Diabetes Mellitus, Hypertension, and Cardiovascular Disease: Which Role for Oxidative Stress?

Dario Giugliano, Antonio Ceriello, and Giuseppe Paolisso

Accelerated atherosclerotic vascular disease is the leading cause of mortality in patients with diabetes mellitus. Endothelium-derived nitric oxide (NO) is a potent endogenous nitrovasodilator and plays a major role in modulation of vascular tone. Selective impairment of endothelium-dependent relaxation has been demonstrated in aortas of both nondiabetic animals exposed to elevated concentrations of glucose in vitro and insulin-dependent diabetic animals. The impaired NO release in experimentally induced diabetes may be prevented by a number of antioxidants. It has been hypothesized that oxygen-derived free radicals (OFR) generated during both glucose autoxidation and formation of advanced glycosylation end products may interfere with NO action and attenuate its vasodilatory activity. The oxidative injury may also be increased in diabetes mellitus because of a weakened defense due to reduced endogenous antioxidants (vitamin E, reduced glutathione [GSH]). A defective endothelium-dependent vascular relaxation has been found in animal models of hypertension and in hypertensive patients. An imbalance due to reduced production of NO or increased production of free radicals, mainly superoxide anion, may facilitate the development of an arterial functional spasm. Treatment with different antioxidants increases blood flow in the forearm and decreases blood pressure and viscosity in normal humans; vitamin E inhibits nonenzymatic glycosylation, oxidative stress, and red blood cell microviscosity in diabetic patients. Long-term randomized clinical trials of adequate size in secondary and primary prevention could support the free-radical hypothesis for diabetic vascular complications and the use of antioxidants to reduce the risk of coronary heart disease.

MUCH ATTENTION has recently been paid to the clustering of powerful cardiovascular risk factors such as hypertension, hyperlipidemia, and diabetes mellitus in the same individuals. A metabolic syndrome, also referred to as syndrome X, has been proposed.[1] In its original description, insulin resistance was believed to be a common causative mechanism that linked hypertension pathogenetically to disorders in both carbohydrate and lipid metabolism. This review will develop the hypothesis that oxidative stress may play a role in the poor cardiovascular outlook of the diabetic patient. The argumentations herein discussed represent an updated and extended version of similar exercises previously published that dealt with nonenzymatic glycation of proteins and the production of free radicals[2] and the role of oxidative injury in the pathogenesis of hypertension.[3]

INSULIN HYPOTHESIS

The role of insulin in macrovascular diabetic complications, which has been extensively studied over the last few years, will be mentioned only briefly. The insulin hypothesis proposes that the compensatory hyperinsulinism that occurs with insulin resistance increases blood pressure through an increase of sodium retention, stimulation of nervous sympathetic activity, or both.[4] Epidemiologic data that link hyperinsulinemia and hypertension seem to be associative rather than causal, but this is inconsistent. As reviewed by Jarrett,[5] the epidemiologic evidence of this association has been flawed by inconsistencies and discrepancies between studies. Moreover, no difference in blood pressure was found between 70 patients with a histologically confirmed diagnosis of insulinoma and matched control subjects.[6]

There is strong evidence that acute physiologic increments in plasma insulin concentrations (euglycemic clamp) stimulate sympathetic noradrenergic activity, which is offset by its vasodilator action on skeletal muscle blood flow. These opposing hemodynamic effects of insulin help explain why acute physiologic hyperinsulinemia does not elevate blood pressure in normal humans.[7] Based on findings that increases in blood flow and glucose uptake after insulin infusion are inversely related to basal blood pressure, Baron et al[8] propose that insulin resistance and hyperinsulinemia are markers of the hypertensive process in the muscle and not contributors to elevated blood pressure. This intriguing hypothesis also seems consistent with the reduced insulin clearance found in hypertensive patients.[9,10]

An attenuation in the balance between the depressor and pressor effects of insulin could influence blood pressure regulation. A decreased insulin-mediated stimulation of skeletal muscle blood flow has been observed in obese subjects[11] and in patients with non–insulin-dependent diabetes mellitus (NIDDM).[12] Feldman and Bierbrier[13] have recently reported that the potency of insulin as a venodilator was reduced by 79% in hypertensive patients and that vascular sensitivity to insulin decreased with increasing body weight and basal systolic blood pressure. Despite evidence that insulin resistance (present in obese subjects and in patients with NIDDM) and a predisposition to hypertension (supposed in hypertensive patients) attenuated the depressor action of insulin, at least four clinical studies failed to show any effect of acute physiologic hyperinsulinemia on blood pressure in obese insulin-resistant hypertensive patients.[7] Many inconsistencies still remain, but insulin seems to be losing importance as a protagonist.

From the Department of Geriatrics and Metabolic Diseases, Second University of Naples, Naples; and Department of Experimental and Clinical Pathology, University of Udine, Udine, Italy.

Submitted February 16, 1994; accepted June 14, 1994.

Address reprint requests to Professor Dario Giugliano, Via Emilia, 1, 80021 Afragola (NA), Italia.

0026-0495/95/4403-0013$03.00/0

GLUCOSE HYPOTHESIS

Hyperglycemia is the distinguishing feature of diabetes. Evidence that high glucose levels may play a role, via oxidative stress, in the cardiovascular complications of diabetic patients will be discussed.

The endothelium has been shown to release a substance that induces smooth muscle relaxation by increasing the production of cyclic guanosine monophosphate. This factor is called endothelium-derived relaxing factor, and nitric oxide (NO) is believed to be one such factor.[14] The strongest evidence for a physiologic role of NO in humans lies in the results of intravenous injection of N^G-monomethyl-L-arginine, an inhibitor of NO formation from L-arginine, into the forearm. Such injections cause substantial vasoconstriction that lasts for 45 to 60 minutes unless it is reversed by L-arginine.[15] Moreover, local forearm infusion of acetylcholine (ACh) causes vasodilation that is primarily due to stimulation of NO synthesis. Thus, vessels of healthy humans are continuously dilated by NO released from endothelial cells.

Exposure to elevated concentrations of glucose in vitro causes selective impairment of endothelium-dependent relaxation.[16] Oxidative stress induced by hyperglycemia is implicated as a source of altered endothelium relaxation in diabetes. Tesfamariam and Cohen[17] have shown, for example, that elevated glucose concentrations impair ACh-stimulated endothelium relaxation in vitro and that this impairment can be reversed by antioxidants, which include superoxide dismutase (SOD) and catalase. Similarly, in diabetic animals impairment of ACh relaxation in aortas was restored to normal by SOD. Others have recently reported that the transient endothelium-dependent relaxation in the aorta of streptozotocin-induced diabetic rats was due to accumulation of superoxide anion (O_2^-),[18] which confirms previous reports that oxygen-derived free radicals (OFR) inactivate endothelium-derived releasing factors[19] and selectively attenuate endothelium-dependent relaxation.[20,21]

The inhibition of Na^+-K^+ adenosine triphosphatase activity seen in endothelium-intact aortas from severely diabetic rabbits, as well as in aortic rings from normal rabbits incubated in high-glucose medium, may be due to a decrease in basal release of NO.[22] High glucose concentrations have been reported to lengthen cell proliferation time and slightly increase cell death in cultured human endothelial cells derived from the umbilical vein.[23] Curcio and Ceriello[24] have shown that a hyperglycemia-induced delay in cell replication time that occurs in cultured human endothelial cells may be reversed by a number of antioxidants with different mechanisms of action, such as SOD, catalase, and reduced glutathione (GSH). A pathophysiologic link between hyperglycemia and development of endothelium dysfunctions through increased synthesis and/or release of OFR may be hypothesized.

FREE RADICALS AND DIABETES

One possible source of OFR in diabetes is autoxidation of glucose.[25] Glucose can oxidize, which generates free radicals, hydrogen peroxide, and reactive ketoaldehydes. These last compounds may largely participate in the formation of glycated proteins, which are themselves a source of OFR.[26,27] The term "glycoxidation products" has been coined by Baynes[28] to indicate autoxidation products, such as carboxymethyllysine and pentosidine, to stress the obligatory role of oxygen in their formation from Amadori products. These markers of glycoxidative damage accumulate on collagen as a function of age and glycemia. Unlike the levels of Amadori products, which may be in part spontaneously reversible, levels of carboxymethyllysine and pentosidine in skin from insulin-dependent diabetes mellitus (IDDM) patients are unaffected by improved glycemic control.[29] Moreover, quenching and inactivation of NO by advanced glycation end products in experimental diabetes has been described.[30]

Impaired generation of naturally occurring antioxidants (vitamin E, vitamin C, and GSH) will also result in increased oxidative injury by failure of protective mechanisms. Increased flux of glucose through the polyol pathway, which is hyperactive in hyperglycemia,[31] may deplete NADPH, which is required for generation of NO from arginine.[32] Furthermore, increased oxidation of sorbitol to fructose increases the ratio of cytosolic NADH/NAD^+. This redox imbalance, also referred to as hyperglycemic pseudohypoxia, augments production of O_2^- via reduction of PGG_2 to PGH_2 by prostaglandin hydroperoxidases that use NADH as a reducing cosubstrate[33] (Fig 1).

The possibility that OFR play a role in the pathogenesis of vascular complications of diabetes is suggested by studies that have shown that antioxidants such as vitamin E, SOD, catalase, GSH, and ascorbic acid are all decreased in diabetic tissues and blood.[34-36] Moreover, elevated levels of OFR products in diabetic patients have been reported.[37-39] In one study, the plasma concentration of O_2^- was elevated

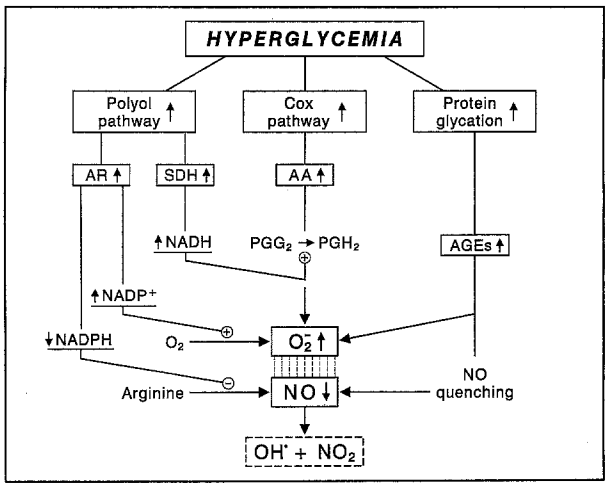

Fig 1. Possible mechanisms by which hyperactivity of some metabolic pathways strictly related to hyperglycemia may lead to upset of the balance between the two radicals O_2^- and NO. AR, aldose reductase; SDH, sorbitol dehydrogenase; Cox, cycloxygenase; AA, arachidonic acid; PGG_2, prostaglandin G_2; PGH_2, prostaglandin H_2; OH, hydroxyl radical; NO_2, nitrogen dioxide. Formation of OH• and NO_2 from decomposition of peroxinitrite has yet to be demonstrated in vivo.

in patients with IDDM, but showed a trend to normalization after strict metabolic control.[38] There was a strong correlation between plasma glucose concentrations and O_2^- concentrations in both normal subjects and diabetic patients over a wide range of glucose concentrations.

With this in mind, one would expect disturbed endothelium-dependent vascular relaxation in diabetes mellitus. Many[40-44] but not all[45] recent studies have shown that endothelium-dependent vasodilation is abnormal in diabetic patients (both IDDM and NIDDM) and that this abnormality is caused by decreased release or activity of NO. Moreover, several properties of NO (inhibition of platelet aggregation, monocyte adhesion to endothelial cells, and smooth muscle cell proliferation) suggest that if its biosynthesis were reduced, this could predispose to atheroma.[46]

Studies on effects of antioxidants in human diabetes are scanty. Pharmacologic vitamin E supplementation (900 mg/d for 4 months) in patients with NIDDM improves insulin action (nonoxidative glucose metabolism, mainly synthesis of glycogen), reduces oxidative stress (oxidized glutathione [GSSG]/GSH ratio decreased by 47%), and ameliorates red blood cell microviscosity.[39] These results confirm and extend previous studies[47] that found that daily oral vitamin E supplementation (600 or 1,200 mg) to IDDM subjects for 2 months reduced oxidative stress and protein glycation. An indirect mechanism by which vitamin E may reduce the unfavorable effects of OFR on endothelial function is through an increased production of the vasodilating prostaglandin PGI_2, as demonstrated in cultured bovine aortic endothelial cells, where specific binding sites for D-alpha-tocopherol have also been found.[48] Interestingly enough and perhaps relevant to the vascular toxicity of hyperglycemia in human diabetes is the finding that high concentrations of glucose (16.8 or 22.4 mmol/L) reduce D-alpha-tocopherol binding through oxidative stress.

FREE RADICALS AND HYPERTENSION

Endothelium-dependent relaxation has been found to be impaired in animal models of hypertension.[49] Moreover, the frequency of endothelial cell death and associated endothelial permeability is significantly increased in the aorta of spontaneously hypertensive rats.[50] The response of forearm blood flow to ACh but not to sodium nitroprusside, an agent that produces vasodilation by direct activation of guanylate cyclase in vascular smooth muscle cells,[51] was significantly reduced in 18 middle-aged hypertensive patients.[52] Since ACh only produces an endothelium-mediated vasodilator response, a defect in endothelium-dependent vascular relaxation has been hypothesized. An acute antihypertensive effect of three structurally unrelated antioxidant agents, such as vitamin C, thiopronine (a substrate for glutathione synthesis), and glutathione, has been reported in hypertensive subjects, regardless of whether they are diabetic.[53] An imbalance due to reduced production of NO, as demonstrated in spontaneously hypertensive rats,[49] or increased destruction by a relative or absolute increase in O_2^- may facilitate the development of an arterial functional spasm. A physiologic role for O_2^- is suggested by results of intravenous infusions of the three different antioxidants that have a vasodilating action in the skeletal muscle of the forearm in normal humans.[54] Thus, it seems likely that a continuous production of O_2^- offsets the vasodilatory action of NO, which allows the maintenance of a proper vascular tone.

FREE RADICALS AND AGING

The prevalence of diabetes (up to 12% after 65 years) and hypertension (up to 30% after 70 years) increases with age. Therefore, age may be a common factor that amplifies the association between diabetes and hypertension.

Paolisso et al[55] found significant increments of serum O_2^- with age associated with increases in both membrane viscosity and the GSSG/GSH ratio, as well as a reduction of total-body glucose disposal evaluated during euglycemic glucose clamp (1 mU/kg/min) with simultaneous D-^{3}H-glucose infusion and indirect calorimetry. In the multivariate analysis, only O_2^- displayed an independent relationship with total-body glucose disposal and nonoxidative glucose metabolism. Elderly NIDDM patients who took vitamin E (900 mg/d) for 3 months demonstrated significant benefits on many parameters of both glucose (glycemia and hemoglobin A_{1c}) and lipid (triglycerides, low-density lipoprotein [LDL] cholesterol, and apoprotein A) metabolism.[56] Moreover, plasma O_2^- production and the GSSG/GSH ratio significantly decreased after vitamin E therapy ($\sim$40%). In another study,[57] elderly atherosclerotic patients showed decreased blood viscosity and improved blood filterability in response to exogenous glutathione (600 mg/d as intravenous infusion per 7 days).

PATHOGENETIC IMPLICATIONS

Treatment with different antioxidants improves many metabolic abnormalities reported to occur in diabetic subjects. Vitamin E and glutathione[58] ameliorate insulin sensitivity mainly by stimulation of nonoxidative glucose metabolism; short-term administrations of vitamin C, thiopronine, and GSH all increase resting blood flow in the forearm of normal and diabetic patients irrespective of the presence of hypertension, which suggests that a continuous basal production of O_2^- offsets the vasodilatory action of NO by quenching it; GSH ameliorates hemorrheologic parameters in diabetic patients, atherosclerotic subjects, and normal people. Moreover, increased blood viscosity and platelet aggregation are found in hypertensive patients.[59] Thus, an increased production of OFR may be a cause of the increased cardiovascular morbidity and mortality found in diabetes mellitus.

CONCLUSIONS

Abnormalities of the NO/O_2^- pathway have been demonstrated in humans with diabetes mellitus,[53,54] essential hypertension,[52] and hyperlipidemia.[60] A dysfunction of the NO/O_2^- system may be a common mechanism by which such apparently diverse conditions result in similar chronic vascular complications. A diminished basal production or effect of NO, as well as an increased concentration or effect of O_2^- would tip the balance in favor of vasoconstriction

and blood hyperviscosity and thus favor the development of vaso-occlusive disorders at the level of coronary, cerebral, and peripheral vessels. Completing this gloomy scenario, OFR are capable of activation of coagulation and oxidation of LDL, and both hemostatic alterations[61] and increased levels of plasma and tissue oxidized lipids[62] are found in diabetic patients. Hyperlipidemia may alter the redox state of vascular smooth muscle[63] and thereby reduce the responsiveness of the vessel wall to nitrovasodilators. In fact, there are sulfhydryl groups associated with guanylate cyclase, the oxidation of which inactivates the enzyme.[64] In humans, the susceptibility of LDL to oxidation correlated with the severity of coronary stenosis in 35 male survivors of myocardial infarction,[65] and LDL from volunteers treated with alpha-tocopherol supplements (200 mg for 6 months) showed increased resistance to oxidation.[66] Although the antioxidant hypothesis of atherosclerosis is supported by experimental and epidemiologic data, it has not yet attained the status of a clinically validated hypothesis.[67]

More than one third of NIDDM patients and half of IDDM patients remain free of hypertension throughout their lives; the suggested chain of events that links high blood glucose to hypertension may only occur in patients who also have other risk factors for hypertension, eg, a family history or increased Na^+-Li^+ countertransport.[68] Moreover, diabetic patients with hypertension seem more susceptible than diabetic subjects without hypertension to the blood pressure–increasing effects of OFR generated by glucose.[69] On the other hand, alterations of blood rheology, blood-flow response to ACh, coagulative parameters, and lipidemic pattern may be present in the majority of diabetic patients.

Recent epidemiologic and clinical evidence supports a role for hyperglycemia in the increased cardiovascular morbidity and mortality found in human diabetes.[70-73] Increased plasma and tissue glucose levels may be one important source of the increased oxidative stress seen in diabetic patients. Although more than one factor may be involved, as the beneficial effects of vitamin E on vasculature unrelated to its antioxidant capability seem to suggest,[48] the evidence that OFR are implicated in the endothelial dysfunction of diabetic patients is growing and may offer novel therapeutic approaches.

REFERENCES

1. Reaven G: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988
2. Ceriello A, Quatraro A, Giugliano D: New insights on non-enzymatic glycosylation may lead to therapeutic approaches for the prevention of diabetic complications. Diabetic Med 9:297-299, 1992
3. Ceriello A, Quatraro A, Giugliano D: Diabetes mellitus and hypertension: The possible role of hyperglycemia through oxidative stress. Diabetologia 36:265-266, 1993
4. Ferrannini E, Natali A: Essential hypertension, metabolic disorders, and insulin resistance. Am Heart J 121:1274-1282, 1991
5. Jarrett RJ: In defence of insulin: A critique of syndrome X. Lancet 340:469-471, 1992
6. Leonetti F, Iozzo P, Giaccari A, et al: Absence of clinically overt atherosclerotic vascular disease and adverse changes in cardiovascular risk factors in 70 patients with insulinoma. J Endocrinol Invest 16:875-880, 1993
7. Anderson EA, Mark AL: The vasodilator action of insulin: Implications for the insulin hypothesis of hypertension. Hypertension 21:136-141, 1993
8. Baron AD, Brechtel-Hook G, Johnson A, et al: Skeletal muscle blood flow: A possible link between insulin resistance and blood pressure. Hypertension 21:129-135, 1993
9. Giugliano D, Salvatore T, Paolisso G, et al: Impaired glucose metabolism and reduced insulin clearance in elderly hypertensives. Am J Hypertens 5:345-353, 1992
10. Giugliano D, Quatraro A, Minei A, et al: Hyperinsulinemia in hypertension: Increased secretion, reduced clearance or both? J Endocrinol Invest 13:315-321, 1993
11. Laakso M, Edelman SV, Brechtel G, et al: Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man: A novel mechanism of insulin resistance. J Clin Invest 85:1844-1853, 1990
12. Laakso M, Edelman SV, Bretchel G, et al: Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. Diabetes 41:1076-1083, 1992
13. Feldman RD, Bierbrier GS: Insulin-mediated vasodilation: Impairment with increased blood pressure and body mass. Lancet 342:707-709, 1993
14. Palmer RM, Ferrige AG, Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327:524-526, 1987
15. Vallance P, Collier J, Moncada S: Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. Lancet 2:997-1000, 1989
16. Tesfamarian B, Brown ML, Deykin D, et al: Elevated glucose promotes generation of endothelium-dependent vasoconstrictor prostanoids in rabbit aorta. J Clin Invest 85:929-932, 1990
17. Tesfamarian B, Cohen RA: Free radicals mediate endothelial cell dysfunction caused by elevated glucose. Am J Physiol 263:H321-H326, 1992
18. Hattori Y, Kawasaki H, Abe K, et al: Superoxide dismutase recovers altered endothelium dependent relaxation in diabetic rat aorta. Am J Physiol 261:H1086-H1094, 1991
19. Gryglewski RJ, Palmer RMJ, Moncada S: Superoxide anion is involved in the breakdown of endothelium-derived relaxing factor. Nature 320:454-456, 1986
20. Pieper GM, Gross G: Oxygen free radicals abolish endothelium-dependent relaxation in diabetic rat aorta. Am J Physiol 255:H825-H833, 1988
21. Rubanyi GM, Vanhoutte PM: Oxygen-derived free radicals, endothelium, and responsiveness of vascular smooth muscle. Am J Physiol 250:H815-H821, 1986
22. Gupta S, Sussman I, McArthur CS, et al: Endothelium-dependent inhibition of Na^+-K^+ ATPase activity in rabbit aorta by hyperglycemia. Possible role of endothelium-derived nitric oxide. J Clin Invest 90:727-732, 1992
23. Lorenzi M, Cagliero E, Toledo S: Glucose toxicity for human endothelial cells in culture. Diabetes 34:621-627, 1985
24. Curcio F, Ceriello A: Decreased cultured endothelial cell proliferation in high glucose medium is reversed by antioxidants: New insights on the pathophysiological mechanisms of diabetic vascular complications. In Vitro Cell Dev Biol 28A:787-790, 1992
25. Hunt JV, Dean RT, Wolff SP: Hydroxyl radical production and autoxidative glycosylation. Glucose autoxidation as the cause

of protein damage in the experimental glycation model of diabetes mellitus and aging. Biochem J 256:205-212, 1988

26. Gillery P, Monboisse JC, Maquat FX, et al: Glycation of proteins as a source of superoxide. Diabete Metab 14:25-30, 1988

27. Sakurai T, Tsuchiya S: Superoxide production from non-enzymatically glycated proteins. FEBS Lett 236:406-410, 1988

28. Baynes JW: Role of oxidative stress in development of oxidative complications in diabetes. Diabetes 40:405-412, 1991

29. Lyons TJ, Baille KE, Dyer DG, et al: Decrease in skin collagen glycation with improved glycemic control in patients with insulin-dependent diabetes mellitus. J Clin Invest 87:1910-1915, 1991

30. Bucala R, Tracey KS, Cerami A: Advanced glycosylation end products quench nitric oxide and mediate defective endothelium-dependent vasodilation in experimental diabetes. J Clin Invest 87:432-438, 1991

31. Greene DA, Lattimer SA, Sima AF: Sorbitol, phosphoinositides and sodium-potassium–ATPase in the pathogenesis of diabetic complications. N Engl J Med 316:599-606, 1987

32. Moncada S, Higgs A: Mechanisms of disease: The L-arginine–nitric oxide pathway. N Engl J Med 329:2002-2012, 1993

33. Williamson JR, Chang K, Frangos M, et al: Hyperglycemic pseudohypoxia and diabetic complications. Diabetes 42:801-813, 1993

34. Karpen CW, Pritchard KA, Arnold JH, et al: Restoration of the prostacyclin/thromboxane A2 balance in the diabetic rat: Influence of vitamin E. Diabetes 31:947-951, 1982

35. Wohaieb SA, Godin DV: Alterations in free radicals tissue-defense mechanisms in streptozotocin-induced diabetes in rat. Effects of insulin treatment. Diabetes 36:1014-1018, 1987

36. Wolff SP, Dean RT: Glucose autoxidation and protein modification. The potential role of oxidative glycosylation in diabetes. Biochem J 245:243-250, 1987

37. Collier A, Wilson R, Bradley H, et al: Free radical activity in type-2 diabetes. Diabetic Med 7:27-30, 1990

38. Ceriello A, Giugliano D, Quatraro A, et al: Metabolic control may influence the increased superoxide generation in diabetic serum. Diabetic Med 8:540-542, 1991

39. Paolisso G, D'Amore A, Giugliano D, et al: Pharmacological doses of vitamin E improve insulin action in healthy subjects and non–insulin-dependent diabetic patients. Am J Clin Nutr 57:650-656, 1993

40. de Tejada S, Goldstein I, Azadzoi K, et al: Impaired neurogenic and endothelium-mediated relaxation of penile smooth muscle from diabetic men with impotence. N Engl J Med 320:1025-1030, 1989

41. Calver AC, Collier JG, Vallance PJT: Inhibition and stimulation of nitric oxide synthesis in the human forearm arterial bed of patients with insulin-dependent diabetes. J Clin Invest 90:2548-2554, 1992

42. McVeigh GE, Brennan GM, Johnston GD, et al: Impaired endothelium-dependent and -independent vasodilation in patients with type-2 (non–insulin-dependent) diabetes mellitus. Diabetologia 35:771-776, 1992

43. Elliott TG, Cockcroft JR, Groop P-H, et al: Inhibition of nitric oxide synthesis in forearm vasculature of insulin-dependent diabetic patients: Blunted vasoconstriction in patients with microalbuminuria. Clin Sci 85:687-693, 1993

44. Johnstone MT, Creager SJ, Scales KM, et al: Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. Circulation 88:2510-2516, 1993

45. Halkin A, Bejamin N, Doktor HS, et al: Vascular responsiveness and cation exchange in insulin-dependent diabetes. Clin Sci 81:223-232, 1991

46. Moncada S, Palmer RMJ, Higgs EA: Nitric oxide: Physiology, pathophysiology and pharmacology. Pharmacol Rev 43:109-142, 1991

47. Ceriello A, Giugliano D, Quatraro A, et al: Vitamin E reduction of protein glycosylation in diabetics: New prospect for prevention of diabetic complications. Diabetes Care 14:68-72, 1991

48. Kunisaki M, Umeda F, Yamauchi T, et al: High glucose reduces specific binding for D-alpha-tocopherol in cultured aortic endothelial cells. Diabetes 42:1138-1146, 1993

49. Auch-Schwalk W, Katusic ZS, Vanhoutte PM: Contractions to oxygen-derived free radicals are augmented in aorta of the spontaneously hypertensive rat. Hypertension 13:859-862, 1989

50. Wu CH, Chi JC, Jerng JS, et al: Transendothelial macromolecular transport in the aorta of spontaneously hypertensive rats. Hypertension 16:154-161, 1990

51. Schultz K, Schultz K, Schultz G: Sodium nitroprusside and other smooth muscle relaxants increase cyclic GMP levels in rat ductus deferens. Nature 265:750-751, 1977

52. Panza JA, Arshed AQ, Brush JE, Epstein SE, et al: Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. N Engl J Med 323:22-27, 1990

53. Ceriello A, Giugliano D, Quatraro A, et al: Antioxidants show an anti-hypertensive effect in diabetic and hypertensive subjects. Clin Sci 81:739-742, 1991

54. Ceriello A, Quatraro A, Caretta F, et al: Evidence for a possible role of oxygen free radicals in the abnormal functional arterial vasomotion in insulin dependent diabetes. Diabete Metab 16:318-322, 1990

55. Paolisso G, D'Amore A, Di Maro G, et al: Evidence for a relationship between free radicals and insulin action in the elderly. Metabolism 42:659-663, 1993

56. Paolisso G, D'Amore A, Galzerano D, et al: Daily vitamin E supplements improve metabolic control but not insulin secretion in elderly type II diabetic patients. Diabetes Care 16:1433-1437, 1993

57. Coppola L, Grassia A, Giunta R, et al: Glutathione (GSH) improves haemostatic and haemorrheological parameters in atherosclerotic subjects. Drugs Exp Clin Res 18:493-498, 1992

58. Paolisso G, Di Maro G, Pizza G, et al: Plasma GSH/GSSG affects glucose homeostasis in healthy subjects and non–insulin-dependent diabetics. Am J Physiol 263:E435-E440, 1992

59. Coppola L, Cozzolino D, Verrazzo G, et al: Cardiovascular and haemorrheological effects of beta-endorphin in humans. J Endocrinol Invest 16:118A, 1993 (suppl 1, abstr)

60. Creager MA, Cooke JP, Mendelsohn SJ, et al: Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. J Clin Invest 86:228-234, 1990

61. Ceriello A: Coagulation activation in diabetes mellitus: The role of hyperglycaemia and therapeutic prospects. Diabetologia 36:1119-1125, 1993

62. Lyons TJ: Oxidized low density lipoproteins: A role in the pathogenesis of atherosclerosis in diabetes. Diabetic Med 8:414-419, 1991

63. Ylä-Herttuala S, Palinski W, Rosenfeld ME, et al: Evidence for the presence of oxidatively modified LDL in human atherosclerotic lesions. Circulation 80:145-160, 1989 (suppl 2)

64. Craven PA, DeRubertis FR: Restoration of the responsiveness of purified guanylate cyclase to nitrosoguanidine, nitric oxide and related activators by heme and heme proteins. J Biol Chem 253:8433-8443, 1978

65. Regnström J, Nilsson J, Tornvall P, et al: Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man. Lancet 339:1183-1186, 1992

66. Abbey M, Nestel PJ, Baghurst PA: Antioxidant vitamins and low density-lipoprotein oxidation. Am J Clin Nutr 58:525-532, 1993

67. Steinberg D: Antioxidant vitamins and coronary heart disease. N Engl J Med 328:1487-1489, 1993

68. Consensus Statement: Treatment of hypertension in diabetes. Diabetes Care 16:1394-1401, 1993

69. Ceriello A, Quatraro A, Motz E, et al: Hypertension, hyperglycemia and diabetes mellitus: The possible role of the oxidative stress. J Endocrinol Invest 17:55A, 1994 (suppl 2)

70. Singer DE, Nathan DM, Anderson KM, et al: Association of HbA_{1c} with prevalent cardiovascular disease in the original cohort of the Framingham Heart Study. Diabetes 41:202-208, 1992

71. Quatraro A, Giugliano D, De Rosa N, et al: Is a family history of diabetes associated with an increased level of cardiovascular risk factors? Studies in healthy people and in subjects with different degree of glucose intolerance. Diabete Metab 19:230-238, 1993

72. Uusitupa MIJ, Niskanen LK, Siitonen O, et al: Ten-year cardiovascular mortality in relation to risk factors and abnormalities in lipoprotein composition in type 2 (non–insulin-dependent) diabetic and non-diabetic subjects. Diabetologia 36:1175-1184, 1993

73. DCCT Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes. N Engl J Med 329:77-86, 1993