

Endothelial Dysfunction and Low-Grade Inflammation Are Associated With Greater Arterial Stiffness Over a 6-Year Period

Citation for published version (APA):

van Bussel, B. C. T., Schouten, F., Henry, R. M., Schalkwijk, C. G., de Boer, M. R., Ferreira, I., Smulders, Y. M., Twisk, J. W., & Stehouwer, C. D. A. (2011). Endothelial Dysfunction and Low-Grade Inflammation Are Associated With Greater Arterial Stiffness Over a 6-Year Period. *Hypertension*, 58(4), 588-595. <https://doi.org/10.1161/HYPERTENSIONAHA.111.174557>

Document status and date:

Published: 01/01/2011

DOI:

[10.1161/HYPERTENSIONAHA.111.174557](https://doi.org/10.1161/HYPERTENSIONAHA.111.174557)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Endothelial Dysfunction and Low-Grade Inflammation Are Associated With Greater Arterial Stiffness Over a 6-Year Period

Bas C. van Bussel, Fleur Schouten, Ronald M. Henry, Casper G. Schalkwijk, Michiel R. de Boer, Isabel Ferreira, Yvo M. Smulders, Jos W. Twisk, Coen D. Stehouwer

Abstract—Endothelial dysfunction and low-grade inflammation are associated with cardiovascular disease. Arterial stiffening plays an important role in cardiovascular disease and, thus, may be a mechanism through which endothelial dysfunction and/or low-grade inflammation lead to cardiovascular disease. We investigated the associations between, on the one hand, biomarkers of endothelial dysfunction (soluble endothelial selectin, thrombomodulin, and both vascular and intercellular adhesion molecules 1 and von Willebrand factor) and of low-grade inflammation (C-reactive protein, serum amyloid A, interleukin 6, interleukin 8, tumor necrosis factor- α and, soluble intercellular adhesion molecule 1) and, on the other hand, arterial stiffness over a 6-year period, in 293 healthy adults (155 women). Biomarkers were combined into mean z scores. Carotid, femoral, and brachial arterial stiffness and carotid-femoral pulse wave velocity were determined by ultrasonography. Measurements were obtained when individuals were 36 and 42 years of age. Associations were analyzed with generalized estimating equation and adjusted for sex, height, and mean arterial pressure. The endothelial dysfunction z score was inversely associated with femoral distensibility (β : -0.51 [95% CI: -0.95 to -0.06]) and compliance coefficients (β : -0.041 [95% CI: -0.076 to -0.006]) but not with carotid or brachial stiffness or carotid-femoral pulse wave velocity. The low-grade inflammation z score was inversely associated with femoral distensibility (β : -0.51 [95% CI: -0.95 to -0.07]) and compliance coefficients (β : -0.050 [95% CI: -0.084 to -0.016]) and with carotid distensibility coefficient (β : -0.910 [95% CI: -1.810 to -0.008]) but not with brachial stiffness or carotid-femoral pulse wave velocity. Biomarkers of endothelial dysfunction and low-grade inflammation are associated with greater arterial stiffness. This provides evidence that arterial stiffening may be a mechanism through which endothelial dysfunction and low-grade inflammation lead to cardiovascular disease. (*Hypertension*. 2011;58:588-595.) • **Online Data Supplement**

Key Words: endothelial dysfunction ■ inflammation ■ arteriosclerosis ■ young adults ■ prospective study ■ epidemiology

From observational studies,¹⁻⁵ it has become increasingly clear that biomarkers of endothelial dysfunction and low-grade inflammation are closely associated with (incident) cardiovascular disease. In part, these associations can be explained by the fact that endothelial dysfunction and low-grade inflammation play key roles in atherothrombosis.⁶ However, other mechanisms may also be important. In this respect, arterial stiffening may constitute one such mechanism. First, greater arterial stiffness has been shown to be independently associated with cardiovascular morbidity and mortality.^{7,8} Second, endothelial dysfunction and low-grade

inflammation affect the composition of the subendothelial matrix, which is an important determinant of arterial stiffness.⁹

Indeed, the association between biomarkers of endothelial dysfunction and low-grade inflammation and greater arterial stiffness is receiving increasing attention.^{10,11} However, previous studies on this topic were cross-sectional,¹⁰⁻²⁶ and most focused on either biomarkers of endothelial dysfunction²⁴ or low-grade inflammation^{12,16-23,25,26} and were done in middle-aged or elderly populations.^{10,11,13-15,18-23,26} Importantly, endothelial dysfunction and low-grade inflammation are closely

Continuing medical education (CME) credit is available for this article. Go to <http://cme.ahajournals.org> to take the quiz.

Received April 12, 2011; first decision May 2, 2011; revision accepted July 27, 2011.

From the Departments of Medicine (B.C.v.B., R.M.H., C.G.S., I.F., C.D.S.) and Clinical Epidemiology and Medical Technology Assessment (I.F.), the Cardiovascular Research Institute Maastricht (R.M.H., C.G.S., I.F., C.D.S.), the School for Public Health and Primary Care (I.F.), and the School for Nutrition, Toxicology and Metabolism (B.C.v.B., C.D.S.), Maastricht University Medical Centre, Maastricht, The Netherlands; Top Institute Food and Nutrition, (B.C.v.B., R.M.H., C.G.S., C.D.S.), Wageningen, The Netherlands; Department of Health Sciences and the EMGO Institute for Health and Care Research (F.S., M.R.d.B., J.W.T.), Faculty of Earth and Life Sciences, VU University Amsterdam, Amsterdam, The Netherlands; Institute for Cardiovascular Research (F.S., Y.M.S.), VU University Medical Center, Amsterdam, The Netherlands; Department of Medicine (Y.M.S., J.W.T.), VU University Medical Center, Amsterdam, The Netherlands.

B.C.v.B. and F.S. contributed equally to this work.

Correspondence to Coen D. Stehouwer, Maastricht University Medical Centre, Department of Medicine, Prof Debye laan 25, 6229 HX Maastricht, The Netherlands. E-mail cda.stehouwer@mumc.nl

© 2011 American Heart Association, Inc.

Hypertension is available at <http://hyper.ahajournals.org>

DOI: 10.1161/HYPERTENSIONAHA.111.174557

linked and difficult to separate conceptually,²⁷ and any relationship with arterial stiffening may, therefore, be interdependent. In addition, the process of arterial stiffening is known to start at an early age.²⁸

In view of the above, we hypothesize that the development of biomarkers of endothelial dysfunction and low-grade inflammation is associated with arterial stiffening in early adulthood. Therefore, we have measured biomarkers of endothelial dysfunction, low-grade inflammation, and arterial stiffness twice in a population-based cohort, that is, for the first time at the age of 36 years and for the second time at the age of 42 years. We have investigated the 6-year longitudinal associations between biomarkers of endothelial dysfunction and low-grade inflammation, on the one hand, and stiffness estimates of the carotid, femoral, and brachial arteries and of the carotid-femoral segment, on the other, in apparently healthy adults of the Amsterdam Growth and Health Longitudinal Study.

Methods

Study Population

Data were derived from the Amsterdam Growth and Health Longitudinal Study, an observational, longitudinal study that started in 1976 with a group of 698 boys and girls (details described elsewhere).²⁹ Briefly, its initial goal was to study the natural development of the growth, health, and lifestyle of adolescents and to investigate longitudinal relationships between biological and lifestyle variables. The mean age of the individuals at the start of the study was 13.1 ± 0.8 years (mean \pm SD). Since then, extensive follow-up measurements have been done, and the cohort is still under investigation. At each follow-up measurement, anthropometric (body height, weight, and skinfolds), biological (blood pressure, serum lipoprotein levels, and physical fitness), lifestyle (nutritional habits, smoking behavior, and daily physical activity), and psychological variables were assessed.²⁹

In addition, in blood samples of the individuals attending the 2000 and 2006 follow-up examinations (1 batch), 5 biomarkers of endothelial dysfunction and 6 biomarkers of low-grade inflammation were measured, and at both examinations arterial properties were assessed by ultrasonography. At the first ultrasound examination, 377 individuals participated, and complete data on stiffness estimates of the carotid, femoral, and brachial arteries were obtained in 373 individuals. From these, complete data on all 3 of the arteries were obtained in 293 individuals during the follow-up ultrasound examination, all of whom had full data on biomarkers of endothelial dysfunction and low-grade inflammation. The present study was, therefore, conducted with these 293 individuals.

The study was approved by the local ethics committee of the Vrije Universiteit University Medical Center, and all of the participants gave their written informed consent.

Assessment of Endothelial Dysfunction and Low-Grade Inflammation

Serum biomarkers of endothelial dysfunction (soluble intercellular adhesion molecule 1 [sICAM-1], soluble vascular cell adhesion molecule 1, soluble endothelial selectin, and soluble thrombomodulin), and of low-grade inflammation (C-reactive protein [CRP], serum amyloid A, interleukin 6, interleukin 8, tumor necrosis factor- α , and sICAM-1) were assessed by an electrochemiluminescence detection system using multiarray technology (SECTOR Imager 2400, Meso Scale Discovery), as described elsewhere³⁰ (please see the online Data Supplement at <http://hyper.ahajournals.org>). In addition, a plasma biomarker of endothelial dysfunction (von Willebrand factor) was determined in citrated plasma by means of ELISA, as described elsewhere³⁰ (please see the online Data Supplement).

Arterial Stiffness

We assessed local arterial stiffness of the carotid, femoral, and brachial arteries and central arterial stiffness (the carotid-femoral pulse wave velocity)^{31,32} (please see the online Data Supplement).

Other Measurements

Weight, height, body mass index, heart rate, and blood pressure were determined according to international standards, and smoking behavior was assessed by questionnaire as described previously.^{29,31} Total and high-density lipoprotein cholesterol, triglycerides, and glycohemoglobin were determined as described previously.^{29,31} Hypertension was defined as a systolic blood pressure >140 mm Hg, and/or a diastolic blood pressure >90 mm Hg, and/or treatment for hypertension.

Statistical Analyses

All of the analyses were performed with SPSS (version 15, SPSS). Variables with a skewed distribution (CRP, serum amyloid A, interleukin 6, and triglycerides) were transformed using a natural logarithm (ln). Overall z scores were calculated for endothelial dysfunction or low-grade inflammation (please see the online Data Supplement). We used generalized estimating equations to examine the associations between either the overall z score for endothelial dysfunction or the overall z score for low-grade inflammation and arterial stiffness over the 6-year study period. In these analyses an exchangeable correlation structure was used. Generalized estimating equation analysis is a method for longitudinal data analyses and takes into account the correlation of repeated measurements within individuals over time.³³

First, we investigated the associations between the overall z scores for either endothelial dysfunction or low-grade inflammation on the one hand and arterial stiffness estimates on the other with adjustments for sex, height, and mean arterial pressure. Second, the analyses were repeated with mutual adjustments for the overall z scores for either low-grade inflammation or endothelial dysfunction. Third, to gain further insight into which of the individual elements of the stiffness formulas might have been primarily responsible for any relationships between endothelial dysfunction or low-grade inflammation and arterial stiffness estimates, the analyses were repeated with the individual elements of the stiffness formulas (diameter, distension, pulse pressure, and intima-media thickness) as dependent variables.

Data are presented as mean (\pm SD) or median (interquartile range) for skewed variables and regression coefficients (β) with their 95% CIs. A 2-sided P value <0.05 was considered statistically significant.

Results

Table 1 shows the general characteristics, the biomarker concentrations, and the stiffness estimates of the study population. Systolic blood pressure remained stable (116 mm Hg), diastolic blood pressure increased (+5.5 mm Hg), pulse pressure decreased (-5.6 mm Hg), and heart rate decreased (-8.9 bpm) during the 6-year follow-up. Biomarkers of low-grade inflammation remained fairly stable over 6-year follow-up, except for a decrease in tumor necrosis factor- α . Biomarkers of endothelial dysfunction remained stable or increased over 6-year follow-up, except for a decrease in soluble endothelial selectin. Pulse pressure and femoral and brachial artery stiffness decreased during 6-year follow-up. Nevertheless, carotid artery stiffness increased (for distensibility coefficient [DC]: -1.00×10^{-3} kPa; for Young elastic modulus: $+0.025 \times 10^3$ kPa) during the 6-year follow-up.

Table 1. Characteristics of the Study Population

Variables	2000	2006	P
Age, y	36.6±0.6	42.6±0.6	<0.001
Women, %	52.9	52.9	...
Smoker, %	64, 21.9	45, 15.4	<0.001
Systolic blood pressure, mm Hg	116.3±11.7	116.2±14.4	0.858
Diastolic blood pressure, mm Hg	64.9±7.2	70.4±8.1	<0.001
Pulse pressure, mm Hg	51.4±6.5	45.8±9.0	<0.001
Mean arterial pressure, mm Hg	82.1±8.7	85.1±9.9	<0.001
Prevalence of hypertension, %	5.1	9.6	0.015
Heart rate, bpm	70.5±11.4	61.6±9.0	<0.001
Body height, cm	177.0±9.2	177.4±9.0	<0.001
Body weight, kg	75.8±12.7	77.9±13.7	<0.001
Body mass index, kg/m ²	24.1±3.2	24.7±3.6	<0.001
Total cholesterol, mmol/L	5.0±0.9	5.0±0.8	0.315
High density lipoprotein cholesterol, mmol/L	1.4±0.4	1.7±0.4	<0.001
Triglycerides, mmol/L	1.0 (0.8 to 1.5)	1.0 (0.7 to 1.4)	0.018
HbA1c, %	5.2±0.4	5.4±0.4	<0.001
Biomarkers			
C-reactive protein, mg/L	0.8 (0.3 to 2.0)	0.8 (0.3 to 1.8)	0.946
Serum amyloid A, mg/L	1.2 (0.7 to 2.2)	1.3 (0.7 to 2.3)	0.099
Interleukin 6, ng/L	2.3 (1.7 to 3.7)	2.4 (1.8 to 3.7)	0.664
Interleukin 8, ng/L	9.2±3.4	9.5±4.1	0.190
Tumor necrosis factor- α , ng/L	9.3±3.4	9.0±3.3	0.006
Soluble intercellular adhesion molecule 1, μ g/L	203.3±52.4	200.3±41.2	0.142
Soluble vascular cellular adhesion molecule 1, μ g/L	332.8±81.2	333.9±69.2	0.767
Soluble endothelial selectin, μ g/L	10.5±4.7	10.1±4.6	0.010
Soluble thrombomodulin, μ g/L	2.47±0.66	2.53±0.62	0.023
von Willebrand factor, %	102.5±40.0	112.8±42.4	<0.001
Carotid artery			
Distensibility coefficient, 10 ⁻³ /kPa	26.7±6.1	25.7±7.2	0.014
Compliance coefficient, mm ² /kPa	1.0±0.3	1.0±0.3	0.143
Young elastic modulus, 10 ³ /kPa	0.44±0.13	0.47±0.16	0.007
Diameter, mm	6.9±0.6	7.1±0.7	<0.001
Distension, mm	0.6±0.1	0.5±0.1	<0.001
Pulse pressure, mm Hg	51.3±7.1	45.9±9.4	<0.001
Intima-media thickness, mm	0.62±0.10	0.66±0.12	<0.001
Femoral artery			
Distensibility coefficient, 10 ⁻³ /kPa	7.1±3.7	8.3±4.5	<0.001
Compliance coefficient, mm ² /kPa	0.51±0.24	0.63±0.33	<0.001
Diameter, mm	9.8±1.3	10.0±1.5	<0.001
Distension, mm	0.22±0.10	0.23±0.10	0.149
Pulse pressure, mm Hg	51.4±7.2	46.5±9.8	<0.001
Brachial artery			
Distensibility coefficient, 10 ⁻³ /kPa	14.6±9.2	17.6±11.2	<0.001
Compliance coefficient, mm ² /kPa	0.17±0.09	0.21±0.11	<0.001
Diameter, mm	4.0±0.7	4.0±0.7	0.187
Distension, mm	0.18±0.09	0.19±0.10	0.205
Pulse pressure, mm Hg	51.7±7.2	45.0±9.5	<0.001
Central arterial stiffness			
Pulse wave velocity, m/s	7.7±1.6*	8.3±1.6†	<0.001

Data are reported as mean±SD, median (interquartile range), or percentage, as appropriate; P value, paired *t* test or McNemar test, as appropriate; n=293.

*n=241.

†n=292.

Table 2. Longitudinal Associations Between, on the One Hand, Endothelial Dysfunction and Low-Grade Inflammation Z Scores During 6-y Follow-Up and, on the Other Hand, Arterial Stiffness Indices During 6-y Follow-Up (n=293)

Variable	Carotid Artery			Femoral Artery			Brachial Artery		
	β	95% CI	P	β	95% CI	P	β	95% CI	P
Endothelial dysfunction									
Distensibility coefficient									
1: +sex, height, MAP	-0.19	-1.02; 0.64	0.656	-0.51	-0.95; -0.06	0.025	-0.94	-2.26; 0.39	0.165
2: 1+low-grade inflammation z score	0.38	-0.51; 1.27	0.402	-0.34	-0.89; 0.20	0.219	-1.46	-3.15; 0.24	0.092
Compliance coefficient									
1: +sex, height, MAP	-0.012	-0.049; 0.026	0.539	-0.041	-0.076; -0.006	0.020	-0.009	-0.023; 0.004	0.163
2: 1+low-grade inflammation z score	-0.001	-0.042; 0.041	0.972	-0.022	-0.065; 0.021	0.322	-0.017	-0.034; 0.001	0.058
Young elastic modulus									
1: +sex, height, MAP	-0.001	-0.020; 0.018	0.919
2: 1+low-grade inflammation z score	-0.005	-0.028; 0.017	0.636
Low-grade inflammation									
Distensibility coefficient									
1: +sex, height, MAP	-0.91	-1.81; -0.008	0.048	-0.51	-0.95; -0.07	0.024	0.19	-1.11; 1.49	0.777
2: 1+endothelial dysfunction z score	-1.12	-2.14; -0.11	0.031	-0.32	-0.86; 0.22	0.247	0.99	-0.64; 2.62	0.232
Compliance coefficient									
1: +sex, height, MAP	-0.022	-0.061; 0.018	0.288	-0.050	-0.084; -0.016	0.004	0.005	-0.008; 0.017	0.480
2: 1+endothelial dysfunction z score	-0.021	-0.066; 0.023	0.353	-0.038	-0.080; 0.004	0.075	0.014	-0.002; 0.030	0.092
Young elastic modulus									
1: +sex, height, MAP	0.006	-0.013; 0.024	0.556
2: 1+endothelial dysfunction z score	0.009	-0.013; 0.030	0.430

β indicates longitudinal regression coefficients that express the relationships between the longitudinal development of endothelial dysfunction and low-grade inflammation, on the one hand, and the longitudinal development of arterial stiffness on the other; 95% CI, 95% CIs and P value; MAP, mean arterial pressure.

Endothelial Dysfunction, Low-Grade Inflammation, and Arterial Stiffness

The endothelial dysfunction overall z score was inversely associated with the femoral artery DC (β : -0.51 [95% CI: -0.95 to -0.06]; $P=0.025$) and compliance coefficient (CC; β : -0.041 [95% CI: -0.076 to -0.006]; $P=0.020$) after adjustment for sex, height, and mean arterial pressure, whereas it was not associated with carotid or brachial artery stiffness (Table 2, model 1). Additional adjustment for the low-grade inflammation overall z score decreased the regression coefficient (β) for the femoral artery DC by 32% and for the femoral artery CC by 46% (Table 2, model 2). In addition, the endothelial dysfunction overall z score was not associated with carotid-femoral pulse wave velocity after adjustment for sex, height, and mean arterial pressure (β : 0.001 [95% CI: -0.22 to 0.22]; $P=0.994$).

The low-grade inflammation overall z score was inversely associated with the carotid artery DC (β : -0.91 [95% CI: -1.81 to -0.008]; $P=0.048$), femoral artery DC (β : -0.51 [95% CI: -0.95 to -0.07]; $P=0.024$), and CC (β : -0.050 [95% CI: -0.084 to -0.016]; $P=0.004$) after adjustment for sex, height, and mean arterial pressure, whereas it was not associated with carotid artery CC, Young elastic modulus, or brachial artery stiffness (Table 2, model 1). Additional adjustment for the endothelial dysfunction overall z score decreased the regression coefficient (β) for the femoral artery DC by 47% and for the femoral artery CC by 24%, whereas the result for the carotid artery DC did not materially change

(Table 2, model 2). In addition, the low-grade inflammation overall z score was not associated with carotid-femoral pulse wave velocity after adjustment for sex, height, and mean arterial pressure (β : -0.074 [95% CI: -0.37 to -0.24]; $P=0.626$).

Endothelial Dysfunction, Low-Grade Inflammation, and Arterial Diameter, Distension, Pulse Pressure, and Intima-Media Thickness

The inverse associations between the endothelial dysfunction overall z score and the femoral artery DC and CC and the low-grade inflammation overall z score and the femoral artery DC and CC were primarily driven through inverse associations with femoral artery distension (for endothelial dysfunction: β : -0.013 [95% CI: -0.025 to -0.001], $P=0.029$; for low-grade inflammation: β : -0.015 [95% CI: -0.027 to -0.004], $P=0.010$; please see the online Data Supplement for Table S1). In contrast, the inverse association between the low-grade inflammation overall z score and the carotid artery DC was not primarily driven through any of the elements of the stiffness formulas (Table S1).

Additional Analyses

Additional adjustment for age (the age range by design being extremely narrow), smoking behavior, total and high-density lipoprotein cholesterol, and hypertension did not materially change the associations, whereas adjustment for weight did attenuate the regression coefficients with $\approx 50\%$ (Table S2). None of the individuals had diabetes mellitus.

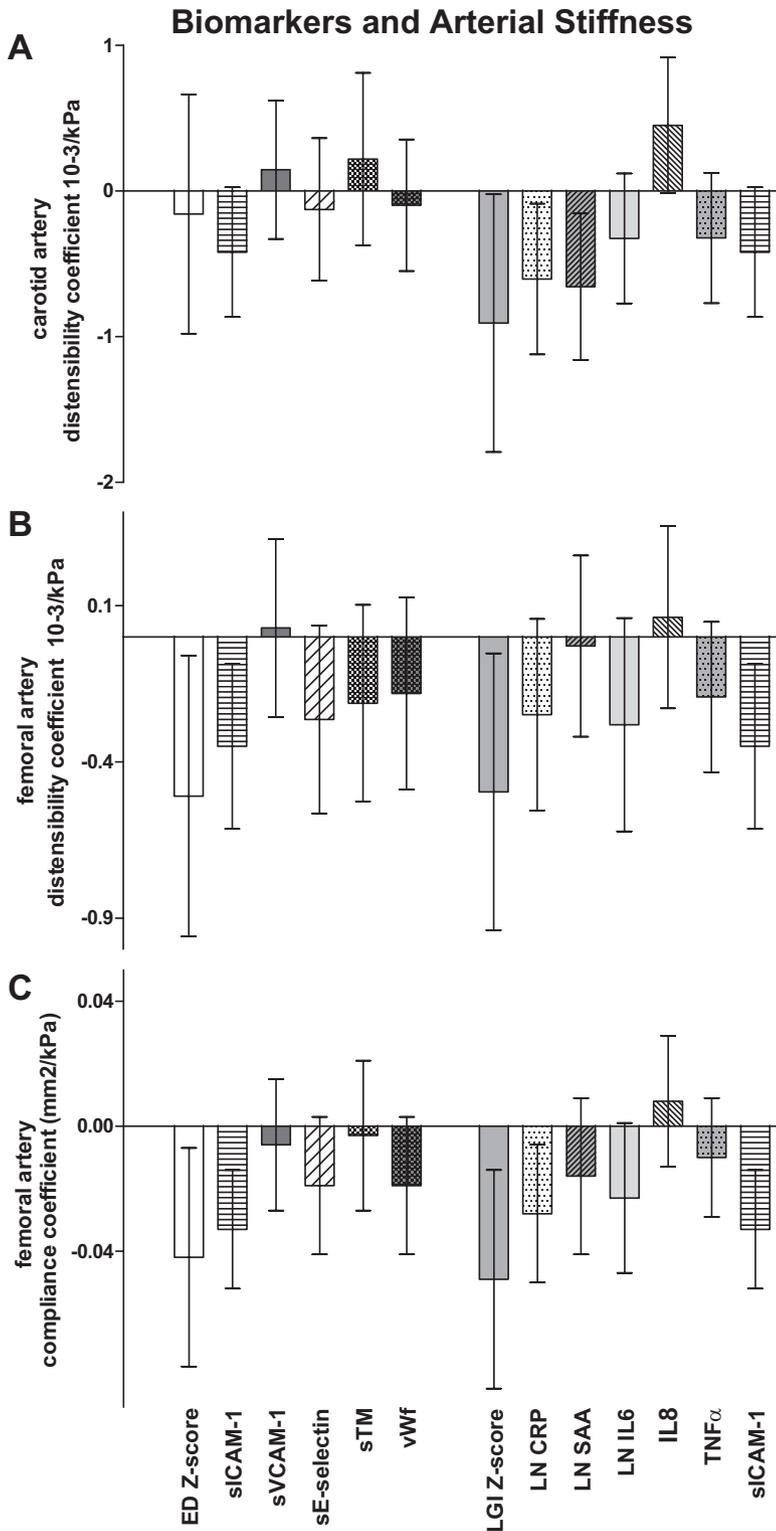


Figure. Bars are regression coefficients that indicate the associations between either the endothelial dysfunction z score (ED), or the low-grade inflammation (LGI) z score or each of the individual biomarker z scores and the carotid distensibility coefficient (A), the femoral distensibility coefficient (B), and the femoral compliance coefficient (C) over the 6-year study period. Whiskers indicate the 95% CIs. All of the results are adjusted for sex, height, and mean arterial pressure. sICAM-1 indicates soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1; sE-selectin, soluble endothelial selectin; sTM, soluble thrombomodulin; vWf, von Willebrand factor; CRP, C-reactive protein; SAA, serum amyloid A; IL-6, interleukin 6; IL-8, interleukin 8; TNF- α , tumor necrosis factor- α ; LN, log normalized.

In the analyses for the individual elements of the low-grade inflammation z score, the results showed that ln serum amyloid A and lnCRP were the strongest determinants of the carotid artery DC (Figure A), whereas sICAM-1 and ln interleukin 6 were the strongest determinants of the femoral artery DC (Figure B) and sICAM-1 and lnCRP were the strongest determinants for the femoral artery CC (Figure C). For the endothelial dysfunction z score, sICAM-1 and soluble

endothelial selectin were the strongest determinants for the femoral artery DC (Figure B) and CC (Figure C).

To investigate whether the longitudinal associations (by generalized estimating equation) were primarily determined by the between- or the within-subject associations over the 6-year study period, we calculated changes (ie, within-subject) in the endothelial dysfunction overall z score, the low-grade inflammation overall z score, and the arterial

stiffness estimates. Then, we reanalyzed the data with the use of linear regression analyses. Changes in the endothelial dysfunction overall z score or in the low-grade inflammation overall z score were not associated with changes in arterial stiffness estimates (data not shown). This suggests that the reported associations were primarily determined by the between-subject associations over the 6-year study period.

Discussion

The present investigation is the first to prospectively evaluate, in apparently healthy adults, the relationship between the development of an extensive array of biomarkers of endothelial dysfunction and low-grade inflammation on the one hand and arterial stiffness on the other. The study had 3 main findings. First, biomarker scores for endothelial dysfunction and low-grade inflammation were associated with greater arterial stiffness over a 6-year period. The biomarker score for endothelial dysfunction was associated with greater femoral artery stiffness, whereas the biomarker score for low-grade inflammation was associated with both greater carotid and femoral artery stiffness. However, both the biomarker scores for endothelial dysfunction and low-grade inflammation were not associated with stiffness of the carotid-femoral segment. Endothelial dysfunction and/or low-grade inflammation may, thus, affect arterial stiffening in a way that depends on the arterial territory under study.³⁴ Second, mutual adjustment for either the biomarker score for low-grade inflammation or endothelial dysfunction showed that the associations between either the biomarker score for endothelial dysfunction or low-grade inflammation with femoral artery stiffness were interdependent. Finally, the associations between each of the biomarker scores and greater femoral artery stiffness were primarily driven through decreased distension, whereas the association between low-grade inflammation and greater carotid stiffness was not primarily driven through any of the arterial parameters.

The endothelium has many functions and is itself heterogeneous.^{35,36} The concept of endothelial dysfunction, therefore, has many dimensions.³⁷ von Willebrand factor, soluble vascular cell adhesion molecule 1, soluble endothelial selectin, soluble thrombomodulin, and sICAM-1 are all synthesized by endothelial cells,^{6,35,36} and higher concentrations of these biomarkers are associated with (incident) cardiovascular disease.^{1–5} Consequently, it is plausible to assume that higher circulating concentrations of these biomarkers reflect greater dysfunction.

A limitation of this study is that a measure of NO-mediated dilation, which represents an important function of the endothelium,^{35–37} was not available. Nevertheless, both biomarkers of the endothelium and NO-mediated dilation (eg, flow-mediated dilation) have been associated with (incident) cardiovascular disease.^{1–5,38}

Furthermore, endothelial dysfunction is closely linked with low-grade inflammation and, therefore, these concepts are difficult to separate.²⁷ We, indeed, show that the associations with femoral artery stiffening are dependent on both endothelial dysfunction and low-grade inflammation, whereas this was not the case for carotid artery stiffening. This suggests that endothelial dysfunction and low-grade inflammation

might affect arterial stiffening in concert or, independently, dependent on the arterial territory under study.

The present investigation was comprehensive and had advantages over previous ones, which investigated either biomarkers of endothelial dysfunction²⁴ or low-grade inflammation,^{12,16–23,25,26} measured a less extensive array of biomarkers of endothelial dysfunction and low-grade inflammation,^{10–26} concerned middle-aged to elderly individuals,^{10,11,13–15,18–23,26} targeted 1 type of artery only,^{10–25} and were cross-sectional by design.^{10–26}

On aggregate, previous studies have shown higher CRP levels to be associated with greater arterial stiffness of elastic,^{10–14,16–23} muscular,²⁵ or both types of arteries²⁶ and higher interleukin 6 levels to be associated with greater elastic arterial stiffness.^{11,15} Taken together, these studies^{10–23,25,26} and the present results support the concept that low-grade inflammation may affect arterial stiffness. In particular, we show that this process is present even in young, apparently healthy adults.

With regard to biomarkers of endothelial dysfunction, previous studies have shown heterogeneous results for von Willebrand factor and sICAM-1, some reporting positive associations with elastic arterial stiffness,^{15,24} whereas others did not.^{11,13,14} We show that the overall endothelial dysfunction z score was significantly associated with femoral artery stiffness (Figure B and C), whereas none of the individual endothelial dysfunction biomarkers was, except for sICAM-1. This may be explained by the fact that, in these young, apparently healthy individuals, endothelial dysfunction may not be very far advanced and, therefore, only the sum of endothelial dysfunction biomarkers reveals its association with greater arterial stiffness.

Endothelial dysfunction may influence arterial stiffening by affecting vascular smooth muscle cell tone because of the reduced availability of NO and the increased activity of vasoconstrictor substances such as endothelin 1⁹ and by affecting the composition of the extracellular matrix.⁹ In addition, inflammation may induce both functional and structural changes in the arterial wall via the increased production of reactive oxygen species,³⁹ which, in turn, triggers an inflammatory process leading to the proliferation of smooth muscle cells, the influx of leukocytes, and the production of proinflammatory substances and chemoattractants.⁶ Our data support the notion that both endothelial dysfunction and low-grade inflammation may cause increased muscular artery stiffness via similar pathophysiological pathways, as in the femoral artery both endothelial dysfunction and low-grade inflammation primarily appeared to affect arterial distension (please see Figure S1). In contrast, only low-grade inflammation affected (elastic) carotid artery stiffness, most likely through a pathophysiological mechanism independent of endothelial dysfunction and not specifically driven through diameter, distension, pulse pressure, or intima-media thickness. This suggests that low-grade inflammation may cause increased arterial stiffness of elastic arteries by affecting multiple elements of the vascular wall. These observations particularly align with the notion that the atherosclerotic and arteriosclerotic processes are, at least in part, inflammatory in origin and that inflammatory changes may lead to an altered matrix homeostasis (affecting vascular smooth muscle cells

and matrix proteins), which, in turn, leads to a decrease in (femoral) distension. Taken together, the above suggests that endothelial dysfunction and low-grade inflammation may affect arterial stiffness of either muscular or elastic arteries differently. This might explain why endothelial dysfunction and low-grade inflammation were not associated with carotid-femoral pulse wave velocity, because the carotid-femoral segment includes both elastic and muscular arterial components. Alternatively, the adults investigated were young and did not have central arterial stiffening, which typically occurs after the age of 60 years.⁷

Additional adjustment for potential confounders did not materially change the results, whereas adjustment for body weight attenuated the associations between biomarkers of endothelial dysfunction or low-grade inflammation and arterial stiffness. However, it is questionable whether body weight should be seen as a true confounder in this relationship. It is biologically more plausible that body weight is part of the causal pathway, that is, body weight influences biomarkers of endothelial dysfunction and low-grade inflammation,⁴⁰ and these influence arterial stiffening.⁹

Finally, the time frame in which endothelial dysfunction or low-grade inflammation have their possible impacts on arterial stiffness is unknown and might differ from the 6-year period of the present investigation. (This might explain why changes in biomarkers of endothelial dysfunction or low-grade inflammation did not parallel changes in arterial stiffness.) The question remains for what period a person should have endothelial dysfunction or low-grade inflammation before it affects the vasculature. In any case, our results may suggest that any inflammatory changes that have led to arterial stiffening have already occurred before the age of 36 years.

The present investigation had some limitations. First, intrinsic to an overall z score is the assumption that each biomarker in the z score carries similar weight. This might have caused us to underestimate the reported associations because of error attributed to a mathematical approach that might not optimally reflect pathophysiology. Second, the increase in diastolic blood pressure of 6 mm Hg and the practically unchanged levels of systolic blood pressure between the ages of 36 and 42 years in this study population led to a decrease in pulse pressure. Although this may seem “unexpected,” increases in pulse pressure with ageing are often observed after the fifth or sixth decades of life as a consequence of arterial stiffening.⁷ In addition, these changes were in line with previous life course trends in changes of the 2 blood pressure components in this cohort, showing steeper increases in (sitting) diastolic (0.6 mm Hg/y) than systolic blood pressure (0.2 mm Hg/y) between late adolescence (age 15 years) and age 36 years (data not shown). Still, because different blood pressure devices were used during the 2 measurement periods, it is possible that diastolic blood pressure data from the year 2000 may have been underestimated as compared with the 2006 data. As a consequence, at the population level, pulse pressure and all arterial stiffness estimates are most likely underestimated in 2006 as compared with 2000. Nevertheless, a systematic underestimation of stiffness does not materially change the reported associations.

Third, on the population level, heart rate was higher in 2000 as compared with 2006. This might be explained by the fact that the ultrasound examination was introduced for the first time in 2000, and, at the second ultrasound examination, individuals might have been more relaxed because they knew what was going to happen. Because a (patho)physiological explanation is not readily at hand, such an effect might explain the difference in heart rate. Again, however, this would not be expected to affect the associations observed. Fourth, renal function was only determined in 2006. Although additional adjustment did not materially change our results, the present study, therefore, can only partly exclude a role for renal function in the reported associations. Finally, our data were obtained in a young, white population, and, therefore, inferences to older individuals and (or) other ethnicities should be made with caution.

Perspectives

Both scores of biomarkers for endothelial dysfunction and low-grade inflammation are associated with greater arterial stiffness in apparently healthy adults over a 6-year period. This suggests that arterial stiffness may be a mechanism through which endothelial dysfunction and low-grade inflammation lead to cardiovascular disease. In addition, the results showed that, early in the development of arterial stiffening, endothelial dysfunction and low-grade inflammation act differentially along the arterial tree. These data may help us to understand the pathophysiology of early arterial stiffening and may provide potential targets for intervention.

Acknowledgments

We thank Marjo van de Waarenburg (Department of Medicine, Maastricht University Medical Centre) for her excellent technical laboratory assistance.

Sources of Funding

This work was supported by the Top Institute Food and Nutrition (Wageningen, The Netherlands); Diet and Endothelial Function (project A1004; to B.C.v.B., R.M.H., C.G.S., and C.D.S.); and by a postdoctoral research grant (2006T050) from The Netherlands Heart Foundation (to I.F.). Throughout the years, the Amsterdam Growth and Health Longitudinal Study was supported by grants from the Foundation for Educational Research; the Dutch Prevention Fund; The Netherlands Heart Foundation; the Dutch Ministry of Public Health, Well Being and Sport; the Dairy Foundation on Nutrition and Health; The Netherlands Olympic Committee/Netherlands Sports Federation; Heineken BV; and the Scientific Board on Smoking and Health.

Disclosures

None.

References

- Blankenberg S, Rupprecht HJ, Bickel C, Peetz D, Hafner G, Tiret L, Meyer J. Circulating cell adhesion molecules and death in patients with coronary artery disease. *Circulation*. 2001;104:1336–1342.
- 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.
- Jager A, van Hinsbergh VW, Kostense PJ, Emeis JJ, Yudkin JS, Nijpels G, Dekker JM, Heine RJ, Bouter LM, Stehouwer CD. Von willebrand factor, C-reactive protein, and 5-year mortality in diabetic and nondi-

- abetic subjects: the Hoorn Study. *Arterioscler Thromb Vasc Biol.* 1999;19:3071–3078.
4. Morange PE, Simon C, Alessi MC, Luc G, Arveiler D, Ferrieres J, Amouyel P, Evans A, Ducimetiere P, Juhan-Vague I. Endothelial cell markers and the risk of coronary heart disease: the Prospective Epidemiological Study of Myocardial Infarction (PRIME) Study. *Circulation.* 2004;109:1343–1348.
 5. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med.* 2000;342:836–843.
 6. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med.* 1999;340:115–126.
 7. Franklin SS, Khan SA, Wong ND, Larson MG, Levy D. Is pulse pressure useful in predicting risk for coronary heart disease? The Framingham Heart Study. *Circulation.* 1999;100:354–360.
 8. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension.* 2001;37:1236–1241.
 9. Stehouwer CD, Henry RM, Ferreira I. Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. *Diabetologia.* 2008;51:527–539.
 10. Lieb W, Larson MG, Benjamin EJ, Yin X, Tofler GH, Selhub J, Jacques PF, Wang TJ, Vita JA, Levy D, Vasani RS, Mitchell GF. Multimarker approach to evaluate correlates of vascular stiffness: the Framingham Heart Study. *Circulation.* 2009;119:37–43.
 11. Schnabel R, Larson MG, Dupuis J, Lunetta KL, Lipinska I, Meigs JB, Yin X, Rong J, Vita JA, Newton-Cheh C, Levy D, Keaney JF Jr, Vasani RS, Mitchell GF, Benjamin EJ. Relations of inflammatory biomarkers and common genetic variants with arterial stiffness and wave reflection. *Hypertension.* 2008;51:1651–1657.
 12. Abramson JL, Weintraub WS, Vaccarino V. Association between pulse pressure and C-reactive protein among apparently healthy US adults. *Hypertension.* 2002;39:197–202.
 13. Amar J, Ruidavets JB, Bal Dit Sollier C, Bongard V, Boccalon H, Chamontin B, Drouet L, Ferrieres J. Soluble CD14 and aortic stiffness in a population-based study. *J Hypertens.* 2003;21:1869–1877.
 14. Amar J, Ruidavets JB, Sollier CB, Bongard V, Boccalon H, Chamontin B, Drouet L, Ferrieres J. Relationship between C reactive protein and pulse pressure is not mediated by atherosclerosis or aortic stiffness. *J Hypertens.* 2004;22:349–355.
 15. Chae CU, Lee RT, Rifai N, Ridker PM. Blood pressure and inflammation in apparently healthy men. *Hypertension.* 2001;38:399–403.
 16. Eklund C, Kivimaki M, Islam MS, Juonala M, Kahonen M, Marniemi J, Lehtimaki T, Viikari J, Raitakari OT, Hurme M. C-reactive protein genetics is associated with carotid artery compliance in men in the Cardiovascular Risk in Young Finns Study. *Atherosclerosis.* 2008;196:841–848.
 17. Juonala M, Jarvisalo MJ, Maki-Torkko N, Kahonen M, Viikari JS, Raitakari OT. Risk factors identified in childhood and decreased carotid artery elasticity in adulthood: the Cardiovascular Risk in Young Finns Study. *Circulation.* 2005;112:1486–1493.
 18. Kampus P, Kals J, Ristimae T, Fischer K, Zilmer M, Teesalu R. High-sensitivity C-reactive protein affects central haemodynamics and augmentation index in apparently healthy persons. *J Hypertens.* 2004;22:1133–1139.
 19. Kampus P, Kals J, Ristimae T, Muda P, Ulst K, Zilmer K, Salonen RM, Tuomainen TP, Teesalu R, Zilmer M. Augmentation index and carotid intima-media thickness are differently related to age, C-reactive protein and oxidized low-density lipoprotein. *J Hypertens.* 2007;25:819–825.
 20. Kullo IJ, Seward JB, Bailey KR, Bielak LF, Grossardt BR, Sheedy PF II, Peyser PA, Turner ST. C-reactive protein is related to arterial wave reflection and stiffness in asymptomatic subjects from the community. *Am J Hypertens.* 2005;18:1123–1129.
 21. Mattace-Raso FU, van der Cammen TJ, van der Meer IM, Schalekamp MA, Asmar R, Hofman A, Witteman JC. C-reactive protein and arterial stiffness in older adults: the Rotterdam Study. *Atherosclerosis.* 2004;176:111–116.
 22. Nakhai-Pour HR, Grobbee DE, Bots ML, Muller M, van der Schouw YT. C-reactive protein and aortic stiffness and wave reflection in middle-aged and elderly men from the community. *J Hum Hypertens.* 2007;21:949–955.
 23. Schumacher W, Cockcroft J, Timpson NJ, McEnery CM, Gallacher J, Rumley A, Lowe G, Smith GD, Wilkinson IB, Ben-Shlomo Y. Association between C-reactive protein genotype, circulating levels, and aortic pulse wave velocity. *Hypertension.* 2009;53:150–157.
 24. Schutte R, Schutte AE, Van Rooyen JM, Huisman HW, Palmer IM, Fourie CM, Peter S, Malan L, Malan NT, Reimann M. Von willebrand factor as marker of vascular function in south african women: the POWIRS Study. *Am J Hypertens.* 2008;21:1298–1303.
 25. Whincup PH, Gilg JA, Donald AE, Katterhorn M, Oliver C, Cook DG, Deanfield JE. Arterial distensibility in adolescents: the influence of adiposity, the metabolic syndrome, and classic risk factors. *Circulation.* 2005;112:1789–1797.
 26. Yasmin, McEnery CM, Wallace S, Mackenzie IS, Cockcroft JR, Wilkinson IB. C-reactive protein is associated with arterial stiffness in apparently healthy individuals. *Arterioscler Thromb Vasc Biol.* 2004;24:969–974.
 27. Stehouwer CD, Gall MA, Twisk JW, Knudsen E, Emeis JJ, Parving HH. Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. *Diabetes.* 2002;51:1157–1165.
 28. Ferreira I, Boreham CA, Twisk JW, Gallagher AM, Young IS, Murray LJ, Stehouwer CD. Clustering of metabolic syndrome risk factors and arterial stiffness in young adults: the Northern Ireland Young Hearts Project. *J Hypertens.* 2007;25:1009–1020.
 29. Kemper HC. *Amsterdam Growth and Health Longitudinal Study (AGALS): A 23-Year Follow-Up From Teenage to Adult About Lifestyle and Health.* Basel, Switzerland: Karger; 2004.
 30. van Bussel BC, Henry RMA, Schalkwijk CG, Ferreira I, Feskens EJM, Streppel MT, Smulders YM, Twisk JWR, Stehouwer CDA. Fish consumption in healthy adults is associated with decreased circulating biomarkers of endothelial dysfunction and inflammation, during a 6-year follow-up. *J Nutr.* 2011;141:1719–1725.
 31. Ferreira I, Henry RM, Twisk JW, van Mechelen W, Kemper HC, Stehouwer CD. The metabolic syndrome, cardiopulmonary fitness, and subcutaneous trunk fat as independent determinants of arterial stiffness: the Amsterdam Growth and Health Longitudinal Study. *Arch Intern Med.* 2005;165:875–882.
 32. Schouten F, Twisk JW, de Boer MR, Stehouwer CD, Serne EH, Smulders YM, Ferreira I. Increases in central fat mass and decreases in peripheral fat mass are associated with accelerated arterial stiffening in healthy adults: the Amsterdam Growth and Health Longitudinal Study. *Am J Clin Nutr.* 2011;94:40–48.
 33. Twisk JWR. *Applied Longitudinal Data Analysis for Epidemiology: A Practical Guide.* Cambridge, United Kingdom: Cambridge University; 2003.
 34. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J.* 2006;27:2588–2605.
 35. Aird WC. Phenotypic heterogeneity of the endothelium: I—structure, function, and mechanisms. *Circ Res.* 2007;100:158–173.
 36. Aird WC. Phenotypic heterogeneity of the endothelium: II—representative vascular beds. *Circ Res.* 2007;100:174–190.
 37. Deanfield J, Donald A, Ferri C, Giannattasio C, Halcox J, Halligan S, Lerman A, Mancina G, Oliver JJ, Pessina AC, Rizzoni D, Rossi GP, Salvetti A, Schiffrin EL, Taddei S, Webb DJ. Endothelial function and dysfunction: part I—methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. *J Hypertens.* 2005;23:7–17.
 38. Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. *Circulation.* 2007;115:2390–2397.
 39. Napoli C, de Nigris F, Palinski W. Multiple role of reactive oxygen species in the arterial wall. *J Cell Biochem.* 2001;82:674–682.
 40. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction—a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol.* 1999;19:972–978.