Endothelial Dysfunction Plays a Key Role in Increasing Cardiovascular Risk in Type 2 Diabetes The Hoorn Study

Endothelial Dysfunction Plays a Key Role in Increasing Cardiovascular Risk in Type 2 Diabetes

The Hoorn Study

Thomas T. van Sloten, Ronald M. A. Henry, Jacqueline M. Dekker, Giel Nijpels, Thomas Unger, Miranda T. Schram, Coen D. A. Stehouwer

See Editorial Commentary, pp 1192–1193

Abstract—In the pathogenesis of cardiovascular events, interaction between risk factors has seldom been identified. However, endothelial dysfunction on the one hand and type 2 diabetes mellitus, impaired glucose metabolism (IGM), and insulin resistance on the other may act synergistically (ie, interact) in the development of cardiovascular disease. We therefore investigated the interaction between endothelial dysfunction and type 2 diabetes mellitus, IGM, and insulin resistance with regard to risk of cardiovascular events. In a prospective population-based cohort (n=445; 69 years; 55% women; 23% type 2 diabetes mellitus, 28% IGM [by design]), endothelial dysfunction (brachial artery flow-mediated dilatation), glucose tolerance (oral glucose tolerance test), and insulin sensitivity (homeostasis model assessment for insulin resistance [HOMA2-IR]) were determined. After a median follow-up of 7.6 years, 106 participants had had a cardiovascular event. After adjustments, 1 SD less flow-mediated dilatation was associated with cardiovascular events in type 2 diabetes mellitus (hazard ratio 1.69 [95% confidence interval, 1.14–2.52]) and IGM (1.50 [0.95–2.37]) and among those in the highest HOMA2-IR tertile (1.92 [1.42–2.60]), but not in normal glucose metabolism (0.85 [0.63–1.16]) or among those in the lower 2 HOMA2-IR tertiles combined (0.85 [0.65–1.12]). Interaction between flow-mediated dilatation and type 2 diabetes mellitus, IGM, or insulin resistance was present on an additive (relative excess risk caused by interaction >0) and on a multiplicative scale (*P* interaction <0.05). Endothelial dysfunction and type 2 diabetes mellitus, IGM, or insulin resistance synergistically increase cardiovascular event risk. This identifies endothelial dysfunction as a key therapeutic target in these individuals. **(*Hypertension*. 2014;64:1299-1305.)** • **Online Data Supplement**

Key Words: cardiovascular diseases ■ endothelium ■ epidemiology ■ insulin resistance ■ prospective studies ■ type 2 diabetes mellitus

In the pathogenesis of cardiovascular events, true interaction (ie, synergy) between risk factors appears rare, that is, most studies find that risk factors act, and, thus, increase cardiovascular event risk, independently of each other.[1–3] However, it has been hypothesized that individuals with type 2 diabetes mellitus (DM2) are particularly prone to the detrimental effects of endothelial dysfunction,[4–6] a key mechanism in the pathogenesis of atherothrombosis, and that this may explain the increased cardiovascular events risk in DM2. If true, this implies that DM2 and endothelial dysfunction interact with regard to the pathogenesis of cardiovascular events. That is, DM2 and endothelial dysfunction may act more strongly in the presence of the other variable than in its absence. From a clinical point of view, detection of interaction between risk factors is important because this identifies key therapeutic targets: interventions aimed at such risk factors are potentially more efficacious than treatment of risk factors that do not interact.

The mechanism that may underlie this phenomenon is a bidirectional association between endothelial dysfunction and DM2, in which endothelial dysfunction may act as both cause[6,7] and consequence[6,8] of DM2. On the one hand, DM2 leads to endothelial dysfunction via, amongst others, formation of advanced glycation end products, intraendothelial accumulation of glucose, and increased oxidative stress.[6,8] On the other hand, endothelial dysfunction causes or aggravates DM2 by impairing the timely access of glucose and insulin to their target tissues.[7] Consequently, a vicious circle may exist between endothelial dysfunction and DM2. In addition, DM2

Received July 7, 2014; first decision July 19, 2014; revision accepted August 11, 2014.

From the Department of Medicine (T.T.v.S., R.M.A.H., M.T.S., C.D.A.S.), Cardiovascular Research Institute Maastricht (T.T.v.S., R.M.A.H., T.U., M.T.S., C.D.A.S.), and School for Nutrition, Toxicology and Metabolism (T.T.v.S.), Maastricht University Medical Centre, Maastricht, the Netherlands; and EMGO Institute for Health and Care Research (J.M.D., G.N.) and Department of Epidemiology and Biostatistics (J.M.D.), VU University Medical Centre, Amsterdam, the Netherlands.

The online-only Data Supplement is available with this article at http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.114.04221/-/DC1.

Correspondence to Coen D. A. Stehouwer, Department of Internal Medicine, Maastricht University Medical Centre, P. Debyelaan 25, 6229HX Maastricht, the Netherlands. E-mail cda.stehouwer@mumc.nl

may amplify the detrimental effects of endothelial dysfunction on atherothrombosis via multiple pathways, including overproduction of reactive oxygen species, low-grade inflammation, and increased procoagulant activity and platelet aggregation.[9] Similar mechanisms may be operative in individuals with impaired glucose metabolism (IGM; ie, impaired fasting glucose and impaired glucose tolerance) or with insulin resistance but with normal glucose tolerance, in whom an increased risk of cardiovascular events is also apparent.[10,11]

If the above hypothesis is correct, then the co-occurrence of endothelial dysfunction and DM2 will increase cardiovascular event risk more than expected on the basis of the presence of these processes alone. This phenomenon is called causal interaction or interaction on an additive scale[12,13] and can be formally tested in observational data through the calculation of the relative excess risk due to interaction (RERI).[12]

To date, 2 previous studies, an earlier report of the Hoorn Study[4] and the Framingham Offspring Study,[5] have evaluated the joint effects of endothelial dysfunction, as determined by plasma biomarkers, and DM2 on incident cardiovascular events. In agreement with the above hypothesis, these studies showed that endothelial dysfunction was most strongly associated with incident cardiovascular events in individuals with DM2 as compared with those without DM2. However, these studies did not evaluate causal interaction (ie, interaction on an additive scale). In addition, these studies did not measure flow-mediated dilatation (FMD), a key functional measure of endothelium-dependent, nitric oxide–mediated dilatation.[14]

In view of the above, we investigated, in a general elderly population, the association between endothelial dysfunction, as determined by FMD, and incident cardiovascular events, and formally tested, for the first time, whether any such association was stronger in individuals with DM2, IGM, and insulin resistance as compared with individuals with normal glucose metabolism (NGM) or normal insulin sensitivity (ie, the presence of causal interaction).

Methods

Study Design

For the present study, we used data from the 2000 Hoorn Study follow-up examination. The Hoorn Study is a population-based cohort study of glucose metabolism and cardiovascular risk among inhabitants of the municipality of Hoorn in the Netherlands. Details of the study have been described elsewhere.[4,14,15] The Hoorn Study was approved by the Ethical Review Committee of the VU University Medical Center. Informed consent was obtained from all participants.

Brachial Artery FMD

A detailed description of the measurement of FMD and nitroglycerin-mediated dilatation (NMD) is provided in the online-only Data Supplement (please see http://hyper.ahajournals.org).

Determination of Glucose Metabolism and Insulin Resistance Status

All participants, except those with previously diagnosed diabetes mellitus, underwent a standard 75-g oral glucose tolerance test and were classified as having either NGM, IGM, or DM2 according to the 1999 World Health Organization criteria.[16] Insulin resistance was estimated using the homeostasis model assessment for insulin resistance (HOMA2-IR) calculator (www.dtu.ox.ac.uk).

Other Measurements

Cardiovascular risk factors and prior cardiovascular disease (CVD) were assessed as described previously.[4,14,15]

Follow-Up

Follow-up was completed until January 1, 2009. Information on cardiovascular morbidity and mortality was extracted from medical records of general practitioners and the local hospital and classified according to the *International Classification of Disease, Ninth Revision*. We defined incident cardiovascular events (nonfatal and fatal combined) as *International Classification of Disease, Ninth Revision* codes: 410 to 414 (coronary heart disease), 427 to 428 (heart failure), 431 to 438 (cerebrovascular disease), 440 to 443 (arterial disease), 798 (sudden death), and *International Classification of Disease, Ninth Revision* clinical modification code 36 (coronary arterial procedures). Data on the participants' vital status were collected from the municipal population register. We determined for each participant whether death had occurred during follow-up and, if so, the date when death occurred.

Statistical Analyses

All analyses were performed with R statistical software (version 2.15.2). For insulin resistance status, individuals were classified by the highest HOMA2-IR tertile versus the lower tertiles combined. The lower tertiles were combined because results for these tertiles did not materially differ from each other in all analyses (ie, there was a nonlinear interaction between FMD and HOMA2-IR levels, see below). In all analyses, FMD was adjusted for baseline diameter, flow increase after cuff release, and NMD. Results were adjusted for NMD, a measure of endothelial-independent vasodilatation, because less FMD may be because of impaired endothelial and endothelial-independent (smooth muscle cell) function.[14] Cox proportional hazard models were used to estimate the association between FMD and incident cardiovascular events. First, the association between FMD and cardiovascular events was evaluated in the total study population. This association was adjusted for age and sex (cohort stratifying variables, model 1) and additionally for prior CVD, body mass index, total/high-density lipoprotein cholesterol, triglycerides, hypertension, estimated glomerular filtration rate, physical activity, smoking habits, the use of antihypertensive and lipid-lowering medication (potential confounders; model 2); glucose metabolism status (ie, DM2, IGM, and NGM; model 3); and insulin resistance status (ie, highest HOMA2-IR tertile and lower 2 tertiles combined; model 4). Second, analyses were repeated after stratification for glucose metabolism or insulin resistance status. Third, we investigated the presence of potentially causal interactions on an additive scale (ie, when risk factors act synergistically in causing disease).[13] In Cox regression analysis, however, statistical interaction is exponential and, therefore, multiplicative. To nevertheless evaluate the presence of additive interaction, we calculated the RERI.[12] RERI represents the risk that is in excess of what would be expected if there had been no additive interaction. An RERI >0 indicates positive additive interaction.[12] In these analyses, adjustments were made for the same sets of potential confounders as described for the Cox regression models. Confidence intervals of the RERI were estimated by using a bootstrap method with 10 000 samples.[12] Finally, we also calculated the presence of any multiplicative interaction by adding, to our Cox regression models, product terms between FMD and DM2, IGM, and insulin resistance.

Results

Study Population

Of the 648 participants, qualitatively sufficient ultrasound examinations were obtained in 492 individuals. Data were missing for logistical reasons (n=49) and poor definition of the arterial wall because of obesity (n=107). In addition, participants were excluded when data on glucose metabolism status (n=8) or cardiovascular event follow-up were missing (n=39; of whom 6 had moved out of town and could not be contacted

Table 1. Clinical Characteristics of the Study Population at Baseline According to Incident Cardiovascular Event Status

Clinical Characteristics	Participants Without a Cardiovascular Event, n=339 (76.2%)	Participants With a Cardiovascular Event, n=106 (23.8%)
General characteristics		
Age, y	68.5±6.2	71.4±6.1
Women	55.5	35.8
Smoking habits		
Current smoker	10.9	21.7
Former smoker	44.0	49.1
Nonsmoker	45.1	29.2
Physical activity (MET hours/week)	82 (49–128)	77 (48–125)
Prior cardiovascular disease	47.5	63.2
Glucose metabolism status		
Type 2 diabetes mellitus	20.9	29.2
Impaired glucose metabolism	26.8	31.1
Normal glucose metabolism	52.3	39.7
Insulin resistance status		
HOMA2-IR	1.00 (0.80–1.50)	1.20 (0.80–1.63)
HOMA2-IR tertile 3	31.0	37.3
HOMA2-IR tertile 2	38.8	31.4
HOMA2-IR tertile 1	30.2	31.3
Body mass index, kg/m^2	26.7±3.3	27.2±3.4
Systolic blood pressure, mm Hg	141±20	148±21
Diastolic blood pressure, mm Hg	82±11	83±11
Hypertension	62.8	79.2
HbA1c, mmol/mol	41±7	44±9
HbA1c, %	5.9±0.7	6.2±0.8
Total cholesterol, mmol/L	5.8±1.0	5.8±1.1
LDL cholesterol, mmol/L	3.7±0.9	3.8±0.9
HDL cholesterol, mmol/L	1.5±0.4	1.3±0.4
Triglycerides, mmol/L	1.2 (0.9–1.7)	1.4 (1.1–1.8)
Estimated glomerular filtration rate, mL/min/1.73 m^2	62.7±10.0	60.2±11.0
(Micro)albuminuria (albumin/creatinine ratio >2 mg/mmol)	10.9	20.0
Medication use		
Lipid-lowering medication	12.4	19.8
Antihypertensive medication	28.9	44.3
Flow-mediated dilatation		
Flow-mediated dilatation, mm	0.19±0.15	0.14±0.20
Flow-mediated percentage change in diameter, %	4.3±3.7	3.1±4.0
Baseline diameter, mm	4.59±0.75	4.83±0.69
Flow increase after cuff release, %	91±45	79±40
Flow increase after cuff release, cm/s	11.3±6.0	9.6±4.8
Nitroglycerin-mediated dilatation, mm	0.44±0.21	0.43±0.23

Data are presented as percentage, mean±SD, or median (interquartile range). CVD indicates cardiovascular disease; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HOMA2-IR, homeostasis model assessment for insulin resistance; LDL, low-density lipoprotein; and MET, metabolic equivalent of task.

and 33 did not give permission to access their medical files or to contact their general practitioner). Thus, 445 participants were eligible for the present analyses. Individuals without follow-up data did not differ from the study population (data not shown).

Clinical Characteristics

Table 1 shows the baseline characteristics of the study population according to cardiovascular event status. The median duration of follow-up was 7.6 years (range 0.2–8.9). A total of 106 participants (42 NGM, 33 IGM, and 31 DM2) had a cardiovascular event; 12 (11.3%) of which were fatal. A total of 48 participants had a coronary heart disease event (16 NGM, 19 IGM, and 13 DM2), 35 had a cerebrovascular event (16 NGM, 10 IGM, and 9 DM2), and 23 had a cardiovascular event other than a coronary heart disease or cerebrovascular event (eg, peripheral arterial disease or heart failure). The incidence rate of cardiovascular events was 3.1% per year. Individuals with an incident cardiovascular event were older and more often men. In addition, these individuals suffered more often from DM2, had greater insulin resistance as determined by HOMA2-IR, and a less favorable cardiovascular risk profile (Table 1). In addition, individuals with a cardiovascular event had less FMD, a smaller baseline diameter, and a lower flow increase after cuff release (Table 1).

Association Between FMD and Incident Cardiovascular Events

Multivariable Cox regression analysis showed that FMD was not significantly associated with incident cardiovascular events in the overall population (Table 2, models 1–4). However, when the analyses were repeated stratified according to glucose metabolism or insulin resistance status, the results showed that, after adjustment for potential confounders, less FMD was associated with cardiovascular events in individuals with DM2 and IGM, but not in individuals with NGM (Table 2, model 2; also illustrated in Figure, panel A). Similarly, less FMD was associated with cardiovascular events among those in the highest HOMA2-IR tertile, but not among those in the lower 2 tertiles combined (Table 2, model 2; also illustrated in Figure, panel B). These results did not materially change when we additionally adjusted glucose metabolism and insulin resistance status for each other (models 3 and 4).

Interaction Analyses

When the analyses were repeated to test for additive interaction between FMD and glucose metabolism status, the results showed that, after adjustment for potential confounders, the RERI, per 1 SD less FMD, was 0.64 (95% confidence interval [CI], −0.35–1.32) for DM2 versus NGM and 0.68 (95% CI, −0.07–1.93) for IGM versus NGM, respectively (Table 2, model 2). This means that the hazard ratios (HRs) for incident cardiovascular events in DM2 and IGM were, per 1 SD less FMD, 0.64 and 0.68 higher, respectively, than if there had been no interaction between FMD and DM2 or IGM. Similarly, the RERI, per 1 SD less FMD, was 0.73 (95% CI, 0.30–1.34) for the highest HOMA2-IR tertile versus the lower 2 tertiles combined (Table 2, model 2).

When the analyses were repeated to test for multiplicative interaction, the results showed that, after adjustment for potential confounders, the HRs, per 1 SD less FMD, for the

Table 2. Association Between Flow-Mediated Dilatation and Incident Cardiovascular Events: Analyses in the Total Study Population and Stratified Analyses and Interaction Analyses With Glucose Metabolism and Insulin Resistance Status

	Analyses in the Total Study Population and Stratified Analyses		Interaction Analyses		
				Additive Scale	Multiplicative Scale
Models		HRs, 95% CIs*		RERIs, 95% CIs*	HRs of Product Terms, 95% CIs*
Total study population					
Model 1		1.31 (1.01–1.70)		…	…
Model 2		1.18 (0.89–1.55)		…	…
Model 3: Model 2 + glucose metabolism status		1.19 (0.90–1.57)		…	…
Model 4: Model 2 + insulin resistance status		1.18 (0.88–1.57)		…	…
Glucose metabolism status (DM2, IGM, and NGM)					
Model 1	DM2	1.56 (1.13–2.17)	DM2 vs NGM	0.69 (–0.34–1.87)	1.61 (1.03–2.53)
	IGM	1.49 (0.97–2.29)	IGM vs NGM	0.68 (–0.17–1.98)	1.54 (0.92–2.59)
	NGM	0.96 (0.68–1.36)			
Model 2	DM2	1.69 (1.14–2.52)	DM2 vs NGM	0.64 (–0.35–1.32)	1.96 (1.21–3.17)
	IGM	1.50 (0.95–2.37)	IGM vs NGM	0.68 (–0.07–1.93)	1.76 (1.03–2.98)
	NGM	0.85 (0.63–1.16)			
Model 3: Model 2 + insulin resistance status	DM2	1.73 (1.14–2.62)	DM2 vs NGM	0.74 (–0.45–1.57)	2.03 (1.23–3.33)
	IGM	1.57 (0.96–2.57)	IGM vs NGM	0.76 (–0.01–2.06)	1.88 (1.07–3.30)
	NGM	0.84 (0.61–1.14)			
Insulin resistance status (HOMA2-IR tertiles)					
Model 1	T3	1.70 (1.32–2.20)	T3 vs T1-2†	0.79 (0.37–2.01)	1.75 (1.21–2.51)
	T1-2†	0.98 (0.73–1.30)			
Model 2	T3	1.92 (1.42–2.60)	T3 vs T1-2†	0.73 (0.30–1.34)	2.25 (1.53–3.32)
	T1-2†	0.85 (0.65–1.12)			
Model 3: Model 2 + glucose metabolism status	T3	1.93 (1.42–2.62)	T3 vs T1-2†	0.73 (0.30–1.39)	2.25 (1.52–3.32)
	T1-2†	0.86 (0.65–1.12)			

Model 1: adjusted for age, sex, baseline diameter, flow increase after cuff release, and nitroglycerin-mediated dilatation.

Model 2: Model 1+prior cardiovascular disease, body mass index, total/HDL cholesterol, triglycerides, hypertension, estimated glomerular filtration rate, physical activity, smoking habits, and the use of antihypertensive and lipid-lowering medication.

CI indicates confidence interval; DM2, type 2 diabetes mellitus; HOMA2-IR, homeostasis model assessment for insulin resistance; HRs, hazard ratios; IGM, impaired glucose metabolism; NGM, normal glucose metabolism; RERIs, relative excess risk due to interactions; and T, tertile.

*Hazard ratios (HRs) and relative excess risk due to interactions (RERIs) are indicated per 1 SD (0.17 mm) less flow-mediated dilatation. RERI >0 indicates the presence of positive additive interaction, and HR of product term >1 indicates positive multiplicative interaction.

†Lower 2 HOMA2-IR tertiles were combined because these tertiles did not materially differ in the analyses.

product terms between FMD and DM2 or IGM versus NGM were 1.96 (95% CI, 1.21–3.17) and 1.76 (95% CI, 1.03–2.98), respectively (Table 2, model 2). This means that the HRs for incident cardiovascular events in DM2 and IGM were, per 1 SD less FMD, 1.96× and 1.76× higher, respectively, than if there had been no multiplicative interaction between FMD and DM2 or IGM. Similarly, the HR, per 1 SD less FMD, for the product terms between FMD and the highest HOMA2-IR tertile versus the lower 2 tertiles combined was 2.25 (95% CI, 1.53–3.32) (Table 2, model 2). These results did not materially change when we additionally adjusted glucose metabolism and insulin resistance status for each other (models 3).

Additional Analyses

There was no additive or multiplicative interaction between FMD and hemoglobin A1c, fasting, or postload glucose in the association with cardiovascular events (data not shown). In addition, the results of the interaction analyses between FMD and DM2, IGM, and insulin resistance did not materially change when we additionally adjusted for hemoglobin A1c, fasting, or postload glucose (data not shown).

All analyses were then repeated with incident cardiovascular events as the outcome, but with incident heart failure (n=9) excluded from the definition of cardiovascular events. The results of these analyses were qualitatively similar to the analyses with total incident cardiovascular events as the outcome (please see http://hyper.ahajournals.org; Table S1 in the online-only Data Supplement).

Next, all analyses were repeated with all-cause mortality as the outcome. These analyses showed that FMD was not associated with all-cause mortality. In addition, there was no additive or multiplicative interaction between FMD and DM2,

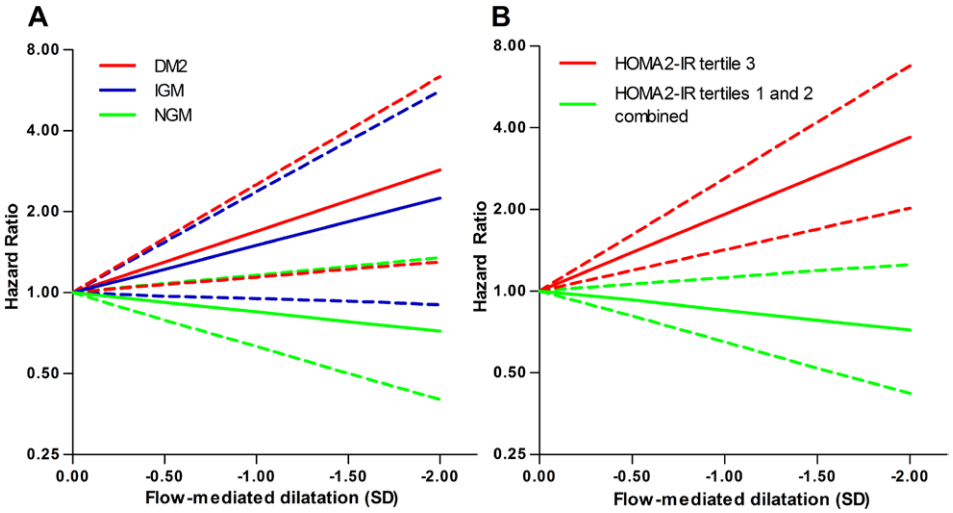

Figure. Associations (hazard ratios) of flow-mediated dilatation with incident cardiovascular events stratified according to (**A**) glucose metabolism status (ie, type 2 diabetes mellitus [DM2], impaired glucose metabolism [IGM], and normal glucose metabolism [NGM]) and (**B**) insulin resistance status (ie, HOMA2-IR tertiles). All results are adjusted for potential confounders (see text). Solid lines indicate estimated effect; dashed lines indicate corresponding 95% confidence intervals.

IGM, or insulin resistance in the association with all-cause mortality (please see http://hyper.ahajournals.org; Table S2).

Endothelial function is closely linked to low-grade inflammation. Any association of endothelial dysfunction with incident cardiovascular events and mortality may, thus, be dependent on low-grade inflammation. We therefore determined circulating biomarkers of low-grade inflammation (high-sensitivity C-reactive protein, serum amyloid A, interleukin-6, interleukin-8, tumor necrosis factor-α, and soluble intracellular adhesion molecule-1) and constructed a summarizing low-grade inflammation Z-score as described previously.[17] When we additionally adjusted the results for this Z-score, results did not materially change (data not shown).

Estimation of insulin resistance based on HOMA2-IR may be less accurate in individuals treated with insulin.[18] When we excluded these individuals (n=10) from the analyses, however, results did not materially change (data not shown).

The associations between FMD and incident cardiovascular events may differ according to the presence of prior CVD. However, no interaction was observed on an additive (RERI −0.17 [95% CI, −1.09–0.74]) or a multiplicative scale (HR for the product term between FMD and prior CVD versus no prior CVD was 0.82 [95% CI, 0.50–1.33]).

Finally, the associations between FMD and incident cardiovascular events may differ according to DM2 duration. However, no interaction was observed on an additive (RERI −0.03 (95% CI, −0.25–0.32)) or a multiplicative scale (HR for the product term between FMD and DM2 duration was 0.96 (95% CI, 0.90–1.04).

Discussion

This population-based study is the first that formally tests the joint effect, on incident cardiovascular events, of FMD on the one hand and DM2, IGM, and insulin resistance on the other. We observed that FMD was most strongly associated with cardiovascular events in individuals with DM2 or insulin resistance, less strongly in IGM, and not associated with incident cardiovascular events in individuals with NGM or normal insulin sensitivity. Importantly, the increased cardiovascular events risk of the joint effect of endothelial dysfunction and DM2, IGM, or insulin resistance was greater than what would have been expected had the effect of FMD on the one hand and glucose metabolism or insulin resistance status on the other acted independently of each other (as indicated by a RERI >0), demonstrating the presence of interaction or synergy between endothelial dysfunction and impairment of glucose metabolism with respect to cardiovascular event risk.

The present study defined endothelial dysfunction as impaired endothelium-dependent FMD, which is a key functional estimate of endothelial function.[14] The study thereby extends previous studies[4,5] on the joint effects of endothelial dysfunction and DM2, IGM, or insulin resistance on incident cardiovascular events, which used plasma biomarkers to define endothelial dysfunction and showed multiplicative interaction between endothelial dysfunction and DM2. No information was, however, provided on an additive interaction scale (ie, potentially causal interaction).[12,13] The present study, therefore, provides additional and strong evidence in favor of a causal interaction between endothelial dysfunction on the one hand and DM2, IGM, and insulin resistance on the other in the pathogenesis of cardiovascular events.

Causal interaction between 2 factors means mutual dependence in causing disease, ie, such factors are component causes in the same causal model. Rothman[13] and others[12] have argued that potentially causal interaction needs to be evaluated as departure from additivity rather than departure from multiplicativity. In the present study, interaction was present on an additive (as indicated by RERI) as well as on a multiplicative scale (as indicated by interaction terms in the regression analyses). Not all interaction tests were, however, statistically significant. This may be because of the fact that, in general, these tests are limited by relatively low statistical power.

The mechanism that may underlie this interaction is the presence of a vicious circle between endothelial dysfunction and DM2, IGM, and insulin resistance,[6] with, on the one hand, DM2, IGM, and insulin resistance causing endothelial

dysfunction and, on the other, endothelial dysfunction causing insulin resistance, IGM, and DM2.[7,8] There is abundant evidence that DM2, IGM, and insulin resistance cause endothelial dysfunction,[9] but evidence for the reverse process is relatively recent.[7] However, insulin normally can redirect blood flow in skeletal muscle from non-nutritive capillaries to nutritive capillaries and, thereby, increase insulin-mediated glucose uptake.[19] These processes are impaired by endothelial dysfunction.[7] In addition, endothelial dysfunction may also cause apoptosis of β-cells in the pancreas,[20] which decreases insulin secretory capacity and, therefore, may, further impair glucose metabolism. In addition, DM2 may amplify the detrimental effects of endothelial dysfunction on atherothrombosis.[9] Hence, the co-occurrence of these processes may accelerate atherothrombosis and, thus, increase cardiovascular event risk more than expected from the presence of these processes alone.

In the present study, glucose metabolism and insulin resistance states interacted with endothelial dysfunction independently of each other. This suggests that mechanisms associated with DM2 and IGM other than insulin resistance may play a role in the interaction with endothelial dysfunction, such as advanced glycation end products, oxidative stress, and diabetic dyslipidemia.[9]

Somewhat surprisingly, estimates of hyperglycemia (ie, hemoglobin A1c, fasting, or postload glucose) did not interact with endothelial dysfunction in the association with cardiovascular events. This finding may have several explanations. First, only a single measurement of (baseline) variables was available in the present study. Baseline glucose levels may not accurately reflect exposure during follow-up. Second, glucose levels may not accurately reflect the mechanisms by which hyperglycemia leads to endothelial dysfunction and cardiovascular events (ie, advanced glycation end products and oxidative stress).[9] Third, it has been suggested that glucose variability[21] and episodes of hypoglycemia[22] may be more strongly associated with endothelial dysfunction than mean blood glucose levels (ie, hemoglobin A1c). Nevertheless, we cannot exclude the play of chance.

From a clinical point of view, the synergistic association between endothelial dysfunction and DM2 is important as endothelial dysfunction may act at least partially as the underlying phenomenon which might explain the 2 to 3× higher cardiovascular events risk seen in DM2. This suggests that endothelial dysfunction is a key therapeutic target for lowering of CVD risk in DM2. In addition, the fact that an interaction was already present in individuals with IGM and insulin resistance identifies endothelial dysfunction as an early therapeutic target even before DM2 is present. This is in accordance with the hypothesis that insulin resistance, IGM, and DM2 are manifestations of a continuous disease process to increase the risk of cardiovascular events (ticking clock hypothesis).[10,11]

Our study had some limitations. First, it is likely that survival bias affected our results, ie, it is probable that individuals who died before the start of the present study were those with the strongest association between endothelial dysfunction and DM2 and incident cardiovascular events. Such bias would, however, have led to an underestimation of the reported associations and may explain why we did not find an association between FMD and incident cardiovascular events in individuals with NGM. Second, the cardiovascular event rate in the present study population at high cardiovascular risk was in accordance with previous studies.[10] However, a relatively low number (11.3%) of participants died of a cardiovascular event, which may reflect a survival effect and may be because of the high quality of CVD management in the Netherlands. This low fatal cardiovascular event rate may explain the lack of an interaction between endothelial dysfunction and glucose metabolism or insulin resistance in the association with all-cause mortality. Third, the present study had insufficient power to evaluate interaction, with regard to specific cardiovascular events, between FMD on the one hand and glucose metabolism status en insulin resistance on the other. Finally, only a single (baseline) measurement of FMD was available. Baseline FMD may not accurately reflect exposure during follow-up, and this may have led to an underestimation of the reported associations.

In conclusion, the present study shows that individuals with DM2, IGM, or insulin resistance are particularly sensitive to the adverse cardiovascular effects of endothelial dysfunction.

Perspectives

In the pathogenesis of cardiovascular events, true interaction between risk factors has rarely been identified. The present study shows, for the first time, the presence of interaction (ie, synergy) between endothelial dysfunction and DM2, IGM, and insulin resistance with respect to cardiovascular event risk. This suggests that endothelial dysfunction is a key therapeutic target for the prevention of cardiovascular events in individuals with DM2, IGM, or insulin resistance.

Sources of Funding

The Hoorn Study was supported by grants from Organization for Health Research and Development, the Netherlands Heart Foundation and the Dutch Diabetes Research Foundation.

Disclosures

None.

References

1. Di Angelantonio E, Gao P, Pennells L, et al. Lipid-related markers and cardiovascular disease prediction. *JAMA*. 2012;307:2499–2506.
2. Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, Danesh J. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet*. 2010;375:132–140.
3. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R; Prospective Studies Collaboration. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*. 2002;360:1903–1913.
4. de Jager J, Dekker JM, Kooy A, Kostense PJ, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD. Endothelial dysfunction and low-grade inflammation explain much of the excess cardiovascular mortality in individuals with type 2 diabetes: the Hoorn Study. *Arterioscler Thromb Vasc Biol*. 2006;26:1086–1093.
5. Frankel DS, Meigs JB, Massaro JM, Wilson PW, O'Donnell CJ, D'Agostino RB, Tofler GH. Von Willebrand factor, type 2 diabetes mellitus, and risk of cardiovascular disease: the framingham offspring study. *Circulation*. 2008;118:2533–2539.
6. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation*. 2006;113:1888–1904.
7. Muris DM, Houben AJ, Schram MT, Stehouwer CD. Microvascular dysfunction is associated with a higher incidence of type 2 diabetes mellitus: a systematic review and meta-analysis. *Arterioscler Thromb Vasc Biol*. 2012;32:3082–3094.

8. Xu J, Zou MH. Molecular insights and therapeutic targets for diabetic endothelial dysfunction. *Circulation*. 2009;120:1266–1286.
9. Paneni F, Beckman JA, Creager MA, Cosentino F. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. *Eur Heart J*. 2013;34:2436–2443.
10. Ford ES, Zhao G, Li C. Pre-diabetes and the risk for cardiovascular disease: a systematic review of the evidence. *J Am Coll Cardiol*. 2010;55:1310–1317.
11. Sarwar N, Sattar N, Gudnason V, Danesh J. Circulating concentrations of insulin markers and coronary heart disease: a quantitative review of 19 Western prospective studies. *Eur Heart J*. 2007;28:2491–2497.
12. Knol MJ, van der Tweel I, Grobbee DE, Numans ME, Geerlings MI. Estimating interaction on an additive scale between continuous determinants in a logistic regression model. *Int J Epidemiol*. 2007;36:1111–1118.
13. Rothman KJ, Greenland S, Lash TJ. *Modern Epidemiology*. Philadelphia: Lippincott Williams and Wilkins; 2008.
14. Henry RM, Ferreira I, Kostense PJ, Dekker JM, Nijpels G, Heine RJ, Kamp O, Bouter LM, Stehouwer CD. Type 2 diabetes is associated with impaired endothelium-dependent, flow-mediated dilation, but impaired glucose metabolism is not; The Hoorn Study. *Atherosclerosis*. 2004;174:49–56.
15. Spijkerman AM, Adriaanse MC, Dekker JM, Nijpels G, Stehouwer CD, Bouter LM, Heine RJ. Diabetic patients detected by population-based stepwise screening already have a diabetic cardiovascular risk profile. *Diabetes Care*. 2002;25:1784–1789.
16. Unwin N, Shaw J, Zimmet P, Alberti KG. Impaired glucose tolerance and impaired fasting glycaemia: the current status on definition and intervention. *Diabet Med*. 2002;19:708–723.
17. van Bussel BC, Henry RM, Schalkwijk CG, Dekker JM, Nijpels G, Stehouwer CD. Low-grade inflammation, but not endothelial dysfunction, is associated with greater carotid stiffness in the elderly: the Hoorn Study. *J Hypertens*. 2012;30:744–752.
18. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care*. 2004;27:1487–1495.
19. Clark MG, Wallis MG, Barrett EJ, Vincent MA, Richards SM, Clerk LH, Rattigan S. Blood flow and muscle metabolism: a focus on insulin action. *Am J Physiol Endocrinol Metab*. 2003;284:E241–E258.
20. Tal MG. Type 2 diabetes: Microvascular ischemia of pancreatic islets? *Med Hypotheses*. 2009;73:357–358.
21. Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, Boemi M, Giugliano D. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. *Diabetes*. 2008;57:1349–1354.
22. Wang J, Alexanian A, Ying R, Kizhakekuttu TJ, Dharmashankar K, Vasquez-Vivar J, Gutterman DD, Widlansky ME. Acute exposure to low glucose rapidly induces endothelial dysfunction and mitochondrial oxidative stress: role for AMP kinase. *Arterioscler Thromb Vasc Biol*. 2012;32:712–720.

Novelty and Significance

What Is New?

- In the pathogenesis of cardiovascular events, true interaction between risk factors has rarely been identified.
- However, it has been suggested that endothelial dysfunction and type 2 diabetes mellitus (DM2) act synergistically (ie, interact) in the development of cardiovascular events.
- Earlier studies indeed showed that endothelial dysfunction, as determined by plasma biomarkers, was most strongly associated with incident cardiovascular events in individuals with DM2 as compared with those without DM2.
- However, previous studies did not evaluate causal interaction (ie, interaction on an additive scale) and did not measure flow-mediated dilatation, a key functional measure of endothelium-dependent, NO-mediated dilatation.

What Is Relevant?

- The present prospective population-based study investigated the association between endothelial dysfunction, as determined by flow-mediated dilatation, and incident cardiovascular events and formally tested whether any such association was stronger in individuals with DM2, impaired glucose metabolism, and insulin resistance as compared with individuals with normal glucose metabolism or normal insulin sensitivity.

Summary

The results of the present study showed that endothelial dysfunction and DM2, impaired glucose metabolism, or insulin resistance synergistically increase cardiovascular event risk. This identifies endothelial dysfunction as a key therapeutic target in these individuals.