Joint Effect of Endothelial Dysfunction and New-onset Diabetes Mellitus in Predicting Cardiovascular Outcomes in Hypertensive Patients: a Cohort Study

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Abstract

Background. Endothelial dysfunction, induced by some cardiovascular risk factors, is the early determinant of atherosclerotic cardiovascular disease. Moreover, it predicts incident type-2 diabetes that is associated with an increased risk of fatal and nonfatal cardiovascular events; however, no data demonstrating a possible interaction between them in predicting cardiovascular outcomes are available. Thus, we designed present study to evaluate the prognostic significance of the joint effect of endothelial dysfunction and new-onset type-2 diabetes in hypertensives.

Methods. We enrolled 653 white never-treated hypertensive outpatients free of cardiovascular events from the CATanzaro Metabolic Risk factors (CATAMERI) study. All subjects were without type-2 diabetes, heart failure and chronic kidney disease. Endothelium-dependent vasodilation was investigated by intra-arterial infusion of acetylcholine, and laboratory determinations were obtained by standard procedures. Follow-up included periodic control visits every six months in our out-patients Clinic and ranged from 26-206 months (median 113). Patients were treated to reduce blood pressure <140/90 mmHg using standard lifestyle and pharmacological treatment. Incident type-2 diabetes and following cardiovascular events were monitored: fatal and nonfatal myocardial infarction and stroke, TIA, unstable angina, coronary revascularization procedures and symptomatic aortoiliac occlusive disease.

Results. We documented 191 new cardiovascular morbid events (3.1%) at the cardiac (n=134), cerebrovascular (n=50), or peripheral (n=7) level. Dividing the study population in progressors and non-progressors, progressors were older and had a higher baseline heart rate, fasting insulin, HOMA, creatinine, and hs-CRP mean values, while e-GFR and acetylcholine-stimulated forearm blood flow (FBF) were lower. Similarly, incident type-2 diabetes was higher in the progressors group. In the multiple Cox regression analysis, incident diabetes (HR=2.015, 95%CI=1.416-2.868), hs-CRP (HR=1.306, 95%CI=1.213-1.407) and acetylcholine-stimulated FBF (HR=0.729, 95%CI=0.633-0.839) maintained an independent association with the outcomes. The interaction between new-onset diabetes and FBF demonstrated that patients with new diabetes and maximal FBF under the median show a higher risk for subsequent cardiovascular events (HR=7.411, 95%CI=4.875-11.265).

Conclusions. Endothelial dysfunction and new-onset diabetes predict cardiovascular events and their joint effect greatly increases this risk. These results highlight the importance of interaction between hemodynamic and metabolic alterations in predicting cardiovascular events.

Background

Essential hypertension, affecting over 1 billion people worldwide, is a chronic disorder with a significant global economic and health impact, and it represents the major risk factor for cardiovascular disease (1). In addition, hypertension is frequently associated with insulin resistance that represents a clinical condition predisposing to a high risk for developing type-2 diabetes that, in turn, is another widespread public health problem, as well as a risk factor for cardiovascular events (2-4). In keeping with this, the

coexistence of these two conditions is very frequent also due to the growing prevalence of obesity and reduced physical activity, thus underlying a cardiovascular risk markedly increased when hypertension and diabetes coexist (5-7).

Endothelial dysfunction, characterized by a reduced nitric oxide (NO) bioavailability as a consequence of the exposure to cardio-metabolic risk factors, has long been proven to be present in both diabetic and hypertensive patients (8,9). Interestingly, both oxidative stress and the activation of pro-inflammatory pathways represent the most important pathogenetic mechanisms responsible of vascular damage (10-12) and the subsequent fatal and nonfatal cardiovascular outcomes (13,14). On the basis of these evidences it is possible to remark that endothelial dysfunction represents an intermediate step in the cardiovascular continuum from risk factors to clinical events and death.

Even if it is well established that both endothelial dysfunction and diabetes are associated with an increased risk of fatal and nonfatal cardiovascular events (3-6,13,14), no data demonstrating a possible interaction between them are available. Thus, we designed this study to assess the prognostic value of the joint effect between endothelial dysfunction, evaluated by strain-gauge plethysmography, and new type-2 diabetes in a cohort of hypertensive subjects free from previous cardiovascular events.

Methods

Study population

From a large cohort of 812 newly diagnosed hypertensive subjects participating to the CATanzaro Metabolic Risk factors (CATAMERI) study, we recruited a total of 653 Caucasian patients [337 men and 316 women aged 22-73 years (mean age 48.5$\pm$10.5 years), with systolic blood pressure (BP) $\geq$140 mmHg and/or diastolic 90 mmHg. From the initial cohort, 17 patients died, 41 were lost to follow-up, 9 refused to continue the study and 82 were excluded because without high-sensitivity C-reactive protein (hs-CRP) determination.

Exclusion criteria were: previous cardiovascular events, diabetes mellitus defined as HbA1c $\geq$6.5% or fasting plasma glucose $\geq$126 mg/dl, chronic kidney disease [estimated-glomerular filtration rate (e-GFR <60 ml/min/1.73m^2)], liver and peripheral vascular disease, and heart failure (diagnosed according to both clinical and echocardiographic criteria).

The CATAMERI study was approved in October 17th, 2012 (approval number 2012.63) by Ethics Committee of the Azienda Ospedaliero-Universitaria Mater Domini of Catanzaro. All the participants gave their informed written consent to study participation and all the investigations were made according with the principles of the Declaration of Helsinki.

Laboratory determinations

At the first eligibility visit, all laboratory measurements were performed after a fasting period of at least 12 h. Plasma glucose was determined by the glucose oxidase method (Glucose Analyzer, Beckman Coulter SpA, Milan, Italy). Triglyceride and cholesterol concentrations were measured by enzymatic methods (Roche Diagnostics GmbH, Mannheim, Germany). Serum creatinine was measured by an automated technique based on the measurement of Jaffe chromogen and by the URICASE/POD method (Boehringer Mannheim, Mannheim, Germany) implemented in an auto-analyzer. Values of e-GFR were calculated by using the equation proposed by CKD-EPI. hs-CRP was measured by a turbidimetric immunoassay (Behring).

Blood pressure measurement

Measurements of clinic BP were obtained in the left arm of seated patients, after 5 min of quiet rest, using a mercury sphygmomanometer, with a minimum of three BP readings on three different occasions at least 2 weeks apart. Baseline BP values were the average of the last two of the three consecutive measurements.

Vascular function

All studies were performed, by the same experienced investigators (R.M., M.P. 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 vascular reactivity, we used the protocol previously described by Panza (8) and subsequently employed by our group (12,13,15-17). All patients underwent measurement of forearm blood flow (FBF) and BP 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 mg $\cdot$ ml$^{-1}\cdot$ min^{-1}, each for 5 min) and sodium nitroprusside infusions (0.8, 1.6, and 3.2 mg $\cdot$ ml$^{-1}\cdot$ min^{-1}, each for 5 min), respectively. Forearm vascular resistance, expressed in arbitrary units (U), was calculated by dividing mean BP by FBF. For the present study, both maximal response to acetylcholine and sodium nitroprusside were considered for statistical analysis.

Follow-up and incident cardiovascular events

Follow-up included periodic control visits at least every six months in the outpatient Clinic. To improve long-term follow-up, a questionnaire was also mailed to family physicians, and patients were contacted by phone every 6 months. All clinical events had to be validated by a local Committee on the basis of source data (hospital records, death certificates or other original documents).

During the follow-up, we assessed the following cardiovascular events: fatal and nonfatal myocardial infarction (MI), fatal and nonfatal stroke, transient cerebral ischemic attack (TIA), unstable angina, coronary revascularization procedures (bypass surgery or angioplasty), and symptomatic aortoiliac occlusive disease documented with angiography. Diagnosis of acute MI was based on chest pain history, cardiac enzyme measurement, and new ST elevation in at least two contiguous leads. Unstable angina

was defined by typical chest pain associated with ischemic electrocardiographic changes and successively documented by provocative tests (treadmill exercise test or/and stress echocardiography, myocardial scintigraphy, or coronary angiography). TIA was defined by physician diagnosis of any sudden focal neurological deficit that cleared completely in <24 hours.

According with American Diabetes Association diagnostic criteria (18), 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 $\geq$7.0 mmol/l or random plasma glucose $\geq$11.1 mmol/l, 2) two or more elevated plasma glucose concentrations (fasting plasma glucose $\geq$7.0 mmol/l, random plasma glucose $\geq$11.1 mmol/l, or 2-h plasma glucose $\geq$11.1 mmol/l during oral glucose tolerance testing), and 3) use of an oral hypoglycemic drug or insulin.

Statistical analysis

Data are expressed as means $\pm$ SD or as percentage frequency, and comparisons between groups were made by one-way ANOVA, Student's t test, or the X^2 test, as appropriate. The responses to acetylcholine and sodium nitroprusside were compared by ANOVA for repeated measurements and, when analysis was significant, Tukey's test was applied. A value of $P \leq 0.05$ was considered statistically significant.

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. Survival curves were estimated by use of the Kaplan-Meier product-limit method and compared by using the mantel (log-rank test).

The association between endothelial function and incidence risk of cardiovascular events was analyzed by univariate and multiple Cox regression analyses. Tested covariates included maximal vasodilatory response to acetylcholine as well as traditional [age, gender, smoking, fasting glucose, serum cholesterol, systolic BP, and body mass index (BMI)] and emerging (fasting insulin, HOMA index and hs-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 cardiovascular events ($P < 0.10$) 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 cardiovascular events occurrence associated with maximal vasodilatory response to acetylcholine were calculated by using the equation derived from the multiple Cox regression analysis. Analysis of biological joint effect between acetylcholine-stimulated FBF and type 2 diabetes was performed, as previously described by Greenland and Rothman (19), by dividing patients into four groups in relation to the median of acetylcholine-stimulated FBF and presence/absence of type 2 diabetes.

Results

Baseline characteristics of patients who suffered from a cardiovascular events (progressors) and those remaining free of cardiovascular disease (non-progressors) are reported in Table 1. There were no statistically significant differences between the two groups in gender distribution, BMI, smoking habit, diastolic BP, glucose, total cholesterol, triglyceride, uric acid and basal FBF. On the contrary, progressors were older, and had a higher baseline systolic BP, heart rate, insulin, HOMA index, creatinine, hs-CRP mean values, while e-GFR values were lower. In addition, in the progressors group there was a higher prevalence of new type-2 diabetes. As expected, the highest response to acetylcholine stimulated FBF was significantly lower in progressors compared with that in non-progressors group (194±107 vs 340±187%; P<0.0001); in contrast, no significant differences were observed in maximal vasodilation induced by sodium nitroprusside (315±108 vs 323±108%; P=0.397).

At the first eligibility visit, none of the patients had been treated with antihypertensive drugs. In the whole study population, baseline blood pressure values were 149.3/91.1±17.0/11.5 mmHg, with a little and significant difference in systolic BP between the two groups (152.2±17.5 vs 148.1±16.6 mmHg). All patients were treated to reduce clinical BP <140/90 mmHg using standard lifestyle and pharmacological treatment. Diuretics, b-blockers, ACE-inhibitors, calcium channel blockers, angiotensin II receptor antagonists, and a1-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 1. No significant differences between groups were observed in the percentage of patients reaching recommended BP target (62 vs 65% in the progressors and non-progressors patients, respectively).

Follow up and incident cardiovascular events

During the follow-up [median 113 months (range 26–206)], there were 191 new cardiovascular morbid events (3.1 events/100 patient-years) at the cardiac (n = 134), cerebrovascular (n = 50) or peripheral vascular (n = 7) level. In particular, there were 51 patients with MI (3 fatal), 53 with unstable angina pectoris, 30 with coronary revascularization procedures, 44 with stroke (5 fatal), 8 with transient cerebral ischemia, and 7 with new onset peripheral occlusive disease. Indications for the revascularization procedures were put forward by physicians not involved in this study.

In addition, during the follow-up period 92 of the 653 initially nondiabetic hypertensive subjects developed new type-2 diabetes; the estimated incidence of new diabetes was 1.6 events/100 patient-years.

Vascular function

In the whole population, intra-arterial acetylcholine infusion caused a significant dose-dependent increase in FBF and decrease in vascular resistance. The FBF increments from basal (3.35±0.66 ml · 100 ml tissue^{-1}· min^{-1}) at the three incremental doses were 1.84±1.21 (55%), 5.25±3.3 (157%), and 10.0±5.9 ml · 100 ml tissue^{-1}· min^{-1} (298%). At the highest dose of acetylcholine (30 mg/min), FBF increased to

13.35±3.9 ml · 100 ml tissue^{-1}· min^{-1} and vascular resistance decreased to 10.1±4.7 units. Interestingly, dividing the study population in progressors and non-progressors, we observed (Table 1) a significant difference in maximal acetylcholine-stimulated FBF: 194±107 vs 340±187 ml · 100 ml tissue^{-1}· min^{-1}. In figure 1 we graphically reported the relationship, expressed as exponential fitting curve, crude and adjusted, between maximal vasodilatory response to acetylcholine and probability of incident cardiovascular events occurrence.

Similarly, sodium nitroprusside infusion induced (Table 1) a significant increase in FBF (maximal increment from the basal, +317%) and a decrease in vascular resistance (-72%), without any significant difference between groups. Intra-arterial infusion of vasoactive substances caused no changes in blood pressure or heart rate values.

Cox regression analyses

On univariate analysis (Table 2), incident risk of cardiovascular events was inversely related with maximal vasodilatory response to acetylcholine [100% increase, HR=0.413 (95% CI=0.295-0.580), P<0.0001], e-GFR [10 ml/min/1.73m^2 increase, HR=0.794 (95% CI 0.732-0.862), P<0.0001], HDL-cholesterol [10 mg/dl increase, HR=0.855 (95% CI 0.756-0.967), P=0.013] and directly with incident type-2 diabetes (HR=3.638 (95% CI 2.698-4.906), P<0.0001], hs-CRP [HR=1.418 (95% CI=1.330-1.512), P<0.0001], HOMA [HR=1.218 (95% CI=1.136-1.306), P<0.0001], age [10 years increase, HR=1.186 (95% CI=1.031-1.364), P=0.017], and systolic BP [10 mmHg increase, HR=1.118 (95% CI=1.027-1.216), P=0.010]. No association was found between occurrence of cardiovascular events and smoking, fasting glucose, gender, LDL cholesterol and BMI.

In the multiple Cox regression analysis, including the variables reaching the statistical significance at univariate analysis, only acetylcholine-stimulated FBF [100% increase, HR=0.674 (95% CI=0.586-0.774), P<0.0001] and serum hs-CRP [HR=1.304 (95% CI=1.214-1.402), P<0.0001] maintained an independent association with the outcomes (Table 2). Interestingly, when we also added type-2 diabetes as independent variable (Table 2, model 2), the latter was retained as the first independent covariate in predicting cardiovascular events, even if both hs-CRP and maximal-stimulated FBF remained in the same model. In this analysis, we included only HOMA index and e-GFR to avoid a possible colinearity with fasting insulin and serum creatinine.

Successively, we tested the possible joint effect between type-2 diabetes and FBF in predicting cardiovascular events. In particular, we observed that patients who have both type-2 diabetes and FBF under the median show a higher risk of developing cardiovascular events [HR=7.411 (95% CI=4.875-11.265), P<0.0001)] (Figure 2).

Discussion

Data obtained in this study, conducted in a very large cohort of never treated and well selected hypertensive patients, demonstrated that endothelial dysfunction, hs-CRP and new-onset type-2 diabetes

are strong and independent predictors of subsequent cardiovascular events after adjustment for some classical and emerging risk factors. In addition, clinically relevant, endothelial dysfunction and type-2 diabetes interact in a significant manner in predicting atherosclerotic vascular outcomes.

The observed adverse prognostic significance of endothelial dysfunction is confirmatory of previously published data (13,14,17) but, interestingly and clinically relevant, the novel finding obtained in the present study is that the predictive significance of baseline endothelial dysfunction also persists after adjustment for some well-established and strong atherosclerotic vascular risk factors such as age, cholesterol, insulin resistance status, systolic BP, renal function and incident diabetes. Thus, the evidence of this strong association, between baseline endothelial function and subsequent development of cardiovascular events in essential hypertension, highlights its relevant pathogenetic role in the appearance and progression of atherosclerotic disease. In this context, because atherosclerosis is characterized by a condition of immune-mediated subclinical inflammation (20,21), it is not surprising that also hs-CRP is emerged as a strong and independent predictor of subsequent clinical outcome. Normally, endothelial regulates vascular tone, thrombogenesis, lipid breakdown, vascular inflammation, and smooth muscle cell proliferation. Nevertheless, in the presence of vascular risk factors and/or specific clinical conditions, the endothelium changes its phenotype promoting vasoconstriction, thrombosis, vascular inflammation, and cell proliferation, playing a key pathophysiological role in the development and progression of the atherosclerotic vascular damage (11,21,22) until the appearance of adverse clinical events.

This study also provides new data on the adverse impact of new-onset diabetes in treated hypertensive patients, negative prognostic effect that significantly increases when it interacts with endothelial dysfunction. It is well demonstrated that type-2 diabetes is a powerful risk factor for cardiovascular events; in fact, individuals with diabetes have a 2- to 4-fold increased risk of developing vascular outcomes than those without diabetes (23). In keeping with this, our hypertensive patients with new-onset type-2 diabetes showed a risk about 4-fold (HR = 3.638) for developing a cardiovascular event; but, for the first time, we demonstrated that the coexistence of type-2 diabetes and endothelial dysfunction increases, in a significant manner, the risk up to over 7-fold (HR = 7.411, CI95% 4.875-11.265) to develop a fatal or non-fatal vascular event. A plausible explanation of this evidence may be recognized in the fact that the coexistence of type-2 diabetes further impairs the vascular wall function, already present in hypertensive patients, accelerating the atherosclerotic disease and promoting the plaque instability and acute vascular syndromes. In this context, we should not forget the impairment of coronary microcirculation which accentuates the global cardiac ischemic burden of diabetic patients. In the other hand, we previously demonstrated that endothelial dysfunction predicts the appearance of incident type-2 diabetes (16), thus activating a vicious circle that worsens both vascular damage and glyco-metabolic control with a reciprocal self-empowerment in the induction of cardiovascular events. On the basis of these evidences, it is plausible to affirm that this bidirectional mechanism between endothelial dysfunction and type-2 diabetes leads to an excess of oxidative stress that reduces the NO bioavailability, release of inflammatory mediators, and activation of proliferative pathways, all involved in atherosclerosis (11,21).

Conclusions

In conclusions, our data demonstrate, over a very long follow-up period, that both endothelial dysfunction and new-onset type-2 diabetes have an increased similar risk for cardiovascular disease but, notably, their joint effect, operating in a synergistic manner, greatly increases the risk to have a subsequent adverse cardiovascular event in comparison with those who remain free from type-2 diabetes. Thus, it is clinically relevant to remark that the occurrence of new-onset type-2 diabetes is an independent predictor of cardiovascular risk in hypertensive patients because these findings emphasize the need to also evaluate insulin resistance status for implementing aggressive strategies to prevent occurrence of new type-2 diabetes in hypertensive subjects.

Additionally, even if the evaluation of endothelial function is not a routine procedure, the relevance of our data is that we directly tested endothelial function by stimulating muscarinic cholinergic receptors by intra-arterial infusion of vasoactive agonists.

Finally, the study has some limitations: it is an observational, nonrandomized, prospective study; our findings were obtained in untreated white hypertensives, so results may not be extended to different racial groups or to subjects receiving antihypertensive treatment at the time of the qualifying evaluation.

Abbreviations

NO = nitric oxide

BP = blood pressure

HbA1c = glycated hemoglobin

e-GFR = estimated glomerular filtration rate

CKD-EPI = chronic kidney disease epidemiology

hs-CRP = high sensitivity C-reactive protein

FBF = forearm blood flow

U = units

MI = myocardial infarction

TIA = transient ischemic attack

SD = standard deviation

BMI = body mass index

HOMA = homeostatic model assessment

HR = hazard ratio

CI = confidence interval

ACE = angiotensin converting enzyme

Declarations

- *Ethics approval:* The CATAMERI study was approved in October 17th, 2012 (approval number 2012.63) by Ethics Committee of the Azienda Ospedaliero-Universitaria Mater Domini of Catanzaro, Italy
- *Consent for publication:* Non applicable
- *Availability of data and materials:* The dataset used and analysed during the current study are available from Raffaele Maio, co-Author of this paper
- *Competing interest:* none
- *Funding:* Not applicable for that section
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- *Author's contributions*:M. P. researched data and wrote the manuscript; R. M. researched data, made statistical analysis and contributed to the discussion: E. S. researched data; V. F. reviewed/edited the manuscript; F. M. contributed the discussion; F. A. reviewed/edited the manuscript; G. S. contributed the discussion; F. P. wrote the manuscript

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Tables

Table 1 – Baseline characteristics of the study population stratified as progressors and non-progressors to cardiovascular events

	All	Progressors	Non-progressors	P
No. (%)	653	191 (29.2)	462 (70.8)	
Gender, male (%)	337 (51.4)	97 (50.8)	240 (51.9)	.786
Age, mean (SD), years	48 (10)	50 (11)	47 (10)	.011
BMI, mean (SD), Kg/m^2	27 (4)	27 (4)	27 (4)	.888
Current smokers, n (%)	107 (16)	37 (19)	70 (15)	.185
Systolic BP, mean (SD), mmHg	149 (17)	152 (17)	148 (17)	.005
Diastolic BP, mean (SD), mmHg	91 (11)	92 (12)	91 (11)	.285
Heart rate, mean (SD), bpm	72 (10)	73 (9)	71 (10)	.013
Fasting glucose, mean (SD), mg/dl	95 (11)	95 (10)	95 (11)	.993
Fasting insulin, mean (SD), U/L	14 (7)	17 (7)	13 (6)	.0001
HOMA, mean (SD)	3.4 (1.7)	4.0 (1.9)	3.2 (1.5)	.0001
Total cholesterol, mean (SD), mg/dl	205 (31)	204 (29)	205 (32)	.899
Triglyceride, mean (SD), mg/dl	115 (39)	117 (38)	115 (40)	.494
Creatinine, mean (SD), mg/dl	0.95 (0.19)	1.05 (0.20)	0.91 (0.17)	.0001
e-GFR, mean (SD), $ml/min/1.7m^2$	85 (20)	75 (20)	89 (19)	.0001
Uric acid, mean (SD), mg/dl	5.0 (1.7)	5.2 (1.6)	5.0 (1.7)	.165
hs-CRP, mean (SD), mg/dl	3.7 (1.7)	4.9 (1.4)	3.2 (1.6)	.0001
new diabetes, No. (%)	92 (14)	66 (34)	56 (12)	.0001
Forearm blood flow				
Basal, mean (SD), $ml \cdot 100\ ml\ tissue^{-1} \cdot min^{-1}$	3.35 (0.66)	3.28 (0.65)	3.38 (0.66)	.080
Acetylcholine, mean (SD), % increase	298 (180)	194 (107)	340 (187)	.0001
Sodium nitroprusside, mean (SD), % increase	317 (110)	315 (109)	323 (110)	.397
Antihypertensive drugs				
ACE-i/ARBs, No. (%)	510 (78)	151 (79)	359 (78)	.703
Calcium antagonists, No. (%)	215 (33)	64 (33)	151 (33)	.838

b-Blockers, No. (%)	59 (9)	16 (8)	43 (9)	.705
a-Blockers, No. (%)	15 (2)	5 (3)	10 (2)	.725
Diuretics, No. (%)	112 (17)	32 (17)	80 (17)	.862
Associations, No. (%)	365 (55.9)	107 (56.1)	258 (55.8)	.966

ACE-i = Angiotensin converting enzyme inhibitors; ARBs = Angiotensin II receptor blockers

Table 2 – Cox regression analysis for incident cardiovascular events

Univariate	Hazard Ratio	95% CI	P
FBF, *100% increase*	0.413	0.295-0.580	.0001
e-GFR, *10 ml/min/1.7m^2*	0.794	0.732-0.862	.0001
HDL cholesterol, *10 mg/ml*	0.855	0.756-0.967	.013
Diabetes, *yes*	3.638	2.698-4.906	.0001
hs-CRP, *mg/dl*	1.418	1.330-1.512	.0001
HOMA	1.218	1.136-1.306	.0001
Age, *10 years*	1.186	1.031-1.364	.017
Systolic BP, *10 mmHg*	1.118	1.027-1.216	.010
Smoking	1.113	0.777-1.593	.561
Fasting glucose, *10 mg/dl*	1.039	0.907-1.190	.583
Gender, *male*	1.019	0.766-1355	.987
LDL cholesterol, *10 mg/ml*	1.009	0.964-1.057	.691
BMI, *Kg/m^2*	0.997	0.959-1.037	.889
Multivariate, model 1			
FBF, *100% increase*	0.674	0.586-0.774	.0001
hs-CRP, *mg/dl*	1.304	1.214-1.402	.0001
Systolic BP, *10 mmHg*	1.500	0.964-1.144	.263
HOMA	1.045	0.963-1.133	.293
e-GFR, *10 ml/min/1.7m^2*	0.922	0.837-1.014	.095
HDL cholesterol, *10 mg/ml*	0.946	0.838-1.068	.369
Age, *10 years*	0.998	0.856-1.165	.984
Multivariate, model 2			
Diabetes	2.015	1.416-2.868	.0001
hs-CRP, *mg/dl*	1.306	1.213-1.407	.0001
FBF, *100% increase*	0.729	0.633-0.839	.0001

Model 2 = model 1 + Diabetes; FBF = forearm blood flow; hs-CRP = high sensitivity C reactive protein; e-GFR = estimated glomerular filtration rate; HOMA = homeostasis model assessment; BP = blood pressure

Figures

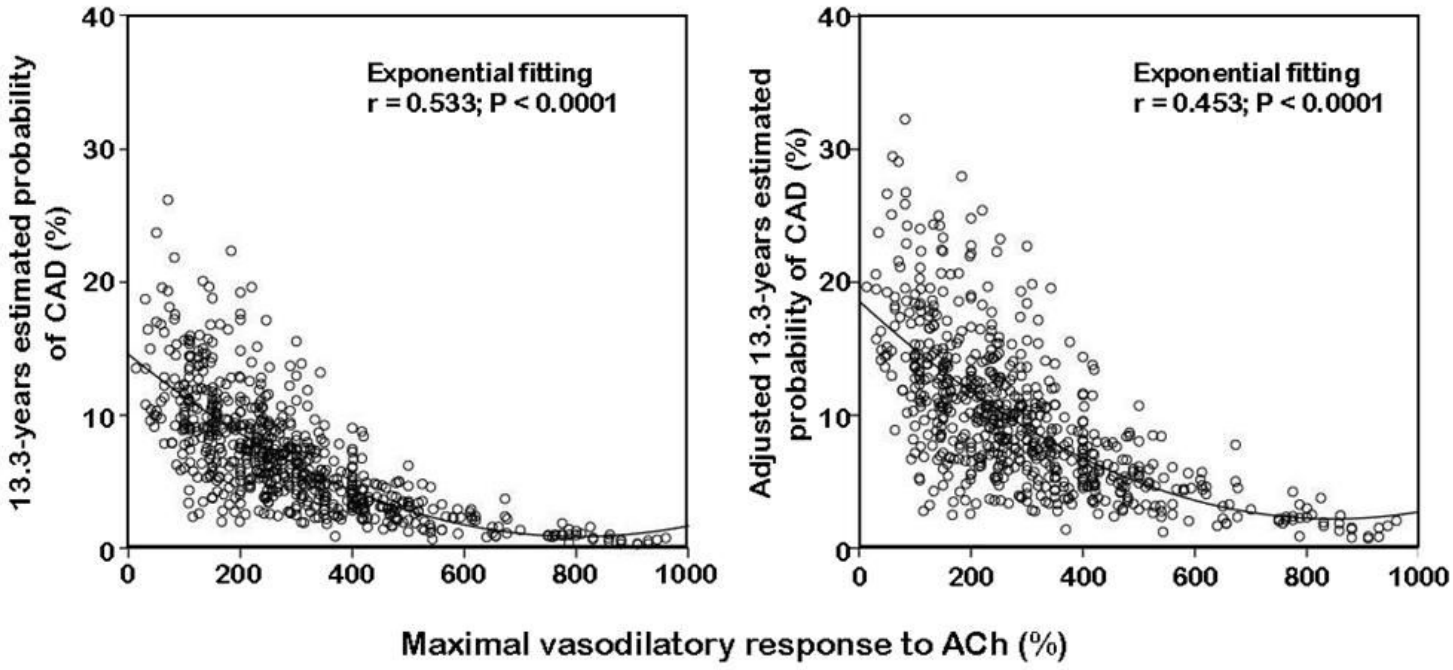

Figure 1

Relationship between endothelial function and risk of cardiovascular events. We graphically reported the relationship, expressed as exponential fitting curve, crude and adjusted, between maximal vasodilatory response to acetylcholine (Ach) and probability of incident cardiovascular events occurrence. Adjusted for age, systolic blood pressure, HDL-cholesterol, HOMA index, e-GFR and hs-CRP

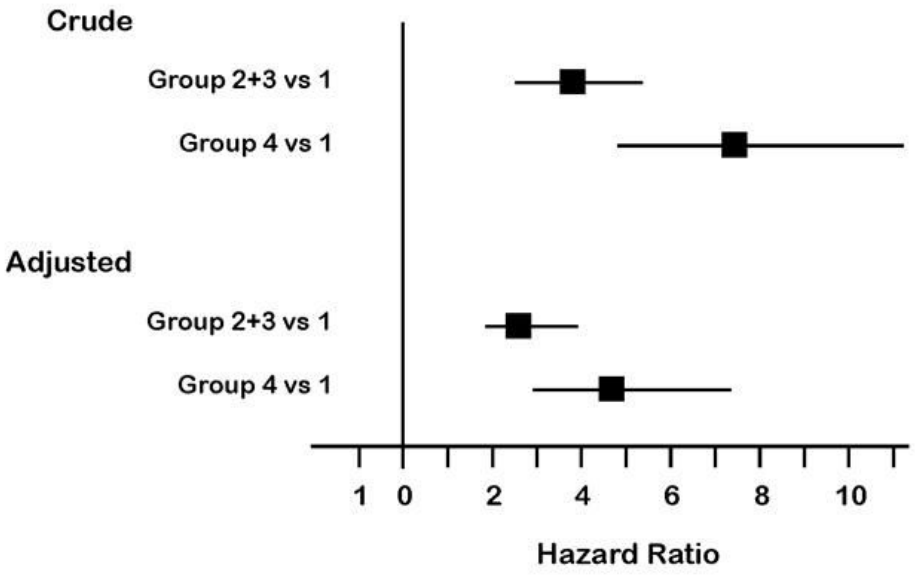

Figure 2

Interaction between endothelial dysfunction and incident diabetes in predicting cardiovascular outcomes. Joint effect of acetylcholine-stimulated forearm blood flow (FBF) and new-onset type-2 diabetes in predicting incident cardiovascular events. Group 1 (reference group) = no diabetes and acetylcholine stimulated FBF above median Group 2 = no diabetes and acetylcholine stimulated FBF under median Group 3 = diabetes and acetylcholine stimulated FBF above median Group 4 = diabetes and acetylcholine stimulated FBF under median Due to the small sample size of group 3, in the analysis this one was unified with group 2. Adjustment was obtained for age, systolic blood pressure, HDL-cholesterol, HOMA index, e-GFR and hs-CRP.