A Cross-Sectional Study of the Effects of Type 2 Diabetes and Other Cardiovascular Risk Factors on Structure and Function of Nonstenotic Arteries of the Lower Limb

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OBJECTIVE — To compare intimal-medial thickness (IMT) and pulse wave conduction velocity (PWCV) in unstenosed arteries of the lower limb in subjects with and without type 2 diabetes and to determine the contribution of a range of cardiovascular risk factors.

RESEARCH DESIGN AND METHODS — IMT and PWCV were determined in lower-limb arteries of 79 subjects with diabetes and 77 euglycemic subjects. Plasma lipids were determined by enzymatic assays, and LDL particle size was measured by gradient gel electrophoresis. Lag time for copper-induced oxidation of LDL was determined. α-Tocopherol, retinol, and ascorbate levels were determined by high-performance liquid chromatography, soluble E-selectin by enzyme-linked immunosorbent assay, and fibrinogen and factor VII by automated assays.

RESULTS — Subjects with diabetes had greater superficial femoral artery (SFA) IMT, popliteal artery (PA) IMT, and SFA PWCV (all $P < 0.0001$). In univariate analysis, IMT and PWCV correlated with increased waist-to-hip ratio, triglycerides, and fibrinogen and inversely with HDL cholesterol and LDL size. Ascorbate was inversely associated with IMT, and LDL lag time was inversely correlated with PWCV. Subjects with the greatest number of features of the metabolic syndrome had the highest IMT and PWCV.

CONCLUSIONS — Adverse changes in the structure and function of unstenosed lower-limb arteries are present in type 2 diabetes and are associated with features of the metabolic syndrome.

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Atherosclerotic peripheral vascular disease is a significant cause of morbidity and mortality, particularly in people with impaired glucose tolerance and type 2 diabetes (1). The current approach to diagnosis and management focuses on patients with plaque, documented by existing imaging modalities, and on complications of the disease. Identification of arterial structural and functional changes before the development of stenoses may be useful, as institution of medical treatment at that stage may be more effective in preventing disease progression.

Intimal-medial thickness (IMT) and pulse wave conduction velocity (PWCV) are structural and functional parameters, respectively, that can be reproducibly and noninvasively determined in large arteries (2,3). Abnormalities in these parameters may precede the formation of atherosclerotic plaque or may be markers for generalized atherosclerosis. To date, the majority of reports have focused on the carotid artery, using high-resolution ultrasonography to measure the IMT of the common and internal carotid arteries. Previous cross-sectional (4–6) and prospective (7) studies have demonstrated an association between common carotid IMT and atherosclerotic disease in other vascular beds. Subjects with type 2 diabetes have been shown to have increased IMT relative to those without diabetes (8,9). Changes in the arteries supplying the lower limb have been less extensively studied. This region is of particular relevance to the diabetic population, which is at high risk of peripheral vasular disease, gangrene, and amputation (1).

We hypothesized that subjects with type 2 diabetes have a greater lower-limb arterial IMT and PWCV than age- and sex-matched subjects without diabetes and that these differences in structure and function precede the formation of stenotic plaque. We also hypothesized that anthropometric, biochemical, hemostatic, and lipid cardiovascular risk factors linked to the metabolic syndrome are associated with increased IMT and PWCV. The aim of this study was therefore to determine these measures of arterial structure and function and of cardiovascular

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Abbreviations: ABI, ankle brachial index; Apo, apolipoprotein; ARIC, Atherosclerosis Risk in Communities; CHD, coronary heart disease; CV, coefficient of variation; CVD, cerebrovascular disease; ECG, electrocardiograph; HPLC, high-performance liquid chromatography; IMT, intimal-medial thickness; PA, popliteal artery; PWCV, pulse wave conduction velocity; SE-selectin, soluble E-selectin; SFA, superficial femoral artery; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Clinical characteristics of nondiabetic and type 2 diabetic subjects in the study*

	Nondiabetic subjects	Diabetic subjects	*P*
n	77	79	
Male/female	50/27	49/30	NS
Age (years)	57 ± 16	60 ± 10	NS
CHD (%)	8	17	0.07
CVD (%)	5	11	NS
Hypertension (%)	21	47	<0.001
Systolic blood pressure (mmHg)	132 ± 22	136 ± 19	NS
Diastolic blood pressure (mmHg)	77 ± 8	79 ± 9	NS
BMI (kg/m^2)	26.0 ± 4.1	30.5 ± 7.4	<0.0001
WHR	0.89 ± 0.08	0.96 ± 0.09	<0.0001
Smoking (pack years)	7 ± 19	18 ± 28	<0.001
Treatment (%)			
Diet alone	0	9	–
Oral agent	0	48	–
Insulin	0	38	–
Insulin + oral agent	0	5	–

Data are means ± SD unless noted otherwise.

risk factors in groups with and without type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects

Seventy-nine subjects with type 2 diabetes (10) and 77 age- and sex-matched nondiabetic subjects were studied. None was taking lipid-modifying medication or antioxidant vitamins or had a major illness requiring hospitalization in the previous 12 months. Diabetic subjects were recruited from the St. Vincent's Hospital Diabetes Clinics, and nondiabetic subjects were recruited from the staff at St. Vincent's Hospital Melbourne, their relatives, and members of Rotary International. Of 193 subjects screened, 157 were suitable for inclusion in the study. The study was approved by the St. Vincent's Hospital Human Research Ethics Committee, and each subject gave written informed consent.

Clinical and anthropometric measures

A detailed clinical history was taken and a physical examination performed. Subjects were considered to have a history of coronary heart disease (CHD) based on the following: a history of prior myocardial infarction confirmed by evaluation of hospital records with electrocardiograph (ECG) (Minnesota codes 1.1, 1.2, 5.1, 7.1, and 9.2 [11]) and cardiac enzyme abnormalities, abnormal result on noninvasive cardiovascular testing, abnormal findings on coronary arteriography, or symptoms of angina pectoris associated with ECG changes (Minnesota code 4.1.1 [11]). Cerebrovascular disease (CVD) was defined as a transient ischemic episode or a cerebrovascular accident. Data were verified from hospital records. Subjects were determined to have hypertension if they had a previously documented elevation in systolic or diastolic blood pressure requiring medication. Smoking history was based on a self-reported estimate in pack-years. Blood pressure was measured in the right arm of the seated subject after 5 min rest, using a mercury sphygmomanometer and determined as the average of three measurements over 10 min. Waist-to-hip ratio (WHR) was assessed using established methods (12). Clinical characteristics of the two groups are detailed in Table 1.

Ultrasonographic assessment

All duplex studies were performed by a single investigator (D.N.O.'N.). Ankle brachial index (ABI) was assessed as previously described (13), using a handheld 5.3-MHz Doppler ultrasonic probe (Medasonics, Mountain View, CA). An ABI <0.9 has up to 95% sensitivity for detection of angiogram-positive disease (14) and such subjects were excluded. For duplex assessment, an Acuson 128 XP/10

Table 2—*A comparison of biochemical, lipoprotein, thrombotic, oxidative, and arterial parameters in nondiabetic and type 2 diabetic subjects in the study*

	Nondiabetic subjects	Diabetic subjects	*P*
n	77	79	
Fasting glucose (mmol/l)	5.5 ± 1.2	11.4 ± 4.4	<0.0001
HbA_{1c} (%)	5.0 ± 0.6	8.9 ± 1.9	<0.0001
UAE (μg/min)	29 ± 70	111 ± 34	<0.05
Total cholesterol (mmol/l)	5.8 ± 1.2	6.1 ± 1.2	NS
Triglyceride (mmol/l)	1.4 (1.0–1.9)	2.5 (2.0–3.6)	<0.0001
HDL cholesterol (mmol/l)	1.4 ± 0.5	1.1 ± 0.3	<0.001
LDL cholesterol (mmol/l)	3.8 ± 1.1	3.8 ± 0.9	NS
VLDL cholesterol (mmol/l)	0.7 ± 0.4	1.1 ± 0.5	<0.0001
ApoA1 (mg/dl)	146 ± 26	131 ± 33	<0.01
ApoB (mg/dl)	123 ± 43	140 ± 46	<0.05
LDL size (nm)	26.1 ± 0.5	25.5 ± 0.7	<0.0001
Apo(a) (units/l)	168 (86–350)	142 (82–388)	NS
Lag time (min)	52 ± 19	47 ± 18	NS
Ascorbate (μmol/l)	62.4 ± 29.2	50.0 ± 21.7	<0.01
Retinol (μmol/l)	0.83 ± 0.20	0.80 ± 0.24	NS
α-Tocopherol (μmol/l)	28 ± 13	30 ± 13	NS
Fibrinogen (g/l)	3.7 ± 1.3	4.2 ± 1.4	<0.05
Factor VII (IU/l)	1.1 ± 0.31	1.1 ± 0.39	NS
sE-selectin (ng/ml)	50 ± 21	83 ± 35	<0.0001
SFA IMT (mm)	0.59 ± 0.17	0.69 ± 0.18	<0.0005
PA IMT (mm)	0.65 ± 0.16	0.78 ± 0.21	<0.0001
SFA PWCV (m/s)	11 ± 3.6	14 ± 4.8	<0.0005

Data are means ± SD or geometric means (95% CI). UAE, urinary albumin excretion rate.

Table 3—*Significant univariate associations of risk factor variables studied with superficial femoral artery (SFA) intimal-medial thickness (IMT), popliteal artery (PA) IMT, and SFA pulse wave conduction velocity (PWCV)*

	SFA IMT		PA IMT		SFA PWCV	
Variable	β	95% CI	β	95% CI	β	95% CI
Diabetes	0.100	0.045–0.155*	0.126	0.067–0.184*	0.259	0.122–0.396*
Male sex	0.061	0.003–0.121†	0.052	−0.011 to 0.116	0.123	−0.24 to 0.27
Age (years)	0.004	0.002–0.007*	0.005	0.003–0.007*	0.011	0.006–0.016‡
Hypertension	0.118	0.017–0.29*	0.125	0.063–0.187*	0.164	0.014–0.314†
Smoking (pack years)	0.002	0.001–0.003§	0.002	0.000–0.003§	0.002	−0.001 to 0.005
WHR	0.575	0.327–0.824*	0.583	0.314–0.852*	1.025	0.384–1.667§
Fasting glucose (mmol/l)	0.009	0.003–0.016§	0.008	0.001–0.015†	0.027	0.011–0.044‡
HbA_{1c} (%)	0.024	0.013–0.036*	0.017	0.004–0.030§	0.004	0.010–0.070§
Triglyceride (mmol/l)	0.014	0.000–0.028†	0.011	−0.004 to 0.027†	0.004	0.004–0.074†
HDL cholesterol (mmol/l)	−0.107	−0.182 to −0.032§	−0.109	−0.191 to −0.027	−0.157	−0.348 to 0.034
VLDL cholesterol (mmol/l)	0.081	0.025–0.138§	0.092	0.032–0.152§	0.132	−0.015 to 0.279
Apo A1 (mg/dl)	−0.001	−0.002 to 0.000§	−0.001	−0.002 to 0.000†	−0.002	−0.005 to 0.000†
LDL size (nm)	−0.050	−0.56 to 0.02†	−0.051	−0.095 to −0.008†	−0.109	−0.210 to −0.007†
LDL lag time (min)	0.000	−0.002 to 0.002	−0.001	−0.004 to 0.001	−0.006	−0.011 to −0.001†
Ascorbate (μmol/l)	−0.002	−0.003 to 0.000†	−0.001	−0.003 to 0.000†	−0.003	−0.006–0.001
Fibrinogen (g/l)	0.030	0.009–0.050§	0.032	0.009–0.054§	0.067	0.014–0.122§
sE-selectin (ng/ml)	0.000	−0.001 to 0.002§	0.001	0.000–0.002	0.002	0.000–0.005†

*$P < 0.0001$; †$P < 0.05$; ‡$P < 0.001$; § $P < 0.01$.

color scanner (Acuson, Mountain View, CA) was used with 7-MHz linear array transducers. The arterial tree was visualized from the common femoral artery to the ankle. Stenosis severity criteria were determined by spectral broadening, flow reversal, and peak flow velocity (15). A stenosis >50% (16) was considered significant, and these subjects were excluded from the study, as we wanted to evaluate arterial parameters early in the disease process.

The superficial femoral artery (SFA) IMT measurement was performed with the patient supine, and that of the popliteal artery (PA), with the patient prone. Images were frozen and the frame magnified. IMT measurements represented the mean of five contiguous measurements performed at 1-mm intervals. IMT of the far arterial wall was determined in the SFA 1 cm distal to the bifurcation of the common femoral artery and at the midpoint of the PA. If plaque was present at either site, a measurement was obtained 1 cm distal to the first point that was free of atheroma.

PWCV (3) was determined between a point 1 cm distal to the common femoral artery bifurcation and the PA midpoint. The peak of the R-wave on a simultaneous ECG trace was used as a reference point. The time interval between the peak of the R-wave and the upstroke of the pulse wave was measured at each point with the heart rate constant. The difference between the two time intervals provided a measurement of the time for the pulse wave to travel the measured distance between the two points, allowing the velocity of the pulse wave to be calculated.

Reproducibilty of the IMT and PWCV measurements was determined by scanning 19 subjects twice at a 4- to 6-week interval. The mean difference (±SD) in IMT for these two examinations was 0.01 (±0.05) mm for the SFA and 0.01 (±0.04) mm for the PA, with correlations of 0.91 and 0.90, respectively. The difference in SFA PWCV for the two measurements was 0.8 (±1.24) m/s, with a correlation of 0.90. Detectable differences between diabetic and nondiabetic subjects at $P < 0.05$ with 80% power for the sample sizes used in the present study were IMT, 0.08 mm, and PWCV, 2.0 m/s.

Biochemical methods

Lipoprotein analysis was performed as previously described on blood collected in EDTA following a 12-h overnight fast (17). Particle size of LDL isolated by ultracentrifugation was measured by 3–13% nondenaturing gradient gel electrophoresis (18). LDL susceptibility to in vitro oxidation was determined by a modification of Esterbauer's method (19,20).

Measurement of lipid-soluble antioxidants, retinol, and α-tocopherol were performed by high-performance liquid chromatography (HPLC) as previously described (21), with interassay coefficients of variation (CVs) of 5.8% and 4.2%, respectively. Ascorbate levels were determined by HPLC with electrochemical detection after precipitation of plasma proteins using 3% metaphosphoric acid containing 1 mg/ml dithiothreitol (22). The CV at 56.8 μmol/l was 5.9%, and at 68.1 μmol/l, 3%.

Soluble E-selectin (SE-selectin) measurements were performed by commercially available enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN) with CVs at 21.9 ng/ml and 115 ng/ml of 5% and 4.7%, respectively. Fibrinogen and factor VII levels were determined on an Automated Coagulation Laboratory analyzer (ACL Instruments Laboratories, Milan, Italy) as previously described (20). HbA_{1c} testing was performed by an in-house HPLC method (23). The CV at 4.9% HbA_{1c} was 4.3%, and at 10.3%, 2.3%. Urinary albumin concentration was measured by immunonephelometry (Beckman-Coulter 360 Array nephelometer; Beckman Instruments, Palo Alto, CA). The interassay CV was 3%. Plasma insulin was estimated by radioimmunoassay, with charcoal separation of bound and free fractions. The

Table 4—*Significant associations with superficial femoral artery (SFA) intimal-medial thickness (IMT), popliteal artery (PA) IMT, and SFA pulse wave conduction velocity (PWCV) following multiple regression analysis*

Variable	Arterial measures	Coefficient (β)	95% CI	Adjusted R^2
SFA IMT	Increased WHR*	0.062	0.001–0.123	0.25
	HbA_{1c} (%)	0.026	0.009–0.043	0.26
PA IMT	Increased WHR	0.107	0.043–0.171	0.28
	High triglyceride/low HDL cholesterol†	0.101	0.027–0.176	0.26
SFA PWCV	Increased WHR	0.197	0.044–0.355	0.21
	LDL lag time (min)	−0.007	−0.002 to −0.062	0.23

Data were adjusted in multiple regression model for age, sex, diabetes, hypertension, and smoking. *Increased WHR defined as men with a ratio >1 and women with a ratio >0.85. †Triglyceride levels ≥2.1 mmol/l in association with an HDL cholesterol <1.1 mmol/l.

interassay CVs were 7.7% and 6.8% at 57.4 and 251.1 pmol/l, respectively.

Statistical analysis

Simple linear univariate and multiple regression analyses were performed using SPSS to estimate predictors of SFA IMT, PA IMT, and SFA PWCV. Multiple linear regression analysis was performed adjusting for age, sex, diabetes status, and history of hypertension and smoking.

To further investigate the relationship of artery structure and function with the metabolic syndrome, four facets of the syndrome were selected: *1*) the presence of type 2 diabetes, *2*) a documented history of hypertension, *3*) an increased WHR (men >1 and women >0.85 [24]), and *4*) triglyceride levels ≥2.1 mmol/l in association with an HDL cholesterol level <1.1 mmol/l (25). The number of these factors present in a subject was plotted against SFA IMT, PA IMT, and SFA PWCV. Statistical analysis was by ANOVA. A similar analysis was performed in the nondiabetic group only, with fasting insulin levels in the highest quartile substituting in the analysis for the presence of type 2 diabetes.

RESULTS

Comparison of diabetic and nondiabetic subjects

Diabetic and nondiabetic groups did not differ with respect to age, sex, or a history of CVD and CHD. Relative to control subjects, those with diabetes had a greater WHR and higher cigarette use. Whereas measured blood pressures did not differ, a greater proportion of subjects with diabetes had hypertension for which they were prescribed medication (Table 1).

Subjects with diabetes had a greater HbA_{1c}, fibrinogen, and SE-selectin levels and a higher urinary albumin excretion rate. The lipid profile of these subjects was characterized by decreased HDL cholesterol, increased triglycerides, and a preponderance of small, dense LDL particles. Ascorbate levels were lower. Relative to nondiabetic subjects, mean IMT in both the SFA and PA and SFA PWCV were greater in those with diabetes (Table 2). The modes of all three of the arterial parameters studied were the same in the diabetic and nondiabetic groups.

Relationship between SFA IMT, PA IMT, and SFA PWCV

SFA PWCV was associated with SFA IMT ($P < 0.0001$; $r = 0.45$) and PA IMT ($P < 0.0001$; $r = 0.36$). An association was also noted when PA IMT and SFA IMT were compared ($P < 0.0001$; $r = 0.61$).

Associations of parameters of arterial structure and function with clinical and biochemical parameters

Subjects with CHD ($n = 20$) were found to have a greater SFA IMT (0.80 ± 0.20 vs. 0.62 ± 0.16 mm; $P < 0.0001$), PA IMT (0.87 ± 0.18 vs. 0.69 ± 0.21 mm; $P < 0.0001$), and SFA PWCV (15 ± 6 vs. 12 ± 4 m/s; $P < 0.01$) versus subjects without CHD. Subjects with CVD ($n = 13$) were found to have a greater SFA IMT (0.83 ± 0.19 vs. 0.62 ± 0.17 mm; $P < 0.0001$), PA IMT (0.90 ± 0.19 vs. 0.70 ± 0.18 mm; $P < 0.001$), and SFA PWCV (17 ± 7 vs. 12 ± 4 m/s; $P < 0.001$) versus subjects without CVD.

Predictors for IMT and PWCV based on univariate analysis are shown in Table 3. Adverse IMT and PWCV profiles were observed with increasing age, increasing WHR, smoking, hypertension, elevated triglyceride and VLDL cholesterol levels, decreased HDL cholesterol and apolipoprotein (Apo)A1 levels, decreased LDL particle size, decreased ascorbate levels, and increased fibrinogen levels. Associations were not observed with total or LDL cholesterol, ApoB, Apo(a), retinol, α-tocopherol, or urinary albumin excretion. SE-selectin was positively associated and LDL lag time was inversely associated with SFA PWCV. Neither of these parameters was associated with either SFA or PA IMT. Ascorbate levels were inversely associated with SFA and PA IMT but not SFA PWCV.

Results of multivariate regression analysis after adjustment for age, sex, diabetes status, hypertension, and smoking are shown in Table 4.

WHR remained associated with SFA IMT, PA IMT, and SFA PWCV. Of the other variables, HbA_{1c} remained associated with SFA IMT, triglyceride levels in conjunction with low HDL cholesterol levels remained associated with PA IMT, and LDL lag time remained associated with SFA PWCV.

Associations with the metabolic syndrome

Figure 1 relates the number of features of the metabolic syndrome (type 2 diabetes, increased WHR, hypertension, and high triglycerides in conjunction with low HDL cholesterol levels) present in a subject to artery structure and function. Those with three or four of the selected features had greater IMT and PWCV than those with none, one, or two of the features. In general, the greater the number of coexisting facets of the metabolic syndrome, the greater the IMT and PWCV.

Figure 2 relates the number of features of the metabolic syndrome (elevated fasting insulin, increased WHR, hyper-

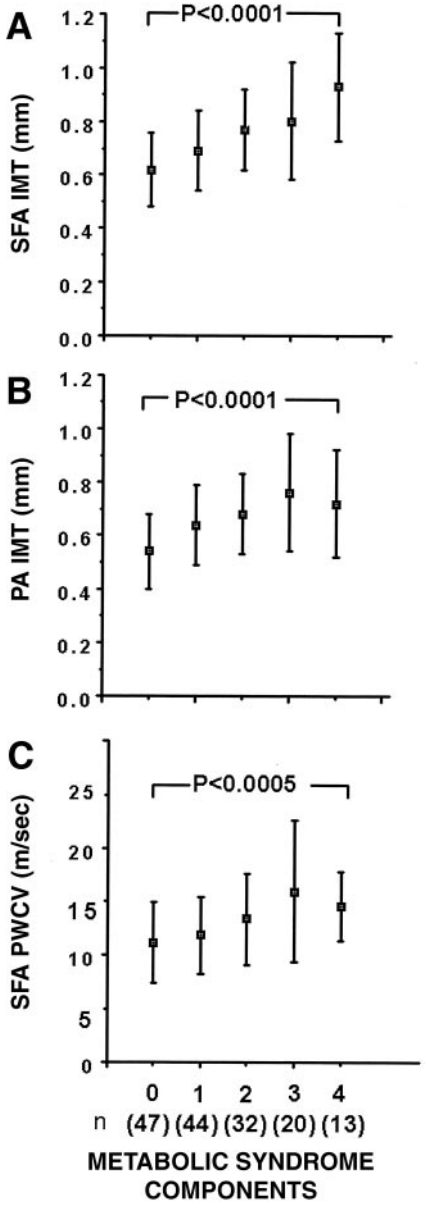

Figure 1—*Plot of SFA IMT (*A*), PA IMT (*B*), and SFA PWCV (C) against the number of factors of the metabolic syndrome present in a subject. The selected factors included hypertension, type 2 diabetes, increased WHR (men >1; women >0.85) (24), and hypertriglyceridemia (≥2.1 mmol/l) in association with decreased HDL cholesterol (<1.1 mmol/l) (25). Plot represents mean ± SD.*

tension, and high triglycerides in conjunction with low HDL cholesterol levels) present in a nondiabetic subject to artery structure and function. Individuals with two or three facets of the metabolic syndrome had greater SFA IMT and PA IMT than those with none. Whereas a trend was observed with SFA PWCV, it did not reach statistical significance.

When subjects with and without type 2 diabetes were analyzed separately, fasting insulin in the nondiabetic group was associated with SFA IMT ($P < 0.001$; $r = 0.61$), PA IMT ($P < 0.001$; $r = 0.049$), and SFA PWCV ($P < 0.01$; $r = 0.34$).

CONCLUSIONS — In a cross-sectional study of subjects with type 2 diabetes and nondiabetic subjects without clinically significant peripheral vascular disease, we have identified abnormalities of lower-limb artery structure and function and related these changes to clinical and biochemical risk factors. We have found that these noninvasively assessed vascular abnormalities are related particularly to features of the metabolic syndrome.

IMT describes a structural parameter of large arteries. PWCV, which is proportional to the square root of Young's modulus of the arterial wall (26), reflects arterial function. The greater the PWCV, the greater the degree of arterial stiffness. These structural and functional parameters of the major lower-limb arteries were significantly correlated. Although correlation does not prove causation, it is likely that changes in structure would lead to changes in function. However, the correlation coefficients suggest that PWCV is only partially determined by IMT. It is of interest that SE-selectin, which in our study was significantly elevated in diabetic subjects as previously reported (27), correlated with PWCV but not with IMT. It may be that cell adhesion molecules exert an influence on arterial function "downstream," possibly at the level of resistance vessels or in the microvascular bed, emphasizing the point that IMT is only one of many possible determinants of large artery function.

Previous studies have found the IMT of carotid arteries in subjects with type 2 diabetes to be greater than in nondiabetic control subjects (8,9). Our results indicate that this relationship also exists in the arterial supply of the lower limbs even in the absence of stenotic peripheral vascular disease. In addition, our data suggest that poor glycemic control may be associated with increased SFA IMT, as the association with HbA_{1c} noted in the univariate analysis persisted after adjusting for the presence of diabetes. Furthermore, our findings indicate that this association extends beyond subjects with diabetes to those with other components of the metabolic syndrome. Central adiposity, in particular, was associated with adverse changes in SFA IMT and PA IMT. In the nondiabetic subjects, fasting insulin was linked to increased lower-limb-artery IMT, suggesting that insulin resistance is associated with adverse changes in this parameter. In the Atherosclerosis Risk in Communities (ARIC) study, abdominal adiposity and abnormal glucose metabolism and fasting plasma insulin were positively associated with carotid IMT in subjects without cardiovascular disease (28). However, not all investigators have found a significant relationship between fasting insulin and carotid IMT (29). It may be that hyperinsulinemia could have a relatively greater detrimental effect on IMT of the more muscular femoral and popliteal arteries than the more elastic carotid artery. Other features of the metabolic syndrome that were linked with increased SFA and PA IMT in the present study were hypertension and dyslipidemia (high triglyceride and low HDL cholesterol levels).

Our findings regarding the relationship between SFA PWCV and the metabolic syndrome were broadly similar to those with SFA and PA IMT as described above. As with IMT, the majority of studies relating to arterial elasticity have focused on the carotid arteries. Our results relating PWCV to fasting insulin are consistent with previous findings by the ARIC group, which related fasting insulin to carotid arterial stiffness (30). The relative impact of the metabolic syndrome on arterial compliance may be greater in the lower-limb arteries than in the carotid artery, as a previous study has reported insulin sensitivity to be associated with arterial stiffness in the femoral but not the common carotid artery (31).

Our findings suggest that increased IMT and PWCV in nonstenosed arteries of the lower limb are markers for generalized atherosclerotic vascular disease, as these parameters correlated with the presence of CHD and CVD. These associations are similar to those noted by previous investigators studying carotid artery IMT (5–7). However, we did not observe a relationship between lower-limb arterial IMT or PWCV and either LDL cholesterol or Apo(a) levels, both risk factors for CHD. The precise relationship of increased IMT and PWCV to the formation of atherosclerotic plaque has not been clearly defined. Although studies to date

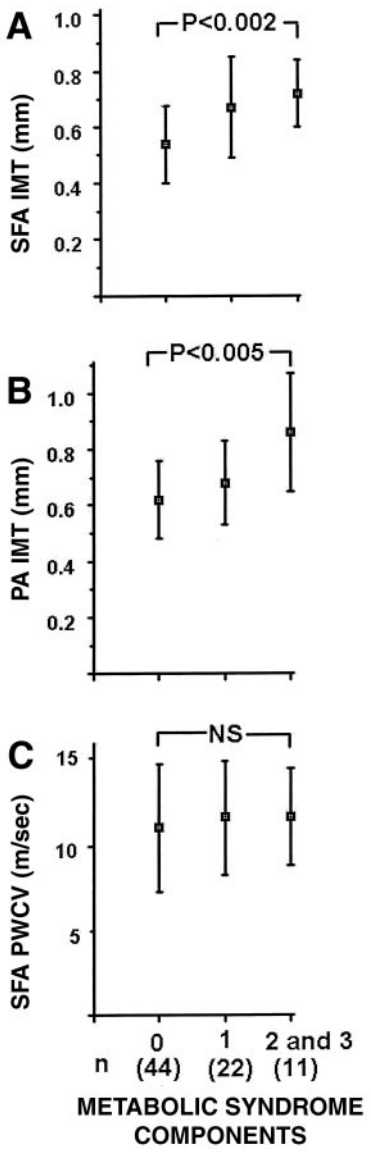

Figure 2—*Plot of SFA IMT* (A)*, PA IMT* (B)*, and SFA PWCV* (C) *against the number of factors of the metabolic syndrome present in nondiabetic subjects. The selected factors included hypertension, increased fasting insulin, increased WHR (men >1; women >0.85) (24), and hypertriglyceridemia (≥2.1 mmol/l) in association with decreased HDL cholesterol (<1.1 mmol/l) (25). Plot represents mean ± SD.*

report (4–7), and our findings indicate, that increased arterial IMT is associated with atherosclerotic macrovascular complications, the atheromatous plaque represents a localized pathological process whereas changes in IMT are diffuse. Focal atherosclerotic plaque develops through processes resulting in lipid accumulation that may have intraplaque hemorrhage superimposed (32). An increase in IMT represents a more diffuse process with smooth muscle proliferation and ground substance accumulation (26). On the other hand, a much higher correlation of LDL cholesterol with carotid IMT than with femoral IMT has been reported in another study (33), again indicating potential regional differences.

Oxidative stress may play a role in vascular disease. Potential mechanisms mediating this role include the LDL oxidation (34), enhanced expression of cell adhesion molecules (35), impairment of endothelial-dependent vasodilatation (36), and the increased formation of advanced glycation end products (37). Dietary antioxidants may be protective against oxidative stress and thereby atherosclerosis. The ARIC study (38) assessed antioxidant intake by food questionnaire and reported an inverse relationship between intake of both vitamin C and α-tocopherol with carotid IMT in subjects >54 years of age. In the prospective Cholesterol Lowering Atherosclerosis Study (CLAS) (39), those subjects with a high intake of vitamin E had a lower incremental increase in carotid IMT. No beneficial effect was observed in subjects with a high intake of vitamin C. By direct measurements of plasma levels, we found that levels of the lipid-soluble vitamins retinol and α-tocopherol did not correlate with either IMT or PWCV. However, there was a significant inverse association between LDL oxidation lag time (which is influenced by lipid-soluble antioxidant content) and PWCV, but not IMT. Ascorbate levels were significantly inversely associated with SFA and PA IMT, but not with PWCV. The relationship of ascorbate with IMT was dependent on age, the presence of diabetes, and cigarette smoking, all of which may be associated with ascorbate depletion.

Potential limitations of the present study include the selection of control subjects. We have not attempted to match diabetic and nondiabetic subjects with respect to socioeconomic factors that may influence cardiovascular disease and thus structure and function. However, health differentials arising from social inequality are at least partly mediated by insulin resistance and behavioral factors that include smoking and dietary intake of fruit and vegetables (40), which we have taken into consideration in our analysis by determining ascorbate and lipid soluble vitamin levels. The ultrasonographic measurements were not blinded, which also may be a source of bias.

In summary, IMT and PWCV are parameters of arterial structure and function that can be measured reliably, safely, and noninvasively. Our study demonstrates that type 2 diabetes is a major factor influencing IMT and PWCV in nonstenosed arteries of the lower limbs. The associations with adverse changes in these arterial parameters extend beyond hyperglycemia to other features of the metabolic syndrome, including central adiposity, hypertension, and dyslipidemia. Adverse changes in arterial structure and function may be forerunners of or risk factors for clinically relevent atherosclerosis. Abnormalities appear to identify those at increased risk of coronary and cerebrovascular disease. Prospective studies in patients with type 2 diabetes and the metabolic syndrome are warranted to determine if IMT and PWCV are good predictors of future clinically significant peripheral vascular disease. If so, then intervention at an early stage to correct the relevant risk factors could be more effective in the preservation of the lower limbs in these patients who are at increased risk of gangrene and amputation.

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