

Preferential Stiffening of Central Over Peripheral Arteries in Type 2 Diabetes

Eiji Kimoto,¹ Tetsuo Shoji,¹ Kayo Shinohara,¹ Masaaki Inaba,¹ Yasuhisa Okuno,² Takami Miki,³ Hidenori Koyama,¹ Masanori Emoto,¹ and Yoshiki Nishizawa¹

Arterial stiffness affects cardiac functions, peripheral circulation, and cardiovascular mortality. We examined whether arterial stiffness in different regions is equally affected by diabetes and other factors. The subjects were 161 patients with type 2 diabetes and 129 healthy subjects comparable in age and sex. Arterial stiffness was evaluated by measuring pulse wave velocity (PWV) in the heart-carotid, heart-brachial, heart-femoral, and femoral-ankle segments using an automatic device. The diabetic patients had greater PWV than the healthy subjects in the four arterial regions, and the effect of diabetes on PWV was greater in the heart-carotid and heart-femoral segments (central) than in the heart-brachial and femoral-ankle regions (peripheral). PWV increased with age in the four arterial regions, and the effect of age on PWV was greater in the central than in peripheral arteries. In multiple regression analysis, age and systolic blood pressure had significant impacts on PWV of the four regions, whereas diabetes was significantly associated only with PWV of the central arteries. In contrast, sex was associated with PWV of the peripheral arteries. Thus, type 2 diabetes had greater impact on PWV of the central arteries, and different factors were involved in PWV among different arterial regions. *Diabetes* 52:448–452, 2003

Atherosclerosis has two key components: thickening (atherosis) and stiffening (sclerosis) of arterial wall (1). Intima-media thickness of carotid and other arteries has been used as a noninvasive index of atherosclerosis, whereas pulse wave velocity (PWV) is one of the classical indexes of arterial stiffness. Aortic PWV is known to be associated with left ventricular hypertrophy (2) and increased pulse pressure (3). Arterial stiffness has gained greater interest because of recent important observations that it is an independent predictor of cardiovascular mortality (4–6). In addition, our recent study (7) showed that stiffness index β of femoral artery was closely associated with symptoms of

lower limb peripheral artery disease independent of femoral artery intima-media thickness in those with type 2 diabetes. Another recent study by Suzuki et al. (8) demonstrated that brachial-ankle PWV correlated inversely with blood flow at the popliteal artery in patients with type 2 diabetes. Thus, arterial stiffness of different regions may have different roles in cardiovascular diseases.

So far, information is limited regarding risk factors involved in arterial stiffness in different regions. Although many studies describe that age and type 2 diabetes are significant factors that affect stiffness of aorta (9,10), carotid (11), brachial (12), femoral (11), and the lower-limb (13) arteries, only a few studies examined the relative impact of age (14) and diabetes (15) on arterial stiffness of different arterial regions. Also, little is known about sex-related difference in regional stiffness of arteries (16). The purpose of the present study was to examine whether arterial stiffness in different segments of arteries is equally affected by diabetes and other factors.

RESEARCH DESIGN AND METHODS

Subjects. The subjects were 290 adults including 161 patients with type 2 diabetes and 129 healthy control subjects comparable in age and sex. All subjects were 40 years old or older. They gave informed consent to participate in the study. Table 1 gives characteristics of the subjects.

The healthy control subjects were recruited from participants of a local health check program at the Osaka Municipal Health Promotion Center. Exclusion criteria were fasting plasma glucose level >7.0 mmol/l (126 mg/dl); renal dysfunction defined as overt proteinuria and/or increased serum creatinine >133 μ mol/l (1.5 mg/dl); liver dysfunction defined as AST >50 IU/l; reduced ankle-brachial pressure index <0.9 ; current medication for diabetes, hypertension, and/or dyslipidemia; and history of myocardial infarction and/or cerebral infarction.

Diagnosis of diabetes was made according to the American Diabetes Association criteria (17). We excluded those with type 1 diabetes who were positive for GAD antibody, who had a history of ketoacidosis, or who were dependent on insulin therapy for survival. Gestational diabetes and diabetes associated with a specific syndrome were carefully ruled out by their history. To avoid the effect of renal dysfunction and peripheral artery disease on arterial stiffness, we excluded those who had overt proteinuria and/or increased serum creatinine, >133 μ mol/l (1.5 mg/dl), and those who had a reduced ankle-brachial pressure index, <0.9 . Antidiabetic treatment was insulin for control ($n = 23$), sulfonylureas ($n = 72$), α -glucosidase inhibitors ($n = 30$), nateglinide ($n = 5$), biguanides ($n = 3$), thiazolidinediones ($n = 2$), and life-style modification alone ($n = 54$). Some patients received combined medications. Antihypertensive medications were calcium channel blockers ($n = 38$); angiotensin-converting enzyme inhibitors ($n = 26$); α , β , and $\alpha\beta$ -blockers ($n = 9$); angiotensin II receptor antagonists ($n = 3$); and loop diuretics ($n = 1$). History of ischemic heart disease, cerebrovascular disease, or both was present in 10, 10, and 3 patients, respectively. Also, deep tendon reflex was absent or reduced in 47 patients.

PWV and blood pressure measurement. PWV and blood pressure were measured in the supine position after 5 min of bed rest using an automatic waveform analyzer (model BP-203RPE; Colin, Komaki, Japan). Pressure waveforms of the brachial and tibial arteries were recorded by an oscillometric method using the occlusion/sensing cuffs adapted to both arms and both

From the ¹Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan; ²Department of Cardiovascular Medicine, Osaka City University Medical School, Osaka, Japan; and ³Department of Geriatrics and Neurology, Osaka City University Medical School, Osaka, Japan.

Address correspondence and reprint requests to Tetsuo Shoji, MD, Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan. E-mail: t-shoji@med.osaka-cu.ac.jp.

Received for publication 20 May 2002 and accepted in revised form 16 October 2002.

AGE, advanced glycation end products; HRT, hormone replacement therapy; PWV, pulse wave velocity.

TABLE 1
Characteristics of the subjects

	Healthy subjects	Diabetic patients	<i>P</i>
No. of subjects	129	161	
Sex (male/female)	56/73	85/76	0.112*
Age (years)	58.7 ± 0.8	60.3 ± 0.6	0.106
BMI (kg/m ²)	22.8 ± 0.2	23.8 ± 0.3	0.015
Fasting glucose (mmol/l)	5.17 ± 0.04	8.64 ± 0.23	<0.0001
HbA _{1c} (%)	4.7 ± 0.1	8.3 ± 0.2	<0.0001
Smoker (%)	26.4	47.2	0.0001*
Total cholesterol (mmol/l)	5.37 ± 0.06	5.29 ± 0.07	0.417
Plasma TG (mmol/l)	1.25 ± 0.06	1.41 ± 0.06	0.061
HDL cholesterol (mmol/l)	1.64 ± 0.04	1.35 ± 0.03	<0.0001
LDL cholesterol (mmol/l)	3.13 ± 0.06	3.29 ± 0.07	0.066
Systolic BP (mmHg)	120 ± 1	125 ± 1	0.008
Diastolic BP (mmHg)	74 ± 1	77 ± 1	0.019
Serum creatinine (μmol/l)	67 ± 1	52 ± 1	<0.0001

Data are means ± SE. *P* values for continuous variables are by one-way ANOVA. BP, blood pressure; TG, triglycerides. **P* value for prevalence is by χ^2 test.

ankles. Pressure waveforms of the carotid and femoral arteries were recorded using multi-element tonometry sensors placed at the left carotid and the left femoral arteries. Electrocardiogram was monitored with electrodes placed to both wrists. Heart sounds S1 and S2 were detected by a microphone set on the left edge of the sternum at the third intercostal space.

The waveform analyzer measures time intervals between S2 and the notch of carotid pulse wave (*Thc*), between S2 and the notch of brachial pulse wave (*Thb*), between pulse waves of the carotid and femoral arteries (*Tcf*), and between pulse waves of the femoral and tibial (ankle) arteries (*Tfa*). The sum of *Thc* and *Tcf* gives the time for pulse waves to travel from the heart (aortic orifice) to the femoral artery (*Thf*). Also, the waveform analyzer estimates the path lengths of the heart-carotid (*Dhc*), the heart-brachial (*Dhb*), the heart-femoral (*Dhf*), and the femoral-ankle (*Dfa*) segments based on the height (HT, in centimeters) using the following formulas; $Dhc = 0.2437 \times HT - 18.999$; $Dhb = 0.2195 \times HT - 2.0734$; $Dhf = 0.5643 \times HT - 18.381$; $Dfa = 0.2486 \times HT + 30.709$. PWV was calculated for each arterial segment as the path length divided by the corresponding time interval.

Reproducibility of the PWV measurement was evaluated by repeating measurements in 17 healthy subjects on two different occasions. The coefficients of variation were 6.0%, 3.3%, 4.9%, and 3.3% for hcPWV, hbPWV, hfPWV, and faPWV, respectively.

For evaluating the effect of age on PWV in the different arterial segments, regional PWV was standardized as percentage relative to the corresponding young adult mean level. The young adult mean obtained from 40 volunteers (21 men and 19 women) with a mean age of 27 years (22–39 years) was 542 cm/s for hcPWV, 666 cm/s for hfPWV, 495 cm/s for hbPWV, and 798 cm/s for faPWV. For evaluating the effect of diabetes independent of age on PWV in the different arterial segments, regional PWV was standardized as percentage relative to the age-adjusted reference value obtained from the healthy control subjects.

Blood sampling and measurements. Blood was drawn in the morning after an overnight fast for at least 12 h. HbA_{1c} was measured by high-performance liquid chromatography, and plasma glucose was measured by a glucose oxidase method. Other measurements were done by routine laboratory methods.

Statistics. Results were summarized as mean ± SE. One-way ANOVA was used to assess difference in mean values between groups. Two-way ANOVA was used to evaluate effects of two categorical variables on one continuous variable. Also, two-way repeated measures ANOVA was applied to paired data. Multiple regression analysis was applied to assess independent associations between one dependent and two or more independent variables. χ^2 test was used for difference in prevalence. *P* < 0.05 was taken as statistically significant.

RESULTS

Effects of diabetes on PWV of the four arterial segments. Figure 1 shows age-stratified comparison of PWV in four arterial segments between the healthy and diabetic groups. PWV of the heart-femoral segment (hfPWV) increased in the presence of diabetes and with age, whereas

no interaction was significant between the effects of diabetes and age. Similarly, the effects of diabetes and age on hcPWV, hbPWV, and faPWV all were significant, whereas no significant interaction was found between the effects of age and diabetes.

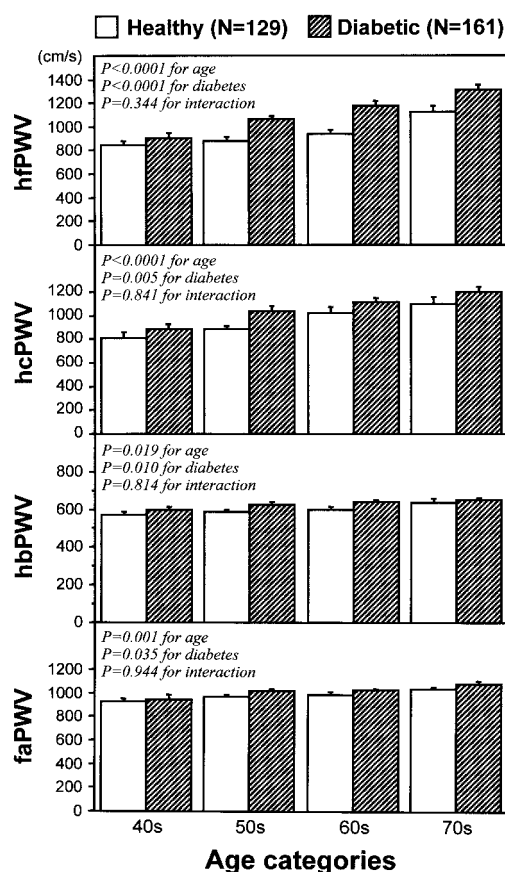


FIG. 1. Effects of age and diabetes on arterial stiffness in four different regions. PWV was compared between the healthy subjects and the patients with type 2 diabetes in each decade of age. Open and shaded columns indicate the healthy subjects and the patients with type 2 diabetes, respectively. Mean ± SE. The effects of age and diabetes were evaluated by two-way ANOVA. hf, heart-femoral segment; hc, heart-carotid segment; hb, heart-brachial segment; fa, femoral-ankle segment.

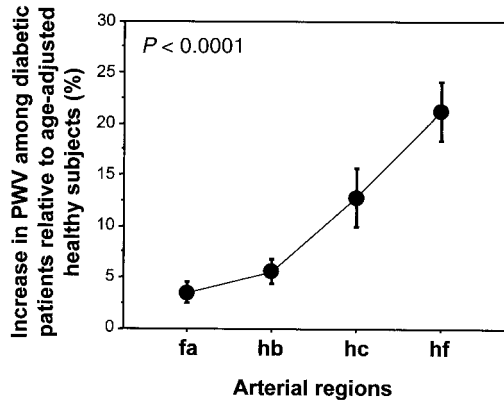


FIG. 2. Different magnitude of influence of diabetes on regional arterial stiffness. For comparing the magnitude of influence of diabetes on arterial stiffness in the four different arterial segments, regional PWV values of the patients with diabetes were expressed as percentages relative to the corresponding mean value of the age-adjusted healthy subjects and compared among the four arterial regions by two-way repeated measures ANOVA.

Different magnitude of influence of diabetes on regional PWV. We intended to examine the possible difference in the magnitude of effect of diabetes on PWV among the four arterial segments. For this purpose, we expressed regional PWV values of the diabetic patients as percentages relative to the corresponding mean value of the age-adjusted healthy subjects, and increases in such an index were compared among the four arterial regions. As shown in Fig. 2, the increase in regional PWV among the patients with diabetes was significantly different among the four arterial segments. The effect of diabetes was smaller in the femoral-ankle and heart-brachial segments and greater in the heart-carotid and heart-femoral segments.

Different magnitude of influence of age on regional PWV. Because absolute values of PWV were different among different regions of artery, it was difficult to compare directly the effects of age on PWV in different arterial segments. Therefore, we expressed regional PWV values as percentages relative to the corresponding young adult mean level and compared them among the four arterial segments. Fig. 3 shows such an index of regional PWV plotted against age. In the healthy subjects, the magnitude of association with age was not the same among the four regions. It was much greater in hcPWV and hfPWV than in hbPWV and faPWV. The effects of age and arterial regions on the standardized PWV both were significant by two-way ANOVA. Also, the effect of age was significantly different among the arterial segments as indicated by the presence of significant interaction between the effects of age and arterial regions. The same was true for the effects of age and arterial segments on regional PWV in patients with type 2 diabetes.

Independent factors that affect regional PWV. Factors associated with PWV in the four arterial segments were evaluated by multiple regression models, including age, sex, smoking, blood pressure, lipids, and presence of diabetes as covariables (Table 2). Age and systolic blood pressure were significant factors associated with increased PWV in the four arterial segments. The effect of diabetes on PWV was significant only in the heart-femoral and the heart-carotid segments. In contrast, the effect of

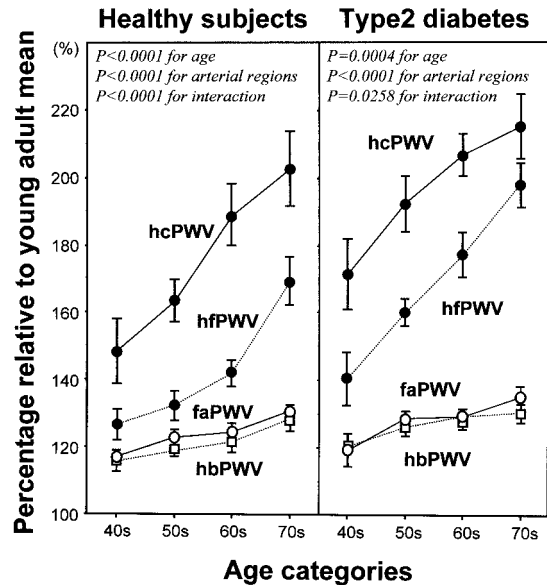


FIG. 3. Different magnitude of influence of age on regional arterial stiffness. For comparing the magnitude of age effect on arterial stiffness in the four arterial segments, regional PWV values were expressed as percentages relative to the corresponding young adult mean level and plotted against age. Mean \pm SE. The effects of age and arterial regions were evaluated by two-way ANOVA. Note that there was significant interaction between the effects of age and the arterial regions, indicating that the effect of age on PWV was different among the arterial regions.

male sex on PWV was significant only in the heart-brachial and the femoral-ankle regions. These models explained 29.0–47.4% of variance in regional PWV of the four arterial segments. The preferential association of diabetes with stiffness of the central arteries remained significant after further adjustment for serum creatinine, BMI, diastolic blood pressure, peripheral neuropathy (absence/reduction of deep tendon reflex), history of ischemic heart disease and/or cerebrovascular disease, and the number of antihypertensive medications (data not shown).

DISCUSSION

In the present study, we measured regional PWV in four segments of arteries in healthy subjects and patients with type 2 diabetes to examine whether arterial stiffness of the different regions was equally affected by diabetes and other factors. The effects of diabetes and age were greater in the heart-carotid PWV and the heart-femoral PWV than in the heart-brachial and the femoral-ankle PWV. In contrast, male sex was significantly associated with the heart-brachial and the femoral-ankle PWV but not with the heart-carotid or the heart-femoral PWV. Age and systolic blood pressure were significant factors associated with PWV of all four arterial segments. These results indicate that type 2 diabetes has different impacts on stiffness of the central and peripheral arteries and that regional PWV is affected differently by various factors.

As compared with healthy subjects, patients with type 2 diabetes have increased arterial stiffness in the aorta (9,10) and the carotid (11), brachial (12), femoral (11), and lower-limb (13) arteries. However, little is known about the effect of diabetes on regional arterial stiffness. According to Scarpello et al. (15), patients with diabetes had increased PWV in the lower-limb arteries but not in the

TABLE 2
Multiple regression analysis of factors that affect regional PWV

	hfPWV	hcPWV	hbPWV	faPWV
Age	0.267*	0.289*	0.176*	0.211*
Male sex	0.015	0.058	0.175†	0.202*
Smoking	0.066	0.096	0.046	-0.053
Systolic blood pressure	0.480*	0.301*	0.537*	0.453*
LDL cholesterol	-0.013	-0.089	-0.092	-0.049
HDL cholesterol	-0.023	-0.105	-0.024	-0.010
Presence of diabetes	0.236*	0.113‡	0.096	0.090
R ²	0.474*	0.290*	0.433*	0.333*

The table gives standard regression coefficients (β values) and level of significance. R², coefficient of determination. *P < 0.001, †P < 0.01, ‡P < 0.05.

upper-limb arteries. In our study, the effects of diabetes on the heart-brachial PWV and the femoral-ankle PWV were significant in the age-stratified comparison. However, the effect of diabetes on peripheral arterial stiffness was only modest, and the presence of diabetes was not a significant factor associated with PWV of the peripheral arteries after adjustment for other confounding variables. More important, the effect of diabetes on PWV was much greater in the heart-femoral and heart-carotid segments, and presence of diabetes was a significant and independent factor associated with increased PWV of these regions. These results demonstrate for the first time that type 2 diabetes has more preferential association with increased PWV of the central over peripheral arteries.

We also found the preferential stiffening of the central over peripheral arteries with age. Avolio et al. (14) examined correlation between age and PWV of the aorta and arteries in the upper and lower extremities among 480 Chinese people and compared the slopes of the regression lines. The slope was the greatest for the aortic PWV, smallest for the upper-limb arteries, and intermediate for the lower-limb arteries. Another study by Benetos et al. (18) found that the effect of age on distensibility was significant in the carotid but not in femoral arteries among 78 untreated normotensive and hypertensive subjects. These results are in good agreement with our data suggesting that the effect of age was greater in the central than the peripheral arteries. Our study extended the findings by measuring PWV of the four different arterial segments simultaneously, by showing the same observation in the presence and the absence of type 2 diabetes, and by adjusting for other confounding variables using multivariate models.

With regard to sex-related difference in PWV, we found that male sex was a significant and independent factor associated with increased PWV of the femoral-ankle and heart-brachial segments, whereas sex was not associated with the heart-femoral or heart-carotid PWV. Similarly, London et al. (16) reported that carotid-radial PWV and femoral-tibial PWV both were lower in premenopausal women than in age-matched men, whereas carotid-femoral PWV was not different between men and women. In addition, no difference was found in aortic PWV (19,20) or carotid artery augmentation index (20) between postmenopausal women with and without hormone replacement therapy (HRT). It is interesting that withdrawal of HRT increases PWV in the femoral-dorsalis pedis region but not in the aorto-femoral regions (21). Taken together,

these studies indicate the sex-related difference and the effect of HRT in arterial stiffness occur preferentially in the peripheral rather than in the central arteries.

The mechanisms by which the influence of these factors on arterial stiffness varies among different arterial regions are not fully understood. Advanced glycation end products (AGE) are deposited on aortic extracellular matrices (22). Aortic AGE content correlates with stiffness of human (23) and rat (22) aorta. The degree of aortic tissue glycation increases with age and in patients with diabetes (24). In animal experiments, age-related increase in aortic wall stiffness was prevented by treatment with aminoguanidine, an inhibitor of AGE formation (25). Also, an AGE cross-link breaker reduced the stiffness of aorta but not systemic arterial resistance (26). Taken together, these studies raise a possibility that AGE are involved in the preferential stiffening of the central (elastic) over peripheral (muscular) arteries as a result of aging and diabetes. In contrast, Shige et al. (27) found that cholesterol-lowering with simvastatin was associated with reduced PWV in the femoral-tibial region but not in the aorto-femoral segment. Because both statins and HRT improve endothelium-dependent vasodilation (28), stiffness of peripheral arteries may be more strongly controlled by the endothelium-dependent mechanism than that of central arteries.

In conclusion, the present study showed that type 2 diabetes has different impacts on stiffness of the central and peripheral arteries and that different factors are involved in PWV among different arterial regions. Additional studies are needed to confirm the results in other ethnic groups and to elucidate the mechanisms and clinical implications of regional arterial stiffness in health and disease.

ACKNOWLEDGMENTS

Part of this study was presented at the 61st annual meeting of the American Diabetes Association, Philadelphia, PA, and published as an abstract: *Diabetes* 50 (Suppl. 2):A458, 2001.

We thank Toshiko Maekawa, Dr. Kyoko Izumotani, and other staff at the Osaka Municipal Health Promotion Center, Osaka, Japan, for kind assistance in this study.

REFERENCES

- O'Rourke M: Mechanical principles in arterial disease. *Hypertension* 26:2-9, 1995
- Bouthier JD, De Luca N, Safar ME, Simon AC: Cardiac hypertrophy and

- arterial distensibility in essential hypertension. *Am Heart J* 109:1345–1352, 1985
3. London GM, Marchais SJ, Safar ME, Genest AF, Guerin AP, Metivier F, Chedid K, London AM: Aortic and large artery compliance in end-stage renal failure. *Kidney Int* 37:137–142, 1990
 4. Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM: Impact of aortic stiffness on survival in end-stage renal disease. *Circulation* 99:2434–2439, 1999
 5. Shoji T, Emoto M, Shinohara K, Kakiya R, Tsujimoto Y, Kishimoto H, Ishimura E, Tabata T, Nishizawa Y: Diabetes mellitus, aortic stiffness, and cardiovascular mortality in end-stage renal disease. *J Am Soc Nephrol* 12:2117–2124, 2001
 6. Meaume S, Benetos A, Henry OF, Rudnichi A, Safar ME: Aortic pulse wave velocity predicts cardiovascular mortality in subjects >70 years of age. *Arterioscler Thromb Vasc Biol* 21:2046–2050, 2001
 7. Taniwaki H, Shoji T, Emoto M, Kawagishi T, Ishimura E, Inaba M, Okuno Y, Nishizawa Y: Femoral artery wall thickness and stiffness in evaluation of peripheral vascular disease in type 2 diabetes mellitus. *Atherosclerosis* 158:207–214, 2001
 8. Suzuki E, Kashiwagi A, Nishio Y, Egawa K, Shimizu S, Maegawa H, Haneda M, Yasuda H, Morikawa S, Inubushi T, Kikkawa R: Increased arterial wall stiffness limits flow volume in the lower extremities in type 2 diabetic patients. *Diabetes Care* 24:2107–2114, 2001
 9. Lehmann ED, Gosling RG, Sonksen PH: Arterial wall compliance in diabetes. *Diabet Med* 9:114–119, 1992
 10. Taniwaki H, Kawagishi T, Emoto M, Shoji T, Kanda H, Maekawa K, Nishizawa Y, Morii H: Correlation between the intima-media thickness of the carotid artery and aortic pulse-wave velocity in patients with type 2 diabetes. Vessel wall properties in type 2 diabetes. *Diabetes Care* 22:1851–1857, 1999
 11. Emoto M, Nishizawa Y, Kawagishi T, Maekawa K, Hiura Y, Kanda H, Izumotani K, Shoji T, Ishimura E, Inaba M, Okuno Y, Morii H: Stiffness indexes beta of the common carotid and femoral arteries are associated with insulin resistance in NIDDM. *Diabetes Care* 21:1178–1182, 1998
 12. Megnien JL, Simon A, Valensi P, Flaud P, Merli I, Levenson J: Comparative effects of diabetes mellitus and hypertension on physical properties of human large arteries. *J Am Coll Cardiol* 20:1562–1568, 1992
 13. Lo CS, Relf IR, Wahlqvist ML: Doppler ultrasound recognition of preclinical changes in arterial wall in diabetic subjects: compliance and pulse-wave damping. *Diabetes Care* 9:27–31, 1986
 14. Avolio AP, Chen SG, Wang RP, Zhang CL, Li MF, O'Rourke MF: Effects of aging on changing arterial compliance and left ventricular load in a northern Chinese urban community. *Circulation* 68:50–58, 1983
 15. Scarpello JH, Martin TR, Ward JD: Ultrasound measurements of pulse-wave velocity in the peripheral arteries of diabetic subjects. *Clin Sci (Lond)* 58:53–57, 1980
 16. London GM, Guerin AP, Pannier B, Marchais SJ, Stimpel M: Influence of sex on arterial hemodynamics and blood pressure. Role of body height. *Hypertension* 26:514–519, 1995
 17. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 24:S5–S20, 2001
 18. Benetos A, Laurent S, Hoeks AP, Boutouyrie PH, Safar ME: Arterial alterations with aging and high blood pressure. A noninvasive study of carotid and femoral arteries. *Arterioscler Thromb* 13:90–97, 1993
 19. Lehmann ED, Hopkins KD, Parker JR, Turay RC, Rymer J, Fogelman I, Gosling RG: Aortic distensibility in post-menopausal women receiving Tibolone. *Br J Radiol* 67:701–705, 1994
 20. Tanaka H, DeSouza CA, Seals DR: Arterial stiffness and hormone replacement use in healthy postmenopausal women. *J Gerontol A Biol Sci Med Sci* 53:M344–M346, 1998
 21. Waddell TK, Rajkumar C, Cameron JD, Jennings GL, Dart AM, Kingwell BA: Withdrawal of hormonal therapy for 4 weeks decreases arterial compliance in postmenopausal women. *J Hypertens* 17:413–418, 1999
 22. Bruel A, Oxlund H: Changes in biomechanical properties, composition of collagen and elastin, and advanced glycation endproducts of the rat aorta in relation to age. *Atherosclerosis* 127:155–165, 1996
 23. Sims TJ, Rasmussen LM, Oxlund H, Bailey AJ: The role of glycation cross-links in diabetic vascular stiffening. *Diabetologia* 39:946–951, 1996
 24. Oimomi M, Igaki N, Hata F, Kitamura Y, Nishimoto S, Baba S, Maeda S: Age- and diabetes-accelerated glycation in the human aorta. *Arch Gerontol Geriatr* 8:123–127, 1989
 25. Cantini C, Kieffer P, Corman B, Liminana P, Atkinson J, Lartaud-Idjouadiene I: Aminoguanidine and aortic wall mechanics, structure, and composition in aged rats. *Hypertension* 38:943–948, 2001
 26. Kass DA, Shapiro EP, Kawaguchi M, Capriotti AR, Scuteri A, deGroot RC, Lakatta EG: Improved arterial compliance by a novel advanced glycation end-product crosslink breaker. *Circulation* 104:1464–1470, 2001
 27. Shige H, Dart A, Nestel P: Simvastatin improves arterial compliance in the lower limb but not in the aorta. *Atherosclerosis* 155:245–250, 2001
 28. Koh KK, Cardillo C, Bui MN, Hathaway L, Csako G, Waclawiw MA, Panza JA, Cannon RO 3rd: Vascular effects of estrogen and cholesterol-lowering therapies in hypercholesterolemic postmenopausal women. *Circulation* 99:354–360, 1999