Endothelial Dysfunction and C-Reactive Protein Are Risk Factors for Diabetes in Essential Hypertension

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OBJECTIVE—Type 2 diabetes and essential hypertension are major risk factors for cardiovascular diseases. Endothelial dysfunction is an early step in the development of atherosclerosis and has been demonstrated in hypertensive and diabetic patients.

RESEARCH DESIGN AND METHODS—We designed this study to determine whether forearm endothelial dysfunction is an independent predictor of type 2 diabetes in patients with essential hypertension. We enrolled 400 white never-treated hypertensive outpatients, free of type 2 diabetes at the time of the first evaluation. Endothelium-dependent vasodilation was investigated by intra-arterial infusion of acetylcholine. Insulin resistance was estimated by homeostasis model assessment.

RESULTS—During the follow-up (4.5 ± 1.6 years), 44 patients developed type 2 diabetes. The event rate was 2.4 events/100 patient-years. In a multivariate Cox regression analysis, the peak percentage increase in acetylcholine-stimulated forearm blood flow (hazard ratio [HR] 0.77 [95% CI 0.61–0.99]; $P = 0.04$) and C-reactive protein (1.16 [1.03–1.32]; $P = 0.01$) resulted in the only independent predictors of type 2 diabetes.

CONCLUSIONS—An impaired vasodilatory response to acetylcholine predicts development of type 2 diabetes in patients with essential hypertension. Present data also extend recent findings regarding a possible inflammatory pathogenesis of type 2 diabetes and suggest a new approach in treatment of essential hypertension. ***Diabetes*** **57:167–171, 2008**

Cardiovascular diseases are the most frequent complications of type 2 diabetes, and often they precede diabetes onset (1). Emerging data suggest that type 2 diabetes and atherosclerotic disease may share common pathogenetic mechanisms. In particular, the condition of insulin resistance may represent this common antecedent even if all mechanisms unifying different effects of insulin resistance are not completely defined.

Several findings demonstrate that insulin resistance and high blood pressure are two often-associated clinical conditions (2), and vascular inflammation probably represents the common soil of this bidirectional association. In addition, subjects with high blood pressure and those with insulin resistance have impaired endothelial-dependent vasodilation, an early step in the atherosclerotic process.

Endothelial dysfunction, detectable in patients with cardiovascular risk factors (3–6), provides prognostic information for future clinical events in different settings of patients (7–9). At this moment, endothelial activation/dysfunction is considered the biological response to vascular injury induced by cardiovascular risk factors (10).

Hypertensive patients are characterized by both endothelial dysfunction (3,6,7) and insulin resistance (2), a clinical condition related to development of diabetes. However, whether endothelial dysfunction precedes the onset of type 2 diabetes independently of underlying risk factors, including insulin resistance, remains an open question. Some studies have demonstrated that biomarkers of endothelial activation are associated with risk of incident type 2 diabetes (11–14), but there are no studies demonstrating that endothelium-dependent vasodilation, tested by pharmacologic stimulation of muscarinic receptor, is able to predict onset of type 2 diabetes. Thus, we designed this study to assess whether endothelial function, evaluated by strain-gauge pletysmography, may be considered an independent predictor of type 2 diabetes onset in a group of never-treated hypertensive patients.

RESEARCH DESIGN AND METHODS

A total of 400 (183 men and 217 women aged 22–60 years [mean age 47.1 ± 9.8 years], all of whom were white) uncomplicated hypertensive patients (systolic blood pressure ≥140 mmHg and/or diastolic 90 mmHg) participated in this study. All patients had newly diagnosed, never-treated essential hypertension without target organ damage or previous cardiovascular events. In accordance with American Diabetes Association diagnostic criteria (15), all patients were nondiabetic at enrollment and did not take any drug known to affect glucose metabolism.

New cases of type 2 diabetes were confirmed on the basis of the following criteria: *1*) presence of more than one classic symptom of hyperglycemia plus either a fasting plasma glucose ≥7.0 mmol/l or random plasma glucose ≥11.1 mmol/l, *2*) two or more elevated plasma glucose concentrations (fasting plasma glucose ≥7.0 mmol/l, random plasma glucose ≥11.1 mmol/l, or 2-h plasma glucose ≥11.1 mmol/l during oral glucose tolerance testing), and *3*) use of an oral hypoglycemic drug or insulin. Follow-up included periodic control visits in the outpatient clinic for most patients. To improve long-term follow-up, a questionnaire was also mailed to family physicians, and patients were contacted by phone every 4 months. Vascular function assessments were performed at the first observation.

The local ethics committee approved the study. All participants gave written informed consent for all procedures and use of all data for successive evaluations.

Insulin resistance. Insulin resistance was estimated by homeostasis model assessment (HOMA) from fasting glucose and insulin concentrations according to the following equation: HOMA = [insulin (μU/ml × glucose (mmol/l)]/22.5 (16).

Vascular function. All studies were performed, by the same experienced investigators (R.M. and A.S.), at 9:00 A.M. after overnight fasting, with the subjects lying supine in a quiet air-conditioned room (22–24°C). To test

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CRP, C-reactive protein; FBF, forearm blood flow; HOMA, homeostasis model assessment.

TABLE 1
Baseline characteristics of the study population stratified as progressors and nonprogressors to diabetes

	Progressors	Nonprogressors	*P*
n	44	356	
Age (years)	49.4 ± 10.5	47.7 ± 11.0	0.332
Sex (male)	24 (54)	159 (45)	0.280
BMI (kg/m^2)	27.6 ± 3.0	27.5 ± 3.8	0.867
Current smokers	6 (14)	69 (19)	0.474
Total cholesterol (mmol/l)	5.5 ± 0.9	5.3 ± 0.8	0.124
Fasting glucose (mmol/l)	5.4 ± 0.7	5.3 ± 0.6	0.307
Fasting insulin (μU/ml)	16.6 ± 7.8	13.7 ± 6.9	0.010
HOMA	4.0 ± 2.0	3.2 ± 1.7	0.004
CRP (mg/l)	5.1 ± 2.6	3.9 ± 2.3	0.001
Systolic blood pressure (mmHg)	152 ± 19	150 ± 16	0.444
Diastolic blood pressure (mmHg)	92 ± 11	92 ± 12	0.999
FBF			
Basal (ml · 100 ml tissue^{-1} · min^{-1})	3.38 ± 0.66	3.34 ± 0.63	0.693
Acetylcholine peak (% increase)	227 ± 149	329 ± 192	0.0001
Vascular resistance			
Basal (units)	34.2 ± 6.9	34.5 ± 7.8	0.808
Acetylcholine peak (% decrease)	−62.0 ± 18.7	−72.1 ± 11.8	0.0001

Data are means ± SD or *n* (%).

vascular reactivity, we used the protocol previously described by Panza (3) and subsequently employed by our group (8,9). All patients underwent measurement of forearm blood flow (FBF) and blood pressure during intra-arterial infusion of saline, acetylcholine, and sodium nitroprusside at increasing doses. Measurements of FBF and vascular resistance were repeated every 5 min until stable. Endothelium-dependent and endothelium-independent vasodilations were assessed by a dose-response curve to intra-arterial acetylcholine infusions (7.5, 15, and 30 μg · ml^{-1} · min^{-1}, each for 5 min) and sodium nitroprusside infusions (0.8, 1.6, and 3.2 μg · ml^{-1} · min^{-1}, each for 5 min), respectively. Forearm vascular resistance, expressed in arbitrary units, was calculated by dividing mean blood pressure by FBF. For the present study, the maximal response to acetylcholine was considered for statistical analysis.

Statistical analysis. Data are expressed as means ± SD or as percentage frequency, and comparisons between groups were made by one-way ANOVA, Student's *t* test, or the χ^2 test, as appropriate. The events rate is reported as the number of events per 100 patient-years based on the ratio of the number of events observed to the total number of patient-years of exposure up to the terminating event or censor. For the patients without events, the date of censor was that of the last contact with the patient.

The association between endothelial function and incidence risk of type 2 diabetes was analyzed by univariate and multiple Cox regression analyses. Tested covariates included maximal vasodilatory response to acetylcholine as well as traditional (age, sex, smoking, glucose, serum cholesterol, systolic blood pressure, and BMI) and emerging (fasting insulin, HOMA index, and serum C-reactive protein [CRP]) cardiovascular risk factors. The multiple Cox regression model was constructed by including all variables that turned out to be associated with incident risk of type 2 diabetes ($P < 0.10$) at univariate Cox regression analysis. By this strategy, we constructed a Cox model of adequate statistical power (at least 10 events for each variable into the final model). Data are expressed as hazard ratio (HR) (95% CI) and *P* value. Point estimates of the probability of type 2 diabetes occurrence associated with maximal vasodilatory response to acetylcholine were calculated by using the equation derived from the multiple Cox regression analysis. Analysis of biological interaction between acetylcholine-stimulated FBF and CRP was performed, as previously described by Greenland and Rothman (17), by dividing patients into four groups in relation to the median of acetylcholine-stimulated FBF and CRP. In two-tailed tests, a value of $P < 0.05$ was considered statistically significant.

RESULTS

In the study population, fasting glucose and insulin ranged from 3.7 to 6.5 mmol/l and from 2 to 40 μU/ml, respectively. During follow-up (median 54 months [range 6–70]), there were 44 new cases of type 2 diabetes (2.4 events/100 patient-years), similar to that observed in the Losartan Intervention For Endpoint reduction in hypertension (LIFE) study (18). Baseline characteristics of patients who progressed toward type 2 diabetes (progressors) and those remaining free of type 2 diabetes (nonprogressors) are reported in Table 1. There were no statistically significant differences between the two groups in age, sex, smoking habit, total cholesterol, fasting glucose, or basal FBF. On the contrary, progressors had a higher baseline fasting insulin and HOMA mean values. Similarly, mean CRP was significantly higher in progressors than in the control group (5.1 ± 2.6 vs. 3.9 ± 2.3 mg/l; $P < 0.001$) (Table 1). In addition, the highest response in acetylcholine-stimulated FBF was significantly lower in progressors compared with that in nonprogressors (227 ± 149 vs. 329 ± 192%; $P < 0.0001$) (Table 1); in contrast, no significant differences were observed in maximal vasodilation induced by sodium nitroprusside (297 ± 117 vs. 317 ± 105%; $P = 0.240$).

At the first eligibility visit, none of the patients had been treated with antihypertensive or other medications known to interfere with insulin sensitivity. Baseline blood pressure values were 150/92 ± 17/12 mmHg and did not differ between the two groups. All patients were treated to reduce clinical blood pressure <140/90 mmHg using standard lifestyle and pharmacological treatment. Diuretics, β-blockers, ACE inhibitors, calcium channel blockers, angiotensin II receptor antagonists, and α_1-blockers were used alone or in various associations without significant differences between the groups. Antihypertensive drugs used in the study population are reported in Table 2.

Vascular function. Intra-arterial acetylcholine infusion caused a dose-dependent increase in FBF ($P < 0.0001$) and decrease in vascular resistance. The FBF increments from basal (3.3 ± 0.6 ml · 100 ml tissue^{-1} · min^{-1}) at the three incremental doses were 1.9 ± 1.3 (+58%), 5.5 ± 3.8 (+164%), and 10.6 ± 6.7 ml · 100 ml tissue^{-1} · min^{-1} (+316%). At the highest dose of acetylcholine (30 μg/min), FBF increased to 13.8 ± 4.2 ml · 100 ml tissue^{-1} · min^{-1} and vascular resistance decreased to 10.2 ± 5.1 units. Similarly, sodium nitroprusside infusion induced a significant increase in FBF (maximal increment from the basal, +325%) and a decrease in vascular resistance (−74%).

TABLE 2
Antihypertensive drugs employed in the study population during the follow-up period

	Progressors	Nonprogressors	*P*
n	44	356	
ACE inhibitors	11	100	0.800
Angiotensin II receptor antagonists	3	28	0.957
α_1-Blockers	2	13	0.900
β-Blockers	3	30	0.940
Calcium channel blockers	6	46	0.917
Diuretics	2	17	0.758
Associations	17	122	0.685

Data are *n*.

Intra-arterial infusion of vasoactive substances caused no changes in blood pressure or heart rate values.

Cox regression analyses. On univariate analysis, incident risk of type 2 diabetes was inversely related with maximal vasodilatory response to acetylcholine (100% increase HR 0.68 [95% CI 0.54–0.86], $P = 0.001$) and directly with serum CRP (1.22 [1.08–1.37], $P = 0.001$), HOMA index (1.21 [1.05–1.39], $P = 0.007$), and fasting insulin (1.05 [1.01–1.09], $P = 0.006$). Serum cholesterol tended to be related to risk of type 2 diabetes (1.36 [0.94–1.95]), but this association did not achieve statistical significance ($P = 0.10$). No association was found between occurrence of type 2 diabetes and fasting glucose ($P = 0.09$), smoking ($P = 0.30$), age ($P = 0.43$), systolic blood pressure ($P = 0.65$), sex ($P = 0.67$), or BMI ($P = 0.86$).

In a multiple Cox regression model (Table 3) including significant univariate predictors of type 2 diabetes, only serum CRP (HR 1.16 [95% CI 1.03–1.32], $P = 0.01$) and maximal vasodilatory response to acetylcholine (100% increase 0.77 [0.61–0.99], $P = 0.04$) maintained an independent association with the outcome (Table 3). Successively, we tested the possible interaction between these variables. In particular, we observed that patients who have CRP values above the median and FBF under the median show a higher risk of developing diabetes (2.80 [1.24–6.30], $P = 0.01$) (Table 3). In this model, we included HOMA index to avoid a possible colinearity with fasting insulin and because the insulin resistance condition is very frequent in essential hypertension.

TABLE 3
Cox regression analysis of diabetes occurrence and interaction between acetylcholine-stimulated FBF and CRP

	Diabetes occurrence		
	Increase	HR (95% CI)	*P*
CRP	1 mg/l	1.16 (1.03–1.32)	0.01
Maximal vasodilatory response to acetylcholine	100%	0.77 (0.61–0.99)	0.04
HOMA index	1	1.13 (0.97–1.33)	0.11
Cholesterol	1 mmol/l	1.31 (0.91–1.89)	0.14

	Interaction between acetylcholine-stimulated FBF and CRP	
	Adjusted HR (95% CI)	*P*
0	1	
1	1.03 (0.35–2.96)	0.95
2	1.36 (0.51–3.63)	0.53
3	2.80 (1.24–6.30)	0.01

CRP median, 3.9 mg/l; acetylcholine-stimulated FBF median, 277%. 0: PCR under median; acetylcholine above median (reference group). 1: PCR above median; acetylcholine above median. 2: PCR under median; acetylcholine under median. 3: PCR above median; acetylcholine under median.

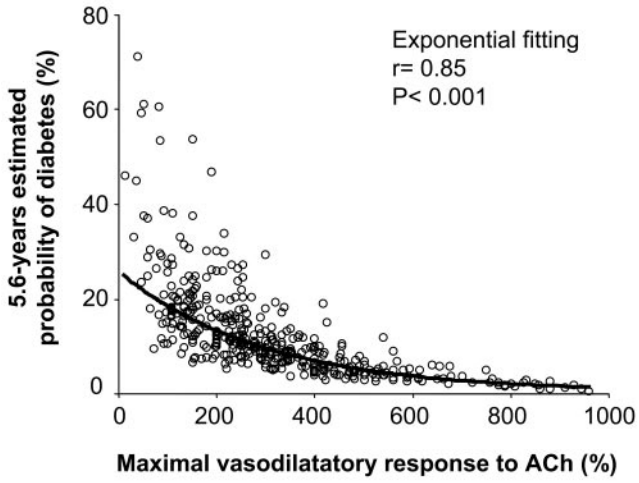

FIG. 1. Adjusted relationship between peak percentage increase in endothelium-dependent vasodilation and the possibility of developing type 2 diabetes. ACh, acetylcholine.

Maximal vasodilatory response to acetylcholine was inversely related with HOMA index ($r = -0.44$, $P < 0.001$), which explains why HOMA index became a significant predictor of type 2 diabetes (HR 1.22 [95% CI 1.06–1.40], $P = 0.006$) in a model excluding endothelial function. This result suggests that insulin resistance and endothelial dysfunction are in the same causal pathway leading to type 2 diabetes in hypertensive patients. The association between maximal vasodilatory response to acetylcholine and probability of type 2 diabetes occurrence is graphically reported in Fig. 1.

DISCUSSION

Hypertensive subjects who developed type 2 diabetes had higher baseline fasting insulin, HOMA, and CRP mean values. However, the major finding of this study is that peak response to acetylcholine-stimulated vasodilation resulted as an independent predictor of new onset of type 2 diabetes in hypertensive patients. After adjustment for well-established type 2 diabetes risk factors, including insulin resistance, HOMA, and pharmacological treatment, vasodilatory response to acetylcholine and CRP remained significant predictors of incident type 2 diabetes. Thus, present data provide evidence for a strong association between baseline endothelial function and development of type 2 diabetes in essential hypertension. Inflammation may be involved in this association, but the inclusion of CRP in the final model did not eliminate this association. Of interest, these two variables interact, increasing in a multiplicative manner the risk of new diabetes. Since inflammation and endothelial dysfunction (3,6,8) are present in hypertensive patients, it could be interesting to know which alteration precedes the appearance of the other. In keeping with this, we suggest a possible pathophysiological mechanism: hypertension-related vascular inflammation at first induces endothelial dysfunction, which in turn promotes insulin resistance and progression

to type 2 diabetes. In fact, essential hypertension is associated with increased oxidative stress, which induces mild vascular inflammation and endothelial dysfunction. This condition impairs insulin sensitivity, activating a vicious circle that contributes to progression to type 2 diabetes (19). This hypothesis is in agreement with previously published data demonstrating that proinflammatory molecules, such as tumor necrosis factor-α, interleukin-6, and CRP, are associated with endothelial dysfunction and insulin resistance (20,21). Accordingly, we observed a significant relationship between CRP and both endothelial dysfunction and type 2 diabetes, supporting the idea that endothelial dysfunction contributes to the development of type 2 diabetes.

On the other hand, insulin resistance is associated with activation of the adrenergic system, which interferes with vascular reactivity. Recently, we demonstrated in human umbilical vein endothelial cells that angiotensin II, acting via the AT1 receptor, exerts an inhibitory effect on the insulin signaling pathway involved in nitric oxide production, which affects insulin resistance (22). In keeping with this, drugs interfering with the renin-angiotensin system are able to significantly improve endothelial dysfunction and to reduce new onset of type 2 diabetes (18,23,24). Taken all together, these data suggest that endothelial dysfunction may contribute to development of type 2 diabetes, preceding—rather than following—its onset.

Data reported by D'Agostino et al. (25) demonstrated that individuals with cardiovascular risk factors are at increased risk of type 2 diabetes, which is only partially mediated by insulin resistance or central adiposity. These results support our hypothesis because it is well established that cardiovascular risk factors are associated with endothelial dysfunction (3–6,8,9). Thus, since cardiovascular risk factors predict the appearance of type 2 diabetes and are associated with endothelial dysfunction, it is plausible that vascular damage precedes the clinical manifestation of type 2 diabetes.

Some previous studies demonstrated that biomarkers of endothelial activation predict type 2 diabetes independently of other known risk factors (11–14). Different from others, the novelty of our data are that we directly tested, in a very large sample of hypertensive patients, endothelial function by stimulating muscarinic cholinergic receptors by intra-arterial infusion of vasoactive agonist. This method allows a direct evaluation of endothelial vasodilating properties. In addition, our data are consistent with those obtained by Rossi et al. (26) in a population of apparently healthy postmenopausal women.

Finally, even if the evaluation of endothelial function is not a routine procedure, our data may have a relevant impact on treatment of essential hypertension. In fact, antihypertensive drugs that improve insulin resistance and endothelial dysfunction should be preferred because of their capability to delay onset of type 2 diabetes and its cardiovascular complications in hypertensive patients (18,23,24). In addition, it would be more appropriate to state that patients with newly diagnosed hypertension should have a basal screening that includes measurement of insulin sensitivity indexes in order to anticipate, and possibly treat, early development of type 2 diabetes.

Study limitations. This is an observational, nonrandomized, prospective study. Our findings have been obtained in initially untreated hypertensive white subjects, so results may not be extended to different racial groups or to subjects receiving antihypertensive treatment at the time of the qualifying evaluation. Finally, the invasiveness, even if minimal, of the method used to evaluate endothelial function may represent another limitation of this study. In fact, the method cannot be easily applicable in a large prospective human study.

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REFERENCES

1. Hu FB, Stampfer MJ, Haffner SM, Solomon CG, Willett WC, Manson JE: Elevated risk of cardiovascular disease prior to clinical diagnosis of type 2 diabetes. *Diabetes Care* 23:1129–1134, 2002
2. Reaven GM, Lithell H, Landsberg L: Hypertension and associated metabolic abnormalities: the role of insulin resistance and the sympathoadrenal system. *N Engl J Med* 334:374–381, 1996
3. Panza JA, Quyyumi AA, Brush JE, Epstein SE: Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 323:22–27, 1990
4. Creager MA, Cooke Cooke JP, Mendelsohn ME, Gallagher SJ, Coleman SM, Loscalzo J, Dzau VJ: Impaired vasodilation of forearm resistance vessel in hypercholesterolemic humans. *J Clin Invest* 86:228–234, 1990
5. Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA: Impaired endothelium-dependent coronary arterial vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 88:2510–2516, 1993
6. Quyyumj AA: Endothelial function in health and disease: new insights into the genesis of cardiovascular disease. *Am J Med* 105:32S–39S, 1998
7. Lerman A, Zeiher AM: Endothelial function and cardiac events. *Circulation* 111:363–368, 2005
8. Perticone F, Ceravolo R, Pujia A, Ventura G, Iacopino S, Scozzafava A, Ferraro A, Chello M, Mastroroberto P, Verdecchia P, Schillaci G: Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* 104:191–196, 2001
9. Sciacqua A, Scozzafava A, Pujia A, Maio R, Borrello F, Andreozzi F, Vatrano M, Cassano S, Perticone M, Sesti G, Perticone F: Interaction between vascular dysfunction and cardiac mass increases the risk of cardiovascular outcomes in essential hypertension. *Eur Heart J* 26:921–927, 2005
10. Ross R: Atherosclerosis: an inflammatory disease. *N Engl J Med* 340:115–126, 1999
11. Meigs JB, Hu FB, Rifai N, Manson JE: Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. *JAMA* 291:1978–1986, 2004
12. Hu FB, Meigs JB, Li TY, Rifai N, Manson JE: Inflammatory markers and risk of developing type 2 diabetes in women. *Diabetes* 53:693–700, 2004
13. Thorand B, Baumert J, Chambless L, Meisinger C, Kolb H, Doring A, Lowel H, Koenig W, the MONICA/KORA Study Group: Elevated markers of endothelial dysfunction predict type 2 diabetes mellitus in middle-aged men and women from the general population. *Arterioscler Thromb Vasc Biol* 26:398–405, 2006
14. Meigs JB, O'donnell CJ, Tofler GH, Benjamin EJ, Fox CS, Lipinska I, Nathan DM, Sullivan LM, D'Agostino RB, Wilson PW: Hemostatic markers of endothelial dysfunction and risk of incident type 2 diabetes: the Framingham Offspring Study. *Diabetes* 55:530–537, 2006
15. The 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
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner R: Homeostatic model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
17. Greenland S, Rothman KJ (Eds.): *In Modern Epidemiology*. Philadelphia, Lippincott Raven, 1998, p. 329–342
18. Dahlöf B, Devereux RB, Kjeldsen SE, Julius S, Beevers G, de Faire U, Fyhrquist F, Ibsen H, Kristiansson K, Lederballe-Pedersen O, Lindholm LH, Nieminen MS, Omvik P, Oparil S, Wedel H, the LIFE Study Group: Cardiovascular morbidity and mortality in the Losartan Intervention for Endpoint Reduction in Hypertension study (LIFE): a randomised trial against atenolol. *Lancet* 359:995–1003, 2002
19. Pinkney JH, Stehouwer CD, Coppack SW, Yudkin JS: Endothelial dysfunc-

tion: cause of the insulin resistance syndrome. *Diabetes* 46 (Suppl. 2):S9–S13, 1997
20. Pradhan AR, Manson JE, Rifai N, Buring JE, Ridker PM: C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 286:327–334, 2001
21. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW: C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 19:972–978, 1999
22. Andreozzi F, Laratta E, Sciacqua A, Perticone F, Sesti G: Angiotensin II impairs the insulin signaling pathway promoting production of NO by inducing phosphorylation of insulin receptor substrate-1 on Ser^{312} and Ser^{616} in human umbilical vein endothelial cells. *Circ Res* 94:1211–1218, 2004
23. 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
24. Pfeffer MA, McMurray JJ, Velazquez EJ, Rouleau JL, Kober L, Maggioni AP, Solomon SD, Swedberg K, Van de Werf F, White H, Leimberger JD, Henis M, Edwards S, Zelenkofske S, Sellers MA, Califf RM, the Valsartan in Acute Myocardial Infarction Trial Investigators: Valsartan, captopril, or both in myocardial infarction complicated by heart failure, left ventricular dysfunction, or both. *N Engl J Med* 349:1893–1906, 2003
25. D'Agostino RB, Hamman RF, Carter AJ, Mikkanen L, Wagenknecht LE, Haffner SM: Cardiovascular risk factors predict the development of type 2 diabetes: the Insulin Resistance Atherosclerosis Study. *Diabetes Care* 27:2234–2240, 2004
26. Rossi R, Cioni E, Nuzzo A, Origliani G, Modena MG: Endothelial-dependent vasodilation and incidence of type 2 diabetes in a population of healthy postmenopausal women. *Diabetes Care* 28:702–707, 2005