S-Adenosylmethionine and 5-Methyltetrahydrofolate Are Associated With Endothelial Function After Controlling for Confounding by Homocysteine

The Hoorn Study

A.M.W. Spijkerman, Y.M. Smulders, P.J. Kostense, R.M.A. Henry, A. Becker, T. Teerlink, C. Jakobs, J.M. Dekker, G. Nijpels, R.J. Heine, L.M. Bouter, C.D.A. Stehouwer

Objective—To explore to what extent homocysteine, S-adenosylmethionine (SAM), S-adenosylhomocysteine, total folate, 5-methyltetrahydrofolate (5-MTHF), vitamin B12, and vitamin B6 are associated with endothelium-dependent, flow-mediated vasodilation (FMD), and whether these associations are stronger in individuals with diabetes or other cardiovascular risk factors.

Methods and Results—In this population-based study of 608 elderly people, FMD and endothelium-independent nitroglycerin-mediated dilation (NMD) were ultrasonically estimated from the brachial artery (absolute change in diameter [μm]). High SAM and low 5-MTHF were significantly associated with high and low FMD, respectively (linear regression coefficient, [95% confidence interval]): 48.57 μm (21.16; 75.98) and −32.15 μm (−59.09; −5.20), but high homocysteine was not (−15.11 μm (−42.99; 12.78). High SAM and low 5-MTHF were also significantly associated with high and low NMD, respectively. NMD explained the association of 5-MTHF with FMD but not of SAM. No interactions were observed for diabetes or cardiovascular risk factors.

Conclusions—In this elderly population, both SAM and 5-MTHF are associated with endothelial and smooth muscle cell function. The effect of homocysteine on endothelial function is relatively small compared with SAM and 5-MTHF. The relative impact of SAM, 5-MTHF, and homocysteine, and the mechanisms through which these moieties may affect endothelial and smooth muscle cell function need clarification. **(*Arterioscler Thromb Vasc Biol*. 2004;25:778-784.)**

Key Words: homocysteine ■ S-adenosylmethionine ■ metabolism ■ folate ■ endothelial function

The mechanisms responsible for the association of hyperhomocystinemia with cardiovascular disease[1,2] are incompletely understood. A leading hypothesis is that homocysteine increases oxidative stress and impairs endothelial function,[3] which is a key factor in the pathogenesis of atherosclerosis.[4] Hyperhomocystinemia has been associated with impaired endothelium-dependent vasodilation,[5–7] although this finding is by no means universal.[5,8,9]

A complementary hypothesis is that the association between hyperhomocystinemia and endothelial dysfunction may be partly explained by factors involved in homocysteine metabolism. Homocysteine is produced from methionine via S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) (Figure 1). Homocysteine can be remethylated to methionine in a reaction that requires folate and vitamin B12, or it can enter the transsulfuration pathway, which requires vitamin B6[10] (Figure 1).

It has been suggested that the association between hyperhomocystinemia and cardiovascular disease may be explained by a low SAM or a high SAH concentration or a low SAM/SAH ratio,[11–13] and/or by low concentrations of folate, vitamin B6, or vitamin B12.[14–17] Another additional hypothesis is that hyperhomocystinemia (or related components) affects vascular function more strongly in the presence of other cardiovascular risk factors,[18,19] particularly type 2 diabetes.[20]

We explored these latter 2 hypotheses in a large population-based study. We determined to what extent homocysteine, SAM and SAH, total folate and 5-MTHF, vitamin B12 and vitamin B6, were associated with brachial artery endothelium-dependent flow-mediated vasodilation (FMD), after controlling for potential confounding. We also assessed whether the associations of these components of

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From the Institute for Research in Extramural Medicine (A.M.W.S., P.J.K., J.M.D., G.N., L.M.B., C.D.A.S.), Institute for Cardiovascular Research (A.M.W.S., Y.M.S., A.B., T.T., C.J.), Department of Internal Medicine (Y.M.S.), Department of Clinical Epidemiology and Biostatistics (P.J.K.), Department of Clinical Chemistry (T.T., C.J.), and Department of Endocrinology (R.J.H.), VU University Medical Center, Amsterdam; Department of Internal Medicine (R.M.A.H.), Hospital Kennemer Gasthuis, Haarlem; and Department of Internal Medicine (C.D.A.S.), Academic Hospital Maastricht, the Netherlands.

Correspondence to Y.M. Smulders, MD, PhD, Department of Internal Medicine, VU University Medical Center, PO Box 7057, 1007 MB, Amsterdam, the Netherlands. E-mail y.smulders @vumc.nl

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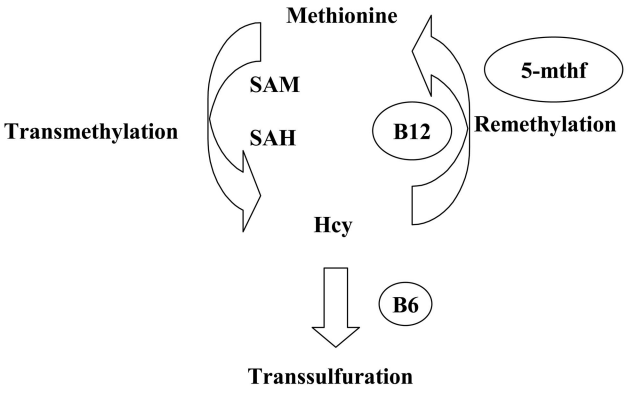

Figure 1. Homocysteine methionine metabolism.

homocysteine metabolism with FMD were stronger in individuals with diabetes and other cardiovascular risk factors, as compared with those without.

Methods

Study Population

For the present investigation, we used data from the 2000 Hoorn Study follow-up examination and the Hoorn Screening Study, both of which were population-based.[21,22] The study population (n=822) consisted of 290 individuals with normal glucose metabolism, 187 with impaired glucose metabolism and 345 type 2 diabetic mellitus patients.[23] The local ethics committee approved the study. All participants gave their written informed consent.

Measurement of Endothelium-Dependent and Endothelium-Independent Dilation

The ultrasound examination was performed according to the guidelines of the International Brachial Artery Reactivity Task Force[24] and has previously been described in detail.[21] Please see http://atvb.ahajournals.org for details. FMD is largely mediated by endothelium-derived nitric oxide and is considered to reflect endothelial function. Nitroglycerin-mediated dilation (NMD) is considered to reflect smooth muscle cell function and to be a control test for FMD. There is evidence that impairment of NMD itself is also associated with an adverse cardiovascular prognosis.[25]

Sample Preparation and Determination of Homocysteine, SAM and SAH, and B Vitamins

All blood sampling and sample processing was previously described in detail.[26,27] Please see online supplement (available at http://atvb.ahajournals.org) for details.

Measurements of Potential Confounding and/or Mediating Factors

Health status, medical history, current medication use, and smoking habits were assessed by a questionnaire.[28] We determined systolic and diastolic pressure, plasma glucose, serum creatinine, serum albumin, serum urea, lipid profiles, body mass index (BMI), and waist-to-hip ratio, as described elsewhere.[28,29] Hypertension and previous cardiovascular disease were defined as described previously,[28] and glomerular filtration rate was estimated according to Levey formula.[30]

Statistical Analyses

All analyses were performed with SPSS 11.0 for Windows 2000 (SPSS, Chicago, Ill). Please see online supplement for details. In brief, we used multiple linear regression analysis to study every single component of homocysteine metabolism in turn as the central determinant of FMD and/or NMD. None of the associations with FMD or NMD was linear and this nonlinearity remained after log-transformation or taking the square root of the independent variables. Because of this consistent nonlinearity, participants were divided in groups of equal size according to levels of components of homocysteine metabolism (tertiles). We compared the highest tertile to the lower 2 tertiles for components for which high levels are known to be associated with an adverse cardiovascular risk profile (for example homocysteine) and vice versa (for example 5-MTHF). The highest tertile of SAM was compared with the lower 2, because of our previous observation of a favorable effect of high SAM on intima-media thickness of the carotid artery.[27] In regression models for FMD, we considered age, sex, glucose tolerance status, baseline diameter, and the increase in peak systolic velocity as standard correction variables (Table 2, standard model)[31,32]. In the models for NMD, we always included age, sex, glucose tolerance status, and baseline diameter (Table 2, standard model). Potential confounding factors were selected through the change in estimate approach.[33] $P<0.05$ was considered statistically significant. Interaction was tested with product terms. Because of the large number of interactions (n=56), the level of significance for interaction was set at $P<0.01$.

Results

The present population consisted of 608 individuals because 214 participants had qualitatively unsatisfactory ultrasound examinations[21] and/or missing data on homocysteine. The group with missing data had a significantly higher BMI, more frequently had type 2 diabetes and hypertension, was significantly older, had higher homocysteine, pyridoxal-5′-phosphate, SAM and SAH levels, a lower glomerular filtration rate, and less favorable lipid profiles (data not shown). The analyses with NMD as the dependent variable were performed in 576 individuals with complete data. We excluded 2 participants with very high 5-MTHF and pyridoxal-5′-phosphate levels for all analyses concerning 5-MTHF and pyridoxal-5′-phosphate.

Tables 1 and 2 show the clinical characteristics and brachial artery characteristics of the study population.

Homocysteine Is Inversely but Not Statistically Significantly Associated With FMD and NMD

Homocysteine in the highest tertile (>12.2mmol/L) was associated with low FMD and low NMD, but not significantly so (Figure 2A and 2B; Table 3, standard model). The weak association of homocysteine with FMD was further attenuated by adjustment for 5-MTHF and plasma SAM (Table 3, model 1). Individual adjustment of the standard model for plasma SAM/SAH ratio, or total folate or pyridoxal-5′-phosphate produced a similar attenuation (data not shown). Adjustment for previous cardiovascular disease and smoking (Table 3, model 3) also further weakened the association of homocysteine with FMD and NMD. Additional adjustment for glomerular filtration rate or plasma SAH or vitamin B12 enhanced the association between high homocysteine and low FMD and low NMD, but the association remained statistically insignificant (data not shown). Lipid levels, statin use, BMI, and waist-to-hip ratio did not have any impact on the association between homocysteine and FMD or NMD, nor did SAM/SAH ratio in erythrocytes, systolic and diastolic blood pressure, hypertension, or microalbuminuria (data not shown). A similar association was observed for homocysteine ≥ 15 μmol/L and FMD (please see online supplement).

TABLE 1. Characteristics of the Study Population

Clinical Characteristics	Homocysteine ≤12.2 (Lower 2 Tertiles), N=402	Homocysteine >12.2 (Highest Tertile), N=206
Age, y	66.6±6.5	69.8±6.8
Sex, male/female (% male)	219/183 (45.5)	88/118 (57.3)
Body mass index, kg/m^2	27.3±3.7	26.9±3.4
Systolic blood pressure, mm Hg	140±19	144±19
Diastolic blood pressure, mm Hg	76±9	77±9
Hypertension >140/90 mm Hg, %	65.7	71.4
Use of ACE-inhibitor/calcium antagonist, %	10.2/9.5	14.1/12.6
Current smoker, %	13.8	16.5
Previous cardiovascular disease, %	46.0	48.3
NGM/IGM/DM, n	156/80/162	76/53/74
Total cholesterol, mmol/L	5.8±1.1	5.6±1.0
Statin therapy, %	15.2	14.6
Glomerular filtration rate, mL/min*	68.1±9.9	63.5±12.6
Homocysteine Metabolism		
Homocysteine, μmol/L	9.5 (8.3–10.6)	14.1 (13.1–16.2)
SAM-plasma, nmol/L	87.2 (76.6–99.3)	89.9 (78.2–101.0)
SAM-erythrocytes, nmol/L	3627 (3226–4111)	3728 (3233–4096)
SAH-plasma, nmol/L	13.3 (11.3–16.2)	16.6 (13.9–19.8)
SAH-erythrocytes, nmol/L	136 (114–164)	140 (109–171)
SAM/SAH-ratio plasma	6.4 (5.6–7.4)	5.5 (4.6–6.3)
SAM/SAH-ratio erythrocytes	26.1 (21.4–33.7)	26.9 (20.6–34.0)
Folate-serum, nmol/L	16.8 (13.3–21.4)	12.6 (10.1–15.9)
Folate-erythrocytes, nmol/L	615 (500–742)	500 (404–642)
Folate deficiency, %, <5.9 nmol/L	—	2.5
5-MTHF, nmol/L	12.2 (9.1–17.1)	8.4 (6.4–10.8)
Vitamin B12, pmol/L	294 (242–362)	246 (202–306)
B12 deficiency, %, <150 pmol/L	1.5	3.0
Pyridoxal-5′-phosphate, nmol/L	40 (28–57)	31 (23–42)
Pyridoxal-5′-phosphate deficiency, %, <20 nmol/L	6.8	16.5

Data are presented as mean±SD, number (percentage), or median (interquartile range).

ACE indicates angiotensin-converting enzyme; DM type 2 diabetes mellitus; IGM, impaired glucose metabolism; NGM, normal glucose metabolism, SAM, S-adenosyl-methionine; SAH, S-adenosyl-homocysteine; 5-MTHF, 5-methyltetrahydrofolate.

*Glomerular filtration rate was estimated by Levey equation and expressed per 1.73m^2.

Plasma SAM and Plasma 5-MTHF Are Directly Associated With FMD and NMD After Controlling for Confounding by Homocysteine or Cardiovascular Risk Factors

Plasma SAM in the highest tertile was associated with higher FMD and NMD, and 5-MTHF in the lowest tertile was associated with lower FMD and NMD compared with the remaining tertiles (Figure 2A and 2B; Table 3, standard model). The associations of high SAM and low 5-MTHF with high and low FMD and NMD, respectively, were statistically significant after controlling for confounding by each other or by homocysteine (Table 3, models 1 and 2). They were also statistically significant after controlling for confounding by cardiovascular risk factors (Table 3, model 3). Adjustment for plasma SAH, SAM/SAH ratio in plasma or erythrocytes, total folate, pyridoxal-5′-phosphate or B12, lipid levels, BMI, or waist-to-hip ratio had little impact on the aforementioned associations (data not shown).

The Association of SAM With FMD Is Significant After Controlling for Confounding by NMD

The large impact of adjustment for NMD on the associations of high homocysteine and low 5-MTHF with FMD (Table 3, model 4) showed that these associations were largely explained by NMD. In contrast, high SAM in plasma remained positively and significantly associated with FMD after adjustment for NMD (Table 3, model 4).

The Associations of SAM and 5-MTHF With FMD Are Stronger Than That of Homocysteine With FMD0

High SAM was associated with a 48.57-μm higher FMD compared with low SAM and low 5-MTHF with a 32.15-μm

TABLE 2. Brachial Artery Characteristics of the Study Population

Right Brachial Artery Characteristics	Homocysteine ≤12.2, N=402	Homocysteine >12.2, N=206
Diameter, μm		
Baseline	4602±746	4771±735
After FMD	4782±746	4921±721
After NMD	5065±739	5155±739
Absolute change in diameter, μm		
After FMD	180±170	150±164
After NMD	464±241	409±216
FMD/NMD ratio, %	40±31	33±57
Peak systolic velocity, cm/s		
Baseline	58±13	59±14
After reactive hyperemia	106±26	106±27

Data are presented as mean±SD.

FMD indicates flow-mediated vasodilation; NMD, nitroglycerin-mediated vasodilation.

lower FMD compared with high 5-MTHF, which reflected stronger associations than the 15.11-μm lower FMD associated with high homocysteine compared with low homocysteine. For comparison, in a previous analysis in the same cohort, diabetes was associated with a 60-μm lower FMD compared with people without diabetes,[21] and smoking, hypertension, and previous cardiovascular disease were associated with a 65.1-μm, 26.1-μm, and 36.6-μm lower FMD, compared with nonsmokers, and to individuals without hypertension or previous cardiovascular disease, respectively (Figure I, available online at http://atvb.ahajournals.org).

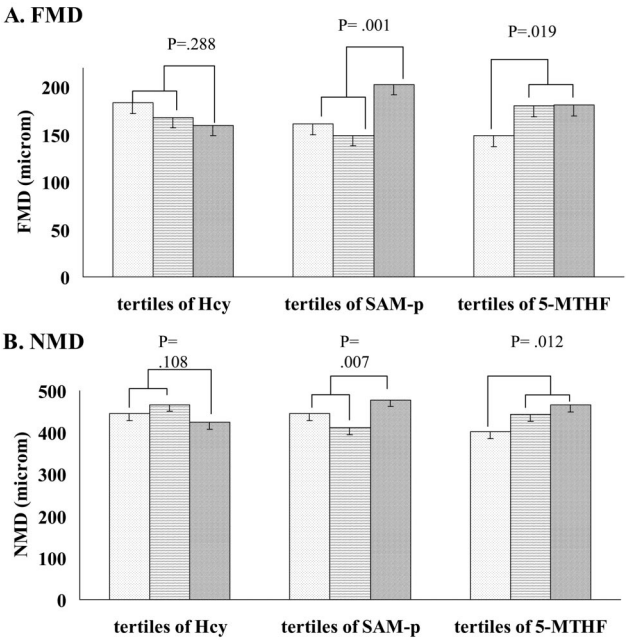

Figure 2. Mean absolute change in diameter (μm) for flow-mediation dilation (A) and nitroglycerine-mediated dilation (B) for tertiles of homocysteine, plasma SAM, and 5-MTHF, adjusted for age, sex, glucose tolerance status, baseline diameter, and, for FMD, increase in peak systolic velocity. Tertile ranges homocysteine: 5.0 to 9.4; 9.5 to 12.2; 12.3 to 38.5 μmol/L. Tertile ranges SAM-plasma: 25.5 to 80.4; 80.5 to 95.0; 95.2 to 193.4 nmol/L. Tertile ranges 5-MTHF: 1.7 to 8.8; 8.59 to 13.4; 13.5 to 79.7 nmol/L.

The Remaining Components of Homocysteine Metabolism Are Not Significantly Associated With FMD or NMD0

The associations of SAM in erythrocytes, SAH in plasma and erythrocytes, SAM/SAH ratio in plasma and erythrocytes, folate in serum and erythrocytes, vitamin B12, and pyridoxal-5′-phosphate with FMD and NMD were not statistically significant, and these associations were further attenuated by adjustment for 5-MTHF, SAM plasma, homocysteine or pyridoxal-5′-phosphate, and for previous cardiovascular disease, smoking, diastolic blood pressure, and glomerular filtration rate, or for NMD (data not shown).

The Associations Between Components of Homocysteine Metabolism and FMD and NMD Are not Stronger in the Presence of Cardiovascular Risk Factors Than in Their Absence

The association of all components of homocysteine metabolism with FMD and NMD did not differ over glucose tolerance categories, for example, *P* interaction (homocysteine × glucose tolerance) for FMD: 0.902 in impaired glucose metabolism and 0.260 in diabetic mellitus, respectively, and *P* interaction (homocysteine × glucose tolerance) for NMD: 0.678 in impaired glucose metabolism and 0.579 in diabetic mellitus, respectively. In addition, no interactions were observed for age, sex, hypertension, smoking, previous cardiovascular disease, and hypercholesterolemia (*P* interaction >0.01) (data not shown).

Discussion

The most important findings of this study are: (1) high SAM plasma levels were associated with high FMD and NMD; (2) low 5-MTHF levels were associated with low FMD and NMD; (3) homocysteine was inversely but not significantly associated with FMD and NMD; (4) NMD largely explained the associations of 5-MTHF and homocysteine (but not SAM) with FMD; and (5) the associations between components of homocysteine metabolism and FMD and NMD were not stronger in the presence of cardiovascular risk factors than in their absence. Taken together, our findings support the concept that components of homocysteine metabolism, such as SAM and 5-MTHF, may affect vascular function. In contrast, our results argue against the notions that the association between homocysteine and vascular function can be explained by other components of homocysteine metabolism or by interactions with other cardiovascular risk factors.

Our study is the first to observe a direct association of high levels of SAM with FMD and NMD after controlling for confounding by homocysteine and other cardiovascular risk factors. Taken together with our previous finding of lower carotid intima-media thickness in nondiabetic individuals with high SAM levels,[27] these data suggest that SAM may have beneficial effects on the vessel wall, possibly by increasing methyl group availability, which is crucial to the biosynthesis and stability of proteins, RNA, and DNA.[34,35] SAH is a potent inhibitor of SAM-dependent transmethyla-

TABLE 3. Homocysteine, SAM, and 5-MTHF as Determinants of Flow-Mediated Vasodilation and Nitroglycerin-Mediated Vasodilation: Multiple Linear Regression Analyses

Central Determinant	Dependent: FMD, μm	Dependent: NMD, μm
Homocysteine (highest tertile vs rest)		
Standard model	−15.11 (−42.99; 12.78)	−31.41 (−71.67; 8.85)
Model 1: standard+low 5-MTHF+high SAM-p	−6.43 (−35.75; 22.90)	−14.23 (−58.57; 30.11)
Model 2: standard+homocysteine	—	—
Model 3: standard+smoking+ prior CVD	−8.86 (−36.20; 18.55)	−26.35 (−66.72; 14.02)
Model 4: standard+NMD	−0.769 (−25.61; 24.07)	—
SAM-plasma (highest tertile vs rest)		
Standard model	48.57 (21.16; 75.98)*	50.05 (10.06; 90.03)*
Model 1: standard+low 5-MTHF	34.70 (7.45; 61.96)*	45.74 (4.20; 87.29)*
Model 2: standard+homocysteine	41.41 (14.81; 68.01)*	46.76 (6.93; 86.60)*
Model 3: standard+smoking+ prior CVD	50.73 (23.64; 77.82)*	47.34 (6.92; 87.75)*
Model 4: standard+NMD	33.89 (9.24; 58.54)*	—
5-MTHF (lowest tertile vs rest)		
Standard model	−32.15 (−59.09; −5.20)*	−52.64 (−93.57; −11.70)*
Model 1: standard+high SAM-p	−31.10 (−58.16; −4.03)*	−52.45 (−93.59; −11.31)*
Model 2: standard+homocysteine	−29.07 (−57.46; −0.69)*	−45.45 (−88.44; −2.47)*
Model 3: standard+smoking+ prior CVD	−28.33 (−55.32; −1.34)*	−55.15 (−96.88; −13.43)*
Model 4: standard+NMD	−10.60 (−34.50; 13.29)	—

Data are presented as linear regression coefficients (95% confidence intervals).

High homocysteine, high SAM, and low 5-MTHF are in turn the central determinant (X) in the regression analysis and either FMD or NMD is the outcome of interest (Y). The coefficient of −15.11 for high homocysteine means that participants with a homocysteine level above 12.2 μmol/l have a 15.11-μm lower FMD compared to people with homocysteine levels below 12.2 μmol/L.

*Significant association ($P<0.05$).

Standard model: determinant under consideration+age, sex, glucose tolerance status, baseline diameter, and peak systolic velocity increase (the latter not included in models for NMD). High SAM-p is highest tertile of plasma SAM. Highest tertiles: Hcy >12.2 μmol/L, SAM-P>95.0 nmol/L, and lowest tertile 5-MTHF <8.8 nmol/L.

tion reactions.[34] Thus, low levels of SAM or high levels of SAH relative to SAM may result in hypomethylation,[34,35] which may lead to impaired endothelial cell regeneration, endothelial dysfunction,[36] and atherosclerosis. To what extent hypomethylation (of DNA) may explain the association between SAM and endothelial and smooth muscle cell function as observed in the present study remains to be established.

Low levels of 5-MTHF, the active form of folate, were associated with low FMD and low NMD in our study after controlling for confounding by homocysteine, suggesting a direct effect of 5-MTHF on endothelial and/or vascular smooth muscle cells. This finding might be explained by the fact that in vitro, 5-MTHF has antioxidant properties,[37,38] directly interacts with endothelial nitric oxide synthase,[37] or has positive effects on tetrahydrobiopterin (BH4), the essential cofactor of endothelial nitric oxide synthase.[38,39] A direct effect of 5-MTHF or folic acid on endothelial function, after controlling for confounding by homocysteine, has also been observed in patients with familial hypercholesterolemia or type 2 diabetes without hyperhomocystinemia[40,41] and in hyperhomocystinemia patients with coronary artery disease.[42,43]

FMD was 49 μm greater in individuals with high SAM compared with individuals with low SAM, which is similar in effect size to the inverse association of diabetes with FMD (−60 μm).[21] FMD was 32 μm smaller in individuals with low 5-MTHF compared with individuals with high 5-MTHF, which is comparable to the inverse association of previous cardiovascular disease with FMD (−37 μm) (Figure I). Thus, the associations of SAM and 5-MTHF with FMD appear to be substantial.

Although we cannot exclude an association of homocysteine with FMD, our data suggest that in a general elderly population with a considerable amount of cardiovascular risk factors, any such association is relatively small ($\approx$−15 μm). In addition, the association of homocysteine with FMD appeared to be explained by NMD. Impairment of either endothelial or vascular smooth muscle cell function will result in impaired FMD; when both FMD and NMD are impaired, dysfunction of the endothelium cannot be separated from that of vascular smooth muscle[44] and an unequivocal interpretation of the FMD result is not possible. There is in vitro evidence that homocysteine can induce smooth muscle cell proliferation,[45,46] which might affect smooth muscle cell function, and that folic acid can reverse some of these effects.[45] Impaired smooth muscle cell function has been associated with an adverse cardiovascular prognosis,[25] and our findings of impaired NMD in individuals with high homocysteine or low SAM and 5-MTHF may be relevant in this regard. Taken together, these findings stress the need to

elucidate the involvement of vascular smooth muscle cells in vascular disease related to components of homocysteine metabolism.

Homocysteine has been found to be a stronger risk factor for cardiovascular events and mortality in the presence of type 2 diabetes.[20,47] However, in the present study, we observed no interactions between homocysteine and glucose metabolism in its relationship with FMD or NMD. Because diabetic individuals with missing ultrasound data had higher homocysteine levels than diabetic patients with complete data, the power to detect such interactions may have been too small. In addition, increased (selective) mortality among participants with diabetes and relatively severe hyperhomocystinemia[20] may have resulted in a "healthy survivor effect," which precluded the observation of an interaction between homocysteine, glucose tolerance status, and FMD in the present study.

Our study had some limitations. First, the population consisted of white individuals in the age range of 50 to 85 with a considerable prevalence of cardiovascular risk factors and we do not know whether our results can be generalized to healthier people of the same age or to people from different age groups or from other ethnic groups. However, as homocysteine concentration increases with age and low to low–normal concentrations or deficiencies of folate, vitamin B12 and pyridoxal-5′-phosphate are relatively common in this age group, we were able to study the association with FMD and NMD over a wide range of concentrations. Second, the study had a cross-sectional design and the observed associations cannot be interpreted as causal relationships. Third, participants who were excluded because of missing or unsatisfactory ultrasound data were older, had higher BMI, higher homocysteine levels, and more frequently had diabetes, which may have resulted in an underestimation of the observed associations. Fourth, there was a discrepancy between the serum levels of total folate (measured by chemiluminescence) levels and of 5-MTHF (measured by high-performance liquid chromatography) in our study. However, total folate and 5-MTHF were inversely and significantly correlated with homocysteine. The discrepancy may be partly explained by the fact that not all (81% to 93%) of total serum folate is methylfolate.[48] In addition, laboratory assays of folate are notoriously difficult and various methods have previously been known to agree poorly.[49,50] We focused on 5-MTHF levels because this folate subform is involved in the methylation cycle, which includes both SAM and homocysteine as metabolic components. Fifth, the assumptions underlying the FMD test, such as the concept that nitroglycerin adequately mimics the effect of endogenous nitric oxide release,[44] still need to established. Nevertheless, there is increasing evidence that both impaired FMD and impaired NMD are associated with an adverse cardiovascular prognosis[25,51] and the FMD test is considered as a reasonable surrogate measure of cardiovascular risk.[52]

In conclusion, this study suggests that in this elderly population, both SAM and 5-MTHF are associated with endothelial and smooth muscle cell function. In addition, our study suggests that the effect of homocysteine on endothelial function may be relatively small compared with SAM, 5-MTHF, or diabetes. Further studies are needed to ascertain the relative impact of SAM, 5-MTHF, and homocysteine, and to elucidate the mechanisms through which these moieties may affect the function of endothelial and vascular smooth muscle cells.

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