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Cardiovascular Risk Factors as Determinants of Endothelium-Dependent and Endothelium-Independent Vascular Reactivity in the General Population

N. Norman Chan, MB, MRCP,* Helen M. Colhoun, MD, MFPHM,* Patrick Vallance, PHD, FRCP†
London, United Kingdom

OBJECTIVES We examined to what extent the variation in risk factors for coronary heart disease (CHD) and the Framingham risk score (FRS) explain the variation in vascular reactivity in adults aged 30 to 53 years.

BACKGROUND The role of risk factors in determining vascular reactivity in the general population has not been quantified.

METHODS Risk factors for CHD were measured, and the FRS was calculated in 69 healthy volunteers. Lipoprotein particle size was measured using proton-nuclear magnetic resonance spectroscopy. Forearm plethysmography was used to assess blood flow responses to acetylcholine (ACh), bradykinin (BK), glyceryl trinitrate (GTN), noradrenaline and N^G-monomethyl-L-arginine (L-NMMA).

RESULTS Lower ACh and BK responses were associated with a higher body mass index (BMI), a higher total cholesterol to high-density lipoprotein (HDL) cholesterol ratio, lower HDL cholesterol and a cigarette smoking habit (all $p < 0.05$). Higher low-density lipoprotein (LDL) cholesterol was also associated with a lower BK response ($p = 0.001$). A decreased GTN response was associated with a higher BMI and total cholesterol to HDL cholesterol ratio (both $p < 0.05$). A decreased L-NMMA response was associated with a smoking habit ($p < 0.001$). Lipoprotein particle sizes did not independently predict any vascular response. A high FRS was associated with a reduced response to ACh ($p = 0.07$), BK ($p = 0.003$) and L-NMMA ($p = 0.003$), and the relationship was stronger in women than in men. Altogether, risk factors explained 13%, 9%, 8% and 15% of the response to ACh, BK, GTN and L-NMMA, respectively.

CONCLUSIONS Lipids, BMI and smoking are important determinants of vascular reactivity. The FRS is predictive of agonist-stimulated, endothelium-dependent vasodilation and basal NO release. However, much of the variation in the vascular responses to these drugs, at this age, remains unexplained. (J Am Coll Cardiol 2001;38:1814–20) © 2001 by the American College of Cardiology

Vascular dysfunction has been implicated as an early event in atherogenesis (1). A particularly important aspect of vascular function is the integrity of the L-arginine–nitric oxide (NO)–cyclic guanosine monophosphate pathway. Endothelium-derived NO is an antiatherogenic molecule that has effects on vascular tone, homeostasis and platelet function (2). Previous studies in humans have suggested that risk factors for coronary heart disease (CHD), such as obesity (3), dyslipidemia (4–7), hypertension (8), cigarette smoking (9) and lack of physical activity (10), are associated with defects in NO-mediated vascular function. In many of these risk-factor studies, subjects with extremely abnormal risk-factor levels were compared with healthy control subjects. Recent studies have also reported a very high correlation between lipoprotein particle size and aspects of vascular function (11,12). However, many risk factors are continuous variables and are not merely present or absent, but present to a greater or lesser extent in every individual.

The purpose of this study was to quantify to what extent the variation in these risk factors, across the range usually found in the general population and not just at the extremes of the range, predict interindividual variations in vascular function. We also examined whether the Framingham risk score (FRS), calculated for each subject, predicts NO release or response.

METHODS

Subjects. The study was approved by the local Ethics Committee, and all participants gave written, informed consent to participate. The participants were recruited from a cohort of 201 healthy subjects who had taken part in a separate study within the preceding 12 months (13). All subjects who had a history of cardiovascular disease were recruited from two general practices in London. Subjects with diabetes mellitus were excluded on the basis of a clinical history and a random blood glucose test taken at the time of the study (all <7 mmol/l) (Table 1). In the

From the *EURODIAB, Department of Epidemiology and Public Health, and †Centre for Clinical Pharmacology, University College London, London, United Kingdom. This study was supported by the British Heart Foundation.

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Abbreviations and Acronyms

ACh = acetylcholine
BK = bradykinin
BMI = body mass index
CHD = coronary heart disease
FRS = Framingham risk score
GTN = glyceryl trinitrate
HDL = high-density lipoprotein
LDL = low-density lipoprotein
L-NMMA = N^G-monomethyl-L-arginine
NO = nitric oxide
PNMR = proton-nuclear magnetic resonance

United Kingdom, general practice registries provide a good sampling frame for the general population. Among this cohort of 201 subjects, 69 took part (34%). All were between 30 and 53 years old, and 50% were women. All participants gave their informed consent. Risk-factor measurements were performed one year before the vascular study. Blood pressure was also measured at the time of the vascular study, and these data are used in this analysis.

Assessment of risk factors. A standardized questionnaire was used. The average weekly consumption of alcohol units was calculated, and cigarette smoking exposure was quantified as pack-years. Obesity was defined as a body mass index (BMI) $\geq$30 kg/m^2. A score combining the duration and intensity of physical activity was calculated and dichotomized as low (weekly score $\leq$10) or high. Before the vascular study, blood pressure in the right arm was measured three times, using an automated digital monitor (Omron 705CP, OMRON, Tokyo, Japan), with the subject seated after a 5-min rest. The mean value of the three measurements is presented. For each subject, the FRS was calculated by using the most recent FRS equation (14).

In vivo vascular function study. Subjects were asked to refrain from drinking alcohol and caffeine-containing beverages 24 h before the study. Studies were performed in a quiet, temperature-controlled (24–27°C) laboratory. Forearm blood flow was measured by venous occlusion plethysmography, as described in detail elsewhere (15). Forearm blood flow was measured in response to intrabrachial infusion of acetylcholine (ACh) (Sigma, St. Louis, Missouri; doses of 25, 50 and 100 nmol/l, each dose for 3 min); bradykinin (BK) (Clinalfa, Laufelfingen, Switzerland; doses of 10, 30 and 100 pmol/min, each dose for 3 min); glyceryl trinitrate (GTN) (David Bull Laboratories, Warwick, U.K.; doses of 4, 8 and 16 nmol/min, each dose for 5 min); noradrenaline (Levophed; Sanofi Winthrop Ltd., Guildford, U.K.; doses of 60, 120 and 240 pmol/min, each dose for 5 min) and N^G-monomethyl-L-arginine (L-NMMA) (Clinalfa, Laufelfingen, Switzerland; doses of 1, 2 and 4 μmol/min, each dose for 5 min). Each drug infusion was separated by a 10-min saline washout period. The order of vasodilator infusions (ACh, BK and GTN) was randomized. L-NMMA was always infused last, because of its long duration of action. Flow was recorded for ~10 s every 15 s, and the mean value of the last four measurements of each recording period was used for data analysis. Blood flow was expressed as milliliter of blood per 100 ml of forearm volume per minute (ml/100 ml per min). At the end of each study, the length and volume of the infused arm were measured.

Laboratory methods. Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were measured using enzymatic colorimetric methods. High-density lipoprotein cholesterol was measured directly after stabilization of other lipoproteins. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. Venous blood samples were also taken before and immediately after vascular studies for plasma glucose analysis. Proton-nuclear magnetic resonance (PNMR) spectroscopy was used to quantify LDL, HDL and very-low-density lipoprotein (VLDL) cholesterol subclasses and the average particle size, as described previously (16). A repeatability study on blinded duplicate samples showed good repeatability; 0.91 for VLDL particle size, 0.92 for LDL particle size and 0.96 for HDL particle size.

Heart rate variability. A 5-min electrocardiographic (ECG) recording was obtained using a lead ensuring a prominent R wave, after 5 to 10 min in the supine position. The ECG signal was digitized at 1,000 Hz, using a computer with an analog-to-digital card (AT-MIO-16E2, National Instruments, Austin, Texas). Autoregressive power spectral analysis was used to determine the spectral power in the following frequency bands: high-frequency power (0.15 to 0.45 Hz), low-frequency power (0.04 to 0.15 Hz) and very-low-frequency power (0.01 to 0.04 Hz), and the total power was the sum of these.

Statistical analysis. The response to drugs was defined as the ratio of flow during drug infusion to flow during baseline saline infusion. We used repeated measures analysis of covariance to define how the response to drugs, across the three doses, varied by risk-factor level, adjusting for age,

Table 1. Characteristics of Subjects

	Men (n = 34)	Women (n = 35)
Age (yr)	37.8 ± 3.9	37.9 ± 3.5
BMI (kg/m^2)	25.0 ± 3.4	25.6 ± 5.6
Smokers (pack-years)	8.2 ± 11.0	6.3 ± 11.0
Systolic blood pressure (mm Hg)	128.9 ± 11.6	119.4 ± 11.9
Diastolic blood pressure (mm Hg)	82.0 ± 9.8	75.2 ± 9.5
Total cholesterol (mmol/liter)	5.7 ± 1.2	5.3 ± 1.2
LDL cholesterol (mmol/liter)	3.3 ± 1.1	3.0 ± 0.8
HDL cholesterol (mmol/liter)	1.57 ± 0.4	1.82 ± 0.4
HDL cholesterol to total cholesterol ratio	0.28 ± 0.09	0.33 ± 0.13
Triglycerides (mmol/liter)	1.72 ± 1.5	1.25 ± 1.9
Plasma glucose (mmol/l)	5.4 ± 0.6	5.0 ± 0.5
Total spectral power (Hz)	1,204 (1,344)	1,106 (1,369)

Data are presented as the mean value ± SD or median value (distance between 25th and 75th centiles).

BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Table 2. Percent Change in Each Drug Response for Per Unit Change in Risk Factor (With 95% Confidence Interval)

	ACh	BK	GTN	NA	L-NMMA
SBP (10 mm Hg)	0.8% (−8, 10)	−0.4% (−1, 0.1)	−2% (−7, 4)	−3% (−9, 3)	0% (−3, 3)
DBP (10 mm Hg)	3% (−7, 15)	−0.1% (−1, 0.6)	−2% (−8, 5)	−4% (−11, 3)	0.4% (−3, 4)
BMI (kg/m^2)	−3%* (−5, 0)	−3%† (−4, −1)	−2%* (−6, −1)	1% (−1, 3)	−1% (−2, 0)
LDL cholesterol (mmol/l)	−3% (−12, 7)	−9%† (−14, −4)	−4% (−10, 2)	6% (−2, 14)	−2% (−5, 1)
HDL cholesterol (mmol/l)	36%* (7, 73)	30%† (11, 51)	11% (−4, 30)	−13% (−18, 4)	0.2% (−8, 9)
Total cholesterol to HDL cholesterol ratio	−9%* (−16, −2)	−9%‡ (−13, −5)	−5%* (−10, −1)	−4% (−9, 1)	2% (−0.5, 5)
Triglycerides (mmol/l)	−3% (−9, 2)	−3% (−7, 0)	−3% (−6, 0.3)	1% (−3, 6)	−1% (−3, 0)
Total spectral power	4% (−8, 18)	4% (−4, 1)	9%* (1, 17)	5% (−3, 14)	3% (−1, 7)
Smoking§	−23%* (−40, −2)	−17%* (−29, −3)	−1% (−16, 15)	−8% (−22, 9)	−13%† (−22, −5)

*$p < 0.05$, †$p < 0.005$, ‡$p < 0.0005$, adjusted for basal flow, flow in control arm, age and gender. §At least 10 pack-years versus no pack-years.
ACh = acetylcholine; BK = bradykinin; DBP = diastolic blood pressure; GTN = glyceryl trinitrate; L-NMMA = N^G-monomethyl-L-arginine; NA = noradrenaline; SBP = systolic blood pressure; other abbreviations as in Table 1.

gender, basal flow and flow in the control arm. Because the flow data had a skewed distribution, they were log-transformed for these analyses. The contribution of each risk factor to the variation in drug response between individuals was quantified by noting the change in the intersubject variance (i.e., the change in the between-subject R^2) when that risk factor was added to the model that already included age, gender, basal flow and flow in the control arm. For ACh and BK, independence from the GTN response was checked by including the GTN response in the models. The models were then repeated by simultaneously including BMI, lipids, systolic and diastolic blood pressure and smoking to examine which factors were independently associated with the response, as well as the combined contribution of the risk factors to the intersubject variation in response. The relationship of lipoprotein particle size and heart rate variability to vascular response was examined in separate models.

RESULTS

Subject characteristics. The background characteristics of the participants are shown in Table 1. Five subjects were non-white. Fourteen subjects (20%) had hypertension, defined as antihypertensive drug use or systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg. Six subjects were taking the oral contraceptive pill, and one was receiving hormone replacement therapy. Three women were postmenopausal. The pattern of risk-factor associations shown subsequently was the same when non-white subjects, postmenopausal women or women using hormone replacement therapy or hormonal contraception were excluded. The majority of women (77%) were in the follicular phase of their menstrual cycle when studied, and the associations noted subsequently were not affected by adjusting for the cycle phase. No participants had a history or symptoms of CHD. There was considerable variation in the response to drugs between subjects. For example, with the highest drug dose, blood flow increased from 1.7- to 4.9-fold for GTN, 1.7- to 9.7-fold for ACh, 1.5- to 6.7-fold for BK and 0.52- to 1.1-fold for L-NMMA.

Associations between vascular response and basal flow, flow in the control arm, age and gender. The differences in basal flow between subjects contributed significantly to the variance in the ratio of flow during drug infusion to flow at baseline, accounting for 16%, 23%, 20% and 15% of the variation in the responses to ACh, BK, GTN and L-NMMA, respectively. Changes in systemic flow during the vascular study, as measured by changes in flow in the control arm, accounted for further variation in the responses to ACh, BK, GTN and L-NMMA between subjects (14%, 17%, 9% and 30%, respectively). Accordingly, all models examining the role of risk factors were first adjusted for basal flow and flow in the control arm. The age range of subjects was narrow, and, accordingly, age did not account for any of the variation in response. Gender was also unrelated to the vascular response independently of basal flow and control arm flow.

Association between classic CHD risk factors and vascular responses. Table 2 shows the association between established CHD risk factors and drug responses adjusted for age, gender, basal flow and flow in the control arm. In addition to the risk factors shown, alcohol intake and physical activity were also examined, and they showed no association with any of the drug responses and were not included in any of the combined models.

Agonist-stimulated, endothelium-dependent vasodilation. A similar pattern of risk-factor associations was found for BK and ACh responses (Table 2), with the responses being lower in those with a higher BMI, total cholesterol to HDL cholesterol ratio, lower HDL cholesterol and in those who smoked cigarettes. Low-density lipoprotein cholesterol was associated with a BK response only. The associations were independent of the response to GTN. Table 2 shows how much of a difference in drug response was associated with a given difference in the risk factors for both genders combined. For example, for every 1 kg/m^2 higher BMI, there was a 3% lower response to ACh and BK. The association between BMI and drug response was independent of forearm volume for BK ($p = 0.04$), but not ACh. Overall, BMI, total cholesterol to HDL cholesterol ratio,

Table 3. Difference in Drug Response Per Quartile Increase in Framingham Risk Score (2000) (95% Confidence Interval) in Men and Women

ACh	BK	GTN	L-NMMA
−10%, p = 0.07 (−2%, 1%)	−11%, p = 0.003 (−17%, −4%)	0.01%, p = 0.9 (−7%, 7%)	−6%, p = 0.003 (−10%, −2%)

Abbreviations as in Table 2.

HDL and LDL cholesterol and smoking accounted for 5%, 7%, 7%, 4% and 7% of the variation in the ACh response, respectively, and 9%, 11%, 9%, 10% and 4% of the variation in the BK response, when each factor was examined separately in the model adjusted for age, gender, basal flow and control arm flow. Because these risk factors cluster with each other, the total variance explained by the entire risk-factor profile is less than the sum of the variance explained by each risk factor examined separately. When entered into the model simultaneously, they accounted for 13% of the ACh response and 19% of the BK response. The only risk factor significantly associated with a drug response, independently of other factors in these models, was smoking, which remained independently associated with the ACh response (p = 0.04).

When each gender was examined separately, the associations of drug response with total cholesterol to HDL cholesterol ratio and HDL cholesterol were less strong in men than in women, although not significantly so (data not shown), and in men, there was no evidence of an association between BMI and BK response. In contrast, LDL cholesterol was associated with a BK response more strongly in men than in women (data not shown).

Endothelium-independent vasodilation. Endothelium-independent vasodilation, as assessed by the GTN response, was associated with BMI and total cholesterol to HDL cholesterol ratio (Table 2). The total cholesterol to HDL cholesterol ratio explained ~6% of the variation in the GTN response and BMI accounted for 5%. When all of the risk factors were entered into the model simultaneously, they accounted for 8% of the variance in response, and no risk factor was statistically significant on its own.

When both genders were examined separately, the association between total cholesterol to HDL cholesterol ratio and GTN response was of a similar magnitude in both genders, but the relationship between BMI and GTN response was only significant in women. Among women, for every for every 1 kg/m^2 increase in BMI, there was a 3% reduction in the GTN response (p = 0.006), and this was independent of forearm volume (p = 0.005 after adjustment).

Basal NO release. The only risk factor that showed a significant association with basal NO release, as assessed by L-NMMA response, was smoking. Subjects who had at least 10 pack-years of smoking had a 13% lower L-NMMA response, as compared with nonsmokers (Table 2). Overall, smoking accounted for ~9% of the interindividual variation in response to L-NMMA. The relationship was apparent in both genders. The relationship was not attributable to a generalized increased vasoconstrictor response in smokers, because there was no difference in the noradrenaline response with smoking (Table 2).

With all risk factors entered into the model simultaneously, they accounted for 15% of the interindividual variation in the L-NMMA response, and the smoking association remained significant (p = 0.001).

Nonclassic CHD risk factors. A higher PNMR spectroscopy-defined HDL particle size was associated with a higher response to BK (18% higher for every nanometer increase in average HDL diameter, p = 0.02 adjusted for basal flow, flow in the control arm, age and gender). The HDL particle size was highly correlated with both HDL cholesterol and total to HDL cholesterol ratio (r = 0.8 and r = −0.7, respectively) and was not associated with the BK response independently of either of these. No other associations were found between average LDL or VLDL particle size and any of the drug responses. In particular, having a pattern B type LDL (average particle size <20.5 nm) was not associated with an altered drug response.

Total spectral power was associated with an ACh response, with those in the bottom quartile of spectral power having a 24% lower response than the remaining subjects (p = 0.03 adjusted for basal flow, flow in the control arm, age and gender). Total spectral power also showed an association with GTN response, with those in the bottom quartile having a 20% lower response than those in the top quartile (p = 0.048). Spectral power accounted for 6% and 5% of the interindividual variation in ACh and GTN responses, respectively. The difference in both ACh and GTN responses with spectral power was independent of BMI and smoking, but was reduced to 18% (p = 0.1) for ACh and 15% (p = 0.1) for adjustment of lipids.

Framingham risk score. The relationship between FRS and vascular response is shown in Table 3. A higher FRS was associated with a reduced response to BK and L-NMMA. These relationships were stronger for women than for men and were nonsignificant in men when examined separately (data not shown). A higher FRS was also associated with a reduced response to ACh in women. There was no relationship between FRS and response to GTN. As shown in Table 4, the percentage of the variance

Table 4. Percent Variance in Drug Response Explained by Framingham Risk Score Beyond That Explained by Age and Gender

ACh	BK	GTN	L-NMMA
4%	7%	0%	7%

Abbreviations as in Table 2.

in drug response explained by the score was much less than that in models in which these risk factors were entered simultaneously, as described earlier. When both the score and risk factors were entered into the models simultaneously, the risk factors explained a further 8% to 10% of the variance in the response to the drugs.

DISCUSSION

Studies that compare vascular function between extremes of a distribution (e.g., obese or hypercholesterolemic patients and control subjects) provide useful information on the pathophysiologic mechanisms that may be involved in vascular dysfunction, with relatively small sample sizes. However, such studies do not give insight into the likely quantitative effect of risk factors in the general population. In contrast, our study, in a sample representative of the range of factors and vascular function found in the general population, provides a different type of information that allows quantification of the role of a given risk factor in the variation in vascular responses in the population. The results show that BMI and lipids are important determinants of stimulated NO release and responsiveness to an NO donor in forearm resistance vessels in the general population. It is clear that HDL cholesterol and the total cholesterol to HDL cholesterol ratio are as important as LDL cholesterol, a finding that is in keeping with the relative importance of these measures as risks factors for cardiovascular events. In the presence of a defective response to an NO donor (GTN), it is not possible to state with certainty whether a reduced response to ACh and BK primarily reflects defective NO release or diminished responsiveness to NO. However, the association of BMI, lipids and smoking with ACh and BK responses was statistically independent of the GTN response, suggesting an effect on stimulated NO release. Cigarette smoking is an important determinant of both stimulated and basal NO release. However, our study also shows that, although these factors are clearly important, they explain relatively little of the variation in the measure of vascular function we have used (13% for ACh, 19% for BK, 8% for GTN and 15% for L-NMMA). Approximately 30% to 45% of the variation in the change in flow in response to drugs between subjects was accounted for by differences in resting basal flow and the concomitant changes in flow in the control arm (data not shown). The rest of the variation must either be due to measurement error in this type of study or as-yet unidentified major determinants of variation in vascular function, that are worth identifying. Previous studies of vascular responsiveness that have focused on more than one risk factor have mostly examined flow-mediated dilation in the brachial artery (17,18) or coronary vessels (19,20), rather than the forearm resistance vessels.

Body mass index and vascular function. Our data are consistent with previous studies showing that higher BMI is associated with reduced endothelium-dependent (BK and ACh responses) vasodilation (3,21). Unlike the previous studies, however, we have also demonstrated that obesity is associated with a reduced response to NO donors (GTN response), at least in women. The mechanisms may be related to the inflammatory effect of obesity (22) or associated oxidative stress (21), but further studies are required to test this directly.

High-density lipoproteins and vascular responses. High HDL cholesterol and a low total cholesterol to HDL cholesterol ratio were associated with a greater response to ACh, BK and GTN in forearm resistance vessels. This is consistent with a recent study in which Li et al. (23) studied the relationship between lipoproteins and conduit vessel response in 63 subjects with CHD and 45 control subjects. They found that only elevated HDL cholesterol was significantly related to flow-mediated vasodilation of the brachial arteries. It has become clear that HDL cholesterol is a major determinant of cardiovascular risk, and it is interesting to note that it is a prime determinant of vascular reactivity in a general, asymptomatic population.

Low-density lipoprotein cholesterol and its subfractions and vascular responses. High LDL cholesterol levels were associated with an impaired BK response, but responses to other agents were unaffected. This is in contrast to studies in which individuals with high or normal total or LDL cholesterol were compared; these showed that increased total or LDL cholesterol is associated with impaired ACh-stimulated, endothelium-dependent vasodilation in forearm resistance vessels (24–26) and coronary arteries (19). It remains to be determined whether the differences in those studies are due to the selection of subjects with high cholesterol, who also have other risk factors (e.g., low HDL cholesterol), which affects the vascular response, or whether the relatively narrow range of LDL cholesterol levels in the present study precluded detection of a weak effect of LDL cholesterol. In a small study (n = 24), Gilligan et al. (25) found no difference in the bradykinin response between hypercholesterolemic and normal subjects, but the study was underpowered (27). We did not replicate previous findings of an association between impaired coronary vasoconstrictor response to L-NMMA in coronary vessels and hypercholesterolemia, and this may partly reflect differences in the vascular bed studied (20).

The relationship between LDL particle size (measured by gradient gel electrophoresis) and endothelial function was examined recently by Vakkilainen et al. (28). They demonstrated that men with small LDL particles (as measured by gradient gel electrophoresis) have a 39% lower forearm vascular response to ACh (but not sodium nitroprusside, an NO donor), as compared with men with large LDL particles. Our study does not support their findings, even when the top and bottom quartile groups for LDL particle size or when male gender alone (n = 34) were compared. The reason for this difference is unclear, but may be related to differences in the study population (younger mean age in our study) or LDL particle measurement methods (gradient

gel electrophoresis versus PNMR), or both. Clearly, further studies are required to address this issue definitively, and the findings of Vakkilainen's study and ours may form the basis for a power calculation for future studies.

Smoking. We found an inverse association between ACh- and BK-stimulated, endothelium-dependent vasodilation with cigarette smoking. In addition, we found that smokers have a lower response to L-NMMA than do nonsmokers. This suggests that either basal NO production is diminished in smoking or there is diminished NO bioavailability, or a combination of these two factors. It has been shown that smoking is associated with depletion of cofactors for NO synthase, such as tetrahydrobiopterin (29) and increased oxidative stress (30). Our finding that there was no significant association between smoking and the noradrenaline response indicates that the defective L-NMMA response in smoking is unlikely to be due to a generalized defect in vasoconstrictor responses. Previous studies of forearm resistance vessel responsiveness in smokers and nonsmokers have not found any defect in stimulated NO production, but they had smaller sample sizes (31–33). The result of a large recent study was consistent with our findings (34). In general, studies are consistent in their finding that smoking decreases basal NO release (33–35).

Blood pressure. With respect to blood pressure, defective endothelium-dependent vasodilation has been demonstrated in subjects with established essential hypertension (8,36), although this association has been challenged (37). In our study, systolic and diastolic blood pressure (measured at the beginning of each study) and pulse pressure were not associated with changes in any drug responses (data not shown), nor was there any association with blood pressure measured one year before the study. This may be due to the low blood pressure range (systolic: 96 to 155 mm Hg) seen in our cohort of participants, as well as the fact that relatively few (14/69) participants had hypertension (defined as systolic blood pressure $\geq$140 mm Hg or diastolic blood pressure $\geq$90 mm Hg or antihypertensive drug use). Alternatively, it is possible that previous associations between hypertension and the vascular response may reflect associated risk factors (e.g., insulin resistance) that were not prevalent in our study group, rather than a direct effect of blood pressure itself or a direct effect of the endothelium on blood pressure.

Heart rate variability and vascular response. We found that lower total reduced heart rate variability was associated with a reduced response to GTN, although not independently of lipids. In the general population, heart rate variability is associated with an increased incidence of CHD and postinfarction mortality (38). The significance of our finding is not clear, but it suggests the role of the autonomic nervous system in altering the vascular response to NO and is worthy of further investigation.

Framingham risk score and vascular responses. Interestingly, the most widely used cardiovascular risk prediction tool—the FRS—is clearly predictive of the responses to ACh, BK and NO, but not to GTN. This suggests that, overall, conventional risk factors are more important for agonist-stimulated, endothelial-dependent vasodilation and basal NO-mediated dilation than for NO responsiveness. This is consistent with flow-mediated vasodilation studies wherein the total number of risk factors was associated with the response during reactive hyperemia, but not the response to GTN (18). The association was clearer in women than in men. This may be because the FRS is more accurate in predicting CHD risk in women than in men. Alternatively, it is possible that NO release is more important to CHD risk in women than in men. Whatever the explanation, it is important that forearm responses are significantly influenced by the score, and this strengthens the use of forearm responses as a surrogate marker in clinical studies of new therapies.

Methodologic considerations. One issue to consider in this study is that, apart from blood pressure, risk factors were measured one year before the vascular study. Contemporaneous measures of these factors might have shown stronger associations with the vascular response. However, most of the risk factors we measured (e.g., HDL and LDL cholesterol and BMI) have high tracking (i.e., the rank order of these values in a group of people will change very little over the course of a year), and we confirmed that smoking status had not changed. Thus, differences in the timing of risk-factor measurement should have little bearing on the pattern of associations seen, unless it is the instantaneous value of these factors, rather than their long-term impact on the vascular response, that matters most. Most of the hypotheses on how these risk factors might affect vascular responsiveness assume that they predict vascular function in the longer term, rather than just instantaneously. As such, our study has the advantages of a cohort study in that what we are measuring is the predictive value of these factors over one-year follow-up.

Conclusions. Lipids and smoking, but not blood pressure or lipoprotein particle size, were associated with demonstrable variations in the NO pathway in early middle age. Many of the changes occur in response to NO, rather than just as endothelium-dependent responses, although for smoking it appears as though there is a true defect in basal NO generation. A high FRS is predictive of reduced agonist-stimulated and basal endothelium-dependent vasodilation. The vast majority of the interindividual variation in vascular function in the general population cannot be explained by classic cardiovascular risk factors.

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Reprint requests and correspondence: Dr. Helen M. Colhoun, EURODIAB, Department of Epidemiology and Public Health, University College London, 1-19 Torrington Place, London WC1E 6BT, United Kingdom. E-mail: Helen@public-health.ucl.ac.uk.

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