

# Diabetes and Vascular Disease Research

<http://dvr.sagepub.com>

---

## **The endothelium and vascular inflammation in diabetes**

Martin M Hartge, Thomas Unger and Ulrich Kintscher

*Diab Vasc Dis Res* 2007; 4; 84

DOI: 10.3132/dvdr.2007.025

The online version of this article can be found at:

<http://dvr.sagepub.com/cgi/content/abstract/4/2/84>

---

Published by:



<http://www.sagepublications.com>

On behalf of:

International Society of Diabetes Vascular Disease

**Additional services and information for *Diabetes and Vascular Disease Research* can be found at:**

**Email Alerts:** <http://dvr.sagepub.com/cgi/alerts>

**Subscriptions:** <http://dvr.sagepub.com/subscriptions>

**Reprints:** <http://www.sagepub.com/journalsReprints.nav>

**Permissions:** <http://www.sagepub.co.uk/journalsPermissions.nav>

**Citations** <http://dvr.sagepub.com/cgi/content/refs/4/2/84>

# The endothelium and vascular inflammation in diabetes

MARTIN M HARTGE, THOMAS UNGER, ULRICH KINTSCHER

## Abstract

**T**he endothelium releases multiple mediators, not only regulators of vasomotor function but also important physiological and pathophysiological inflammatory mediators. Endothelial dysfunction is caused by chronic exposure to various stressors such as oxidative stress and modified low-density lipoprotein (LDL) cholesterol, resulting in impaired nitric oxide (NO) production and chronic inflammation. Biomechanical forces on the endothelium, including low shear stress from disturbed blood flow and hypertension, are also important causes of endothelial dysfunction. These processes seem to be augmented in patients with diabetes. In states of insulin resistance and in type 2 diabetes insulin signalling is impaired. Increased vascular inflammation, including enhanced expression of interleukin-6 (IL-6), vascular cellular adhesion molecule-1 (VCAM-1) and monocyte chemoattractant protein (MCP-1) are observed, as is a marked decrease in NO bioavailability. Furthermore, hyperglycaemia leads to increased formation of advanced glycation end products (AGE), which quench NO and impair endothelial function.

In summary, during the development of diabetes a number of biochemical and mechanical factors converge on the endothelium, resulting in endothelial dysfunction and vascular inflammation. In the presence of insulin resistance, these processes are potentiated and they provide a basis for the macrovascular disease seen in diabetes.

*Diabetes Vasc Dis Res* 2007;4:84–8  
doi:10.3132/dvdr.2007.025

**Key words:** diabetes, endothelial dysfunction, endothelium, inflammation, obesity.

## Endothelial function

The endothelium is the innermost layer of blood vessels, thus it is the largest organ in the body. It has many important

biological functions additional to its role as a mechanical lining. These involve the regulation of leucocyte extravasation, adhesion and subendothelial accumulation; the prevention of platelet adhesion that could result in thrombotic processes; and the regulation of blood vessel patency for maintenance of appropriate blood flow. Numerous vasoactive substances control these functions tightly, completing a regulatory network with mechanical stimuli such as shear stress and pressure. The reaction of the endothelium to these stimuli results in the release of agents which affect vasomotor function through endothelium-mediated relaxation of vascular smooth muscle, through inhibition of platelet aggregation and through the promotion of fibrinolysis, resulting in the dissolution of possible microthrombi and maintenance of normal blood flow.

One of the key functions of the endothelium is to ensure adequate blood flow, which is regulated by the secretion of diverse substances. Prostacyclin I<sub>2</sub> (PGI<sub>2</sub>) and nitric oxide (NO) are the two main vasodilators; others include endothelium-derived hyperpolarising factor and C-type natriuretic peptide.<sup>1–3</sup> PGI<sub>2</sub> and NO show additional effects in that they act to inhibit platelet aggregation.<sup>4,5</sup> So-called vasoconstrictors are also secreted by the endothelium, including endothelin-1 (ET-1), angiotensin II (Ang II), thromboxane A<sub>2</sub> and reactive oxygen species (ROS).<sup>6,7</sup>

The release of NO and PGI<sub>2</sub> increases the activity of guanylate- and adenylate-cyclase, respectively, raising c-GMP and c-AMP levels. This is followed by inhibition of platelet aggregation and thrombosis.<sup>3,8</sup> Likewise, the endothelial expression and presentation of the cell surface protein thrombomodulin leads to inhibition of thrombosis. Thrombomodulin binds thrombin, causing a configurational change that inhibits the conversion of fibrinogen into fibrin<sup>9</sup> and permits the activation of protein C by thrombin, followed by inactivation of factor Va and VIIIa.<sup>10</sup>

Moreover, the endothelium enables fibrinolysis in order to ensure vascular patency and perfusion. After secretion of tissue plasminogen activator (tPA) by the endothelium, active plasmin is formed and this leads to fibrin degradation. In contrast, the endothelium and other tissues secrete plasminogen-activator inhibitor-1 (PAI-1), which inhibits tPA and functions as an anti-fibrinolytic agent.

When the endothelium is functioning normally, all these functions are tightly balanced, whereas major imbalances in these processes arise during endothelial dysfunction.

## Endothelial dysfunction

The maintenance of balanced vascular pressure, patency and perfusion, the inhibition of thrombosis and induction of

Center for Cardiovascular Research, Charité-Universitätsmedizin Berlin, Hessische Strasse 3-4, 10115 Berlin, Germany.

**Martin M Haertge**, Pharmacist

**Thomas Unger**, Professor of Pharmacology

**Ulrich Kintscher**, Professor of Pharmacology

**Correspondence to: Professor Ulrich Kintscher**

Center for Cardiovascular Research, Charité-Universitätsmedizin Berlin, Hessische Strasse 3-4, 10115 Berlin, Germany.

Tel: +49 30 450525002; Fax: +49 30 450525901

E-mail: ulrich.kintscher@charite.de

fibrinolysis characterise normal endothelial function. In contrast, interactions of numerous proinflammatory processes, reduced vasodilation and prothrombotic properties distinguish endothelial dysfunction. Multiple diseases and conditions, including hypertension, coronary artery disease,<sup>11</sup> congestive heart failure<sup>12</sup> and chronic renal failure,<sup>13</sup> are initiated or associated with endothelial dysfunction. It is also seen in type 1 and 2 diabetes<sup>14-18</sup> and in the normotensive, normoglycaemic, first-degree relatives of patients with type 2 diabetes.<sup>19</sup> Finally, endothelial dysfunction has been shown to occur in the metabolic syndrome, dyslipidaemia,<sup>20</sup> insulin resistance,<sup>21</sup> obesity,<sup>22</sup> hyperhomocysteinemia,<sup>23</sup> sedentary lifestyle<sup>24</sup> and smoking.<sup>25</sup> In summary, the pathophysiology of endothelial dysfunction is complex, involving multiple mechanisms.

### **The link between inflammation, type 2 diabetes, obesity and endothelial dysfunction**

The association of the inflammatory state with obesity and insulin resistance<sup>26</sup> was described in 1993 by Hotamisligil *et al.*<sup>27</sup> In this study, adipocyte expression of the pro-inflammatory cytokine tumour necrosis factor alpha (TNF $\alpha$ ) was observed to be markedly increased in obese mice, and neutralisation of TNF $\alpha$  led to an improvement in insulin resistance. Additional studies have shown that obesity is a state of chronic inflammation significantly associated with increased plasma concentrations of C-reactive protein (CRP),<sup>28</sup> interleukin-6 (IL6)<sup>29</sup> and plasminogen-activator inhibitor-1 (PAI-1).<sup>30</sup> Likewise, TNF $\alpha$  levels in obese patients correlate significantly with body mass index (BMI).<sup>31</sup>

In this inflammatory condition, the two adipocyte-specific proteins adiponectin and leptin play major roles. An inverse relationship with adiposity has been observed for plasma adiponectin concentrations, and similarly with insulin resistance, diastolic pressure, triglyceride concentration and TNF $\alpha$  receptor concentrations.<sup>32</sup> Leptin has pro-aggregatory effects on platelets and it regulates immune function by stimulation of inflammatory responses in immune cells; leptin levels are elevated in obese humans. It has also been shown to induce oxidative stress and inflammation in endothelial cells<sup>33</sup> and it may induce hypertension through centrally-mediated mechanisms.<sup>34-36</sup>

The inflammatory site in endothelial dysfunction may be where the processes of inflammation in obesity and type 2 diabetes begin. The inhibition of autophosphorylation of the insulin receptor (IR) by TNF $\alpha$  on tyrosine residues results in induction of serine phosphorylation of insulin receptor substrate-1 (IRS-1). In turn, this causes adipocyte IR serine phosphorylation and inhibits IR tyrosine phosphorylation.<sup>37</sup> These processes in endothelial cells contribute to impairment of the normal insulin response and normal stimulation of NO synthesis, resulting in endothelial dysfunction. This enhancement of inflammation by a diminished endothelial insulin response could in itself be one possible explanation for the close link between obesity, type 2 diabetes, inflammation and endothelial dysfunction, because insulin exerts anti-inflammatory effects at the cellular and molecular level both *in vitro* and *in vivo*. A low-dose infusion of insulin has been shown to suppress NADPH oxidase expression and

plasma intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein (MCP-1) concentrations and to reduce reactive oxygen species (ROS) generation. Conversely, a longer-term insulin infusion (over four hours) in normal subjects was associated with induction of endothelial dysfunction.

One of the most important substances for the normal function of blood vessels is endothelial NO. It inhibits abnormal growth and inflammation, exerts anti-aggregatory effects on platelets and promotes vasodilatation. In the presence of impaired endothelial function, reduced endothelium-derived NO expression has frequently been reported. This may be caused by reduced activity of endothelial NO synthase (eNOS) as a result of increased levels of endogenous or exogenous inhibitors or by reduced availability of the substrate, L-arginine. The cytotoxic oxidant ROS quenches NO to form peroxynitrite<sup>38</sup> and affects protein function, causing endothelial dysfunction through nitration of proteins. Peroxynitrite is an important mediator of LDL oxidation, and thus has a proatherogenic role.<sup>39</sup> Furthermore, peroxynitrite leads to degradation of the eNOS cofactor tetrahydrobiopterin (BH<sub>4</sub>),<sup>40</sup> resulting in an uncoupling of eNOS activity. Studies with diabetic mice have shown that treatment with the novel peroxynitrite decomposition catalyst FP15 can prevent endothelial and cardiac dysfunction.<sup>41</sup> Oxidant excess also results in reduction of BH<sub>4</sub> to 7,8-dihydrobiopterin, which leads to decreased formation of the active dimer of eNOS, oxygenase activity and curtailed production of NO. Under these conditions, the reductase function of eNOS is activated to produce more ROS: eNOS shifts from an oxygenase that produces NO to a reductase that produces ROS, with consequent exaggeration of oxidant excess and its deleterious effects on endothelial and vascular function.<sup>42</sup>

Oxidative excess in hypertension studies seems to be correlated with endothelial dysfunction, as confirmed by monitoring of impaired endothelium-dependent vasodilatation after use of antioxidants.<sup>43</sup> Human studies in hypertensive populations investigating effects of antioxidants such as vitamin C and E have reported antihypertensive effects.<sup>44,45</sup> In contrast, clinical data received from the Heart Outcomes Prevention Evaluation (HOPE) trial<sup>46</sup> and the Collaborative Group of the Primary Prevention Project,<sup>47</sup> in which hypertensive patients were treated with vitamin E (400 IU/d), did not demonstrate any clinically relevant blood pressure-reducing effects. The reason for these conflicting data may be the higher doses of vitamin E used in the experimental studies (800 to 1,000 IU/d) compared to those used in the clinical trials (300 to 500 IU/d).

### **The influence of diabetes on endothelial dysfunction**

In industrialised westernised countries, the incidence of diabetes, particularly type 2 diabetes, is rising at a dramatic rate.<sup>48-51</sup> This rise is combined with an increased prevalence of diabetes closely related to ageing and obesity. Endothelial dysfunction, although triggered by additional mechanisms, is the result of oxidative excess, which is closely linked to diabetes.<sup>52,53</sup> Insulin signalling is altered in states of insulin resistance, differently affecting the two major pathways emerging

from the insulin receptor. The phosphoinositide 3-kinase/ Akt/ protein kinase B signalling pathway is significantly altered, resulting in a marked decrease of eNOS activation. However, the mitogen-activated protein kinase pathway leading to mitogenic effects and growth is unaffected.<sup>54-57</sup>

An inflammatory vascular state is induced by hyperglycaemia, which promotes the formation of advanced glycation end-products (AGEs). This leads to an induction of ROS and promotes endothelial expression of IL-6, VCAM-1 and MCP-1.<sup>58</sup>

NO availability can be reduced by acute hyperglycaemia,<sup>59</sup> which also attenuates endothelium-dependent vasodilation in humans *in vivo*.<sup>60</sup> AGEs play an important role in these processes; inhibition of AGE formation with aminoguanidine prevents NO depletion and sustains endothelial function.<sup>61</sup>

### Endothelial dysfunction – a major mediator of diabetic macrovascular disease

In patients with type 2 diabetes mellitus, the major cause of mortality and morbidity is cardiovascular disease (CVD). Hypertension is present approximately twice as frequently in people with diabetes mellitus compared to individuals without diabetes, and is accompanied by dyslipidaemia, hyperglycaemia, hypercoagulation and hyperinsulinaemia.<sup>62</sup>

The metabolic syndrome and type 2 diabetes are characterised by several haemodynamic and metabolic abnormalities. Among these abnormalities, endothelial dysfunction plays a central role and is evident prior to the onset of diabetes. Moreover, in the increased CVD risk found in persons with diabetes and hypertension<sup>49,63</sup> dysfunction of the vascular endothelium plays an important role. Compared to diabetes alone, the co-existence of hypertension and diabetes seems to correlate with decreased coronary flow responses.<sup>64</sup> Alterations in the vascular endothelium linked to diabetes that contribute to endothelial dysfunction include elevated expression and plasma levels of vasoconstrictors such as angiotensin II and endothelin-1, increased expression of adhesion molecules and associated enhanced adhesion of platelets and monocytes to vascular endothelium, plus impairment of NO release and reduced NO responsiveness. Endothelial expression of adhesion molecules is enhanced by exposure to dyslipidaemia, hypertensive plasma vasoconstrictor concentrations and elevated adipose-derived proinflammatory cytokine levels, and promotes leukocyte adhesion and vascular extravasation.

In conclusion, endothelial dysfunction seems to be the trigger in atherogenesis and diabetes-associated vascular disease and explains, at least in part, the enhanced progression of CVD in type 2 diabetes.

### Effects of glitazone treatment on endothelial dysfunction and CVD

The glitazones, chemically defined as thiazolidinediones (TZDs), exert their effects as insulin sensitisers through the peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) by enhancing the effects of insulin in metabolic target tissues (such as skeletal muscle, liver and fat) and directly improving peripheral insulin resistance. The expression of genes

involved in carbohydrate and lipid metabolism is, at least in part, regulated by PPAR $\gamma$ , which also plays a role in adipocyte differentiation. The receptor is expressed in all major cell types involved in vascular lesions: monocytes and macrophages, endothelial cells and vascular smooth muscle cells.

Because of their reductive effect on insulin resistance, the possible role of TZDs in improvement of endothelial dysfunction has been studied. The endothelial function of patients with diabetes is directly improved by PPAR $\gamma$  agonists, which block one of the earliest steps in atherogenesis.<sup>65</sup> The glitazones mediate their beneficial effects on endothelial function in a number of ways, including molecular effects related to PPAR $\gamma$  agonist actions, such as improvement of glycaemic control and decreasing the levels of circulating free fatty acids, and via important anti-inflammatory effects on endothelial cells and leukocytes.

Other beneficial antiatherogenic effects have been reported in several studies using PPAR $\gamma$  ligands,<sup>66</sup> such as potent inhibition of inflammation, blockade of macrophage differentiation<sup>67</sup> and cytokine secretion. Inhibition of vascular smooth muscle cell proliferation and migration have also been shown. Glitazone treatment additionally improves several risk factors for atherosclerosis, including plasma cytokine and C-reactive protein levels and intima-media thickness. Pioglitazone has been shown recently to reduce stroke, total mortality and non-fatal myocardial infarction in high-risk patients with diabetes, proving that such treatment can be effective.<sup>68</sup>

### Conclusions

The foundation for possible subsequent diseases is laid when the normal endothelial function is altered to a pathological degree. One of the major characteristics of endothelial dysfunction is a state of chronic subclinical systemic and vascular inflammation which is associated with reduced vasodilatation and a pro-thrombotic state. Subsequently, endothelial dysfunction is strongly associated with cardiovascular morbidity and mortality. In states of insulin resistance and in type 2 diabetes, endothelial dysfunction is markedly enhanced, providing a significant pathophysiological basis for the massively increased cardiovascular risk observed in patients with diabetes. Future therapeutic approaches for the treatment of diabetic cardiovascular disease should target the dysfunctional endothelium first.

### Conflict of interest declaration

None declared.

### References

1. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; **288**:373-6.
2. Rovati GE, Giovanazzi S, Negretti A, Nicosia S. Prostacyclin effects on adenylate cyclase in platelets and vascular smooth muscle: interaction with an inhibitory receptor or partial agonism? *Adv Prostaglandin Thromboxane Leukot Res* 1995; **23**:263-5.
3. Nicosia S, Oliva D, Bernini F, Fumagalli R. Prostacyclin-sensitive adenylate cyclase and prostacyclin binding sites in platelets and smooth muscle cells. *Adv Cyclic Nucleotide Protein Phosphorylation Res* 1984; **17**: 593-9.
4. Grodzinska L, Marcinkiewicz E. The generation of TXA2 in human

- platelet rich plasma and its inhibition by nictindole and prostacyclin. *Pharmacol Res Commun* 1979;**11**:133-46.
5. Barrett ML, Willis AL, Vane JR. Inhibition of platelet-derived mitogen release by nitric oxide (EDRF). *Agents Actions* 1989;**27**:488-91.
  6. Verma S, Anderson TJ. Fundamentals of endothelial function for the clinical cardiologist. *Circulation* 2002;**105**:546-9.
  7. Schiffrin EL. A critical review of the role of endothelial factors in the pathogenesis of hypertension. *J Cardiovasc Pharmacol* 2001;**38**(suppl 2):S3-S6.
  8. Riddell DR, Owen JS. Nitric oxide and platelet aggregation. *Vitam Horm* 1999;**57**:25-48.
  9. Wu KK, Thiagarajan P. Role of endothelium in thrombosis and hemostasis. *Annu Rev Med* 1996;**47**:315-31.
  10. van't Veer C, Golden NJ, Mann KG. Inhibition of thrombin generation by the zymogen factor VII: implications for the treatment of hemophilia A by factor VIIa. *Blood* 2000;**95**:1330-5.
  11. Monnink SH, van Haelst PL, van Boven AJ et al. Endothelial dysfunction in patients with coronary artery disease: a comparison of three frequently reported tests. *J Investig Med* 2002;**50**:19-24.
  12. Landmesser U, Spiekermann S, Dikalov S et al. Vascular oxidative stress and endothelial dysfunction in patients with chronic heart failure: role of xanthine-oxidase and extracellular superoxide dismutase. *Circulation* 2002;**106**:3073-8.
  13. Bolton CH, Downs LC, Victory JG et al. Endothelial dysfunction in chronic renal failure: roles of lipoprotein oxidation and pro-inflammatory cytokines. *Nephrol Dial Transplant* 2001;**16**:1189-97.
  14. Beckman JA, Goldfine AB, Gordon MB, Garrett LA, Keaney JF Jr, Creager MA. Oral antioxidant therapy improves endothelial function in Type 1 but not Type 2 diabetes mellitus. *Am J Physiol Heart Circ Physiol* 2003;**285**:H2392-H2398.
  15. Rizzoni D, Porteri E, Guelfi D et al. Structural alterations in subcutaneous small arteries of normotensive and hypertensive patients with non-insulin-dependent diabetes mellitus. *Circulation* 2001;**103**:1238-44.
  16. Schofield I, Malik R, Izzard A, Austin C, Heagerty A. Vascular structural and functional changes in type 2 diabetes mellitus: evidence for the roles of abnormal myogenic responsiveness and dyslipidemia. *Circulation* 2002;**106**:3037-43.
  17. Endemann DH, Pu Q, De Ciuceis C et al. Persistent remodeling of resistance arteries in type 2 diabetic patients on antihypertensive treatment. *Hypertension* 2004;**43**:399-404.
  18. Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990;**323**:22-7.
  19. Balletshofer BM, Rittig K, Enderle MD et al. Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. *Circulation* 2000;**101**:1780-4.
  20. Engler MM, Engler MB, Malloy MJ et al. Antioxidant vitamins C and E improve endothelial function in children with hyperlipidemia: Endothelial Assessment of Risk from Lipids in Youth (EARLY) Trial. *Circulation* 2003;**108**:1059-63.
  21. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation* 2006;**113**:1888-904.
  22. Raitakari M, Ilvonen T, Ahotupa M et al. Weight reduction with very-low-caloric diet and endothelial function in overweight adults: role of plasma glucose. *Arterioscler Thromb Vasc Biol* 2004;**24**:124-8.
  23. Viridis A, Ghiadoni L, Cardinali H et al. Mechanisms responsible for endothelial dysfunction induced by fasting hyperhomocystinemia in normotensive subjects and patients with essential hypertension. *J Am Coll Cardiol* 2001;**38**:1106-15.
  24. Green DJ, Walsh JH, Maiorana A, Best MJ, Taylor RR, O'Driscoll JG. Exercise-induced improvement in endothelial dysfunction is not mediated by changes in CV risk factors: pooled analysis of diverse patient populations. *Am J Physiol Heart Circ Physiol* 2003;**285**:H2679-H2687.
  25. Oida K, Ebata K, Kanehara H, Suzuki J, Miyamori I. Effect of cilostazol on impaired vasodilatory response of the brachial artery to ischemia in smokers. *J Atheroscler Thromb* 2003;**10**:93-8.
  26. Lehrke M, Lazar MA. Inflamed about obesity. *Nat Med* 2004;**10**:126-7.
  27. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- $\alpha$ : direct role in obesity-linked insulin resistance. *Science* 1993;**259**:87-91.
  28. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 1999;**19**:972-8.
  29. Mohamed-Ali V, Goodrick S, Rawesh A et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor- $\alpha$ , in vivo. *J Clin Endocrinol Metab* 1997;**82**:4196-200.
  30. Lundgren CH, Brown SL, Nordt TK, Sobel BE, Fujii S. Elaboration of type-1 plasminogen activator inhibitor from adipocytes. A potential pathogenetic link between obesity and cardiovascular disease. *Circulation* 1996;**93**:106-10.
  31. Griending KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part II: animal and human studies. *Circulation* 2003;**108**:2034-40.
  32. Fernandez-Real JM, Lopez-Bermejo A, Casamitjana R, Ricart W. Novel interactions of adiponectin with the endocrine system and inflammatory parameters. *J Clin Endocrinol Metab* 2003;**88**:2714-18.
  33. Matarese G, La Cava A, Sanna V et al. Balancing susceptibility to infection and autoimmunity: a role for leptin? *Trends Immunol* 2002;**23**:182-7.
  34. Rahmouni K, Correia ML, Haynes WG, Mark AL. Obesity-associated hypertension: new insights into mechanisms. *Hypertension* 2005;**45**:9-14.
  35. Hall JE, Hildebrandt DA, Kuo J. Obesity hypertension: role of leptin and sympathetic nervous system. *Am J Hypertens* 2001;**14**:1035-1155.
  36. Dunbar JC, Hu Y, Lu H. Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. *Diabetes* 1997;**46**:2040-3.
  37. Hotamisligil GS, Budavari A, Murray D, Spiegelman BM. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor- $\alpha$ . *J Clin Invest* 1994;**94**:1543-9.
  38. Koppol WH, Moreno JJ, Pryor WA, Ischiropoulos H, Beckman JS. Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. *Chem Res Toxicol* 1992;**5**:834-42.
  39. Griending KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS. *Circulation* 2003;**108**:1912-16.
  40. Milstien S, Katusic Z. Oxidation of tetrahydrobiopterin by peroxynitrite: implications for vascular endothelial function. *Biochem Biophys Res Commun* 1999;**263**:681-4.
  41. Szabo C, Mabley JG, Moeller SM et al. Part I: pathogenetic role of peroxynitrite in the development of diabetes and diabetic vascular complications: studies with FP15, a novel potent peroxynitrite decomposition catalyst. *Mol Med* 2002;**8**:571-80.
  42. Landmesser U, Dikalov S, Price SR et al. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest* 2003;**111**:1201-09.
  43. Chen X, Touyz RM, Park JB, Schiffrin EL. Antioxidant effects of vitamins C and E are associated with altered activation of vascular NADPH oxidase and superoxide dismutase in stroke-prone SHR. *Hypertension* 2001;**38**:606-11.
  44. Duffy SJ, Gokce N, Holbrook M et al. Treatment of hypertension with ascorbic acid. *Lancet* 1999;**354**:2048-9.
  45. Fotherby MD, Williams JC, Forster LA, Craner P, Ferns GA. Effect of vitamin C on ambulatory blood pressure and plasma lipids in older persons. *J Hypertens* 2000;**18**:411-15.
  46. Hoogwerf BJ, Young JB. The HOPE study. Ramipril lowered cardiovascular risk, but vitamin E did not. *Cleve Clin J Med* 2000;**67**:287-93.
  47. Palumbo G, Avanzini F, Alli C et al. Effects of vitamin E on clinic and ambulatory blood pressure in treated hypertensive patients. Collaborative Group of the Primary Prevention Project (PPP) - Hypertension study. *Am J Hypertens* 2000;**13**:564-7.
  48. Amos AF, McCarty DJ, Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabet Med* 1997;**14**(suppl 5):S1-S85.
  49. Sowers JR. Diabetes mellitus and cardiovascular disease in women. *Arch Intern Med* 1998;**158**:617-21.
  50. Muggeo M, Verlato G, Bonora E et al. The Verona diabetes study: a population-based survey on known diabetes mellitus prevalence and 5-year all-cause mortality. *Diabetologia* 1995;**38**:318-25.
  51. Berger M, Jorgens V, Flatten G. Health care for persons with non-insulin-dependent diabetes mellitus. The German experience. *Ann Intern Med* 1996;**124**:153-5.
  52. Frisbee JC, Stepp DW. Impaired NO-dependent dilation of skeletal muscle arterioles in hypertensive diabetic obese Zucker rats. *Am J Physiol Heart Circ Physiol* 2001;**281**:H1304-H1311.
  53. Kim YK, Lee MS, Son SM et al. Vascular NADH oxidase is involved in impaired endothelium-dependent vasodilation in OLETF rats, a model of type 2 diabetes. *Diabetes* 2002;**51**:522-7.
  54. Cusi K, Maezono K, Osman A et al. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. *J Clin Invest* 2000;**105**:311-20.
  55. Montagnani M, Ravichandran LV, Chen H, Esposito DL, Quon MJ. Insulin receptor substrate-1 and phosphoinositide-dependent kinase-1

- are required for insulin-stimulated production of nitric oxide in endothelial cells. *Mol Endocrinol* 2002;**16**:1931-42.
56. Osman AA, Pendergrass M, Koval J et al. Regulation of MAP kinase pathway activity in vivo in human skeletal muscle. *Am J Physiol Endocrinol Metab* 2000;**278**:E992-E999.
  57. Federici M, Menghini R, Mauriello A et al. Insulin-dependent activation of endothelial nitric oxide synthase is impaired by O-linked glycosylation modification of signaling proteins in human coronary endothelial cells. *Circulation* 2002;**106**:466-72.
  58. Zhang L, Zalewski A, Liu Y et al. Diabetes-induced oxidative stress and low-grade inflammation in porcine coronary arteries. *Circulation* 2003;**108**:472-8.
  59. Giugliano D, Marfella R, Coppola L et al. Vascular effects of acute hyperglycemia in humans are reversed by L-arginine. Evidence for reduced availability of nitric oxide during hyperglycemia. *Circulation* 1997;**95**:1783-90.
  60. Williams SB, Goldfine AB, Timimi FK et al. Acute hyperglycemia attenuates endothelium-dependent vasodilation in humans in vivo. *Circulation* 1998;**97**:1695-701.
  61. Bucala R, Tracey KJ, Cerami A. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. *J Clin Invest* 1991;**87**:432-8.
  62. Sowers JR. Hypertension in Type II Diabetes: Update on Therapy. *J Clin Hypertens (Greenwich)* 1999;**1**:41-7.
  63. Sowers JR, Epstein M. Diabetes mellitus and associated hypertension, vascular disease, and nephropathy. An update. *Hypertension* 1995;**26**:869-79.
  64. Prior JO, Quinones MJ, Hernandez-Pampaloni M et al. Coronary circulatory dysfunction in insulin resistance, impaired glucose tolerance, and type 2 diabetes mellitus. *Circulation* 2005;**111**:2291-8.
  65. Hsueh WA, Lyon CJ, Quinones MJ. Insulin resistance and the endothelium. *Am J Med* 2004;**117**:109-17.
  66. Collins AR, Meehan WP, Kintscher U et al. Troglitazone inhibits formation of early atherosclerotic lesions in diabetic and nondiabetic low density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 2001;**21**:365-71.
  67. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 1998;**391**:79-82.
  68. Dormandy JA, Charbonnel B, Eckland DJ et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* 2005;**366**:1279-89.