabetes (21), such evidence in human diabetes is necessarily indirect (22), and until recently no direct evidence existed for capillary hypertension in human IDDM condition. The development of a technique for measuring human nailfold capillary pressure involving microcannulation of individual capillaries and a dynamic electronic recording system (23) has allowed the testing of this pivotal component of the hemodynamic hypothesis. In a cross-sectional study of patients with IDDM, capillary pressure was found to be elevated compared with age- and sex-matched control subjects (24). Furthermore, the characteristics of capillary pressure elevation in IDDM conformed to certain criteria that could legitimately be regarded as requisite features of any prime moving mechanism, namely: related to prevailing glycemic control; reduced after intensification of control; operative from very early in the course of diabetes; enhanced by passage through puberty (25); more evident in individuals with a high risk of complications yet relatively normal in subjects who avoid significant microangiopathy despite a long disease duration (26); and compounded by coexistent arterial hypertension, a recognized risk factor for microangiopathy (27), and reduced by pharmacological measures that are known to retard the development of certain microangiopathic complications (28). The above observations relate to capillary pressure estimated at rest in supine subjects under carefully controlled laboratory conditions. In health, capillary pressure appears to be a tightly regulated variable in response to, for example, changes in local venous (29) or arterial pressure (30) as might occur during normal physiological activity. In diabetes, such regulatory mechanisms appear to be disturbed (J.E.T., unpublished observations), implying that resting values may underestimate the everyday capillary blood pressure load in IDDM.

Several observations point to capillary hydrostatic pressure being an important determinant of capillary basement membrane thickening (31,32), and recent experiments by Riser et al. (33) have linked glomerular capillary distension to the expression of mRNA for the extravascular matrix proteins that contribute to the mesangium.

With increasing duration of IDDM, maximal microvascular vasodilatation becomes impaired. Best demonstrated using laser Doppler flowmetry, the maximum microvascular blood flow in foot skin in response to local heating or injury is blunted (34) and is negatively correlated with diabetes duration in the absence of significant arterial disease. Indeed, the same phenomenon may even be observed in diabetic children before passage through puberty (35). Although the nature of this defect is likely to be complex, involving impaired release of vasoactive mediators, a limited response of vascular smooth muscle to such mediators (36), and the structural limits imposed by microvascular sclerosis, the hyperemic response of foot skin has been negatively correlated with the degree of local capillary basement membrane thickening, suggesting that this is an important component (37). Furthermore, the autoregulatory capacity of the peripheral microcirculation has been correlated with the degree of arteriolar hyalinosis (38).

How does this array of microvascular hemodynamic abnormalities in human IDDM sit with the concept that the propensity to accelerated microangiopathy relates to (inherited) differences in the endothelial cell layer charge barrier (39)? Not only does this theory provide an explanation for the occurrence of microalbuminuria in high-risk subjects but it also provides a mechanism for increased lipoprotein accumulation in the arterial wall, thereby explaining the high rate of atherosclerosis to which such patients are prone. Resolution of these apparently alternative perspectives is made more problematic by the limited scope for measuring capillary permeability directly in humans; the transfer of a given solute across the capillary wall is determined not only by the intrinsic permeability properties of that barrier but also by flow rate, hydrostatic pressure gradient, intra- and extraluminal solute concentration (in variable proportions according to molecular size and charge), and the surface

area available for exchange. It is seldom, if ever, in human studies that all such variables are known at the time of permeability measurement. Thus, the potential for misinterpretation is considerable, particularly as it has been demonstrated that control-related increases in capillary pressure occur, which are likely to favor macromolecular (protein) flux. Further, the demonstration that capillary hypertension is more marked in subjects with microalbuminuria compared with subjects without complications despite similar age, sex, diabetes duration, and levels of glycemic control (26) suggests that at the very least capillary hypertension must be a compounding variable.

Single capillary studies have revealed an increased passage of sodium fluorescein from nailfold capillaries in IDDM (40). Using a sophisticated plethysmographic technique, which avoids compounding changes in hemodynamics, Jaap et al. have described increased limb capillary filtration coefficient in patients with IDDM (41), which appears to be more marked in those with microalbuminuria compared with a long duration complication-free group (42).

There is thus some evidence for increased permeability in the susceptible subset, but it remains a possibility that this is, at least in part, a consequence of altered hydrostatic pressure, which is known to influence barrier synthetic and secretory function as well as the permeability characteristics of the basement membrane (43). At the present time, it is safe to assume that accelerated microangiopathy is associated with the presence of capillary hypertension plus inherited or acquired changes in capillary permeability. An understanding of the relative contribution of each component is likely to stem from the use of in vitro models, in which prevailing hydrostatic pressure and the biophysical characteristics of the capillary wall can be manipulated independently.

The recognition of these physiological perturbations, which are undoubtedly linked in some way to the metabolic derangement of diabetes, begs the question as to the link between hyperglycemia and vascular cell dysfunction.

Endothelial cell culture studies have established that prolonged exposure to high D-glucose concentration results in increased formation of endothelium-derived relaxing factor (44), which could underlie the pathological peripheral vasodilatation observed in early IDDM. Considerable debate surrounds the mechanisms that mediate the glucose effect with much attention focusing on the increased activity of the polyol pathway and the generation of reactive oxygen species with resultant oxidative damage (45) to proteins and lipids. It is conceivable that many of these processes are orchestrated through the key enzyme protein kinase C, with a fundamental derangement being an imbalance in the vascular cells' redox state (46) resulting from increased sorbitol pathway activity. In addition, the accumulation of advanced glycation end products (47) may result in changes in the tertiary structure and biophysical properties of basement membrane and other extravascular matrix proteins with possible resultant effects on permeability and vasodilatory reserve. An important potential link between the polyol pathway and nonenzymatic glycation is suggested by the ability of aldose reductase inhibitors to simulate the action of aminoguanidine (which inhibits the accumulation of advanced glycation end products in a rat model) (48) and the fact that both increased polyol pathway activity and nonenzymatic glycation processes increase the rate of free radical production (46,49).

IS NIDDM THE SAME?

The foregoing observations relate to IDDM, in which the duration of disease is relatively securely known. Given that retinopathy, nephropathy, and neuropathy also occur in NIDDM, it may at first sight appear incongruous to suggest that the pathophysiological mechanisms may differ in the two major forms of diabetes. However, closer scrutiny reveals that important differences exist in the clinical expression of large and small vessel disease and hypertension in IDDM and NIDDM, which raises the possibility that the physiological framework may also differ. This evidence has recently been reviewed in detail (14) and hence will only be summarized here.

At a clinical level, large vessel disease is particularly important in NIDDM, and hypertension is common in this patient group, occurring in 40% in several studies (50). In contrast, in IDDM in the absence of nephropathy, the prevalence of hypertension is similar to that observed in the nondiabetic population (51). Subtle yet potentially important differences also occur in the expression of microangiopathy in the retina and kidney; for example, maculopathy is the common cause of visual loss in NIDDM whereas proliferative retinopathy is much more common in IDDM. Although nephropathy is an important problem in NIDDM (and in view of the relative size of this patient population accounts for an increasing proportion of the work of renal support services), the disease process is more complex, being compounded by hypertension and ischemia, and is probably more slowly progressive in this form of diabetes.

There have been relatively few studies of microvascular function in NIDDM, but those that have been performed contrast with findings in IDDM. Capillary pressure is normal in normotensive patients with NIDDM (52) as is capillary filtration coefficient (53); the transcapillary escape rate of albumin is also probably normal in the absence of arterial hypertension (54). There is, however, a profound reduction in maximum microvascular blood flow evident at diagnosis of the disease (55). Although this abnormality could represent many years of clinically silent diabetes preceding the diagnosis, the changes observed are equivalent to those seen after a diabetes duration of 18 years in IDDM, raising the possibility that changes in the hormonal and metabolic milieu before the emergence of glucose intolerance modify microvascular functions. A new hypothesis can thus be generated, suggesting that prediabetic changes occur, perhaps related to insulin resistance, that act to increase vascular resistance at the arteriolar level. Not only will this limited vasodilatory capacity explain the early diminution in maximal microvascular blood flow, but it may also serve to modulate the increase in capillary flow, pressure, and hyperfiltration that accompanies the development of hyperglycemia in acute IDDM. Furthermore, the relative increase in peripheral resistance implied would contribute to the increased risk of hypertension to which insulin-resistant subjects and patients with NIDDM are prone. It is important to stress that such a hypothesis does not negate the hemodynamic hypothesis in NIDDM, for it is likely that capillary hypertension will still occur in the presence of arterial hypertension, either pathologically or physiologically in-

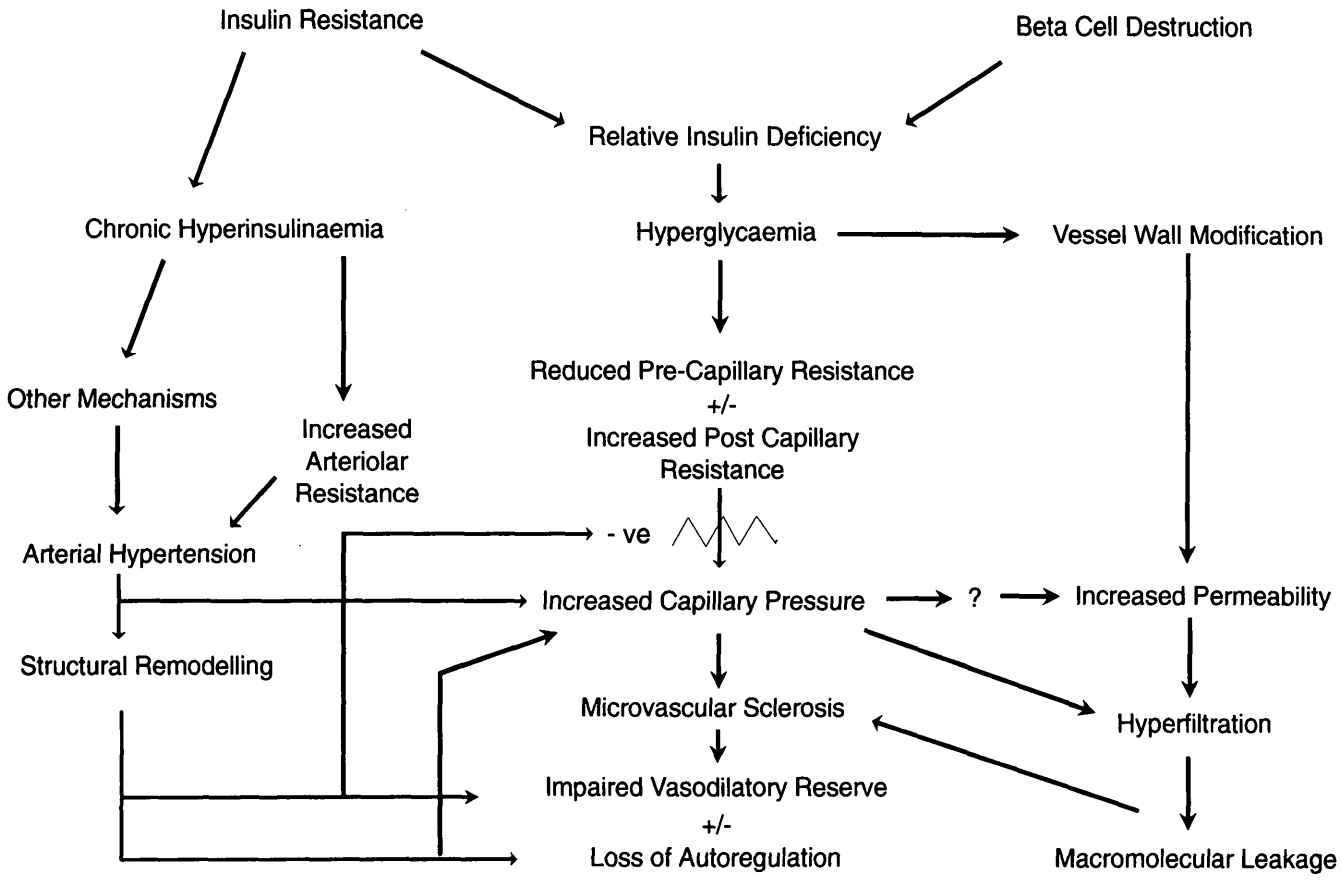

FIG. 1. A unifying physiological framework describing the evolution of microangiopathy in functional terms.

duced, due to incomplete regulation of capillary pressure—a concept supported by epidemiological evidence that arterial hypertension is a risk factor for microangiopathy in this form of the disease.

The rational test of this hypothesis is to study microvascular vasodilatory reserve in subjects at risk of development of NIDDM. From population screening, subjects with mild fasting hyperglycemia yet nondiabetic glucose tolerance tests have been identified. Maximum microvascular hyperemic responses are profoundly impaired in this group compared with age- and sex-matched normoglycemic subjects with no family history of diabetes and with similar body mass index and arterial blood pressure (56). A reduction in microvascular vasodilatation has also been observed in normotensive nondiabetic subjects who are insulin-resistant due to the presence of high levels of growth hormone (57). Studies in prediabetes using iontophoresis of acetylcholine as a stimulus to the release of endothelium-derived relaxing factor (EDRF) as well as direct nitro donors point to an endothelial cell defect in prediabetes (58). This finding is consistent with earlier observations in human (59) and experimental diabetes (60–62), all of which point to a defect in the generation of EDRF, although it should be emphasized that other observations implicate increased production of nitric oxide as the potential mediator of hyperfiltration in the renal circulation (63). The rationale for these apparently conflicting observations almost certainly relates to the physiological context and stage of the microangiopathic process and perhaps, in the human condition, to the type of diabetes considered.

A UNIFYING PHYSIOLOGICAL FRAMEWORK FOR DIABETIC MICROANGIOPATHY

It is possible to combine the various physiological observations in the two major types of diabetes to produce a unifying functional framework for the development of diabetic microangiopathy, summarized in Fig. 1. Such a framework does not presuppose the underlying cellular and molecular mechanisms responsible for these processes but merely highlights the pivotal links and their interrelationships, an understanding of which is likely to be the source of therapeutic advance.

Mechanistic and therapeutic implications. It is clear from the complexity of physiological processes involved that susceptibility to microangiopathy in an individual is likely to be determined by a large number of factors, including initial microvascular architecture, the susceptibility to hyperglycemia of key enzymes governing the various stages of microangiopathy, the impact of age on gene expression, and the coexisting degree of insulin resistance and propensity for arterial hypertension. Against this background, it is perhaps not surprising that attempts to define susceptibility to microangiopathy in terms of single genetic markers have proven disappointing, and identical diabetic twins do not show complete concordance for the development of the clinical complications of diabetes.

If the analysis of the physiological status of the microvasculature in patients with NIDDM is correct, in view of the heterogeneous nature of this disorder it will become increasingly important to understand and characterize the metabolic and hormonal defect and, where possible, the molecular genetic abnormality, in patients undergoing such studies

if the physiological features and their implications are to be properly interpreted. A corollary of this suggestion is that microvascular pathophysiology in IDDM may be modified by independent inherited or acquired differences in insulin resistance. It is interesting in this regard that capillary hypertension at rest does not rise higher in frankly nephropathic patients compared with microalbuminuric patients despite an increase in arterial blood pressure, which perhaps relates to the increased insulin resistance described in this population (64) influencing arteriolar structure/function.

Finally, it is accepted that fundamental to an understanding of microangiopathy in its various stages and guises will be the application of cellular and molecular techniques to unravel the mechanisms of key physiological processes. Important among such processes are the manner in which hyperglycemia results in precapillary vasodilatation; the nature of the relative increase in postcapillary resistance in IDDM; the transduction of capillary hypertension resulting in changes in basement membrane production and perhaps permeability; the interaction of chemical processes, particularly advanced glycosylation and free radical attack and hydrostatic pressure, on the biophysics of the basement membrane; and the nature of impaired vasodilatory capacity in NIDDM and insulin-resistant states. The successful completion of such a formidable research agenda is likely to rely on focused application of the newer biological sciences in carefully defined clinical phenotypes with continued regard for the need to reintegrate findings into a physiological perspective as outlined above or modified in the light of future knowledge.

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REFERENCES

1. Shore AC, Tooke JE: Microvascular function in human essential hypertension. *J Hypertens* 12:717–728, 1994
2. Hassan AAK, Tooke JE: Mechanism of the postural vasoconstrictor response in the human foot. *Clin Sci* 75:379–387, 1988
3. Mahy IR, Tooke JE: The peripheral microcirculation in human heart failure. *Clin Sci* 88:501–508, 1994
4. Weinberg JR, Boyle P, Thomas K, Murphy K, Tooke JE, Guz A: Capillary blood cell velocity is reduced in fever without hypotension. *Int J Microcirc Clin Exp* 10:13–19, 1991
5. Jacobs MJHM, Ubbink DT, Kitslaar PJEHM, Tordoir JHM, Slaaf DW, Reneman RS: Assessment of the microcirculation provides additional information in critical limb ischaemia. *Eur J Vasc Surg* 6:135–141, 1992
6. Franzeck UK, Bollinger A, Huch R, Huch A: Transcutaneous oxygen tension and capillary morphologic characteristics and density in patients with chronic venous incompetence. *Circulation* 70:806–811, 1994
7. Tooke JE, Milligan DW: Capillary flow in haematological disorders: the role of the red cell, white cell, and plasma viscosity. *Clin Hemorheol* 7:311–319, 1987
8. Tooke JE: Methodologies used in the study of the microcirculation in diabetes mellitus. *Diabetes Metab Rev* 9:57–70, 1993
9. Flynn MD, Tooke JE: Aetiology of diabetic foot ulceration: a role for the microcirculation? *Diabetic Med* 8:320–329, 1992
10. Yarom R, Zirkin H, Stammler G, Rose AG: Human coronary microvessels in diabetes and ischaemia: morphometric study of autopsy material. *J Pathol* 166:265–270, 1992
11. Malik RA, Newrick PG, Sharma AK, Jennings A, Ah See, AK, Mayhew TM, Jakubowski J, Boulton AJM, Ward JD: Microangiopathy in human diabetic neuropathy: relationship between capillary abnormalities and the severity of neuropathy. *Diabetologia* 32:92–102, 1989
12. Tooke JE: Microcirculation and diabetes. *Br Med Bull* 45:206–223, 1989
13. Froguel PH, Zouali H, Vionnet N: Familial hyperglycemia due to mutations in glucokinase: definition of a subtype of diabetes mellitus. *N Engl J Med* 328:697–702, 1983
14. Jaap AJ, Tooke JE The pathophysiology of microvascular disease in non-insulin-dependent diabetes (Editorial). *Clin Sci.* In press
15. Parving H-H, Viberti GC, Keen H, Christiansen JS, Lassen NA: Hemodynamic factors in the genesis of diabetic microangiopathy. *Metabolism* 32:943–949, 1983
16. Zatz R, Brenner BM: Pathogenesis of diabetic microangiopathy: the hemodynamic view. *Am J Med* 80:443–453, 1986
17. Tooke JE: Microvascular hemodynamics in diabetes mellitus. *Clin Sci* 70:119–125, 1986
18. Duker JS, Brown GC, Bosley TM, Colt CA, Reber R: Asymmetric proliferative diabetic retinopathy and carotid artery disease. *Ophthalmology* 97:869–874, 1990
19. Berkman J, Rifkin H: Unilateral nodular diabetic glomerulosclerosis (Kimmel-Steel-Wilson): report of a case. *Metabolism* 22:715–722, 1973
20. Tooke JE: Editorial: Pathogenesis of diabetic microangiopathy. *Br Med J* 1:1555–1556, 1977
21. Hostetter TH, Troy JL, Brenner BM: Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int* 19:410–415, 1981
22. Paulsen EP, Pauly FL, Croft BY, Teates CD: Simultaneous measurement of glomerular filtration rate and effective renal plasma flow reveals increased glomerular capillary pressure among teenage diabetic subjects. *Contrib Nephrol* 79:52–57, 1990
23. Williams SA, Wassermann S, Rawlinson DW, Kitney RI, Smaje LH, Tooke JE: Dynamic measurement of human capillary blood pressure. *Clin Sci* 74:507–512, 1988
24. Sandeman DD, Shore AC, Tooke JE: Relation of skin capillary pressure in patients with insulin-dependent diabetes mellitus to complications and metabolic control. *N Engl J Med* 327:760–764, 1992
25. Shore AC, Price KJ, Sandeman DD, Tripp JH, Tooke JE: Posturally induced vasoconstriction in diabetes mellitus. *Arch Dis Child* 70:22–26, 1994
26. Shore AC, Jaap AJ, Tooke JE: Capillary pressure in insulin dependent diabetic patients of long disease duration with and without microangiopathy. *Diabetic Med* 9 (Suppl. 2):S11, 1992
27. Williams SA, Boolell M, MacGregor GA, Smaje LH, Wasserman SM, Tooke JE: Capillary hypertension and abnormal pressure dynamics in patients with essential hypertension. *Clin Sci* 79:5–8, 1990
28. Shore AC, Donohoe M, Jaap AJ, Tooke JE: The effect of increasing doses of an angiotensin converting enzyme inhibitor on capillary pressure levels in patients with insulin-dependent-diabetes mellitus and microalbuminuria (Abstract). *Diabetic Med* 10 (Suppl. 1):55, 1993
29. Mahy IR, Tooke JE, Shore AC: Capillary pressure during and after incremental venous pressure elevation in man. *J Physiol.* In press
30. Shore AC, Sandeman DD, Tooke JE: Effect of an increase in systemic blood pressure on nailfold capillary pressure in humans. *Am J Physiol* 265:H820–H823, 1993
31. Williamson JR, Kilo C: Current status of capillary basement-membrane disease in diabetes mellitus. *Diabetes* 26:65–73, 1977
32. Vracko R: Skeletal muscle capillaries in diabetics. *Circulation* 41:271–283, 1970
33. Riser BL, Cortes P, Zhao X, Bernstein J, Dumler F, Narins RG, Hassett CC, Sury-Sastry KS, Atherton J, Holcomb MA: Intraglomerular pressure and mesangial stretching stimulate extracellular matrix formation in the rat. *J Clin Invest* 90:1932–1943, 1993
34. Rayman G, Williams SA, Spencer PD, Smaje LH, Wise PH, Tooke JE: Impaired microvascular hyperaemic response to minor skin trauma in type I diabetes. *Br Med J* 292:1295–1298, 1986
35. Shore AC, Price KJ, Sandeman DD, Green EM, Tripp JH, Tooke JE: Impaired microvascular hyperaemic response in children with diabetes mellitus. *Diabetic Med* 8:619–623, 1991
36. Boolell ML, Tooke JE: The skin hyperaemic response to local injection of substance P and Capsaicin in diabetes mellitus. *Diabetic Med* 7:898–901, 1990
37. Rayman G, Malik RA, Metcalfe J, Sharma AK, Day JL: Reduced skin capillary size in the feet of insulin-dependent diabetic patients (Abstract). *Diabetic Med* 7 (Suppl. 1):9A, 1990
38. Kastrup J, Norgaard T, Parving H-H, Henriksen O, Lassen NA: Impaired autoregulation of blood flow in subcutaneous tissue of long-term type 1 (insulin-dependent) diabetic patients with microangiopathy: an index of arteriolar dysfunction. *Diabetologia* 28:711–717, 1985
39. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed Enevoldsen, A: Albuminuria reflects widespread vascular damage: the Steno hypothesis. *Diabetologia* 32:219–226, 1989
40. Bollinger A, Frey J, Jäger K, Furrer J, Seglias J, Siegenthaler W: Patterns of diffusion through skin capillaries in patients with long-term diabetes. *N Engl J Med* 307:1305–1310, 1982
41. Jaap AJ, Shore AC, Gartside IB, Gamble J, Tooke JE: Increased microvascular fluid permeability in young type 1 (insulin-dependent) diabetic patients. *Diabetologia* 36:648–652, 1993
42. Jaap AJ, Shore AC, Tooke JE: Capillary filtration coefficient is different in long duration type 1 diabetic patients with and without microvascular complications (Abstract). *Diabetic Med* 11 (Suppl. 1):S13, 1994
43. Robinson GB, Walton HA: Glomerular basement membrane as a compressible ultrafilter. *Microvasc Res* 38:36–48, 1989
44. Graier WF, Wascher TC, Lackner L, Toplak H, Krejs GJ, Kukovetz WR: Exposure to elevated D-glucose concentrations modulates vascular endothelial cell vasodilatory response. *Diabetes* 42:1497–1505, 1993
45. Larkins RG, Dunlop ME: The link between hyperglycaemia and diabetic nephropathy. *Diabetologia* 35:499–504, 1992

46. Williamson JR, Chang K, Frangos M, Hasan KS, Ido Y, Kawamura T, Nyengaard JR, van den Enden M, Kilo C, Tilton RG: Perspectives in Diabetes: Hyperglycemic pseudohypoxia and diabetic complications. *Diabetes* 42:801–813, 1993
47. Brownlee M, Cerami A, Vlassara H: Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 318:1315–1321, 1988
48. Suarez G, Rajarani R, Bhurjan KC, Oronsky L, Goidi JA: Administration of aldose reductase inhibitor induces a decrease of collagen fluorescence in diabetic rats. *J Clin Invest* 82:624–627, 1988
49. Mullarkey CJ, Edelstein D, Brownlee M: Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. *Biochem Biophys Res Commun* 173:932–939, 1990
50. UKPDS Group: United Kingdom Prospective Diabetes Study III: prevalence of hypertension and hypotensive therapy in patients with new diagnosed diabetes. *Hypertension* 7 (Suppl. 2):8–13, 1985
51. Norgaard K, Feldt-Rasmussen B, Borch-Johnsen K, Saelan H, Deckert T: Prevalence of hypertension in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 33:407–410, 1990
52. Shore AC, Jaap AJ, Tooke JE: Capillary pressure in patients with NIDDM. *Diabetes* 43:1198–1202, 1994
53. Jaap AJ, Shore AC, Gamble J, Gartside IB, Tooke JE: Capillary filtration coefficient in type II (non-insulin-dependent) diabetes. *J Diabetes Complications* 8:111–116, 1994
54. O'Hare JA, Ferriss JB, Twomey B, O'Sullivan DJ: Poor metabolic control, hypertension and microangiopathy independently increase the transcapillary escape rate of albumin in diabetes. *Diabetologia* 25:260–263, 1983
55. Sandeman DD, Pym CA, Green EM, Seamark C, Shore AC, Tooke JE: Microvascular vasodilatation in feet of newly diagnosed non-insulin dependent diabetic patients. *Br Med J* 302:1122–1123, 1991
56. Jaap AJ, Hammersley MS, Shore AC, Tooke JE: Reduced microvascular hyperaemia in subjects at risk of developing type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 37:214–216, 1994
57. Jaap AJ, Donohoe M, Shore AC, Tooke JE: Limited microvascular vasodilation in patients with acromegaly (Abstract). *J Endocrinol* 137 (Suppl.):P45, 1993
58. Jaap AJ, Shore AC, Tooke JE: Diminished endothelium-dependent microvascular vasodilation in subjects with impaired glucose tolerance. *Diabetic Med* 11 (Suppl. 2):S22, 1994
59. McVeigh GE, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Henry LT, Henry WR, Andrews JW, Hayes JR: Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 35:771–776, 1992
60. Tesfamariam B, Brown ML, Cohen RA: Elevated glucose impairs endothelium-dependent relaxation by activating protein kinase C. *J Clin Invest* 87:1643–1647, 1991
61. Wang YX, Brooks P, Edwards RM: Attenuated glomerular cGMP production and renal vasodilation in streptozotocin-induced diabetic rats. *Am J Physiol* 264:952–956, 1993
62. Craven PA, Studer RK, DeRubertis FR: Impaired nitric oxide-dependent cyclic guanosine monophosphate generation in glomeruli from diabetic rats. *J Clin Invest* 93:311–320, 1994
63. Bank N, Aynedjian HS: Role of EDRF (nitric oxide) in diabetic renal hyperfiltration. *Kidney Int* 43:1306–1312, 1993
64. Yip J, Mattock MB, Morocutti A, Sehti M, Trevisan R, Viberti GC: Insulin resistance in insulin-dependent diabetic patients with microalbuminuria. *Lancet* 342:883–887, 1993