REVIEW ARTICLE

FUNCTIONAL STATUS OF VASCULAR ENDOTHELIUM IN DIABETES MELLITUS

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Normal endothelial cells synthesize and release biologically active substances. These substances maintain homeostasis through adequate blood flow, delivery of nutrients, activation and inhibition of coagulation proteins, prevention of thrombosis and diapedesis of leukocyte. Endothelial dysfunction implies failure of vascular endothelium to perform its normal functions of vasodilatation and vasoconstriction. It results from an imbalance between endothelium derived constricting and relaxing factors. Altered endothelial cell activity predisposes to increased production of vasoconstrictors, i.e., prostaglandins, endothelins, glycated proteins, endothelial adhesion molecules and platelet and vascular growth factors. These changes enhance vasomotor tone, vascular permeability, growth and remodeling of the vessels. Diabetes is associated with abnormalities of vascular endothelium. Several regulatory vasodilators and vasoconstrictors are altered in diabetes leading to diabetic vascular complications. Balance between dilating and constricting substances is altered and is shifted towards vasoconstriction in diabetes. Disturbances in the endothelial functions lead to increased platelet adhesion and aggregation in patients with diabetes. Activated platelets interact with endothelial cells and leukocytes in the genesis of atherosclerosis. High level of Von Willebrand factor(vWF) is a consistent finding in diabetes. Increased vWF level is one of the major risk factors for the development of micro vascular complications. High levels of vWF may predict cardiovascular disease progression in diabetes mellitus.

Keywords: Vascular, endothelium, diabetes mellitus

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INTRODUCTION

Endothelial cells regulate vascular function and structure due to their strategic anatomic position between the blood and vessels. Normal endothelial cells synthesize and release biologically active substances. These substances maintain homeostasis through adequate blood flow, delivery of nutrients, activation and inhibition of coagulation proteins, prevention of thrombosis and diapedesis of leukocyte.[1]

Regulatory functions of the vascular endothelium include vasodilatation, vasoconstriction, antiplatelet and anticoagulant effects.[2] Endothelium produces dilating factors e.g. nitric oxide, prostacyclin and bradykinin and constricting factors like endothelin, superoxide ions, endothelium derived constricting factor, angiotensin II and thromboxane. Many other endothelium derived factors are involved in the regulation of blood coagulation and fibrinolysis such as von Willebrand factor (vWF), tissue factor (TF), tissue factor pathway inhibitor (TFPI), thrombomodulin and plasminogen activator inhibitor-1 (PAI-1).

Endothelial dysfunction implies failure of vascular endothelium to perform its normal functions of vasodilatation and vasoconstriction. It results from an imbalance between endothelium derived constricting and relaxing factors. Altered endothelial cell activity predisposes to increased production of vasoconstrictors, i.e., prostaglandins, endothelins, glycated proteins, endothelial adhesion molecules and platelet and vascular growth factors. These changes enhance vasomotor tone, vascular permeability, growth and remodeling of the vessels. [3]

Diabetes is associated with abnormalities of vascular endothelium. Several of the above mentioned regulatory factors, i.e., vasodilators and vasoconstrictors are altered in diabetes leading to diabetic vascular complications.[4,5] Balance between dilating and constricting substances is altered and is shifted towards vasoconstriction in diabetes.[6] Following are some of the factors and mechanisms that predispose to vascular abnormalities in diabetes mellitus.

Nitric Oxide and Reactive Oxygen Species

Nitric oxide is one of the most important platelet inhibiting substances. Nitric oxide synthase (eNOS) in the endothelial cells synthesizes nitric oxide through oxidation of the guanidine-nitrogen terminal of L-arginine utilizing O_2 as a cofactor and nicotinamide adenine diucleotide phosphate (NADP) as a coenzyme.[7]

Nitric oxide performs many important functions. It plays a major role in the vascular integrity. It also causes vasodilation by activating guanylate cyclase on adjacent vascular smooth muscle cells. [12] GTP degradation releases cGMP by the action of guanylate cyclase which regulates cytoplasmic Ca^{2+} level. Increase in the cytosolic Ca^{2+} causes smooth muscle cells relaxation and consequent vasodilatation.[8]

Nitric oxide protects blood vessels from endogenous injury by mediating molecular signals that prevent platelet and leukocyte interaction with the vessel wall. It also inhibits vascular smooth muscle cells proliferation, migration and growth.[9,10]

Nitric oxide inhibits platelet aggregation, procoagulants activation, tissue oxidation, inflammation

and release of pro-atherogenic and pro-inflammatory cytokines. It also facilitates fibrinolysis.[8]

Reduced vascular synthesis and sensitivity to prostacyclin and decreased synthesis and release of nitric oxide are the two consistent features in diabetes.[6,11] Impaired endothelial dependent nitric oxide mediated relaxation in patients with diabetes is an indication of endothelial dysfunction and seems to precede atherosclerosis in diabetes mellitus.[12–13]

Decreased availability of endothelium derived nitric oxide in diabetes is due to various reasons. Hyperglycemia inhibits production of nitric oxide by blocking endothelial nitric oxide synthase activation. This inhibition increases the production of reactive oxygen species (ROS), especially superoxide ions (O_2^-) in the endothelium and smooth muscles.[14] Peroxynitrite ions are formed from superoxide ions. Peroxynitrite separates tetrahydrobiopterin by oxidation which is a cofactor of eNOS and produces O_2.[15] Production of peroxynitrite decreases the synthesis of prostacyclin that causes vasodilation and inhibits platelet functions.[16] Nitric oxide synthesis is inversely proportional to the production of peroxynitrite; this further impairs the production of vasodilators.

Diabetic endothelial dysfunction is also expressed by increased vascular permeability related to hyperglycemia-induced free oxygen radical production.[17] Diabetes induced microvascular permeability is responsible for the expression of endothelial mitogen growth factor which is the main promoter of angiogenesis and neovascularization in diabetic microangiopathy.[18]

Endothelin Production

Diabetes induced endothelial cell dysfunction is characterized not only by decreased nitric oxide production but also by increased synthesis of vasoconstrictor prostanoids and endothelin-1.[19,20]

Endothelin-1 promotes inflammation and acts on endothelin-A receptors on vascular smooth muscle cells to induce vascular smooth muscle contraction. These functions of endothelium play a pivotal role in vascular diseases in diabetes.[14] Response to endothelin-1 is also reduced in diabetes mellitus.[21] Increased renal salt and water retention, stimulation of renin-angiotensin system and induction of vascular smooth muscles hypertrophy are some of the other functions of endothelin-1.

Activation of Protein Kinase C

Protein kinase C (PKC) pathway is activated by hyperglycemia. Like endothelial nitric oxide synthase pathway, PKC system also plays an important role in the development of diabetic complications.[22,23] PKC is involved in the endothelium dependent vasodilatation in diabetes mellitus. PKC increases the production of superoxide ions (O_2^-). These react with nitric oxide to produce peroxynitrite (ONOO─) which damage tissues and activates monocyte and macrophages. Apart from peroxynitrite production, activated PKC also increases the expression of platelet derived growth factor-β receptor on endothelium and smooth muscle cells.[24]

Transforming growth factor-β1 (TGF-β1) regulates the production of endothelial cell matrix. Hyperglycemia induced activation of PKC increases the expression of TGF-β1 which activates gene expression of proteoglycans, collagen type IV and fibronectin causing thickening of the basement membrane. TGF-β1 also decreases the synthesis of proteolytic enzymes (e.g. matrix metalloproteinase) that degrade matrix proteins. Early structural abnormalities in pre diabetic animals show increased expression of TGF-β1 that causes thickening of the basement membranes.[25]

Nuclear Transcription Factor

Hyperglycemia induced endothelial damage is also associated with excessive formation of advanced glycation end products (AGEs) and increased activation of pro-inflammatory nuclear transcription factor, i.e., nuclear factor-kappa B (NF-κB).[26,27] NF-κB activated by hyperglycemia induces inflammatory gene expression which causes increased production of leukocyte attracting chemokines, inflammatory cytokines and expression of cell adhesion molecules.[28] These changes in the endothelial cells lead to increased production of tissue factor (TF), platelet activation and aggregation, alterations in coagulation and fibrinolytic factors. They also promote monocyte and vascular smooth muscle cells migration into the intima and formation of macrophage foam cells characterizing the initial morphological changes of atherosclerosis.[29,30]

Advanced Glycation End Products

Diabetes is associated with increased oxidative stress, enhanced leukocyte–endothelial interaction and glycation of proteins and phospholipids in the body.[31] Glycation is a process of binding glucose to amino group of proteins or phospholipids in the absence of enzymes. In the early phase non enzymatic reactive products such as Maillard, Schiff base and Amadori products are formed. These biochemical changes are initially reversible. Continued hyperglycemia subsequently leads to the formation of more stable products known as advanced glycation end products.[32]

AGEs have a potential to cause certain pathological changes. They disrupt the molecular conformation of proteins and lipids, reduce the enzymatic activity, degradative capacity and receptor recognition of protein and lipids.[33] AGEs are also associated with the development of vascular dysfunctions and diabetic complications.[34,35] AGEs bind with receptors for advanced glycation end products (RAGE) present on endothelial cells and initiate a series of reactions. These include; induction of oxidative stress, reduction in the vascular barrier function, increased vascular permeability and enhanced

expression of vascular cell adhesion molecules (VCAM-1). These molecules when expressed on the surface of monocytes act as chemokines which facilitate monocyte recruitment.[28]

AGEs produce superoxide ions leading to increased oxidative damage of the endothelium.[11] AGEs cross link collagen and extra cellular matrix proteins in the vessel wall. This cross linking leads to accumulation of low density lipoproteins in the vessel wall. Low density lipoproteins, being more susceptible to oxidative stress, impair endothelial cell function, initiate inflammation and adhesion, and promote vascular smooth muscles cell changes.[36] Hyperglycemia delays endothelial cell replication, increases apoptosis and accelerates atherosclerosis.[37]

Endothelial Effects on Platelets

Disturbances in the endothelial functions lead to increased platelet adhesion and aggregation. Altered platelet metabolism and changes in intra platelet signaling pathways contribute to the overall increased platelet hyperactivity.[38] Platelets-derived growth factor is released from the platelet granules which stimulates proliferation of smooth muscle cells and subsequently results in the thickening and vasoconstriction of vessel wall. Platelet adhesion and aggregation may occur in diabetes even when the endothelium is injured but is still intact. Platelet-endothelial cell adhesion molecule-1 (PECAM-1) and decrease NO synthesis facilitate platelet adhesion and aggregation.[39]

Hyperactive platelets act by three major mechanisms for the development of atherosclerosis; these include micro embolisation of the capillaries, local progression of vascular lesions and triggering of acute arterial thrombosis.[40]

Increased Expression of Adhesion Molecules

Activated platelets interact with endothelial cells and leukocytes in the genesis of atherosclerosis. This process is dependent on a group of receptors and binding proteins termed adhesion molecules. These adhesion molecules are expressed by several cells. L-selectin is expressed by leukocytes. P-selectin is expressed both by activated platelets and endothelial cells while E-selectin (also known as endothelial leukocyte adhesion molecule-1, ELAM-1) is expressed by endothelial cells. Expression of various members of the immunoglobulin super family, i.e., intercellular adhesion molecules (ICAM-1), (ICAM-2), vascular cell adhesion molecule-1 (VCAM-1) and PECAM-1 by endothelial cells, leukocytes and platelets or smooth muscle cells are the other adhesion molecules.[4, 41] Hyperglycemia causes increased expression of adhesion molecules like ICAM-1 and reduce PECAM-1 expression. These numerical aberrations facilitate the development of atherosclerotic lesions.[42]

Endothelial cells produce soluble E-selectin (sE-selectin) when activated by cytokines. Elevated levels of sE-selectin have been reported in patients with diabetes mellitus. Positive correlation of sE-selectin with hyperglycemia,[43] macro as well as microangiopathy has been shown in patients with diabetes.[44] sE-selectin and cell adhesion molecules ICAM-1 and VCAM-1 predict the development of type 2 diabetes mellitus in initially non-diabetic women.[45]

Adhesion molecules mediate the process of platelet activation. Platelets activation is regulated by the biochemical signal transduction mechanisms. P-selectin from the outer membrane of activated platelets and endothelial cells is responsible for the recruitment and activation of leukocytes. It also acts as a receptor for sialic acid containing oligosaccharides in the cell membranes of monocytes and neutrophils. Leukocytes become ligands for the adherence of P-selectin of platelets. Leukocytes can further adhere to locally activated endothelium and cause further adherence of P-selectin containing cells.[42] Platelet adhesion molecules e.g. PECAM-1 act as ligands for the αIIbβ3 integrin (vitronectin receptor) expressed on activated endothelial cells and leukocytes. αIIbβ3 mediates not only platelet-platelet interaction, it also interacts with neutrophil CD11/CD18 complex that enhances platelet activation.[46]

ICAM-2 present on the surface of the activated platelets anchors platelets irreversibly to leukocytes. In this interaction of platelets, leukocytes and endothelial cells platelets play their role as a component of the cellular hemostasis and immune system.[46]

Levels of von Willebrand Factor

von Willebrand factor is a multimeric glycoprotein stored in the Weibel-Palade bodies of endothelial cells and α-granules of platelets. vWf carries factor VIII in the circulation, mediates platelet adhesion and participates in platelet aggregation. vWF is a well recoganised plasma marker of endothelial cell damage and dysfunction.[41] High level of vWF is a consistent finding in diabetes. vWF levels correlate with the age of the patient and duration of diabetes mellitus which is one of the major risk factors for the development of micro vascular complications. High levels of vWF may predict cardiovascular disease progression in diabetes mellitus.[47] Glycosylation of vWF does not alter its function since vWF antigens are not affected by acute changes in blood glucose level or the degree of glycemic control.

Insulin Resistance and its Effects on Vascular Endothelium

More than 80% of the individuals with type 2 diabetes show insulin resistance. Insulin resistance leads to abnormal endothelial functions. It precedes the development of type 2 diabetes and is associated with

increased plasma concentrations of endothelin and vWF even in the absence of diabetes.[48]

Insulin Resistance and Nitric Oxide

Physiologically insulin stimulates NO production in the endothelial cells by increasing the activity of nitric oxide synthase via activation of phosphatidylinositol-3 kinase and protein kinase (Akt kinase).[49] Insulin increases endothelium dependent NO mediated vasodilation in healthy individuals. As type 2 diabetes mellitus is characterized by insulin resistance, endothelial dependent vasodilation is reduced in insulin resistant subjects. Insulin-mediated glucose disposal correlates inversely with the severity of impairment of endothelial dependent vasodilation.[50]

Abnormal vasodilation in insulin resistant state may be explained by changes in intracellular signaling that reduces the production of NO. Insulin signal transduction via phosphatidylinositol-3 kinase pathway is impaired and insulin is less able to activate nitric oxide synthase and produce NO.[51]

Insulin Resistance and Inflammation

Insulin signaling via mitogen activated protein kinase pathway remains intact. Mitogen dependent protein kinase activation is associated with increased endothelin production and a greater level of inflammation and thrombosis.[51]

Matrix Metalloproteinase and Insulin Resistance

Insulin resistance is associated with the up-regulation of CD36 protein of macrophages. This increases the uptake of oxidized low density lipoprotein by macrophages.[52] Hyperglycemia increases macrophage matrix metalloproteinase (MMP) expression in human fibroblasts in patients with diabetes mellitus.[53] Increased MMP-2 is an indicator of microangiopathy in children with type 1 diabetes.[54]

Production of Free Fatty Acids

Circulating levels of free fatty acids are elevated in diabetes because of their excess liberation from adipose tissue and diminished uptake by skeletal muscles.[55] Free fatty acids impair endothelial function through several mechanisms. These include increased production of oxygen-derived free radicals, activation of protein kinase C and exacerbation of dyslipidemia.[56]

Elevation of free fatty acid levels activate PKC and decrease insulin receptor substrate-1-associated phosphatidylinosital-3 kinase activity.[56] These may decrease nitric oxide synthase activity through their effect on signal transduction.

Liver responds to free fatty acid flux by increasing very low density lipoprotein (VLDL) production and cholesterol ester synthesis.[57] Increased production of triglyceride rich proteins and diminished clearance by lipoprotein lipase results in hyper triglyceridemia typically observed in diabetes. Elevated triglyceride level decreases high density lipoprotein by promoting cholesterol transport from HDL to VLDL. These abnormalities change structure of LDL, increasing the amount of the more atherogenic, small, dense LDL. Hypertriglyceridemia and low HDL are both associated with endothelial cell dysfunction.[58]

REFERENCES

1. Kinlay S, Libby P, Ganz P. Endothelial function and coronary artery disease. Curr Opin Lipidol 2001;12:383–9.
2. Rahman S, Rahman T, Ismail AA, Rashid AR. Diabetes-associated macrovasculopathy: pathophysiology and pathogenesis. Diabetes Obes Metab 2007;9:767–80.
3. Paston L, Talor PD. Glaxo/MRS Young Investigator Prize. Endothelium-mediated vascular function in insulin-dependent diabetes mellitus. Clin Sci (Lond) 1995;88:245–55.
4. Carter AM, Grant P. Vascular homeostasis, adhesion molecules and macrovascular disease in non-insulin dependent diabetes mellitus. Diabet Med 1997;14:423–32.
5. Galajda P, Martinka E, Mokan M, Kubisz P. Endothelial markers in diabetes mellitus.. Thromb Res 1997;85:63–5.
6. Martina V, Bruno GA, Trucco F, Zumpano E, Tagliabue M, Di Bisceglie C, *et al.* Platelet cNOS activity is reduced in patients with IDDM and NIDDM. Thromb Haemost 1998;79:520–2.
7. Moncada S, Higgs A. The L-arginine–nitric oxide pathway. N Engl J Med 1993;329:2002–12.
8. Esper RJ, Nordaby RA, Vilarino JO, Paragano A, Cacharron JL, Machado RA. Endothelial dysfunction: a comprehensive appraisal. Cardiovasc Diabetol 2006;5:4. doi:10.1186/1475-2840-5-4
9. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA 1991;88:4651–5.
10. Sarkar R, Meinberg EG, Stanley JC, Gordon D, Webb RC. Nitric oxide reversibly inhibits the migration of cultured vascular smooth muscle cells. Circ Res 1996;78:225–30.
11. Queen LR, Ji Y, Goubareva I, Ferro A. Nitric oxide generation mediated by beta-adrenoceptors is impaired in platelets from patients with type 2 diabetes mellitus. Diabetologia 2003;46:1474–82.
12. Bohlen HG, Lash JM. Topical hyperglycemia rapidly suppresses EDRF-mediated vasodilation of normal rat arterioles. Am J Physiol 1993;265(1 Pt 2):H219–25.
13. Pieper GM, Meier DA, Hager SR. Endothelial dysfunction in a model of hyperglycemia and hyperinsulinemia. Am J Physiol 1995;269(3 Pt 2):H845–50.
14. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: Epidemiology, pathophysiology and management. JAMA 2002;287:2570–81.
15. Milstien S, Katusic Z. Oxidation of tetrahydrobiopterin by peroxynitrite. Biochem Biophys Res Commun 1999;63:681–4.
16. Zou M, Yesilkaya A, Ullrich V. Peroxynitrite inactivates prostacyclin synthase by heme-thiolatecatalyzed tyrosine nitration. Drug Metab Rev 1999;31:343–9.
17. El-Remessy AB, Behzadian MA, Abou-Mohamed G, Franklin T, Caldwell RW, Caldwell RB. Experimental diabetes causes breakdown of the blood-retina barrier by a mechanism involving tyrosine nitration and increases in expression of vascular endothelial growth factor and urokinase plasminogen activator receptor. Am J Pathol 2003;162:1995–2004.
18. Hammes HP, Lin J, Bretzel RG, BrownleeM, Breier G. Upregulation of the vascular endothelial growth factor/vascular endothelial growth factor receptor system in experimental background diabetic retinopathy of the rat. Diabetes 1998;47:401–6.
19. Golovchenko I, Goalstone ML, Watson P, Brownlee M, Draznin B. Hyperinsulinemia enhances transcriptional activity of nuclear factor-kappa B induced by angiotensin II, hyperglycemia, and advanced glycosylation end products in vascular smooth muscle cells. Circ Res 2000;87:746–52.
20. Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular

disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol 2004;24:816–23.
21. Nugent AG, McGurk C, Hayes JR, Johnston GD. Impaired vasoconstriction to endothelin 1 in patients with NIDDM. Diabetes. 1996;45:105-7.
22. Feener EP, King GL. Vascular dysfunction in diabetes mellitus. Lancet 1997; 350(Suppl 1):SI9–3.
23. Koya D, King GL. Protein kinase C activation and the development of diabetic complications. Diabetes 1998;47:859–66.
24. Inaba T, Ishibashi S, Gotoda T, Kawamura M, Morino N, Nojima Y, *et al*. Enhanced expression of platelet-derived growth factor-beta receptor by high glucose. Involvement of platelet-derived growth factor in diabetic angiopathy. Diabetes 1996;45:507–12.
25. Blankenberg FG[1], Wen P, Dai M, Zhu D, Panchal SN, Tait JF, *et al*. Detection of early atherosclerosis with radiolabeled monocyte chemoattractant protein 1 in prediabetic Zucker rats. Pediatr Radiol 2001;31:827–35.
26. Creager MA, Luscher TF, Cosentino F, Beckman JA. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. Circulation 2003;108:1527–32.
27. Du X, Matsumura T, Edelstein D, Rossetti L, Zsengeller Z, Szabo C. et al. Inhibition of GAPDH activity by poly (ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. J Clin Invest 2003;112:1049–57.
28. Zeiher AM, Fisslthaler B, Schray-Utz B, Busse R. Nitric oxide modulates the expression of monocyte chemoattractant protein 1 in cultured human endothelial cells. Circ Res 1995;76:980–6.
29. Collins T, Cybulsky MI. NF-kappaB: pivotal mediator or innocent bystander in atherogenesis? J Clin Invest 2001;107:255–64.
30. Libby P. Changing concepts of atherogenesis. J Intern Med 2000;247:349–58.
31. Morigi M, Angioletti S, Imberti B, Donadelli R, Micheletti G, Figliuzzi M, et al. Leukocyte-endothelial interaction is augmented by high glucose concentrations and hyperglycemia in a NF-kB-dependent fashion. J Clin Invest 1998;101:1905–15.
32. Shah P, Kaul S, Nilsson J, Cercek B. Exploiting the vascular protective effects of high-density lipoprotein and its apolipoproteins. An idea whose time for testing is coming. Part I. Circulation 2001;104:2376–83.
33. Lieberman EH, Gerhard MD, Uehata A, Walsh BW, Selwyn AP, Ganz P, et al. Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. Ann Intern Med. 1994;121:936–41.
34. Ehara S, Ueda M, Naruko T, Haze K, Itoh A, Otsuka M, *et al*. Elevated levels of oxidated low density lipoprotein show a positive relationship with the severity of acute coronary syndrome. Circulation 2001;103:1955–60.
35. Landmesser U, Harrison DG. Oxidant stress and marker for cardiovascular events. Ox marks the spots. Circulation 2001;104:2638–40.
36. Lyons TJ. Glycation and oxidation: a role in the pathogenesis of atherosclerosis. Am J Cardiol 1993;71:26B–31B.
37. McMillen DE. Development of vascular complications in diabetes. Vasc Med 1997;2:132–42.
38. Mazzanti L, Mutus B. Diabetes-induced alterations in platelet metabolism. Clin Biochem 1997;30:509–15.
39. Rosenblum WI. Platelet adhesion and aggregation without endothelial denudation or exposure of basal lamina and:or collagen. J Vasc Res 1997;34:409–17.
40. Tschoepe D, Roesen P, Schwippert B, Gries FA. Platelets in diabetes: the role in the hemostatic regulation in atherosclerosis. Semin Thromb Hemost 1993;19:122–8.
41. Blann AD, Lip GY. Endothelial integrity, soluble adhesion molecules and platelet markers in type 1 diabetes mellitus. Diabet Med 1998;15:634–42.
42. Maugeri N, Evangelista V, Celardo A, Dell'Elba G, Martelli N, Piccardoni P et al., Polymorphonuclear leukocyte-platelet interaction: role of P-selectin in thromboxane B2 and leukotriens C4 cooperative synthesis. Thromb Haemost 1994;72:450–6.
43. Ryysy L, Yki-Jarvinen H. Improvement of glycemic control by 1 year of insulin therapy leads to a sustained decrease in sE-selectin concentrations in type 2 diabetes. Diabetes Care 2001;24:549–54.
44. Yngen M, Ostenson CG, Hu H, Li N, Hjemdahl P, Wallen NH. Enhanced P-selectin expression and increased soluble CD40 ligand in patients with type 1 diabetes mellitus and microangiopathy: evidence for platelet hyperactivity and chronic inflammation. Diabetologia 2004;47:537–40.
45. Meigs JB, Hu FB, Rifai N, Manson JE. Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. JAMA 2004;291:1978–86.
46. Tschoepe D, Roesen P, Kaufmann L, Schauseil S, Kehrel B, Ostermann H, et al. Evidence for abnormal platelet glycoprotein expression in diabetes mellitus. Eur J Clin Invest 1990;20:166–70.
47. Thompson SG, Kienast J, Pyke SD, Haverkate F, van de Loo JC. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. N Engl J Med 1995;332:635–41.
48. Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Targher G, et al. Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. Diabetes 1998;47:1643–9.
49. Kuboki K, Jiang ZY, Takahara N, Ha SW, Igarashi M, Yamauchi T, et al. Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and in vivo: a specific vascular action of insulin. Circulation 2000;101:676–81.
50. Mather K, Laakso M, Edelman S, Hook G, Baron A. Evidence for physiological coupling of insulin-mediated glucose metabolism and limb blood flow. Am J Physiol Endocrinol Metab 2000;279:E1264–70.
51. Montagnani M, Golovchenko I, Kim I, Koh GY, Goalstone ML, Mundhekar AN, et al. Inhibition of phosphatidylinositol 3-kinase enhances mitogenic actions of insulin in endothelial cells. J Biol Chem 2002;277:1794–9.
52. Liang CP, Han S, Okamoto H, Carnemolla R, Tabas I, Accili D, *et al.* Increased CD36 protein as a response to defective insulin signaling in macrophages. J Clin Invest 2004;113:764–73.
53. Death AK, Fisher EJ, McGrath KC, Yue DK. High glucose alters matrix metalloproteinase expression in two key vascular cells: potential impact on atherosclerosis in diabetes. Atherosclerosis 2003;168:263–9.
54. Derosa G, Avanzini MA, Geroldi D, Fogari R, Lorini R, De Silvestri A, *et al*. Matrix metalloproteinase 2 may be a marker of microangiopathy in children and adolescents with type 1 diabetes. Diabetes Care 2004;27:273–4.
55. Fujimoto WY. The importance of insulin resistance in the pathogenesis of type 2 diabetes mellitus. Am J Med 2000;108(Suppl 6a):9S–14S.
56. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, et al. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells. Diabetes 2000;49:1939–45.
57. Sniderman AD, Scantlebury T, Cianflone K. Hypertriglyceridemic hyperapob: the unappreciated atherogenic dyslipoproteinemia in type 2 diabetes mellitus. Ann Intern Med 2001;135:447–59.
58. de Man FH, Weverling-Rijnsburger AW, van der Laarse A, Smelt AH, Jukema JW, Blauw GJ. Not acute but chronic hypertriglyceridemia is associated with impaired endothelium-dependent vasodilation: reversal after lipid-lowering therapy by atorvastatin. Arterioscler Thromb Vasc Biol 2000;20:744–50.

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