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Assessment of Vascular Function in Systemic Sclerosis

Indications of the Development of Nitrate Tolerance as a Result of Enhanced Endothelial Nitric Oxide Production

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Objective. **To investigate the relationship between endothelium-dependent and endothelium-independent functions and the stiffness of conduit arteries as well as levels of endothelial activation markers in patients with systemic sclerosis (SSc).**

Methods. **Endothelium-dependent (i.e., flow-mediated) and endothelium-independent (i.e., nitroglycerin-induced) dilation of the brachial artery was measured as the percentage of change from baseline (FMD% and NTG%, respectively) in 24 SSc patients and 24 age- and sex-matched healthy controls by high-resolution ultrasound imaging. The maximum increase in systolic pressure per unit of time (dP/dt_{max}), as a measure of arterial wall stiffness, was assessed in the radial artery by pulse applanation tonometry. Plasma nitrate, the most important metabolite of nitric oxide, and 24-hour urinary excretion of nitrate were measured by gas chromatography mass spectrometry. Soluble E-selectin and soluble vascular cell adhesion molecule 1 (sVCAM-1) were measured by enzyme-linked immunosorbent assay.**

Results. **Brachial artery FMD% and NTG% did not differ between SSc patients and controls. Radial artery dP/dt_{max} was significantly increased in the patients and correlated significantly with elevated levels of plasma nitrate and sVCAM-1. Twenty-four–hour urinary nitrate excretion tended to be elevated. Brachial artery NTG% was significantly inversely correlated with levels of plasma nitrate and soluble endothelial adhesion molecules.**

Conclusion. **The ability of the brachial arteries to dilate in response to hyperemia and nitroglycerin challenge is preserved in SSc. Stiffness of the radial artery is increased, however. Endothelial activation seems to determine the extent of the brachial artery NTG% and the radial artery dP/dt_{max}. The data are compatible with the hypothesis that nitrate tolerance is present in the vascular smooth muscle cells of the brachial artery wall in SSc.**

Vascular instability with Raynaud's phenomenon (RP) (1) and vasospastic transient interruption of inner organ perfusion are hallmarks of systemic sclerosis (SSc) (2,3). Several studies have been performed to pinpoint the mechanism behind the vascular instability, particularly that appearing as increased sensitivity to cold challenge and emotional distress, in SSc. Mediators of endothelium-dependent and endothelium-independent vasodilation (mainly, acetylcholine and nitroprusside, respectively) (4–8) have been used to test these func-

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tions in the fingers and in the skin of the fingers and forearm. The effects of mediators of α-adrenergic stimulation and inhibition (6,7,9) have also been investigated. The results of these investigations, however, have been divergent.

In an inflammatory state such as SSc, the endothelium produces increased amounts of the vasoactive substances nitric oxide (NO) (10–12) and endothelin (13,14), substances which may influence the effects of neurohumoral mediators on vascular tone. Furthermore, the endothelium produces leukocyte-homing molecules and other mediators of the inflammatory process (12,15,16) that lead to structural, inflammatory changes. There are no reports on the effects of these endothelial-generated substances on vascular function in SSc.

Structural changes in the wall of arterioles, or resistance vessels, are well recognized in SSc (17). These changes consist of intimal proliferation, intimal and medial hypertrophy, disruption of the internal elastic lamina, and fibrous scarring. Less is known about structural changes in the vessel wall of small elastic conduit arteries, such as the brachial and radial arteries, which are readily accessible to measurement by noninvasive means.

Recently, a noninvasive peripheral pulse wave analysis system, pulse applanation tonometry, was developed. By means of this method, the maximum increase in arterial pressure per unit of time during systolic rise (dP/dt_{max}) in the radial artery can be computed. This parameter is a reliable measure of arterial wall stiffness (18).

Furthermore, using high-resolution ultrasound, small changes in the diameter of the brachial artery produced by reperfusion hyperemia (flow-mediated dilation [FMD%], which is endothelium-dependent) or by nitroglycerin challenge (nitroglycerin-induced dilation [NTG%], which is endothelium-independent) can now be measured (19). FMD% is caused by the release of endogenous vasodilator substances from the endothelium, primarily, NO. The role of NO release is corroborated by the finding that FMD% may be blocked by ablation of the endothelium or by administration of N^G-monomethyl-L-arginine, a specific antagonist of NO (20).

We hypothesized that vascular wall properties are dependent on the degree of ongoing inflammation. To test our hypothesis, we measured radial artery dP/dt_{max} and brachial artery FMD% and NTG% (defined as the percentage of change from baseline), and we correlated the findings with levels of plasma nitrate, a soluble marker of endothelial activation, as well as with levels of the endothelial adhesion molecules soluble E-selectin (sE-selectin) and soluble vascular cell adhesion molecule 1 (sVCAM-1).

PATIENTS AND METHODS

Patients and controls. Twenty-four consecutive patients (20 women and 4 men) with SSc, defined according to the criteria of the American College of Rheumatology (formerly, the American Rheumatism Association) (21), were studied. Twenty patients (18 women and 2 men) had limited cutaneous SSc (lcSSc) and 4 (2 women and 2 men) had diffuse cutaneous SSc (dcSSc) (22). Three patients (all of whom were women) fulfilled the Alarcón-Segovia and Cardiel criteria for mixed connective tissue disease (MCTD) (23).

Twenty-four age- and sex-matched healthy subjects were randomly selected from within the age cohort in the population registry of the county of Västerbotten, Sweden. These subjects served as controls.

At the time of investigation, 8 patients with lcSSc had anticentromere antibodies, 2 patients with lcSSc and 1 with dcSSc had Scl-70 antibodies, and all 3 patients who fulfilled the criteria for MCTD had RNP antibodies in high titers. The mean ± SD disease duration from the time of diagnosis to study entry was 13.6 ± 10.5 years (range 1–38 years). All patients except 1 had RP (24). Eight of these patients were severely affected, with digital pitting scars or ulcers, and 2 of the 8 patients with severe RP had undergone amputation of fingers. A skin score was assessed according to the modified Rodnan model C method, with 8 unilateral sites and a maximum of 16 points (25), which resulted in a mean ± SD score of 2.2 ± 1.7 points. Esophageal dysmotility (diagnosed by radiographic examination) was found in 60% of the patients. Eleven patients showed decreased (>2 SD below the expected value) single-breath diffusing capacity for carbon monoxide.

Eight patients were taking corticosteroids. Six were being treated with angiotensin-converting enzyme (ACE) inhibitors. Two patients with dcSSc had experienced renal crisis, with partial restitution of renal function (creatinine clearance 43 and 23 ml/minute, respectively). Six of the patients and 5 of the controls were current smokers.

The findings in the corticosteroid-treated and the ACE inhibitor–treated groups were not significantly different from those in the untreated groups, although plasma nitrate levels tended to be lower in the corticosteroid-treated group. Moreover, the findings in the dcSSc group did not differ significantly from those in the lcSSc group, nor did the findings in current smokers differ from those in nonsmokers.

Measurements of arterial functions. All measurements were performed in a quiet room with the subject supine. Endothelium-dependent and endothelium-independent functions of the right brachial artery were assessed according to the method described by Celermajer et al (19). Ultrasound scans were performed with a high-resolution ultrasonographic scanner (Acuson XP; Acuson Computed Sonography, Mountain View, CA) equipped with a 7.5-MHz linear-array transducer. The brachial artery was scanned over a longitudinal section 3–5 cm above the antecubital fossa. When an

adequate image was obtained, both the patient's arm and the ultrasound probe were secured in position by use of a stereotactic clamp.

The arterial diameter was measured at rest, after reactive hyperemia with increased flow causing endothelium-dependent vasodilation (see below), then again at rest, and finally, after sublingual administration of 0.5 mg of nitroglycerin (an endothelium-independent vasodilator). Post-ischemic artery diameter was recorded at 1 minute after rapid deflation of a blood pressure cuff that had been held inflated around the forearm at a pressure of 250 mm Hg for 4.5 minutes. Twenty SSc patients had a second baseline measurement performed after a subsequent 10 minutes; 0.5 mg of NTG was then administered sublingually, and after an additional 4.5 minutes, a final brachial diameter was measured.

Ultrasound images were recorded on videotape, digitized by a high-resolution frame grabber, and stored in a computer. Offline measurements were performed using dedicated software, averaging the arterial diameter along a 10-mm segment. Diameters from 4 consecutive end-diastolic frames (identified by the electrocardiographic R wave) were averaged to yield the brachial artery diameter during respective experimental stages. Brachial artery FMD% and NTG% values were obtained as the percentages of change from the preischemia and pre-NTG baseline values, respectively.

Radial artery stiffness was assessed by pulse applanation tonometry, using a commercially available device (SphygmoCor PX; AtCor Medical, Sydney, New South Wales, Australia). The system was composed of a signal processing module, a dedicated software system, and a pressure tonometer. A transducer (tonometer) held in contact with the radial pulse at the wrist received the pulse pattern signal. The system recognized dynamic pulse patterns and calculated the peak systolic, positive pressure derivative (dP/dt_{max}),

Table 1. Characteristics of the SSc patients and the age- and sex-matched healthy control subjects*

	SSc patients (n = 24)	Healthy controls (n = 24)
Age, years	57.9 ± 15.1	57.7 ± 14.6
Height, cm	166.5 ± 8.6	166.6 ± 9.3
Weight, kg	68.9 ± 11.8	67.1 ± 14.3
Systolic blood pressure, mm Hg	141.6 ± 25.2	137.0 ± 22.9
Diastolic blood pressure, mm Hg	78.0 ± 13.5	80.5 ± 9.1
Resting heart rate, beats/minute	73.2 ± 11.0	70.7 ± 12.4
Brachial artery diameter, mm	3.4 ± 0.7	3.5 ± 0.5
Creatinine clearance (per 1.73 m^2), ml/minute	59.3 ± 28.1	62.0 ± 21.2
Cholesterol, mmoles/liter	5.3 ± 1.2	5.2 ± 0.9
Triglycerides, mmoles/liter	1.5 ± 0.7†	1.3 ± 0.9
HDL cholesterol, mmoles/liter	1.3 ± 0.4	1.4 ± 0.5
Lipoprotein(a), mg/liter	237.2 ± 218.6	208.2 ± 220.2

* Values are the mean ± SD. See ref. 27 for methods and for reference values. SSc = systemic sclerosis; HDL = high-density lipoprotein.
† $P = 0.045$ versus controls.

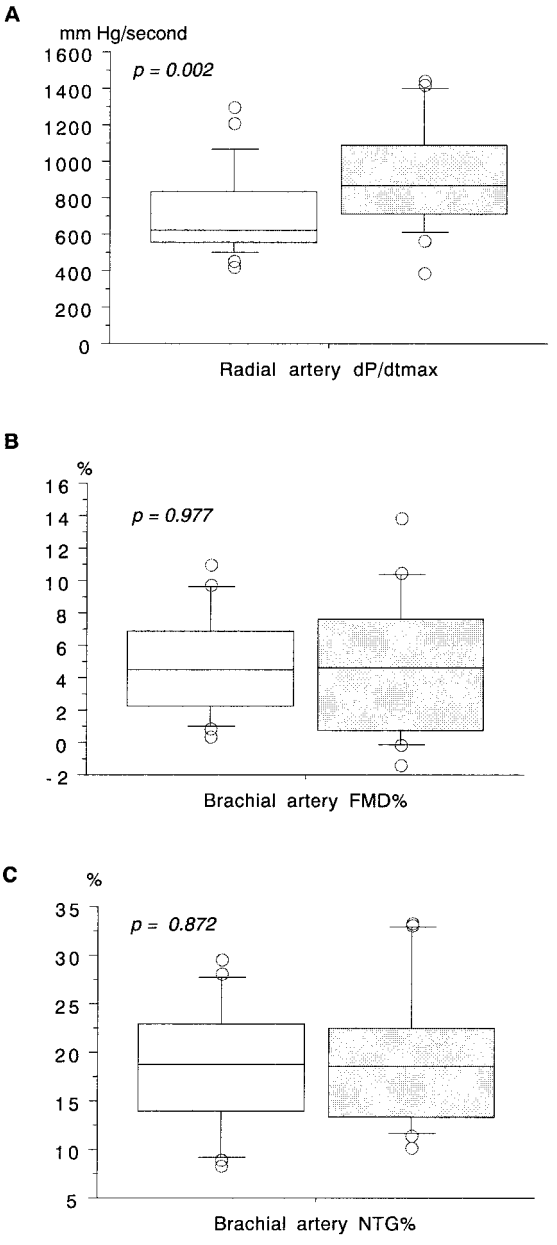

Figure 1. Box plots showing **A,** maximum rise in pulse pressure per second (dP/dt_{max}) in the radial artery, **B,** flow-mediated dilation (FMD%; % change from baseline) of the brachial artery, and **C,** nitroglycerin-induced dilation (NTG%; % change from baseline) of the brachial artery in patients with systemic sclerosis (shaded boxes) and in age- and sex-matched healthy control subjects (open boxes) (n = 24 subjects per group for **A** and **B**; n = 20 subjects per group for **C**). Boxes show the 25th and 75th percentiles; horizontal lines within the boxes show the median; bars above and below the boxes show the 10th and 90th percentiles; open circles show outlying values. *P* values were determined by Wilcoxon's signed rank test.

which is a reliable measure of the stiffness of the radial artery wall (18).

Determination of levels of nitrate in plasma and urine, cGMP in urine, and sE-selectin and sVCAM-1 in plasma. Plasma and urinary nitrate (26), urinary cGMP, and plasma sE-selectin and sVCAM-1 levels in these patients have been previously reported (12). Three of the 27 SSc patients in the original cohort were not included in the present study because their arterial function had not been measured.

Determination of cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and lipoprotein(a) levels. Levels of these lipids were measured in plasma essentially as described elsewhere (27).

Statistical analysis. Wilcoxon's signed rank test for continuous data was used to evaluate differences between patients and their matched controls. Differences between 2 independent groups were analyzed with the Mann-Whitney nonparametric test for continuous data. Spearman's rank correlation was used to test correlations between variables.

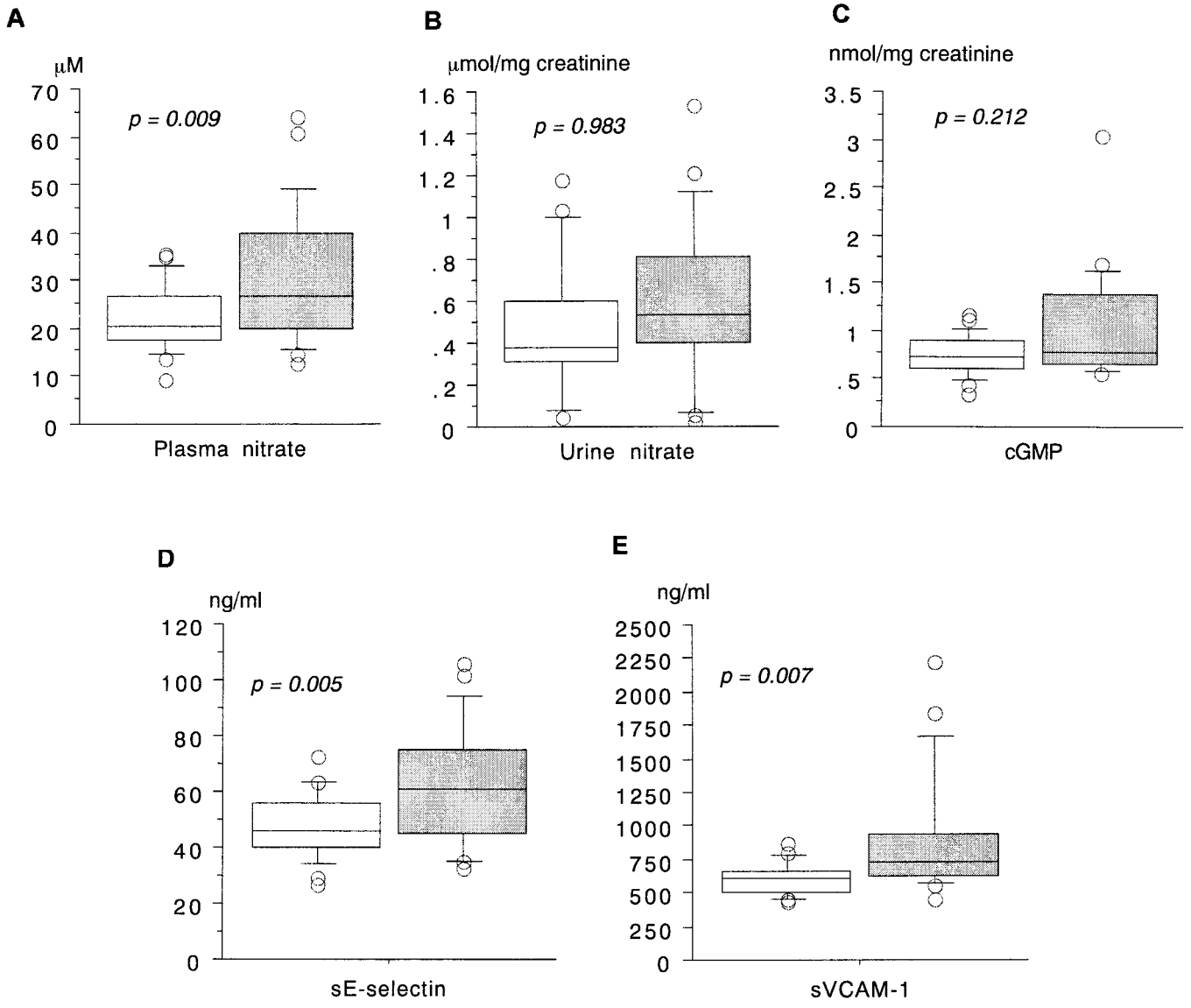

Figure 2. Box plots showing the levels of **A,** plasma nitrate, **B,** urinary nitrate, **C,** urinary cGMP, **D,** soluble E-selectin (sE-selectin), and **E,** soluble vascular cell adhesion molecule 1 (sVCAM-1) in 24 patients with systemic sclerosis (shaded boxes) and in 24 age- and sex-matched healthy control subjects (open boxes). Boxes show the 25th and 75th percentiles; horizontal lines within the boxes show the median; bars above and below the boxes show the 10th and 90th percentiles; open circles show outlying values. *P* values were determined by Wilcoxon's signed rank test.

Table 2. Correlations between plasma nitrate levels, soluble endothelial adhesion molecule levels, radial artery wall stiffness, and brachial artery dilation and diameter in 24 SSc patients and 24 age- and sex-matched healthy control subjects*

	sE-selectin	sVCAM-1	Radial artery wall stiffness, by dP/dt_{max}	Brachial artery dilation		Brachial artery diameter
				By FMD%	By NTG%†	
SSc patients						
Plasma nitrate	0.472‡	0.465‡	0.506‡	−0.202	−0.697§	0.394
sE-selectin		0.623§	0.388	−0.490‡	−0.505‡	0.381
sVCAM-1			0.487‡	−0.342	−0.555‡	0.198
Radial artery wall stiffness, by dP/dt_{max}				−0.295	−0.474‡	0.182
Brachial artery dilation						
By FMD%					0.528‡	−0.416‡
By NTG%						−0.583§
Healthy controls						
Plasma nitrate	0.176	0.061	−0.075	−0.280	−0.016	0.211
sE-selectin		0.035	0.115	0.368	0.116	0.199
sVCAM-1			−0.264	−0.025	0.057	0.213
Radial artery wall stiffness, by dP/dt_{max}				−0.049	−0.217	0.150
Brachial artery dilation						
By FMD%					0.417	−0.172
By NTG%						−0.281

* Correlation coefficients were determined by Spearman's rank correlation. Radial artery wall stiffness was measured as the maximum increase in systolic pulse pressure over time (dP/dt_{max}). Endothelium-dependent (i.e., flow-mediated) dilation (FMD%) and endothelium-independent (i.e., nitroglycerin-induced) dilation (NTG%) of the brachial artery were measured as the percentage of change from baseline. See Patients and Methods for details. sE-selectin = soluble E-selectin; sVCAM-1 = soluble vascular cell adhesion molecule 1; SSc = systemic sclerosis.
† Measured in 20 SSc patients.
‡ $P < 0.05$.
§ $P < 0.01$.

Factor analysis was performed with principal components analysis as the factor extraction method and orthotran/varimax as the transformation method. The calculations were performed using StatView 5.0.1 software (Brain Power, Calabasas, CA). All P values were 2-sided; P values less than 0.05 were considered significant.

RESULTS

Clinical characteristics. Characteristics of the patients and controls are summarized in Table 1. For ethical reasons, medication was not withdrawn prior to study participation. Patients did not differ from controls in terms of their mean height, weight, systolic and diastolic blood pressure (BP), resting heart rate, brachial artery diameter, or levels of cholesterol or HDL cholesterol. Elevated levels of triglycerides were found exclusively in patients who were receiving corticosteroid treatment.

Radial artery dP/dt_{max} and brachial artery FMD% and NTG%. Arterial wall stiffness, as assessed by the radial artery dP/dt_{max}, was significantly greater in the SSc patients than in the controls (Figure 1A). The median dP/dt_{max} in the radial artery of the patients was 871 mm Hg/second (interquartile range [IQR] 708–1,093) and 624 mm Hg/second (IQR 553–832) in the controls ($P = 0.002$). The median FMD% in the brachial artery of the patients was 4.63% (IQR 0.71–7.58), which was not significantly different from the value in the controls (4.55% [IQR 2.23–6.82]; $P = 0.977$) (Figure 1B). The median NTG% in the brachial artery of the patients was 18.60% (IQR 13.39–22.55); this value was not significantly different from that in the controls (18.68% [IQR 13.88–22.84]; $P = 0.872$) (Figure 1C).

Plasma and urinary nitrate and urinary cGMP levels. Plasma nitrate levels were significantly elevated in the patients, with a median of 26.90 μM (IQR 19.90–39.65) compared with 20.60 μM (IQR 17.65–26.55) in the controls ($P = 0.009$) (Figure 2A). Levels of nitrate and cGMP excreted in the urine were elevated in the SSc patients compared with the controls, with a median nitrate level of 0.538 μmoles/mg of creatinine (IQR 0.400–0.812) versus 0.377 μmoles/mg of creatinine (IQR 0.307–0.595) and a median cGMP level of 0.784 nmoles/mg creatinine (IQR 0.667–1.386) versus 0.729 nmoles/mg creatinine (IQR 0.611–0.894) (Figures 2B and C). The differences, however, were not statistically significant.

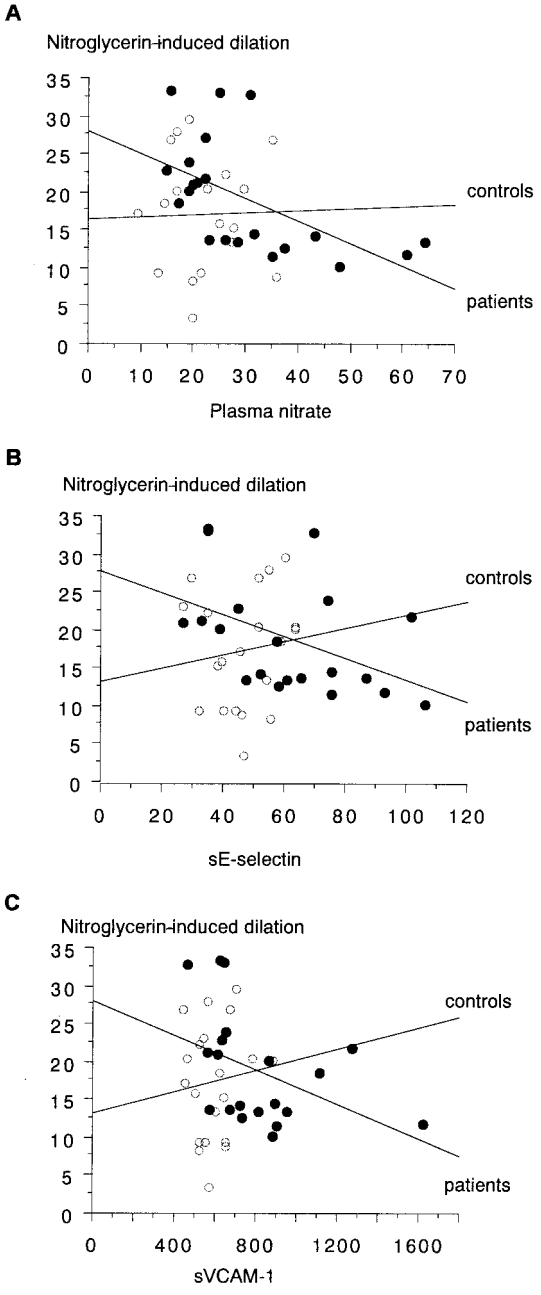

Figure 3. Scattergrams showing the correlations (Spearman's rank correlation coefficients) between nitroglycerin-induced dilation of the brachial artery and levels of **A,** plasma nitrate (patients $r_s = -0.697$, $P = 0.0018$; controls $r_s = 0.003$, $P = 0.9911$; all $r_s = -0.269$, $P = 0.0889$), **B,** soluble E-selectin (sE-selectin) (patients $r_s = -0.505$, $P = 0.0276$; controls $r_s = 0.111$, $P = 0.6275$; all $r_s = -0.193$, $P = 0.2213$), and **C,** soluble vascular cell adhesion molecule 1 (sVCAM-1) (patients $r_s = -0.555$, $P = 0.0156$; controls $r_s = 0.072$, $P = 0.7530$; all $r_s = -0.185$, $P = 0.2421$) in 20 systemic sclerosis patients (●) and 20 age- and sex-matched healthy control subjects (○).

Levels of adhesion molecules. Soluble E-selectin and sVCAM-1 levels were significantly increased in the patients compared with the controls. The median levels of sE-selectin and sVCAM-1, respectively, were 60.5 ng/ml (IQR 45.2–75.3) in the patients versus 46.2 ng/ml (IQR 39.9–56.2) in the controls and 733.0 ng/ml (IQR 633.0–939.0) in the patients versus 607.5 ng/ml (IQR 508.5–661.5) in the controls (Figures 2D and E).

Findings of the correlation analysis. In the patients, but not in the controls, radial artery wall stiffness (as determined by the radial artery dP/dt_{max}) was significantly positively correlated, whereas endothelium-dependent dilation (as determined by the brachial artery FMD%) and endothelium-independent dilation (as determined by the brachial artery NTG%) were significantly inversely correlated, with levels of one or more soluble markers of endothelial activation (i.e., plasma nitrate, sE-selectin, and sVCAM-1) (Table 2 and Figure 3). Moreover, brachial artery NTG% correlated inversely and significantly with radial artery dP/dt_{max} and positively and significantly with brachial artery FMD% in the SSc patients, but not in the controls (Table 2).

Radial artery dP/dt_{max} correlated strongly with systolic BP and age in both the patient and control groups, but not with the duration of SSc (data not shown). It is well known from previous investigations that FMD tends to be inversely correlated with vessel diameter (28). Indeed, we found that brachial artery FMD% and NTG% correlated inversely with brachial artery diameter as well as with systolic BP in the patients, but not in the controls (data not shown). Brachial artery NTG%, but not FMD%, correlated inversely with age in the patients, but neither of the values correlated with disease duration. There were no significant correlations between either radial artery dP/dt_{max}, brachial artery FMD%, or brachial artery NTG% and values for cholesterol, HDL cholesterol, triglycerides, lipoprotein(a), erythrocyte sedimentation rate, haptoglobin, orosomucoid, and the skin score in either the patients or the controls (data not shown).

Exploratory data analysis. We performed factor analysis of the patient data, seeking fundamental, latent, inherent patient qualities, or factors, influencing brachial artery NTG% variability. In factor analysis, the loading of a hypothetical factor onto a variable expresses how much a change of the factor would affect the variable. We included the data for plasma nitrate, sE-selectin, sVCAM-1, radial artery dP/dt_{max}, brachial artery diameter, brachial artery NTG%, age, and creati-

nine clearance as variables in our analysis. The analysis yielded 4 factors with eigenvalues >1, which explained 82.5% of the total variation of these variables. The eigenvalue is the square of the sum of the loadings on the variables for a particular factor, and it is a measure of how well the factor explains the variability. To be of importance, the eigenvalue ought to be >1. Moreover, to be of interest, a loading should be >0.3.

The 4 factors we identified included one factor showing large loadings on plasma nitrate, brachial artery NTG%, and both soluble adhesion molecules (sE-selectin and sVCAM-1), a second factor showing large loadings on age and creatinine clearance, a third factor showing large loadings on plasma nitrate, brachial artery NTG%, and radial artery dP/dt_{max}, and a fourth factor showing large loadings on brachial artery diameter and brachial artery NTG%. In the factor analysis, the third factor loaded negatively on brachial artery NTG% and positively on plasma nitrate and radial artery dP/dt_{max}. There were no important loadings of the third factor on the endothelial adhesion molecules sE-selectin and sVCAM-1. These findings indicate a role for NO production in the determination of brachial artery NTG% beyond the role as a marker of structural vascular inflammatory changes (Table 3).

DISCUSSION

In the present study, we used pulse tonometry to demonstrate an increased radial artery dP/dt_{max}, signifying increased radial artery stiffness, in patients with SSc. In addition, we found a significant positive correlation between radial artery stiffness and elevated plasma levels of soluble markers of endothelial inflammation, that is, plasma nitrate and the soluble endothelial adhesion molecules, sE-selectin and sVCAM-1. The correlation may be explained by the assumption that dysfunctional endothelial changes, such as edema, leukocyte infiltration, and endothelial cell conformational changes, increase the stiffness of the arterial wall. Moreover, the demonstrated increase in radial artery stiffness in SSc patients is consistent with findings of macroangiopathy, as demonstrated angiographically, in the ulnar and radial arteries of SSc patients (29). Our study was based on the presumption that plasma nitrate levels in inflammatory states are mainly dependent on the rate of production of NO by inducible nitric oxide synthase (30), which may become induced by inflammatory cytokines in monocytes, endothelial cells, and fibroblasts. Plasma nitrate may therefore be regarded as a marker of the inflammatory process, beyond just being a metabolite of the vasodilator substance NO, in SSc.

The discrepancy between the data provided in the present study supporting preserved dilation of the brachial artery, by FMD% and NTG%, in SSc patients and the previously reported impairment of dilation in dcSSc patients (31,32) may be explained by the more extensive vascular manifestations of disease in patients with dcSSc than in patients with lcSSc, since 20 of our 24 patients had lcSSc. This is supported in a report of a study evaluating forearm arteries by digital subtraction angiography in patients with dcSSc and lcSSc (29). Endothelium-dependent and endothelium-independent dilation of arteries of the finger as well as of the skin of the finger and forearm have been studied by means of infusions of acetylcholine, a substance that mediates endothelial NO release, and by nitroprusside infusions. In one study, both measures were found to be decreased (8). In another study, decreased endothelium-dependent dilation was demonstrated (4). In other studies, no aberrations were found (5–7,9).

The median FMD% in the brachial artery of our SSc patients was about one-fourth of the median NTG% in the brachial artery, a finding that is consistent with those of previous reports (31,32). The two measures were significantly correlated. The correlation analysis also showed a closer relationship between brachial artery NTG% and plasma levels of endothelial activation markers than between brachial artery FMD% and plasma levels of endothelial activation markers. Thus, brachial artery NTG% correlated significantly and inversely with all markers, while brachial artery FMD% correlated significantly and inversely with sE-selectin

Table 3. Factor analysis for brachial artery dilation, plasma nitrate levels, soluble endothelial adhesion molecule levels, radial artery wall stiffness, brachial artery diameter, age, and creatinine clearance in 20 patients with SSc*

	Factor			
	1	2	3	4
Brachial artery dilation, by NTG%	−0.421	–	−0.555	−0.510
Plasma nitrate	0.552	–	0.531	–
sE-selectin	0.828	–	–	–
sVCAM-1	0.874	–	–	–
Radial artery wall stiffness, by dP/dt_{max}	–	–	0.962	–
Brachial artery diameter	–	–	–	0.906
Age	–	0.522	–	–
Creatinine clearance	–	0.765	–	–

* The factor extraction method used was principal components. The extraction rule used was roots greater than one. The transformation method used was orthotran/varimax. See Table 2 for definitions.

only. The correlation pattern may be explained by the assumption that inflammatory changes in the arterial wall become increasingly important restricting factors when ultimate limits of vascular dilation are approached.

Our finding of an inverse correlation between brachial artery NTG% and plasma nitrate levels in SSc patients may have another explanation aside from the fact that plasma nitrate is a marker of inflammatory changes in the arterial wall. Considering the growing evidence that the endothelium in SSc probably produces increased amounts of NO (10–12), it seems possible that NO synthesis may become elevated to an extent sufficient to generate changes in the homeostatic reactivity of vascular smooth muscle cells (VSMCs) to NO. Our finding of an inverse correlation between brachial artery NTG% and plasma nitrate levels thus tempts us to hypothesize that high levels of endothelial NO in SSc patients may have rendered the VSMCs tolerant. This hypothesis is supported in the present study by the results of the factor analysis. Furthermore, the recent demonstration of nitrate tolerance in transgenic mice with inherently increased production of endogenous nitrate supports our hypothesis (33).

While the mechanisms of nitrate tolerance are still not fully established (34,35), the phenomenon is known to cause decreased response to organic nitroesters, a supersensitivity to neurohumoral vasoconstrictor stimuli, and a tendency to rebound vasoconstriction. The abnormal sensitivity of the VSMCs to neurohumoral vasoconstrictory agents (36) might be facilitated by an increase in endothelin in SSc patients, as has been reported previously (37).

The effects of the administration of phenylephrine, a selective α_1-adrenergic receptor agonist, on blood flow in digital and forearm skin of SSc patients have been reported to be normal (6,7,9). However, it was recently demonstrated that the VSMCs in arterioles dissected from the affected skin of the upper arm of SSc patients are supersensitive to stimulation with selective α_2-adrenergic agents (9), a finding that supports our hypothesis of the development of nitrate tolerance in SSc. Due to variations in disease activity, fluctuations in the endogenous production of NO are likely to occur in SSc. Such fluctuations may result in rebound vasoconstriction, accentuating the vascular instability in SSc.

The FMD% is thought to be mediated via increased Ca^{2+} influx into the endothelial cell, activating the Ca^{2+}-dependent constitutive endothelial nitric oxide synthase (eNOS) (38). Reports on eNOS expression in lesional forearm skin from scleroderma patients are divergent (11,39). In a state of nitrate tolerance, development of both FMD- and NTG-induced dilations would be expected to be affected. However, the inverse correlation between brachial artery FMD% and plasma nitrate in our study was not significant.

In summary, we found evidence that inflammatory vascular changes in SSc are not restricted to arterioles, but may even engage small elastic conduit arteries, such as the radial artery. The significant correlation between a measure of radial artery stiffness and markers of endothelial activation indicates the utility of the easily performed technique of pulse applanation tonometry in future monitoring of disease activity in SSc patients. Furthermore, the indications we identified for the development of nitrate tolerance in SSc may open up whole new aspects in the understanding of the vascular pathophysiology of SSc. Further investigations of the relationship between endothelial NO production and vasoreactivity are needed, however, to fully substantiate the presence of this phenomenon in SSc.

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