Review

ENDOTHELIAL FUNCTION, TYPE 2 DIABETES MELLITUS AND CARDIOVASCULAR DISEASE: A CLINICAL PERSPECTIVE

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INTRODUCTION

The worldwide prevalence of diabetes has continued to increase. In the year 2000, there were 151 million people with diabetes. This number is expected to reach 239 million in 2010, and to 300 million in 2025 (1,2). Presently, India leads the world with the largest number of adults with diabetes, estimated to be 32.7 million (2).

Ischemic heart disease is the leading cause of morbidity and mortality in patients with type 2 diabetes. Several studies have demonstrated that patients with type 2 diabetes have an increased risk for cardiovascular disorders (3-5). The increased risk is even more significant for women with diabetes because they seem to lose the protection against cardiovascular disease afforded to their gender without diabetes (6,7). Recent data suggests that patients with diabetes without previous myocardial infarction have as high a risk of myocardial infarction as non-diabetic patients with previous myocardial infarction (8).

Type 2 diabetes is a heterogeneous disorder characterized by insulin resistance and β cell dysfunction (9, 10). Insulin resistance in turn is associated with several risk factors including hypertension, hypertriglyceridemia, low HDL cholesterol, small LDL particles, visceral obesity, increased fibrinogen, and plasminogen activator inhibitor 1 (PAI-1). This constellation of factors has been variously called insulin resistance syndrome, metabolic syndrome, and more recently, cardiovascular dysmetabolic syndrome (11).

Atherosclerosis, the pathologic basis of development of macrovascular disease, occurs prematurely and is more severe in patients with diabetes. The emerging data suggests that endothelial cell dysfunction plays a key role in the initiation of atherosclerotic vascular disease (12). This article reviews briefly, the basic pathophysiology of endothelium and alteration of endothelial function in patients with type 2 diabetes from a clinical perspective.

PATHOPHYSIOLOGY OF ENDOTHELIUM

It is now generally accepted that vascular endothelium is not a simple barrier between the lumen and the vessel wall. In addition to providing a smooth nonthrombogenic surface and a permeability barrier, it also synthesizes and releases a number of vasoactive substances that modulate smooth muscle function and structure (13). Vasodilating and vasoconstricting agents produced by vascular endothelium not only control and alter vascular tone, but can also affect platelet adhesion and aggregation, influence thrombogenecity of blood, smooth muscle cell proliferation and ultimately the development of atherosclerosis (14,15). Some of the vasodilator factors produced by the endothelium include nitric oxide (NO), prostacyclin (PGI_2), bradykinin, and the endothelial derived hyperpolarizing factor. Constricting agents include endothelin, superoxide anion, locally produced angiotensin II, and thromboxane (14).

Injury to the endothelial cell resulting primarily from the increased oxidative stress within the endothelium (16) leads to activation of cytokines, and expression of adhesion molecules such as vascular cell adhesion molecule (VCAM-1), intracellular adhesion molecule (ICAM-1) and endothelial

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leukocyte adhesion molecule (ELAM), which attracts monocytes to adhere to the surface of the endothelium (17). This is followed by infiltration of mononuclear cells into the vessel wall, formation of lipid laden foam cells and development of atherosclerotic plaque (18). The endothelium thus plays a critical role in the development of atherosclerotic plaques.

ASSESSMENT OF ENDOTHELIAL FUNCTION

Early experiments of Furchgott and Zawadzki (19) established that the vasodilation of isolated rabbit aortic rings induced by acetylcholine was mediated by an endothelium derived relaxing factor (EDRF). Denudation of endothelial cells abolished acetylcholine mediated vasodilation. Subsequently the EDRF was identified as nitric oxide (20). Since then, a variety of agents have been shown to stimulate the synthesis and release of NO from the endothelium, including serotonin, thrombin, bradykinin, vasopressin, and norepinephrine (21). The action of these agents is mediated by activation of the enzyme NO synthase. In contrast, the enzyme NO synthase can be inhibited by a variety of arginine analogs such as N^G-monomethyl L-arginine (L-NMMA) (22). Endothelium dependent relaxation can be studied in human coronary circulation by injecting acetylcholine or L-NMMA directly in to the coronary arteries (23, 24). In normal, healthy vessels, intra-arterial administration of acetylcholine results in vasodilatation and is indicative of the normally functioning intact endothelium. In patients with vessels diseased by atherosclerosis, or in the presence of risk factors for atherosclerosis infusion of acetylcholine may paradoxically cause vasoconstriction (23, 25). Endothelial function may also be studied by direct intra-arterial infusion of acetylcholine in a limb artery. At least a modest correlation exists between the endothelial function in the coronary and peripheral circulations (14, 26). Endothelial independent vasodilation can be evaluated by administration of direct vasodilators such as nitroglycerin or sodium nitroprusside.

However, intra-arterial injection into a peripheral or coronary vessel is at least moderately invasive. In clinical studies, therefore, the ability of brachial artery to dilate in response to reactive hyperemia has been used as an index of the endothelial function (27). In this procedure, brachial artery blood flow is determined by ultrasound at rest (baseline). The brachial artery blood flow is then stopped for a brief period (3 to 5 minutes) by inflating a blood pressure cuff above the systolic pressure. After the cuff is deflated, brachial artery diameter and blood flow is determined. The increase in brachial artery blood flow and the increase in the brachial artery diameter is an endothelial dependent function. Impairment of endothelial function results in decreased dilatation of brachial artery, and a diminished (or no) increase in blood flow.

MECHANISMS FOR ENDOTHELIAL DYSFUNCTION IN DIABETES

A number of changes occur in the endothelial cell function in patients with diabetes, which results in alterations of vascular tone, facilitate plaque formation and promote thrombosis. These changes include decreased nitric oxide activity and decreased prostacyclin release, whereas there is increased level of endothelin-1, increased adhesion molecule expression, increased platelets and monocyte adhesion, increased procoagulant activity, advanced glycosylated end products, impaired fibrinolytic activity, and impaired degradation of glycosylated fibrin (28). Increased endothelin-1 levels leading to vasoconstriction have been demonstrated in patients with diabetes mellitus. Endothelin-1 levels are also directly related to plasma glucose levels. NO release and function are also impaired, which seems to be dependent on hyperglycemia and genetic factors. In addition, enhanced vascular permeability is seen in diabetes. The latter appears to be related to impaired endothelial cell relaxation, reactive oxygen species, as well as advanced glycation products (29). Role of the renin angiotensin system in the regulation of endothelial function deserves a special mention because the beneficial role of angiotensin converting enzyme (ACE) inhibitors in the treatment of diabetic nephropathy is well established (30). Presence of microalbuminuria has a close association with endothelial dysfunction (31). This had lead Deckert to propose that albuminuria reflects a widespread vasculopathy of the micro and macrocirculation that is a consequence of generalized endothelial dysfunction (31). Initial microalbuminuria is now regarded as an independent predictor of subsequent progression of nephropathy and risk for cardiovascular morbidity and mortality (32, 33). There are two renin angiotensin systems (RAs) that

regulate the endothelial function, the circulating RAs and the tissue RAs. Approximately 90% of ACE is found in tissues, where as 10% of ACE circulates in plasma (34, 35). Angiotensin II is a potent vasoconstrictor and has been directly implicated in the generation of superoxide anion in the smooth muscle cells (36). Angiotensin II regulates a membrane bound flavin containing NADH/NADPH oxidase that produces oxygen radicals (37). It has been postulated that ACE inhibitors, besides their well known hemodynamic effects, could participate in the reduction of oxidative stress in the vessel wall (36).

ENDOTHELIAL FUNCTION IN INSULIN RESISTANCE AND DIABETES

It is now generally accepted that several years may elapse during which abnormalities of insulin secretion and action can be detected before a clinical diagnosis of diabetes is made. During this "prediabetic" phase, hyperinsulinemia (38, 39) and increased risk factors for coronary artery disease are present (40, 41). In agreement with this concept of "ticking clock", as suggested by Haffner et. al. (41) abnormalities of endothelial dysfunction can be detected before the carbohydrate intolerance becomes evident (42, 43). Anastasious et. al. (42) demonstrated that a group of women with previous gestational diabetes, but with normal glucose tolerance at the time of testing, had impaired endothelial function as demonstrated by flow mediated dilatation. Defects in the vasodilatory reserve have been observed in normoglycemic women for an average of eight years after gestational diabetes (43). It has been hypothesized that impaired endothelial function may be part of the insulin resistance syndrome, which may persist in women with previous gestational diabetes, despite the presence of euglycemia. This is consistent with the observations that women with a history of gestational diabetes continue to exhibit defects in insulin secretion and action (44).

As previously mentioned, insulin resistance is an integral metabolic abnormality in patients with type 2 diabetes (9,10). Insulin resistance can be demonstrated in obese individuals (45) and in individuals who are destined to become diabetic (41). Steinberg et. al. have demonstrated impaired endothelial function in patients with obesity (46). More recently, Caballero et. al. (47) demonstrated reduced vasodilatory response to acetylcholine in healthy normoglycemic subjects with a history of diabetes in one or both parents (relatives) and subjects with impaired glucose tolerance. Results of studies by Steinberg and Caballero suggest that abnormalities in vascular reactivity are present early in individuals at risk of developing type 2 diabetes.

Williams et. al. (48) demonstrated that hyperglycemia induced by infusion of 50% glucose in normal individuals resulted in impaired endothelial function as determined by methacholine induced vasodilation. However, since glucose infusion was also accompanied by an increase in insulin levels, the insulin could have been a confounding factor. Even when insulin levels were held constant by an octreotide infusion, hyperglycemia was still accompanied by impaired vasodilation. The same group (49) and others (50) have also demonstrated impaired NO mediated vasodilatation in patients with type 2 diabetes. Hogikyan et. al. also demonstrated specific endothelial cell dysfunction in patients with type 2 diabetes independent of obesity (51).

Some studies evaluating endothelial function have also demonstrated impaired endothelial function in patients with type 1 diabetes (52, 53, 54). Johnstone et. al. (52) observed impaired vasodilation in response to methacholine, while Calver et. al. (53) and Elliott et. al. (54) observed reduced vasoconstrictor response to NO synthase antagonist L-NMMA. On the other hand, some studies failed to demonstrate endothelial dysfunction in patients with type 1 diabetes (55, 56). In the studies which failed to demonstrate endothelial dysfunction, patients with detectable microalbuminuria were excluded. Since the presence of microalbuminuria correlates with severity of endothelial dysfunction, exclusion of these patients may explain the discrepancy noted among these studies.

Studies of patients with type 2 diabetes mellitus being treated with various therapeutic agents have suggested an improvement in endothelial function. In a study by Murakami et. al. (57) ten patients with spontaneous or provoked coronary vasospasm, who were receiving conventional medications for vasospastic angina pectoris, were also treated with 400 mg of troglitazone daily (an insulin sensitizer) for four months. Anginal episodes and anginal duration were significantly reduced. Flow mediated endothelium dependent vasodilatation was improved after use of troglitazone. This study, therefore, suggests that reduction of insulin resistance is accompanied by improvement in endothelial function, which in turn may

lead to improvement in vasospastic angina. Avena et. al. (58) demonstrated in patients with peripheral vascular disease and impaired glucose tolerance an abnormal response to hyperemia, which was normalized after treatment with troglitazone. Comancini et. al. (59) also reported a beneficial effect of troglitazone, which was accompanied by resistance of LDL to be oxidized compared with placebo. These investigators concluded that in type 2 diabetes, troglitazone may slow down the development of atherosclerosis by modifying LDL-related atherogenic events. The beneficial effect of troglitazone, however, has not been confirmed in all the studies. Tack et. al. (60) evaluated the effects of troglitazone in fifteen, obese subjects in a double-blind cross over trial. The obese subjects were insulin resistant, but had normal endothelial function. Although treatment of troglitazone resulted in improved insulin sensitivity, it had no effect on endothelium dependent or independent vascular responses. Therefore, it appears that the insulin sensitizers may have a beneficial effect on the endothelial function although this finding is not universal. The role of insulin sensitizers in the development of atherosclerosis, therefore, deserves further study. Moreover, troglitazone has now been removed from the U.S. and other markets and studies with other thiazolidinediones (Pioglitazone and Rosiglitazone) are also needed to determine if this effect is mediated through improvement in insulin resistance or is it specific for troglitazone.

The effect of metformin on the endothelial function has also been studied. Although metformin lowers blood glucose predominantly by decreasing hepatic glucose output, it also improves insulin sensitivity (61, 62). Mather et. al. (63) studied the effect of treatment with metformin in patients with type 2 diabetes mellitus and observed that metformin treatment improved both insulin resistance and endothelial function.

Treatment with insulin is essential in patients with type 1 diabetes and is frequently also needed in type 2 patients. Insulin mediated glucose uptake is dependent on the arteriovenous glucose difference and blood flow into the muscle. Insulin is known to have a specific action to vasodilate skeletal muscle vasculature (64, 65). The vasodilatory action of insulin is mediated by endothelium dependent nitric oxide (66, 67). The decreased insulin sensitivity in patients with insulin resistance such as obesity is not only due to lower glucose extraction in insulin sensitive tissues, but also to lower blood flow in these tissues. Administration of insulin partially restores endothelium dependent relaxation (68).

Many of the components of the "cardiac dysmetabolic syndrome" also exert their atherosclerotic effects through their actions on the endothelium. Endothelial dysfunction has been demonstrated in hypertension (69, 70), and hyperlipidemia (71, 72). It has been speculated that loss of endothelial dependent vasodilatation and increased vasoconstriction may be etiological factors in hypertension (73). In regards to lipids, a single high fat meal demonstrated an adverse affect on endothelial function (72) and improvement in forearm blood flow was observed in hypercholesterolemic subjects after LDL apheresis (71). Therapies that reduce lipid levels have also been shown to improve endothelial function (74). Andersen et. al. (74) randomized hypercholesterolemic subjects to three different treatment groups; diet alone, a group receiving lovastatin and probucol, and the third group receiving lovastatin plus cholestyramine. The two groups receiving pharmacologic therapy showed a significant reduction in LDL-C levels and a significant improvement in vasomotor response to administration of acetylcholine. A review of literature reveals substantial evidence that a group of lipid lowering agents (statins) can improve endothelial function (75, 76, 77). However, each specific agent may have variable effects. At least one trial with simvastatin in patients with type 2 diabetes, however, failed to show any effect on flow mediated vasodilatation (78). The Armed Forces Regression Study (79) also failed to show an improvement in flow mediated vasodilatation when hyperlipidemic subjects were treated with gemfibrozil and if necessary with niacin and/or cholestyramine. On the other hand, treatment with ciprofibrate for three months was accompanied by improvement in fasting and postprandially measured endothelial function with an improvement in serum triglyceride and HDL cholesterol concentration (80).

CLINICAL STUDIES WITH ACE INHIBITORS

Several clinical studies have demonstrated that treatment with ACE inhibitors leads to improvement of endothelial function. The TREND (Trial on Reversing Endothelial Dysfunction) in a six-month, double-blind placebo controlled study in 129 normotensive adults with coronary artery disease demonstrated that subjects randomized to quinapril showed a net increase in the mean diameter of target segment of coronary arteries in

response to acetylcholine, where as placebo group showed no response (81). In a sub-group of TREND patients, coronary blood flow was measured using a doppler flow wire. At baseline, placebo and quinapril groups had similar endothelium dependent flow responses. After treatment with quinapril, endothelium dependent flow increased in the treatment group, but remained unchanged in the placebo group (82).

The BANFF trial was an open-label, short-term trial comparing the effects of quinapril, enalapril, losartan, and amlodipine on endothelial function assessed by reactive hyperemia to determine flow mediated dilation of brachial artery. The quinapril group had significantly improved flow mediated vasodilatation. None of the other groups showed significant improvement although some improvement was seen with losartan and amlodipine (83).

More recently, HOPE study (84), conducted at 267 research centers in 19 countries, assessed the role of an ACE inhibitor, ramipril, in patients at high risk for cardiovascular events but who did not have left ventricular dysfunction (ejection fraction >40%). A total of 9297 patients, age = 55 years, who had evidence of vascular disease or had diabetes plus one other risk factor, were randomized to ramipril 10 mg daily, vitamin E 400 IU daily or placebo. There was a 4.5-year follow up. In this study, ACE inhibition (with ramipril) was associated with a significant risk reduction for cardiovascular death (25%), stroke (31%), revascularization (16%) and new onset of diabetes (32%). A significant decrease in the new onset of diabetes was an unexpected finding and will be further investigated in subsequent studies. It was postulated that beneficial effects may have been mediated by improved insulin sensitivity, a decrease in hepatic clearance of insulin, an anti-inflammatory effect, improved blood flow to the pancreas, or an effect on the abdominal fat.

However, the effects of ACE inhibition on endothelial function in type 2 diabetes and/or insulin resistance are not clear. In a short-term study, O'Driscoll et. al. (85) evaluated the effects of enalapril in 10 subjects with type 2 diabetes. Enalapril increased the endothelium dependent vasodilatory response to acetylcholine and L-NMMA and sodium nitroprusside. In this study, ACE inhibition improved basal and stimulated NO dependent, endothelial function. On the other hand, Bijlstra et. al. (86) were unable to document a beneficial effect on endothelium dependent blood flow in a 6-month study in patients with type 2 diabetes and hypertension. Further studies are needed to clarify the effects of ACE inhibitors on the endothelial function in patients with diabetes mellitus.

SUMMARY AND CONCLUSION

Macrovascular disease is the major cause of morbidity and mortality in patients with type 2 diabetes. Endothelium not only serves as a barrier between the lumen and vessel wall, but also secretes several vasoactive substances. Injury to endothelium plays a critical role in the development of atherosclerosis, a process which occurs prematurely and at an accelerated rate in patients with diabetes. Endothelial dysfunction has been demonstrated in patients with insulin resistance, "prediabetic individuals (i.e. subjects who subsequently became diabetic and in women with a previous history of gestational diabetes), patients with overt type 2 diabetes independent of obesity, as well as in patients with hyperlipidemia and hypertension. Several drugs associated with lowering cardiovascular risk, such as statins and ACE inhibitors have been shown to improve endothelial function and may exert their beneficial effects through their effects on the endothelium.

REFERENCES

1. Eschwège E, Simon D, Balkau B. The growing burden of diabetes in the world population. IDF bulletin 1997; 42: 14-9.
2. The rising global burden of diabetes estimates for the year 2000. Diabetes Atlas 2000, International Diabetes Federation 2000; P. 19-70.
3. American Diabetes Association. Role of cardiovascular risk factors in prevention and treatment of macro-vascular disease in diabetes. Diabetes Care 1993; 16: (Suppl 2) 72-8.
4. Donahue RP, Abbott RD, Reed DM, Yaho K. Post challenge glucose concentration and coronary heart disease in men of Japanese ancestry. Honolulu Heart Program. Diabetes 1987; 36: 689-92.
5. Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12 year cardiovascular mortality in men screened in multiple risk factor intervention trial. Diabetes Care 1993; 16: 434-44.
6. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation 1998; 97: 1837-47.
7. Brezinka V, Padmos J. Coronary heart disease risk factors in women. Eur Heart J 1994; 15: 1571-84.

8. Haffner S, Lehto S, Ronnemma T, Pyorolak K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in non-diabetic subjects with and without prior myocardial infarction. N Engl J Med 1998; 339: 229-34.

9. Olefsky JM. Pathogenesis of insulin resistance and hyperglycemia in non-insulin dependent diabetes mellitus (type 2). *In* Endocrinology 2nd Edition DeGroot LJ, Besser GM, Cahill JC *(eds)*. WB Saunders Co. Philadelphia PA 1989; pp1369-88.

10. Reaven GM. Insulin secretion and insulin action in non-insulin dependent diabetes mellitus: Which defect is primary. Diab Care 1984; 7(Suppl 1) 17-24.

11. Fagan TC, Deedwania PC. The cardiovascular dysmetabolic syndrome. Am J Med 1998; 105 (1A) 775-855.

12. Cosentino F, Luscher TF. Endothelial dysfunction in diabetes mellitus. J Cardiovasc Pharmacology 1998; 32 (Suppl 3) S54-S61.

13. Dyexler H. Factors involved in the maintenance of endothelial function. Am J Cardiol 1998; 82 (10A) 35-45.

14. Quyyumi AA. Endothelial function in health and disease. Am J Med 1998; 105 (1A) S32-S39.

15. Gibbons GH, Dzau VJ. Mechanism of disease: The emerging concept of vascular remodeling. N Eng J Med 1994; 330: 1431-8.

16. Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide function. J Clin Invest 1993; 91: 2546-51.

17. Cybulsky MI, Gimbrone MA. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science 1991; 251: 788-91.

18. Berliner JA, Navab M, Fogelman AM, Frank SJ, Demer LL, Edwards PA, Watson AD, Lusis AJ. Atherosclerosis: basic mechanisms. Oxidation, inflammation and genetics. Circulation 1995; 91: 2488-96.

19. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 288: 373-6.

20. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium derived relaxing factor. Nature 1987; 327: 524-6.

21. Vanhoutte PM, Rubanyi GM, Miller VM, Houston DS. Modulation of vascular smooth muscle contractions by the endothelium. Ann Rev Physiol 1986; 48: 307-20.

22. Vallance P, Collier J, Moncada S. Effect of endothelium derived nitric oxide on peripheral arteriolar tone in man. Lancet 1989; 2: 997-1000.

23. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Eng J Med 1980; 315: 1046-51.

24. Lefroy DC, Crake T, Uren NG, Davis GJ, Maseri A. Effect of inhibition of nitric oxide synthesis on the epicardial coronary artery caliber and coronary blood flow in humans. Circulation 1993; 88: 43-54.

25. Vita JA, Treasure CB, Nabel EG, McLenachan JM, Fish RD, Young AC, Vekshtein VI, Selwyn AP, Ganz P. The coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. Circulation 1990; 811: 491-7.

26. Anderson TJ, Uehata A, Gerard MD et al. Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 1995; 26: 1235-41.

27. Celermajer DS, Sorensen KE, Cooch VM, Spregelhalter GM, Miller OI, Sullivan ID, Lloyd JK, Dramfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk for atherosclerosis. Lancet 1992; 340: 1111-5.

28. Sowers JR. Diabetes mellitus and cardiovascular disease in women. Arch Int Med 1998; 158: 617-21.

29. Schiekofer S, Balletshofer B, Andrassy M, Bierhaus A, Nawroth PP. Endothelial dysfunction in diabetes mellitus. Seminars in Thrombosis and Hemostasis 2000; 26: 503-11.

30. Lewis EJ, Hunsicker LG, Bain RP et. al. The effect of angiotensin converting enzyme inhibition on diabetic nephropathy. N Eng J Med 1993; 329: 1456-62.

31. Deckert T, Feldt-Rasmussen B, Borche Johnsen K et. al. Albuminuria reflects widespread vascular damage, the Steno hypothesis. Diabetologia 1989; 32: 219-26.

32. Neil A, Hawkins M, Potok M, Thorogood M, Cohen D, Mann J. A prospective population based study of microalbuminuria as a predictor of mortality in NIDDM. Diabetes Care 1993; 16: 996-1003.

33. Dinneen SF, Gerstein H. The association of mortality and non-insulin dependent diabetes mellitus – A systematic overview of the literature. Arch Int Med 1997; 157: 1413-8.

34. Dzau VJ. Tissue renin angiotensin system physiologic and pharmacologic implications. Circulation 1988; 77 (Suppl I); 11-3.

35. Johnstone CI. Tissue angiotensin converting enzyme in cardiac and vascular hypertrophy repair and remodeling. Hypertension 1994; 23: 258-68.

36. Hayoz D, Ziegler T, Brunner H, Ruiz J. Diabetes mellitus and vascular lesions. Metabolism 1998; 47: 16-9.

37. Fukui T, Ishizaka N, Rajgopalan S et. al. P^{22} phox mRNA expression and NADPH oxidase activity are increased in aortas from hypertensive rats. Circ Research 1997; 80: 45-51.

38. Sicree RA, Zimmet PZ King HOM, Coventry JS. Plasma insulin response among Naurans: Prediction of deterioration in glucose tolerance over 6 years. Diabetes 1987; 36: 179-86.

39. Haffner SM, Stern MP, Mitchell BD, Hazuda HP, Patterson JK. Incidence of type 2 diabetes in Mexican Americans predicted by fasting insulin and glucose levels, obesity and body fat distribution. Diabetes 1990; 39: 283-8.

40. McPhillips JB, Barrett-Connor E, Wingard DL. Cardiovascular disease risk factors prior to the diagnosis of impaired glucose tolerance and non-insulin dependent diabetes. Am J Epidemiol 1990; 131: 443-53.

41. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK. Cardiovascular risk factors in confirmed pre-diabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes. JAMA 1990; 263: 2893-8.

42. Anastasion E, Lekakis JP, Alevizaki M, Papamichael CM, Megas J, Souvatzoglou A, Stamatelopoulos SF. Impaired endothelium dependent vasodilatation in women with previous gestational diabetes. Diabetes Care 1998; 21: 2111-5.

43. Tooke JE, Goh KL. Editorial. Endotheliopathy precedes type 2 diabetes. Diabetes Care 1998; 12: 2047-9.

44. Ryan EA, Imes S, Liu D, McManus R, Finegood DT, Polonsky KS, Sturis J. Defects in insulin secretion and action in women with a history of gestational diabetes. Diabetes 1995; 44: 506-12.

45. Bjorntorp P. Endocrine abnormalities in obesity. Diabetes Reviews 1997; 5: 52-68.

46. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/Insulin resistance is associated with endothelial dysfunction: Implications for the syndrome of insulin resistance. J Clin Invest 1996; 97: 2601-10.

47. Caballero AE, Arora S, Saouaf R, Lim SC, Smakowski P, Park JY, King GL, LoGerfo FW, Horton ES, Veves A. Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. Diabetes 1999; 48: 1856-62.

48. William SB, Goldfine AB, Timimi FK et.al. Acute hyperglycemia attenuates endothelium dependent vasodilatation in humans in vivo. Circulation 1998; 97: 1695-1701.

49. Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA. Impaired nitric oxide mediated vasodilation in patients with non-insulin dependent diabetes. J Am Coll Cardiol 1996; 27: 567-74.

50. McVeigh GE, Brennan GM, Johnston GD, McDermot BJ, McGrath LT, Henry WR, Andrews JW, Hayes JR. Impaired endothelium dependent and independent vasodilation in patients with type 2 (non-insulin dependent) diabetes mellitus. Diabetologia 1992; 35: 771-6.

51. Hogikyan RV, Galecki AJ, Pitt B, Halter JB, Greene DA, Supano MA. Specific impairment of endothelium dependent vasodilation in subjects with type 2 diabetes independent of obesity. J Clin Endocrinol Metab 1998; 83: 1946-52.

52. Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA. Impaired endothelium dependent vasodilatation in patients with insulin dependent diabetes mellitus. Circulation 1993; 88: 2510-16.

53. Calver A, Collier J, Vallance P. Inhibition and stimulation of nitric oxide synthesis in human forearm arterial blood of patients with insulin dependent diabetes. J Clin Invest 1992; 90: 2548-54.

54. Elliott TG, Cockcroft JR, Groop PH, Viberti GC, Ritter JM. Inhibition of nitric oxide synthesis in forearm vasculature of insulin dependent diabetic patients: Blunted vasoconstriction in patients with microalbuminuria. Clin Sci (Colch)1993; 85: 687-93.

55. Halkin A, Benjamin N, Doktor HS, Todd SD, Viberti G, Ritter JM. Vascular responsiveness and cation exchange in insulin dependent diabetes. Clin Sci (Colch) 1991; 81: 223-32.

56. Smits P, Kapma JA, Jacobs MC, Lutterman J, Thein T. Endothelium dependent vascular relaxation in patients with type 1 diabetes. Diabetes 1993; 42: 148-53.

57. Murakami T, Mizuno S, Ohsata K, Moriuchi I, Arai Y, Nio Y, Kaku B, Takahashi Y, Ohnaka M. Effects of troglitazone on frequency of coronary vasospastic-induced angina pectoris in patients with diabetes mellitus. Am J Cardiol 1999; 84: 92-4.

58. Avena R, Mitchell ME, Nylen ES, Curry KM, Sidway AN. Insulin action enhancement normalizes brachial artery vasoactivity in patients with peripheral vascular disease and occult diabetes. J Vasc Surg 1998; 28: 1024-31.

59. Cominacini L, Garbin U, Fratta Pasini A, Campagnola M, Davoli A, Foot E, Sighieri G, Sironi AM, Locascio V, Ferranini E. Troglitazone reduces LDL oxidation and lowers plasma E selectin concentration in NIDDM patients. Diabetes 1998; 47: 130-3.

60. Tack CJ, Ong MK, Lutterman JA, Smits P. Insulin-induced vasodilatation and endothelial function in obesity/insulin resistance. Effects of troglitazone. Diabetologia 1998; 41: 569-76.

61. Bell PM, Hadden DR. Metformin. Endocrinol Metab Clin North Am 1997; 26: 523-37.

62. Bailey CJ. Biguanides and NIDDM. Diab Care 1992; 15: 755-72.

63. Mather KJ, Verma S, Anderson TJ. Improved endothelial function with metformin in type 2 diabetes mellitus. J Am Coll Cardiol 2001; 37: 1344-50.

64. Laasko M, Edelman SV, Brechtel G, Baron AD. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese men. J Clin Invest 1990; 85: 1844-52.

65. Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark Al. Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. J Clin Invest 1991; 87: 2246-52.

66. Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P. Nitric oxide release accounts for insulin's vascular effects in humans. J Clin Invest 1994; 94: 2511-5.

67. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin mediated skeletal muscle vasodilation is nitric oxide dependent. J Clin Invest 1994; 94: 1172-9.

68. Sobrevia L, Mann GE. Dysfunction of the endothelial nitric oxide signaling pathway in diabetes and hyperglycemia. Exp Physiol 1997; 82: 423-52.

69. Li J, Zhao SP, Lix P, et. al. Non-invasive detection of endothelial dysfunction in patients with essential hypertension. Int J Cardiol 1997; 61: 165-9.

70. Iiyama K, Nagano M, Yo Y et. al. Impaired endothelial function with essential hypertension assessed by ultrasonography. Am Heart J 1996; 132: 779-82.

71. Tamai O, Hidehiro M, Hiroyuki I, Yoshifumi W, Keisuke K, Tsutomu I. Single LDL apheresis improves endothelial dependent vasodilation in hypercholesterolemic humans. Circulation 1997; 95: 76-82.

72. Vogel RA, Correti MC, Plotnick GD. Effect of a single high fat meal on endothelial function in healthy subjects. Am J Cardiol 1997; 79: 350-4.

73. Calles-Escandon J, Cipolla M. Diabetes and endothelial dysfunction: A clinical perspective. Endocrine Reviews 2001; 22: 36-52.

74. Andersen TJ, Meredith IT, Yeung AC, Free B, Selwyn AP, Ganz P. The effect of cholesterol lowering and antioxidant therapy on endothelium dependent coronary vasomotion. N Eng J Med 1995; 332: 488-93.

75. Rosenson RS, Tangney EC. Antithrombotic properties of statins. JAMA 1998; 279: 1643-50.

76. Simons LA, Sullivan D, Simons J, Celermajer DS. Effects of atorvastatin monotherapy and simvastatin plus cholestyramine on hypercholesterolemia. Atherosclerosis 1998; 137: 197-203.

77. Koh KK, Cardillo C, Bui MN et. al. Vascular effects of estrogen and cholesterol lowering therapies in hypercholesterolemic postmenopausal women. Circulation 1999; 94: 345-60.

78. Sheu Wh, Juang BL, Chen YT, Lee WJ. Endothelial dysfunction is not reversed by simvastatin treatment in type 2 diabetic patients with hypercholesterolemia. Diabetes Care 1999; 22: 1224-5.

79. Andrews TC, Whitney EJ, Green G, Kalenian R, Personius BE. Effect of gemfibrozil +/- niacin +/- cholestyramine on endothelial function in patients with serum low-density lipoprotein cholesterol levels <160 mg/dL and high-density lipoprotein cholesterol levels <40 mg/dL. Am J Cardiol 1997; 80(7): 831-5.

80. Evans M, Anderson RA, Graham J, Ellis GR, Morris K, Davies S, Jackson SK, Lewis MJ, Fienneaux MP, Rees A. Ciprofibrate therapy improves endothelial function and reduces postprandial lipemia and oxidative stress in type 2 diabetes mellitus. Circulation 2000; 101: 1773-9.

81. Mancini GBJ, Henry GC, Macaya C, et. al. Angiotensin converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease TREND (Trial on Reversing Endothelial Dysfunction) Study. Circulation 1996; 94: 258-65.

82. Schlaifer JD, Wargovich TJ, O'Neill B, Mancini GV, Haber EV, et. al. Effects of quinapril on coronary blood flow in coronary artery disease patients with endothelial function. Am J Cardiol 1997; 80: 1594-7.

83. Anderson TJ, Elstein E, Haber H, Charbonneau F. Comparative study of ACE-inhibition, angiotensin II antagonist and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF study). J Am Coll Cardiol 2000; 35 (1): 67-70.

84. The HOPE study investigators. Effect of an angiotensin converting enzyme inhibitor, ramipril, on death from cardiovacular causes, myocardial infarction and stroke in high risk patients. N Engl J Med 2000; 342:145-53.

85. O'Driscoll G, Green D, Maiorana A, Stanton K, Colreavy F, Taylor R. Improvement in endothelial function by angiotensin converting enzyme inhibition in non-insulin dependent diabetes mellitus. J Am Coll Cardiol 1999; 33: 1506-11.

86. Bijlstra PJ, Smits P, Lutterman JA, Thien T. Effect of long-term angiotensin-converting enzyme inhibition on endothelial function in patients with the insulin- resistance syndrome. J Cardiovasc Pharmacol 1995; 25 (4): 658-64.