Endothelial-Dependent Vasodilation and Incidence of Type 2 Diabetes in a Population of Healthy Postmenopausal Women

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OBJECTIVE — Both postmenopausal state and diabetes are associated with endothelial dysfunction and are well-known risk factors for atherosclerosis. However, the relationship of endothelium-dependent vasodilation and diabetes has never been prospectively evaluated. This study provided the opportunity to assess the association between endothelial vasodilation function and the incidence of diabetes in a cohort of apparently healthy postmenopausal women.

RESEARCH DESIGN AND METHODS — We conducted a prospective cohort study that began in 1997 with 840 apparently healthy, nonobese, postmenopausal women, aged 53 ± 6 years, initially with normal glucose tolerance at the oral glucose tolerance test. All participants were followed up for a mean period of 3.9 ± 0.7 years (range 0.5–6.9). Endothelial function was measured as flow-mediated dilation (FMD) of the brachial artery, using high-resolution ultrasound.

RESULTS — There were no significant differences in demographic, blood pressure, and biochemical profiles among each tertile group at baseline or at follow-up review. During follow-up, 102 women developed type 2 diabetes. The adjusted relative risk (RR) for women with FMD ≤4.3 (lowest tertile) was 5.87 (95% CI 4.34–8.10) versus women with FMD ≥5.6 (highest tertile reference). Each 1-unit decrease of FMD was associated with a significant 32% (22–48%) increase in the multiple-adjusted RR of incident diabetes.

CONCLUSIONS — These prospective data indicate a significant increase in the RR of diabetes with each unit decrease of FMD. This could suggest that an impaired endothelial function may play a fundamental role in diabetogenesis in postmenopausal women.

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Postmenopause is a physiological condition known to be associated with endothelial dysfunction. It is due to a lack of estrogen that is typical in this phase of a woman's life (1,2). There is considerable evidence that the impairment of endothelial function is a predicting factor in the development of atherosclerosis (3).

Type 2 diabetes represents a very important public health problem in all industrialized countries, mainly in the U.S. (4,5), that involves conspicuous cardiovascular consequences and high costs in terms of mortality, morbidity, and financial resources (6). Endothelial dysfunction is a very frequent occurrence among diabetic patients (7–10).

Because endothelial dysfunction is also present in nondiabetic postmenopausal women (1,2), it is not clear whether endothelial dysfunction is a consequence or rather the cause of diabetes, thus preceding its onset. Although there are both references relating to the fact that endothelial dysfunction may precede insulin resistance (11,12) and unique recent work concerning the relationship between the spillover markers of endothelial dysfunction and incident diabetes (13), a clear relationship between endothelium-dependent vasodilation and diabetes has, to our knowledge, never been demonstrated. This study provided the opportunity to prospectively assess the association between endothelial vasodilation function, evaluated by ultrasound study of the brachial artery, and the incidence of diabetes among apparently healthy initially nondiabetic postmenopausal women.

RESEARCH DESIGN AND METHODS

— Patients enrolled in the study were selected from women referred to the Bene Essere Donna center, an institution dedicated to the study of menopause-related disorders. This center is open to all postmenopausal women aged ≤60 years. Menopause was defined as the absence of menstruation from >6 months or a plasma level of 17β-estradiol <120 pmol/l and/or follicle-stimulating hormone >40 IU/l.

At baseline, each participant underwent fasting blood testing for levels of total cholesterol and triglycerides as well as a 75-g oral glucose tolerance test (OGTT), as required by our protocol. Participants were eligible for this study if they had a normal OGTT (fasting plasma glucose <110 mg/dl and 2-h plasma glucose <140 mg/dl) (14). Physical examination

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Abbreviations: eNOS, endothelial nitric oxide synthase; FMD, flow-mediated dilation; MAPK, mitogen-activated protein kinase; OGTT, oral glucose tolerance test; PI3K, phospatidylinositol 3-kinase.

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

variables measured at baseline included body weight, waist circumference, and systolic and diastolic blood pressure. Patient history, 12-lead electrocardiogram, and echocardiogram were used to exclude past or present cardiac diseases. At baseline, all women underwent a vascular reactivity test on the brachial artery to measure the endothelium-dependent vasodilatation using a noninvasive ultrasound method. Participants provided questionnaire data concerning lifestyle practices and potential risk factors for cardiovascular disease.

Hypertension (blood pressure ≥140/90 mmHg or use of antihypertensive medications), hyperlipidemia (total cholesterol plasma levels ≥200 mg/dl and/or triglyceride levels ≥170 mg/dl), smoking habits, and obesity (BMI ≥30 kg/m^2) were the exclusion criteria of the study; women treated with hormone replacement therapy were also excluded.

A total of 840 healthy postmenopausal women (mean age 53 ± 6 years) with normal glucose tolerance satisfied the above-mentioned criteria. Enrollment started in 1997, and the results were finally elaborated at the end of April 2004, when the last women to enroll had been followed for at least 0.5 years; the mean follow-up was 3.9 ± 0.7 years (range 0.5–6.9). All participants gave written informed consent to participate in this prospective study, which was approved by the ethics committee of the University of Modena and Reggio Emilia.

Ultrasound study of the brachial artery

The technique for assessing brachial artery flow-mediated dilation (FMD) has been described in detail elsewhere (15–18). Briefly, FMD was assessed in the subject's right arm in the recumbent position after a 15-min equilibration period in a temperature-controlled room (22–25°C) by means of a Acuson 128 XP/10 mainframe (Acuson, Mountain View, CA) with a 7.0-MHz linear array transducer. The brachial artery was longitudinally imaged ~5 cm proximal to the antecubital crease, where the clearest image was obtained, and the diameter at end-diastole was measured (the mean of three measurements was used in the analysis). A pressure cuff, placed on the forearm (proximal to the target artery), was inflated until no blood flow was detected through the brachial artery with the Doppler probe. After 5 min, the cuff was released, and this was followed by an increase in blood flow. This phenomenon increased shear stress, which served as the stimulus to induce vasodilation. After cuff release, the diameter of the brachial artery was measured at 45, 60, 90, 180, and 300 s. The maximum diameter in any of these measurements was used in the calculation of FMD according to the following formula: (maximum diameter during reactive hyperemia − diameter at baseline)/diameter at baseline × 100%.

Measurement reproducibility was assessed in 50 randomized women who were examined twice, 1 h apart, by two different investigators. The intraobserver intersession coefficient of variation (CV) (evaluated as the SD of the mean difference/$\sqrt{2}$ × pooled mean) (19) was 3.3% for basal brachial artery diameter and 12.4% for FMD.

Definition and ascertainment of cases

All women were seen in our outpatient clinics at regular intervals (every 6 months). Telephone contact was used every 3 months to reduce the drop-out rate. At baseline and every 6-month follow-up visit, women underwent an interview, an examination, and blood collection. Fasting serum glucose measurements were performed at each visit. New cases of diabetes were identified in accordance with the criteria used in the Atherosclerosis Risk in Communities (ARIC) study (20,21), as follows: *1*) self-reported use of hypoglycemic medications, *2*) fasting (>8 h) serum glucose level >126 mg/dl, *3*) nonfasting serum glucose level >200 mg/dl, or *4*) self-report of physician diagnosis. For individuals classified by physician diagnosis or medication use, the date of the onset of diabetes was considered to be the midpoint between the last visit when a woman was not diabetic and the first visit when a woman was diabetic. For those diagnosed by fasting or nonfasting glucose level, the date of the onset of diabetes was the estimated date at which blood glucose levels crossed the above-mentioned threshold, assuming a linear increase in glucose levels between visits. All of the patients with diabetes were telephoned in August through September 2004 in order to avoid false diagnosis of diabetes.

Statistical analysis

Descriptive statistics were tabulated as the mean ± SD or frequency percentage. Differences in baseline characteristics between the groups were examined by an ANOVA and χ^2 test, when appropriate. We used the Cox proportional hazards regression model to analyze the association between endothelial function and incident diabetes. Person-time was calculated from enrollment until the date of diabetes onset, death, drop out, or the end of the study, whichever occurred first. FMD was evaluated in the following two ways: divided into tertiles and as continuous term. We computed crude and multiple-adjusted hazard ratios (and 95% CIs) as a measure of the relative risk (RR) of incident diabetes for decreasing FMD, with the highest tertile as the reference. Adjusted estimates of risk were calculated by use of the Cox proportional hazards regression model, which controlled for a range of potential confounders, including age (continuous), family history of diabetes (yes/no), duration of postmenopausal period (continuous), alcohol consumption (never, 1–5 drinks per month, 2–6 drinks per week, and ≥1 drink per day), physical activity (never, 1–2 times per week, and ≥3 times per week), baseline systolic and diastolic blood pressure (continuous), BMI (continuous), waist circumference (continuous), fasting plasma levels of glucose, and total cholesterol and triglycerides (continuous), as well as the changes from baseline to the last follow-up visit (continuous). A two-sided *P* value <0.05 was considered statistically significant.

RESULTS — A total of 840 women were followed for 3.9 ± 0.7 years (3,252 person-years). During follow-up, 102 incident cases of diabetes occurred (equivalent of 7.9 cases/1,000 women-years). Of these, 58 of 102 (56.9%) patients were diagnosed by their own personal physician, 41 of 102 (40.2%) patients were diagnosed at our clinic, and 3 (2.9%) patients stated that they were taking hypoglycemic drugs. All diabetic patients were informed by telephone, and none died. All of them were being administered at least one hypoglycemic drug; of 102 patients, 27 (26.5%) were taking an association of two hypoglycemic drugs and only 6 (5.9%) were being treated with insulin. Six patients among the general population died during follow-up, three from

Table 1—*Baseline characteristics of the study participants according to tertiles of endothelial-dependent FMD of the brachial artery*

	3rd tertile	2nd tertile	1st tertile
n	280	280	280
Levels of FMD (%)	≥5.6	4.4–5.5	≤4.3
Median of FMD (%)	6.2	5.0	3.9*
Brachial artery diameter (mm)	3.99 ± 0.57	3.97 ± 0.58	3.98 ± 0.60
Age (years)	53 ± 7	52 ± 6	54 ± 5
BMI (kg/m^2)	26.4 ± 3.0	26.3 ± 2.8	26.2 ± 2.9
Waist circumference (%)	83 ± 13	82 ± 16	82 ± 14
Family history of diabetes (%)	35.3 (*n* = 99)	35.0 (*n* = 98)	33.9 (*n* = 95)
Systolic blood pressure (mmHg)	126 ± 12	125 ± 14	126 ± 13
Diastolic blood pressure (mmHg)	83 ± 7	83 ± 7	82 ± 8
Time from menopause (months)	28 ± 11	25 ± 10	30 ± 11
Exercise frequency			
Never (%)	51.8 (*n* = 145)	47.1 (*n* = 132)	48.2 (*n* = 135)
1–2 times/week (%)	40.0 (*n* = 95)	35.7 (*n* = 100)	36.4 (*n* = 102)
≥3 times/week (%)	8.2 (*n* = 40)	17.2 (*n* = 48)	15.4 (*n* = 43)
Alcohol consumption			
Never (%)	36.4 (*n* = 102)	35.7 (*n* = 100)	37.5 (*n* = 105)
>1 drink/day (%)	8.2 (*n* = 23)	8.9 (*n* = 25)	7.2 (*n* = 20)
2–6 drinks/week (%)	21.4 (*n* = 60)	25.0 (*n* = 70)	31.4 (*n* = 88)
1–5 drinks/month (%)	34.0 (*n* = 95)	30.4 (*n* = 85)	23.9 (*n* = 67)
Biochemical profile			
Fasting total cholesterol (mg/dl)	189 ± 19	190 ± 17	188 ± 19
Fasting triglycerides (mg/dl)	149 ± 16	148 ± 19	154 ± 16
Plasma glucose (mg/dl)			
Basal	88 ± 8	87 ± 8	85 ± 9
2-h	121 ± 9	119 ± 12	121 ± 11

Data are means ± 1 SD or %. *$P < 0.0001$.

non–cardiac-related causes (one accident and two neoplasm) and three from acute myocardial infarction. We lost contact with four patients.

Table 1 gives the baseline characteristics of the participants according to FMD tertiles. No significant differences were registered across tertiles. In Table 2, the changes of the parameters detected both at baseline and during follow-up are shown. Only systolic blood pressure revealed a significant increase in every tertile considered. Nevertheless, we found no significant differences in the intertertile comparisons.

The RRs of diabetes, according to FMD tertiles, are summarized in Table 3. The RR decreased steadily across FMD categories compared with the reference tertile (FMD ≥5.6%). Adjustment for age and various confounders attenuated the RR only slightly. When FMD was examined as a continuous variable, each 1-unit decrease of FMD was associated with a significant 32% (95% CI 22–48%) increase in the multiple-adjusted RR of incident diabetes.

CONCLUSIONS — The results of our prospective study clearly demonstrate that endothelial function significantly influences the future development of diabetes, independently of age and several other well-known diabetes risk factors. In our opinion, this is a very important tool because it radically changes the way endothelial dysfunction is considered. Endothelial dysfunction is usually explained as being the consequence of the endothelium being exposed to damaging factors, e.g., high blood pressure, high cholesterol, high blood glucose, smoking, etc.—the response-to-injury theory (3). Our data revolutionize the concept because they indicate that endothelial dysfunction may influence the development of diabetes. The present results have been obtained by studying a population of postmenopausal women who represent a unique model of studying endothelial dysfunction consequences. In fact, the decrease in estrogens that physiologically follows menopause does in itself compromise the endothelial function in women, even in the absence of other cardiovascular risk factors (1,2).

The current data support the conclusions of Meigs et al. (13), who demonstrated, in the large cohort of postmenopausal women examined in the Nurses'

Table 2—*Changes in clinical and biochemical parameters detected at baseline and during follow-up*

	3rd tertile (*n* = 280)		2nd tertile (*n* = 280)		1st tertile (*n* = 280)	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Clinical parameters						
Systolic blood pressure (mmHg)	126 ± 12	131 ± 14*	125 ± 14	132 ± 13*	126 ± 13	130 ± 14*
Diastolic blood pressure (mmHg)	83 ± 7	84 ± 8	83 ± 7	84 ± 7	82 ± 8	84 ± 7
BMI (kg/m^2)	26.4 ± 3.0	26.9 ± 2.8	26.3 ± 2.8	27.1 ± 2.7	26.2 ± 2.9	27.0 ± 3.0
Waist circumferences (cm)	83 ± 13	85 ± 12	82 ± 16	84 ± 13	82 ± 14	85 ± 15
Biochemical parameters (fasting plasma levels)						
Total cholesterol (mg/dl)	189 ± 19	190 ± 21	190 ± 17	193 ± 16	188 ± 19	192 ± 19
Triglycerides (mg/dl)	149 ± 17	151 ± 15	148 ± 19	150 ± 17	154 ± 16	153 ± 19
Glucose (mg/dl)	88 ± 8	90 ± 8	87 ± 8	91 ± 7	85 ± 9	89 ± 8

Data are means ± 1 SD. *$P < 0.05$ vs. baseline. No intertertile significant differences.

Table 3—*Crude and multiple-adjusted RR of incident diabetes by tertiles of endothelial-dependent FMD of the brachial artery*

	3rd tertile (referent)	2nd tertile	1st tertile
Levels of FMD (%)	≥5.6	4.4–5.5	≤4.3
Median FMD (%)	6.2	5.0	3.9
Incident diabetes (no. of cases)	9	35	58
Person-years	1,132	1,112	1,008
Incidence rate (no. of cases per 1,000 person-years)	2.0	7.9	14.4
Crude RR (95% CI)	1.00	3.95 (2.85–8.21)	7.20 (5.88–12.6)*
Mutiple-adjusted RR (95% CI)†	1.00	2.85 (2.14–5.10)	5.40 (3.35–7.99)*

*$P < 0.0001$. †Cox proportional hazards regression model adjusted for age (continuous); family history of diabetes (yes/no); level of plasma glucose (continuous); BMI (continuous); waist circumference (continuous); duration of postmenopausal period (continuous); alcohol consumption (never, 1–5 drinks/month, 2–6 drinks/week, ≥1 drink/day); physical activity (never, 1–2 times/week, ≥3 times/week); baseline systolic and diastolic blood pressure (continuous); BMI (continuous); waist circumference (continuous); and fasting plasma levels of glucose, total cholesterol, and triglycerides (continuous) and their changes from baseline to the last follow-up visit (continuous).

Health Study, that elevated plasma levels of molecular biomarkers of endothelial dysfunction were significant predictors of incident diabetes. The difference between this and the present study is that endothelial function has been evaluated, in our experience, directly on a large conduit artery rather than indirectly by evaluating the concentration of spillover markers whose overproduction reveals a suffering of the endothelial cells in the capillary and arteriolar microcirculation. The concentration of the previously mentioned biomarkers shows a modest but also meaningful correlation with the endothelial function assessed by brachial artery FMD (22,23). The data of our study are, therefore, in line with the hypothesis that endothelial dysfunction, however it is determined and independent of which vascular district is involved, is always associated with a meaningful risk of developing diabetes in postmenopausal women.

The explanation of this has not been completely clarified and is still a focus of research. One of the most interesting hypotheses, in our opinion, is that of Pinkney et al. (11), who identified the endothelium as the principal controlling factor of insulin concentrations in the interstitium and, therefore, the amount of insulin effectively reaching the target cells. This hypothesis considers the endothelium as a "barrier," which, when dysfunctional, limits the contact between the insulin and the insulin-sensitive cells. In other words, normal endothelial function would be a fundamental prerequisite for normal insulin action, and therefore endothelial dysfunction would be accompanied by a progressive insulin resistance. Moreover, experimental and clinical studies suggest that a major determinant of insulin effectiveness is the *trans* endothelium insulin transport (24,25). Contrarily, studies in humans and animals and in vitro experiences have generated many hypotheses on the possible link between insulin resistance and endothelial dysfunction. Insulin stimulates NO production in cultured endothelial cells extracted from human umbilical vein (26). However, insulin is a vasodilator that stimulates endothelial NO production in humans (27,28). Stimulation of NO production by insulin is mediated by signaling pathways involving phospatidylinositol 3-kinase (PI3K) and activation of endothelial NO synthase (eNOS) (29,30) and mitogen-activated protein kinase (MAPK) (31). These data provide a molecular basis for the dependency of insulin action on endothelial function or vice versa. In vitro, inhibition of PI3K blocks the ability of insulin to stimulate increased expression of eNOS and increases the expression of vascular cellular adhesion molecule-1 and E-selectin, as well as the rolling interaction of monocytes with endothelial cells (30). The MAPK pathway appears to mediate not only the ability of monocytes to migrate, but also the expression of the prothrombotic, profibrotic factor plasminogen activator inhibitor-1 (32).

It is well known that FMD induced by reactive hyperemia is endothelium dependent (33,34); in other words, it depends on the ability of the endothelium to produce vasodilator-endowed substances, mainly NO. It is well demonstrated that postmenopausal status is associated with a significant reduced arterial NO activity (35). It is reasonable to conclude that our patients with a lower level of FMD had worse endothelial dysfunction, implying a lower production of NO. Some important biological phenomenon, such as the glucose uptake from the insulin target cells and the production of glucose-stimulated insulin secretion, are influenced by mechanisms involving the production of NO from the endothelial cells (36,37). A mutation of the eNOS able to determine a lower production of NO is associated, in fact, with a reduction in glucose uptake, hyperlipidemia, and hypertension in animals (36), and the administration of an inhibitor of the eNOS leads to a significant reduction in glucose uptake in humans (37). On the other hand, it is well known that the activity of glucokinase, the enzyme that plays a key role in glucose-stimulated insulin secretion from pancreatic β-cells, is controlled in its action from endothelium-derived NO (38). These data explain why drugs able to improve endothelial function, such as pravastatin and ramipril, are also able to reduce the risk of developing diabetes (39,40). For the same reason, some insulin-sensitizing drugs, such as troglitazone and metformin, even when used in patients without diabetes, improve endothelium-dependent FMD and reduce the risk of developing diabetes (41,42).

Our study has some limitations in that it is an observational, nonrandomized study. For this reason, results must be considered with caution. We cannot exclude, for example, that endothelial dysfunction may be simply an epi-phenomenon, resulting from a hidden confounding etiological cause, leading to both diabetes and endothelial dysfunction. However, our study presents several strengths, including the prospective design and the large and homogeneous cohort. All of these factors encourage us to hypothesize a causal link between impairment of endothelium-dependent vasodilation and incident diabetes.

In conclusion, our data support the idea that endothelial dysfunction is a fundamental step in the diabetogenesis process. Today it is possible to study, in a noninvasive way, the endothelial function

through ultrasound. This allows us to prospectively identify those patients with a poor endothelium-dependent FMD. The latter should be regarded as a marker of diabetes risk in postmenopausal women. Close follow-up and "aggressive" management of other diabetic risk factors should be justified in these subjects.

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References

1. Taddei S, Virdis A, Ghiadoni L, Mattei P, Sudano I, Bernini G, Pinto S, Salvetti A: Menopause is associated with endothelial dysfunction in women. *Hypertension* 28: 576–582, 1996
2. Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE: Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol* 24:471–476, 1994
3. Ross R: The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362:801–809, 1993
4. National Diabetes Data Group: Summary. In *Diabetes in America*. 2nd ed. Harris MI, Cowie CC, Stern MP, Boyko EJ, Reiber GE, Bennett PH, Eds. Bethesda, MD, National Institute of Health, National Institute of Diabetes and Digestive and Kidney Disease, 1995, p. 1–13 (DHHS publ. no. 95-1468)
5. Harris MI, Flegal KM, Cowie CC, Eberhard MS, Goldstein DE, Little RR, Wieldmeyer HM, Byrd-Holt DD: Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults: the Third National Health and Nutrition Survey, 1988–1994. *Diabetes Care* 21: 518–524, 1998
6. Manson J: Risk modification in the diabetic patients. In *Prevention of Myocardial Infarction*. Manson J, Ridker P, Gaziano J, Hennekens C, Eds. New York, Oxford University Press, 1996, p. 241–273
7. Cosentino F, Luscher FA: Endothelial dysfunction in diabetes mellitus. *J Cardiovasc Pharmacol* 32 (Suppl. 3):S54–S61, 1998
8. Steinberg HO, Paradisi G, Cronin J, Crowde K, Hempfling A, Hook J, Baron AD: Type 2 diabetes abrogates sex differences in endothelial function in premenopausal women. *Circulation* 101:2040–2046, 2000
9. Ihlemann N, Stokholm KH, Eskildsen PC: Impaired vascular reactivity is present despite normal levels of von Willebrand factor in patients with uncomplicated type 2 diabetes. *Diabet Med* 19:476–481, 2002
10. Tan KC, Chow WS, Ai VH: Advanced glycation end products and endothelial dysfunction in type 2 diabetes. *Diabetes Care* 25:1055–1059, 2002
11. Pinkney JH, Stehouwer CDA, Coppack SW, Yudkin JS: Endothelial dysfunction: cause of the insulin resistance syndrome. *Diabetes* 46 (Suppl. 2):S9–S13, 1997
12. Hsueh WA, Quinones MJ: Role of endothelial dysfunction in insulin resistance. *Am J Cardiol* 92 (Suppl.):J10–J17, 2003
13. Meigs JB, Hu FB, Rifai N, Manson JE: Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. *JAMA* 291:1978–1986, 2004
14. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183–1197, 1997
15. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE: Non-invasive detection of endothelial dysfunction in children and in adult at risk of atherosclerotis. *Lancet* 340:1111–1115, 1992
16. Sorensen KE, Celermajer DS, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Thomas O, Deanfield JE: Non-invasive measurement of human endothelium dependent responses: accuracy and reproducibility. *Br Heart J* 74:247–253, 1995
17. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC: Close relationship of endothelial function in the human coronary and peripheral circulation. *J Am Coll Cardiol* 26:1235–1241, 1995
18. Modena MG, Bonetti L, Coppi F, Bursi F, Rossi R: Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. *J Am Coll Cardiol* 40:505–510, 2002
19. Henry RMA, Ferreira I, Kostense PJ, Dekker JM, Nijpels G, Heine RJ, Kamp O, Bouter LM, Stehouwer CDA: Type 2 diabetes is associated with impaired endothelium-dependent, flow-mediated dilation, but impaired glucose metabolism is not: the Hoorn Study. *Atherosclerosis* 174: 49–56, 2004
20. The ARIC Investigators: The Atherosclerosis Risk in Communities (ARIC) study: design and objectives. *Am J Epidemiol* 129: 687–702, 1989
21. Folsom AR, Szklo M, Stevens J, Liao F, Smith R, Eckfeldt JH: A prospective study of coronary heart disease in relation to fasting insulin, glucose, and diabetes: the Atherosclerosis Risk in Communities (ARIC) study. *Diabetes Care* 20:935–942, 1997
22. 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* 48:1856–1862, 1999
23. Nawawi H, Osman NS, Annuar R, Khalid BA, Yusoff K: Soluble intercellular adhesion molecule-1 and interleukin-6 levels reflect endothelial dysfunction in patients with primary hypercholesterolaemia treated with atorvastatin. *Atherosclerosis* 169:283–291, 2003
24. Yang YJ, Hope ID, Ader M, Bergman R: Insulin transport across capillaries is rate limiting for insulin action in dogs. *J Clin Invest* 84:1620–1628, 1989
25. Castillo C, Bogardus C, Bergman R, Thuillez P, Lillioja S: Interstitial insulin concentrations determine glucose uptake rates but not insulin resistance in lean and obese men. *J Clin Invest* 93:10–16, 1994
26. Zeng G, Quon MJ: Insulin-stimulated production of nitric oxide is inhibited by wortmannin: direct measurement in vascular endothelial cells. *J Clin Invest* 98: 894–898, 1996
27. Scherrer U, Randin D, Vollenweiden P, Vollenweiden L, Nicod P: Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 94:2511–2515, 1994
28. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD: Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent: a novel action of insulin to increase nitric oxide release. *J Clin Invest* 94:1172–1179, 1994
29. Montagnani M, Chen H, Barr VA, Quon MJ: Insulin-stimulated activation of eNOS is independent of Ca2+ but requires phosphirilation by Akt at Ser(1179). *J Biol Chem* 276:392–398, 2001
30. Zeng G, Nystrom FH, Ravichandran LV, Cong LN, Kirby M, Mostowski H, Quon MJ: Role of insulin receptor, PI3-kinase, and Akt in insulin-signaling pathways related to production of nitric oxide in human vascular endothelial cells. *Circulation* 101:1539–1545, 2000
31. Montagnani M, Golovchenko I, Kim I, Koh GY, Goalstone ML, Mundhekar AN, Johansen M, Kucik DF, Quon MJ, Draznin B: Inhibition of phosphatidylinositol 3-kinase enhances mitogenic actions of insulin in endothelial cells. *J Biol Chem* 277:1794–1799, 2001
32. Takeda K, Ichiki T, Tokunou T, Iino M, Fujii S, Kitabatake A, Shimokawa H, Takeshita A: Critical role of Rho-kinase and MEC/ERK pathways for angiotensin II-induced plasminogen activator inhibitor type-1 gene expression. *Arterioscler Thromb Vasc Biol* 21:868–873, 2001

33. Rubanyi GM, Romero JC, Vanhoutte PM: Flow-induced release of endothelium-derived relaxing factor. *Am J Physiol* 250: 1145–1149, 1986
34. Sagach VF, Tkachenko MN: On the mechanisms of the involvement of endothelium in reactive hyperemia. *Experientia* 47:828–830, 1991
35. Majmudar NG, Robson SC, Ford GA: Effects of the menopause, gender, and estrogen replacement therapy on vascular nitric oxide activity. *J Clin Endocrinol Metab* 85:1577–1583, 2000
36. Duplain H, Burcelin R, Sartori C, Cook S, Egli M, Lepori M, Vollenweider P, Pedrazzini T, Nicod P, Thorens B, Scherrer U: Insulin resistance, hyperlipidemia, and hypertension in mice licking endothelial nitric oxide synthase. *Circulation* 104:342–345, 2001
37. Baron AD, Steiberg HO, Chaker H, Leaming R, Johnson A, Brechtel G: Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. *J Clin Invest* 96:786–792, 1995
38. Rizzo MA, Piston DW: Regulation of beta cell glucokinase by S-nitrosylation and association with nitric oxide synthase. *J Cell Biol* 161:243–248, 2003
39. Freeman DJ, Norrie J, Sattar N, Neely RD, Cobbe SM, Ford I, Isles C, Lorimer AR, Macfarlane PW, McKillop JH: Pravastatin and the development of diabetes mellitus: evidence for a protective treatment effect in the West of Scotland Coronary Prevention Study. *Circulation* 103:357–362, 2001
40. The Heart Outcomes Prevention Evaluation Study Investigators: Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 342:145–153, 2000
41. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM: Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 346:393–403, 2002
42. Caballero AE, Saouaf R, Lim SC, Hamdy O, Abou-Elenin K, O'Connor C, Logerfo FW, Horton ES, Veves A: The effects of troglitazone, an insulin-sensitizing agent, on the endothelial function in early and late type 2 diabetes: a placebo-controlled randomized clinical trial. *Metabolism* 52: 173–180, 2003