Impaired Endothelium-Dependent Vasodilatation in Women With Previous Gestational Diabetes

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OBJECTIVE — To assess whether otherwise healthy women with a history of gestational diabetes mellitus (GDM) may have abnormalities in endothelial function at a very early stage, before glucose intolerance occurs.

RESEARCH DESIGN AND METHODS — A total of 33 women with previous GDM (17 nonobese [BMI <27] and 16 obese [BMI ≥27]) and 19 healthy nonobese women were examined. A 75-g oral glucose tolerance test was performed, and insulin levels and biochemical parameters were also measured. Using high-resolution ultrasound, we measured vasodilatory responses of the brachial artery during reactive hyperemia (endothelium-dependent vasodilatation), and after nitroglycerin administration, an endothelium-independent vasodilator.

RESULTS — Flow-mediated dilatation (FMD) was significantly and equally decreased in both groups of women with previous GDM, compared with control subjects (1.6 ± 3.7% in the nonobese GDM group and 1.6 ± 2.5% in the obese GDM group vs. 10.3 ± 4.4% in control subjects, $P < 0.001$). FMD correlated inversely with serum uric acid levels, BMI, serum total cholesterol, and basal insulin resistance (homeostasis model assessment). Nitrate-induced dilatation was significantly decreased only in the obese GDM group compared with control subjects (21.4 ± 5.1 vs. 27.9 ± 9.5, $P < 0.05$).

CONCLUSIONS — Endothelial dysfunction, which is considered as a very early index of atherogenesis, is already present in both obese and nonobese women with a history of GDM, even when they have normal glucose tolerance.

Diabetes Care 21:2111–2115, 1998

Pregnancy is characterized by the development of significant insulin resistance which, when superimposed on preexisting defects in insulin action, unmasks even the slightest defects in insulin secretion, resulting in glucose intolerance. Thus gestational diabetes mellitus (GDM) develops, which is considered a prediabetic state (1). In this way, GDM offers the opportunity to study abnormalities that may appear very early in the natural history of NIDDM (2).

It is well known that cardiovascular complications are the principal causes of mortality and morbidity in patients with NIDDM, mainly as a result of an acceleration of atherosclerosis and increased thrombosis (3). One of the first signs in the development of atherosclerosis is endothelial dysfunction. It is thought that abnormal endothelial function could lead to abnormal vasomotor function, increased platelet aggregability, increased monocyte adhesion to the vascular wall, and eventually atherogenesis (4). There is evidence that endothelial function is abnormal in diabetic animal models (5,6) as well as in patients with NIDDM (7).

We conducted this study to assess whether normoglycemic normotensive women with a history of GDM may have abnormalities in endothelial function at a very early stage, even before glucose intolerance occurs. It has been reported that obesity alone may contribute to endothelial dysfunction (8); therefore, both obese and nonobese women were studied.

RESEARCH DESIGN AND METHODS

Patient population

A total of 33 women with a history of GDM were enrolled in the study; of these women, 17 were nonobese (BMI <27) and 16 were obese (BMI ≥27). In the majority of the women, waist-to-hip ratios (WHRs) were also available (Table 1). Obese women with previous GDM had significantly higher WHRs than control subjects ($P = 0.004$); WHRs of nonobese GDM women tended to be higher ($P = 0.075$ vs. control subjects). The American Diabetes Association criteria were used to define GDM (9). All women had been followed up at the Diabetes Clinic of Alexandra University Hospital, Athens, Greece. During pregnancy in the nonobese group, 14 women had been treated with diet only and 3 with diet plus insulin; in the obese group, 8 were treated with diet and 8 with diet plus insulin. The control group included 19 healthy nonobese women (BMI <27) with normal oral glucose tolerance tests (OGTTs) during pregnancy. Characteristics of the women are shown in Table 1. In the vast majority of subjects, endothelial function was evaluated 3–6 months after delivery. The length of time (mean ± SD) of the tests from delivery for the three groups was as follows: nor-

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Received for publication 9 March 1998 and accepted in revised form 18 August 1998.

Abbreviations: FMD, flow-mediated dilatation; GDM, gestational diabetes mellitus; HOMA, homeostasis model assessment; NID, nitrate-induced dilatation; OGTT, oral glucose tolerance test; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Subject characteristics*

	Normal	Nonobese GDM	Obese GDM
n	19	17	16
Age (years)	32.5 ± 4.7	33.8 ± 5.2	38 ± 4.1*†
BMI (kg/m²)	24.2 ± 2.6	24.0 ± 2	31.7 ± 3.6*
Blood pressure (mmHg)			
Systolic	109.1 ± 8.9	106.5 ± 11.1	116.3 ± 12.6†
Diastolic	68.2 ± 7.3	62.9 ± 8.5	73.4 ± 7.5‡
Heart rate (beats/min)	75 ± 9	70 ± 6.6	76 ± 6
Parity	1.6 ± 0.6	1.4 ± 0.6	1.7 ± 0.8
WHR§	0.77 ± 0.06 (14)	0.83 ± 0.09 (15)	0.88 ± 0.08 (10)*

Data are means ± SD. *$P < 0.01$ vs. normal group; †$P < 0.05$ vs. nonobese GDM group; ‡$P < 0.01$ vs. nonobese GDM group; §*n* in parentheses.

mal 6.7 ± 2.1, nonobese GDM 6.6 ± 2.0, obese GDM 7.2 ± 1.9 months (NS). Care was taken that lactation had ceased and the menstrual cycle had resumed. Women were examined at random with respect to phase of the menstrual cycle. All women were healthy and were not taking any medication at the time of the study. Only nonsmoking women were enrolled. Each subject gave informed consent before entering the study.

Biochemical evaluation

On the 1st day, a 75-g OGTT test was performed (World Health Organization criteria), and a biochemical profile taken, which included total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, uric acid (Analyser Falcor, Menarini, Florence, Italy), and glycosylated hemoglobin HbA_{1c} (high-pressure liquid chromatographic method [Hi-Auto A1C Analyser HA-8140 Menarini]). Insulin was measured at baseline and during OGTT by enzyme-linked immunosorbent assay (Boehringer Mannheim, Mannheim, Germany); the intra- and interassay coefficients of variation were 7.8 and 6.4, respectively, at 100 μU/ml. Plasma glucose concentration at baseline and during OGTT was measured with an auto-analyzer (Vision, Abbott, Rockford, IL) using the hexokinase method. To evaluate basal insulin resistance and β-cell function, we used homeostasis model assessment (HOMA) (10), applying the following formulas: insulin resistance = FI × G/22.5 and β-cell function (%) = 20 × FI/(G – 3.5), where FI is fasting insulin (microunits per milliliter) and G is fasting glucose (millimoles per liter).

Brachial artery endothelial function

On the 2nd day, brachial artery endothelial function was tested. Each subject was studied in the morning after abstaining from alcohol, caffeine, and food for 8 h. High-resolution echo-Doppler ultrasound (Acuson 128xP, Mountain View, CA) with a 7.0-MHz transducer was used to measure vasodilator responses of the right brachial artery by a previously validated and reproducible technique (11). The intra- and interobserver variability in our laboratory for repeated measurements of artery diameter are 0.1 ± 0.12 and 0.08 ± 0.19 mm, respectively. Reactive hyperemia studies were performed on two separate days; the mean difference in brachial vasodilator response was 1.1 ± 1%.

In all studies, scans were taken at rest, during reactive hyperemia (an endothelium-dependent stimulus to vasodilatation), again at rest, and after 0.4 mg sublingual nitroglycerin (an endothelium-independent vasodilator). The subject rested quietly for 10 min before the scan; when a satisfactory position was found, the skin was marked, a resting scan was recorded, and arterial flow velocity was measured using a pulsed Doppler signal at a 60° angle in the center of the artery. Blood flow through the brachial artery was altered for 5 min with a blood pressure cuff inflated to 250 mmHg on the forearm ~8 cm distal to the site of brachial artery measurement. After the cuff was deflated, reactive hyperemia occurred; the brachial artery was scanned continuously for 30 s before and 90 s after cuff deflation. After 10 min, a second rest scan was recorded, 0.4 mg sublingual nitroglycerin was given, and 4 min later the brachial artery was imaged.

All images were recorded on videotape and later analyzed by two observers (J.P.L., C.M.P.) who did not know the identity of the subjects, the scan sequence, or the stage of the experiment. Artery diameter measurements were made at end-diastole (R-wave peak of electrocardiogram) using electronic calipers; five cardiac cycles were analyzed and measurements were averaged. Blood flow at baseline and during hyperemia was calculated as the product of velocity and πr^2 ($\pi = 3.14$, r = vessel diameter/2) multiplied by the corresponding heart rate. Flow-mediated dilatation (FMD) was calculated as the percent increase in arterial diameter during hyperemia. Nitrate-induced dilatation (NID) was calculated similarly.

Statistical analysis

Descriptive data are expressed as mean values ± SD. All variables were found to follow a normal distribution. The sample size for FMD was calculated for power 0.95 (assuming a difference in means of ~6 and SD 4.5 from preliminary findings); for this sample size, the power for NID was calculated to be ~58%.

Statistical analysis was performed using analysis of variance; the least significant difference test was used to determine significant differences between any two of the groups. Pearson's bivariate correlation coefficients were computed for the total population.

RESULTS — Metabolic characteristics of the control women and the two groups with previous GDM are shown in Table 2. Total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol were significantly higher in the obese GDM group. Uric acid was significantly higher in both obese and nonobese GDM women. Glycosylated hemoglobin levels were similar in all groups. OGTT was within the normal range in all groups, although glucose concentrations at 30 and 60 min were significantly higher in both GDM groups, and glucose at 90 min was significantly higher in obese GDM women (Fig. 1*A*). Insulin levels at baseline and at 90 and 120 min during the OGTT were significantly higher in the obese GDM group (Fig. 1*B*). Basal insulin resistance, calculated using HOMA, was significantly higher in the obese GDM group when compared with both the normal and the nonobese GDM groups (Table 2).

Vascular studies

Baseline brachial artery diameter (vessel size) was similar in the three groups (Table 3). Baseline and hyperemic velocity and blood flow were comparable in all groups. FMD (i.e., endothelium-dependent dilatation) was 1.6 ± 3.7% in the nonobese GDM group (range −5 to 10%) and 1.6 ± 2.5% in the

Table 2—*Comparison of metabolic characteristics between control subjects and women with a history of GDM*

Variable	Normal	Nonobese GDM	Obese GDM
n	19	17	16
Cholesterol (mmol/l)	4.8 ± 0.7	5.5 ± 0.8	5.8 ± 1.4*
Triglycerides (mmol/l)	0.84 ± 0.47	0.96 ± 0.35	1.62 ± 0.94†‡
HDL cholesterol (mmol/l)	1.6 ± 0.4	1.9 ± 0.3	1.5 ± 0.5§
LDL cholesterol (mmol/l)	2.4 ± 0.6	2.6 ± 0.7	3.3 ± 1.7*
Uric acid (mmol/l)	172.5 ± 23.8	220 ± 36†	244 ± 42†
HbA_{1c} (%)	4.3 ± 0.4	4.4 ± 0.4	4.6 ± 0.5
Insulin resistance	2.77 ± 0.95	3.43 ± 1.56	4.64 ± 2.05†§
β-Cell function (%)	1.7 ± 0.6	1.79 ± 0.79	2.41 ± 1.51

Data are means ± SD. *$P < 0.05$, †$P < 0.01$ vs. normal group; ‡$P < 0.01$, §$P < 0.05$ vs. nonobese group.

obese GDM group (range −3 to 7%), which is significantly lower ($P < 0.001$) compared with control subjects (10.3 ± 4.4%, range 3–19%). It has to be noted that 7 of the 33 GDM women (4 obese and 3 nonobese) were examined longer than 1 year after delivery; their FMD was 0.86 ± 1.46, not different from the whole group. NID (i.e., endothelium-independent dilatation) was significantly decreased in the obese GDM group compared with control subjects and nonobese GDM women. To exclude the possibility that this difference could be due to the older age and the higher blood pressure of obese GDM women, we carried out a simple factorial analysis of variance of NID with diagnosis (normal or GDM) as the main effect and BMI, age, and blood pressure as covariates. The main effect of diagnosis on NID was not significant. The effects of the above covariates were examined before the main effect, and only the effect of BMI was found to be significant ($P < 0.05$).

The bivariate correlations of FMD with several variables are shown in Table 4. FMD was inversely correlated with serum uric acid concentration, BMI, serum total cholesterol, and basal insulin resistance. NID was inversely correlated only with BMI ($r = -0.39$, $P < 0.01$).

In a subgroup of eight GDM women (four obese and four nonobese), vascular studies were repeated at a later stage (13 ± 1 months after delivery). There was no significant difference between the first and second measurements in either FMD (1.75 ± 2.5 vs. 1.8 ± 2.1%) or NID (23 ± 2.5 vs. 24.5 ± 6.0%).

CONCLUSIONS — The present study showed that FMD is impaired in the large systemic arteries of both obese and nonobese women with previous GDM, a finding not previously reported. FMD is known to depend on the ability of the endothelium to release nitric oxide in response to shear stress (12) and can be used reliably as an estimate of endothelial function in various disease states. Peak reactive hyperemia was not different between GDM women and control subjects; however, we cannot preclude an impairment of vasodilatation of the small resistance vessels, since flow debt repayment after ischemia was not measured in this study.

It is known (13,14), and was further confirmed in this study, that women with normal glucose tolerance and a history of GDM (especially those who are obese) have impaired insulin sensitivity. A plausible explanation for the early endothelial dysfunction in GDM women could be insulin resistance. It has been suggested that there is an inverse relationship between insulin sensitivity and endothelial function (15); this finding was further confirmed with the rough estimation of basal insulin resistance used in this study. Furthermore, it has recently been reported (16) that the phosphatidylinositol 3-kinase signaling pathway

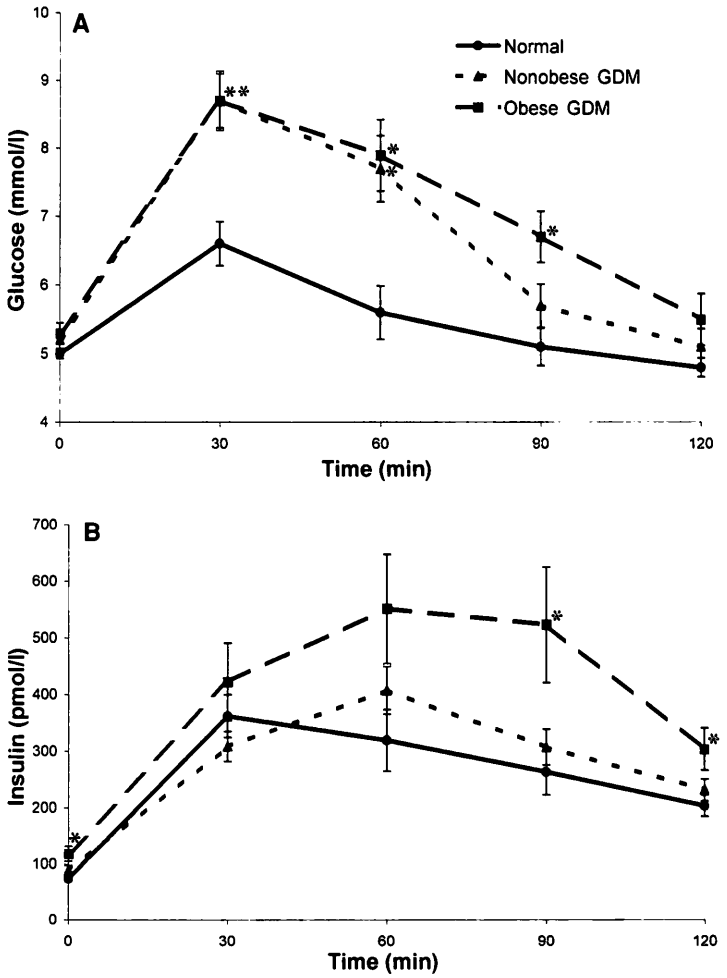

Figure 1—*Serum concentrations (mean ± SEM) of glucose (A) and insulin (B) during the 75-g OGTT in the three groups (*P < 0.05, **P < 0.01 vs. normal group).*

Table 3—*Comparison of vascular studies between control subjects and women with a history of GDM*

Variable	Normal	Nonobese GDM	Obese GDM
n	19	17	16
Vessel size (mm)	3 ± 0.5	3 ± 0.4	3.2 ± 0.5
FMD (%)	10.3 ± 4.4	1.6 ± 3.7*	1.6 ± 2.5*
NID (%)	27.9 ± 9.5	26.2 ± 7.8	21.4 ± 5.1†
Baseline velocity (m/s)	0.24 ± 0.13	0.25 ± 0.11	0.25 ± 0.16
Hyperemic velocity (m/s)	0.66 ± 0.18	0.63 ± 0.22	0.61 ± 0.16
Baseline flow (ml/min)	111 ± 43	129 ± 71	130 ± 57
Hyperemic flow (ml/min)	432 ± 159	365 ± 204	405 ± 139

Data are means ± SD. *$P < 0.001$, †$P < 0.05$ vs. normal group.

in insulin action may be common for the production of nitrous oxide in the endothelium and for glucose uptake in muscle cells.

It has been reported that obesity per se is associated with endothelial dysfunction (8). This was further confirmed in this study: a significant linear negative correlation between FMD and BMI was found. However, nonobese GDM women had a degree of endothelial dysfunction similar to that of obese GDM women. We assume that this subgroup of nonobese GDM women probably present a slight decrease of insulin sensitivity not clearly detectable with the rough method employed in this study, which uses plasma insulin concentrations as a surrogate measure of insulin sensitivity. Furthermore, it is known that there is a wide range of insulin sensitivity in both lean and obese normal individuals detected with the use of more sophisticated methods such as the euglycemic-hyperinsulinemic clamp; some of these values even overlap with the values of the diabetic population (17,18). The trend toward a higher WHR and the significantly higher uric acid levels found in the group of nonobese GDM women support the presence of some degree of insulin resistance in those patients. Uric acid levels have indeed been associated with insulin resistance (19,20). It is interesting that the most significant inverse correlation of endothelium-dependent vasodilatation found in this study was with serum uric acid levels, which were in the normal range.

Table 4—*Pearson's bivariate correlation coefficients of FMD with other variables*

Variable	*R*	*P*
Uric acid	−0.63	<0.001
BMI	−0.34	<0.05
LDL cholesterol	−0.20	NS
HDL cholesterol	0.16	NS
Total cholesterol	−0.31	<0.05
Triglycerides	−0.19	NS
Insulin resistance	−0.32	<0.05

Other factors that have been independently demonstrated to attenuate endothelium-dependent vasodilatation include hypertension (21), total cholesterol, and LDL cholesterol (22). In this study, plasma total cholesterol, triglycerides, and LDL cholesterol concentrations were significantly different only in obese GDM women compared with control subjects. FMD showed a significant inverse continuous correlation with total cholesterol, although within the normal range, as has been reported (8).

A moderate impairment of nitrate-induced dilatation, which correlated with obesity, was observed in the obese GDM group, a finding different from that reported in another study, where nondiabetic obese subjects were studied with a different method (8). We cannot exclude the possibility that there is a concomitant dysfunction of the vascular smooth muscle in these subjects, such as has been reported in other groups affected by atherogenic risk factors such as smoking, hypercholesterolemia, or diabetes (7,23).

Finally, the repeated studies performed after a 1-year period in some of our patients showed similar results for both FMD and NID, suggesting that endothelial dysfunction in GDM women is a consistent finding.

The findings of this study support the conclusion that even a prediabetic state such as GDM may be—independently of obesity—one of the conditions associated with endothelial dysfunction and possible increased risk for atherogenesis (24,25). Restoration of endothelial function has important clinical implications for reducing the risk of vascular disease in this population. Therefore, eliminating other risk factors for endothelial dysfunction, such as inactivity, obesity, hypercholesterolemia, and smoking, could be very important. Finally, these findings provide a further valid reason for the necessity to screen women during pregnancy, especially for the identification of the subgroup of nonobese women who, according to the results reported here, may have a risk of developing cardiovascular disease equal to that of obese women.

References

1. Buchanan T, Catalano P: The pathogenesis of GDM: implication for diabetes after pregnancy. *Diabetes Rev* 3:584–601, 1995
2. Pendegrass M, Fazioni E, DeFronzo R: NIDDM and GDM: same disease, another name? *Diabetes Rev* 3:566–583, 1995
3. Zimmet PZ, Alberti KGMM: The changing faces of macrovascular disease in non-insulin dependent diabetes mellitus: an epidemic in progress. *Lancet* 350 (Suppl.):1–4, 1997
4. Ross R: The pathogenesis of atherosclerosis: an update. *N Engl J Med* 8:488–500, 1986
5. Durante W, Sen AK, Sunahara FA: Impairment of endothelium dependent relaxation in aortae from spontaneously diabetic rats. *Br J Pharmacol* 94:463–468, 1988
6. Mayhan WG: Impairment of endothelium-dependent dilatation of cerebral arterioles during diabetes mellitus. *Am J Physiol* 256:H621–H625, 1989
7. Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA: Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 27:567–574, 1996
8. 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* 97:2601–2610, 1996
9. National Diabetes Data Group: Classification and diagnosis of diabetes and other categories of glucose intolerance. *Diabetes* 18:1039–1057, 1979
10. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
11. Celermajer DS, Sorensen KE, Gooch VM, Spregelhalter GM, Miller OI, Sullivan ID, Lloyd JK, Dranfield JE: Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 340:1111–1115, 1992

12. Joannides, R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, Luscher TF: Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation* 91:1314–1319, 1995
13. Catalano PM, Berstein IM, Wolfe RR, Srikanta S, Tyzbir E, Sims EAH: Subclinical abnormalities of glucose metabolism in subjects with previous gestational diabetes. *Am J Obstet Gynecol* 155:1255–1263, 1986
14. Ryan EA, Imes S, Liu D, McManus R, Finegood DT, Polonski KS, Sturis J: Defects in insulin secretion and action in women with a history of gestational diabetes. *Diabetes* 44:506–512, 1995
15. Petrie JR, Ueda S, Webb DJ, Elliott HL, Connell JM: Endothelial nitric oxide production and insulin sensitivity: a physiological link with implications for pathogenesis of cardiovascular disease. *Circulation* 93:1331–1333, 1996
16. Hsueh WA, Quinines MJ, Creager MA: Endothelium in insulin resistance and diabetes. *Diabetes Rev* 5:343–352, 1997
17. Ferranini E, Natali A, Bell P, Cacallo-Perin P, Lalic N, Migrone G: Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR). *J Clin Invest* 100:1166–1173, 1997
18. American Diabetes Association: Consensus Development Conference on Insulin Resistance: 5–6 November 1997. *Diabetes Care* 21:310–314, 1998
19. Facchini F, Chen YD, Hollenbeck CB, Reaven GM: Relationship between resistance to insulin-mediated glucose uptake, urinary uric acid clearance, and plasma uric acid concentration. *JAMA* 266:3008–3011, 1991
20. Vuorinen-Markkola H, Yki Jarvinen H: Hyperuricemia and insulin resistance. *J Clin Endocrinol Metab* 78:25–29, 1994
21. Panza JA, Quyyumy A, Brush JE, Epstein SE: Abnormal endothelium dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 323:22–27, 1991
22. Creager MA, Cooke JP, Mendelsohn MP, Gallagher SJ, Coleman SM, Loscalzo J, Dzau VJ: Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. *J Clin Invest* 86:228–234, 1990
23. Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J, Deanfield JE: Cigarette smoking is associated with dose related and potentially reversible impairment of endothelium dependent dilatation in healthy young adults. *Circulation* 88:2149–2155, 1993
24. Vita JA, Treasure CB, Nabel EG, McLenachan JM, Fish RD, Yeung AC, Vekshtein VI, Selwyn AP, Ganz P: Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. *Circulation* 81:491–497, 1990
25. Zeiher AM, Drexler H, Wollschlager H, Just H: Modulation of coronary vasomotor tone in humans. Progressive endothelial dysfunction with different early stages of coronary atherosclerosis. *Circulation* 83:391–401, 1991