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Heterogeneity of Vasomotor Response to Acetylcholine Along the Human Coronary Artery

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Objectives. **In view of the segmental occurrence of coronary atherosclerosis, we postulated that acetylcholine may cause heterogeneous vasomotion, depending on the extent of vessel analyzed, criteria for change in vessel caliber and dose of drug administered.**

Background. **Previous studies have reported that acetylcholine causes constriction of atherosclerotic arteries. This dysfunction of endothelium-dependent dilation may be seen without angiographically detectable disease.**

Methods. **We developed algorithms to quantitate the dimensions of a single coronary artery over virtually its entire length during a control state and during graded doses of intracoronary acetylcholine. On the basis of triplicate control angiograms, the limit of detection of a change from control diameter was 0.31 mm (≥2 SD).**

Results. **Analysis of multiple segments (each 5.6 ± 1.1 [mean ± SD] mm) along a single coronary artery revealed a heterogeneous response to acetylcholine in 27 of 31 patients at the 10^{-4} mol/liter dose and in 29 of 31 patients when responses at 10^{-6}, 10^{-5} and 10^{-4} mol/liter doses were combined; in this latter analysis, constriction and dilation in the same vessel occurred in 45% of the patients. With acetylcholine, most of 349 segments demonstrated no change, but the greatest frequency of vasoconstriction (24.6%) and vasodilation (6.9%) was seen at the 10^{-4} mol/liter dose. Inducible vasomotion was observed as far distally as 7.3 cm from the site of acetylcholine infusion.**

Conclusions. **Response to intracoronary acetylcholine with mild coronary disease is heterogeneous; disparate dimensional responses may occur in different segments of the same vessel. Inclusion of all analyzable regions of a coronary artery and the use of a reproducibility limit for quantitative angiography are optimal for assessment of segmental coronary vasomotion.**

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After the observation by Ludmer et al. (1) that acetylcholine causes paradoxic vasoconstriction of atherosclerotic coronary arteries in humans, others (2–5) have observed that both mild and severe degrees of atherosclerosis are associated with dysfunction of endothelium-dependent vasodilation. Impairment of this normal physiologic function has also been correlated with elevated serum cholesterol, family history of coronary artery disease, hypertension, male gender, age and enhanced sensitivity to the vasoconstrictor effects of catecholamines (6–11). Further, in subjects with significant risk factors for coronary disease, endothelium-dependent dilation of coronary arteries is compromised before changes in intimal morphology are seen by intravascular ultrasound (12). An abnormal, vasoconstrictor response to acetylcholine is particularly prone to occur in the proximal segment of the artery as well as at branch points (13). Some investigators have suggested that endothelial-dependent vasodilator dysfunction occurs in a hierarchic fashion, with acetylcholine and serotonin dilator responses becoming impaired before flow-mediated dilation or the dilator responses to complex sympathetic stimuli, such as cold exposure (14).

In view of the segmental occurrence of coronary atherosclerosis, we postulated that in any given coronary artery there may be a relatively heterogeneous vasomotor response to the administration of acetylcholine, depending on the region and extent of the vessel analyzed. Moreover, the reproducibility of serial measurements could affect the categorization of an individual patient or individual arterial segment as one that demonstrates coronary dilation or constriction.

Accordingly, we developed cineangiographic processing methods aimed at quantitating the dimensions of a coronary artery over virtually its entire length. To maximize correspondence of diameter measurements from multiple sequential contrast injections, fiducial loci based on branch points were utilized to interpolate any pharmacologically stimulated artery against its control injection. These computer algorithms were then applied to subjects with mild degrees of obstructive

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coronary atherosclerosis to assess the response to acetylcholine over multiple sequential segments of the same coronary artery. The results of this study have been presented previously in preliminary form (15).

Methods

Patient selection, demographics and characteristics. Patients with a history of chest pain and mildly positive exercise treadmill tests were enrolled in a protocol approved by the Investigational Review Board of the University of California San Diego, School of Medicine and the Veterans Affairs Medical Center, San Diego. Informed consent was obtained in writing from all subjects. Subjects with a recent myocardial infarction, unstable angina, variant angina, congestive heart failure, severe peripheral vascular disease or severe coronary disease by coronary angiography were excluded.

The study group comprised 31 patients (28 men, 3 women; mean age 60 years, range 32 to 73), 16 of whom had a history of hypertension, 26 were tobacco smokers (5 active, 22 former), and 4 had a history of diabetes mellitus. Mean total cholesterol was 244 mg% (range 192 to 308), mean low density lipoprotein cholesterol was 175 mg% (range 105 to 226), and mean high density lipoprotein cholesterol was 48 mg% (range 33 to 67). A single coronary artery was selectively studied in each patient (left anterior descending coronary artery in 26 patients, left circumflex coronary artery in 5). All patients had mild atherosclerotic coronary disease, as manifested by lumen irregularities or significant stenosis of a studied or nonstudied coronary artery, respectively. No artery studied had a diameter stenosis >50% by angiography.

Protocol and methods. All antianginal therapy was discontinued at least 24 h before the catheterization and angiographic procedure. After diagnostic catheterization, a 3F infusion catheter was placed subselectively through a standard 8F guiding catheter into the proximal segment of the coronary artery to be studied; right atrial pacing at 80 beats/min was then established by an electrode catheter. Biplane coronary angiography was performed in the control state and after serial 3-min infusions by a Harvard pump of acetylcholine at concentrations of 10^{-6}, 10^{-5} and 10^{-4} mol/liter. Assuming a coronary artery flow of 80 ml/min, intracoronary acetylcholine concentrations were 10^{-8}, 10^{-7}, and 10^{-6} mol/liter, respectively. All 31 patients received the three doses of acetylcholine. Coronary arteriography was performed by an electrocardiographic (ECG)-gated power injection of 9 ml of nonionic contrast agent (Omnipaque 350, Winthrop-Breon Laboratories) at 6 ml/s using a Medrad Mark IV injector (Medrad Inc.). Multiple control injections during saline infusion were performed in 17 patients (11 of whom subsequently participated in the acetylcholine protocol) for determination of the reproducibility limits of repeated measurements of coronary artery dimensions.

All images were acquired maintaining identical X-ray gantry, table height and source-to-image intensifier distance (i.e., the magnifications and projections were maintained constant throughout the imaging sequences). Projections were selected to 1) optimally display the maximal length of the vessel parallel to the image plane so as to minimize foreshortening (Fig. 1); 2) minimize superimposition of branches and other arteries; and 3) minimize overlap of the artery with the diaphragm, spine and lung field. Before injection of iodinated contrast agent, fluoroscopic images were used to confirm visualization of the guiding catheter tip in the image field. The catheter was initially imaged while unopacified by contrast. At study end, the catheter tip was measured using electronic calipers to calibrate the magnification for each contrast injection.

Cine frame selection and image digitization. All cineangiographic images were acquired during held inspiration at 30 frames/s in the 6-in (15-cm) magnification mode of the image intensifier. An ECG trigger was used to identify the QRS onset and to mark the corresponding cine frames. Images were displayed by a General Electric CAP-35 cine projector interfaced to a Cohu CCD camera with a Fujinon zoom lens (10:1). The camera system's focus, iris and zoom were remotely controlled. For each patient, frames from each contrast injection (after control and acetylcholine infusions) were selected to be in the same phase of the cardiac cycle (usually end-diastole) so as to visualize the whole or nearly all of the selected coronary artery. Frames that showed optimal opacification of the vessel were then digitized using a Gould DeAnza IP8500 image-processing system interfaced to a micro VAX II (Digital Equipment Corp.) at a resolution of 512 × 512 pixels (magnification of 112 ± 23 [mean ± SD] μm/pixel, 8 bits, 256 shades of gray). The artery under study was ultimately constituted by capturing different but overlapping frames from the same contrast injection. Video gain and offset were adjusted to utilize maximally the analog voltage range of the video digitizer. Oversaturation and undersaturation of video levels were avoided by utilizing a special video look-up table.

Quantitative coronary angiography: edge detection, frame splicing and annotation. Coronary artery quantification in the present study was based on a geometric approach utilizing single-plane views. From the biplane images, the images that optimized the projection described earlier were chosen for analysis. An automatic detection routine was utilized to determine vessel edges, but manual editing was utilized when necessary. A gradient image was created using a Sobel operator; then a maximal likelihood matrix, based on the gradient and the raw X-ray images, was created and used to define an estimate of the edges. Based on weighted first- and second-derivative thresholds, a correction was applied to define the edges. This algorithm is based on a method proposed by Dijkstra (16). Once edges were established, a centerline was computed as the midpoints of the shortest distances between corresponding points on either edge as the interrogation proceeds along the coronary artery. The lengths of chords perpendicular to the centerline between both edges were defined and plotted as successive diameters. We validated the edge detection algorithm on Plexiglas, contrast-filled vessel phantoms with diameters ranging from 0.5 to 6 mm; the

JACC Vol. 25, No. 5
April 1995:1046–55

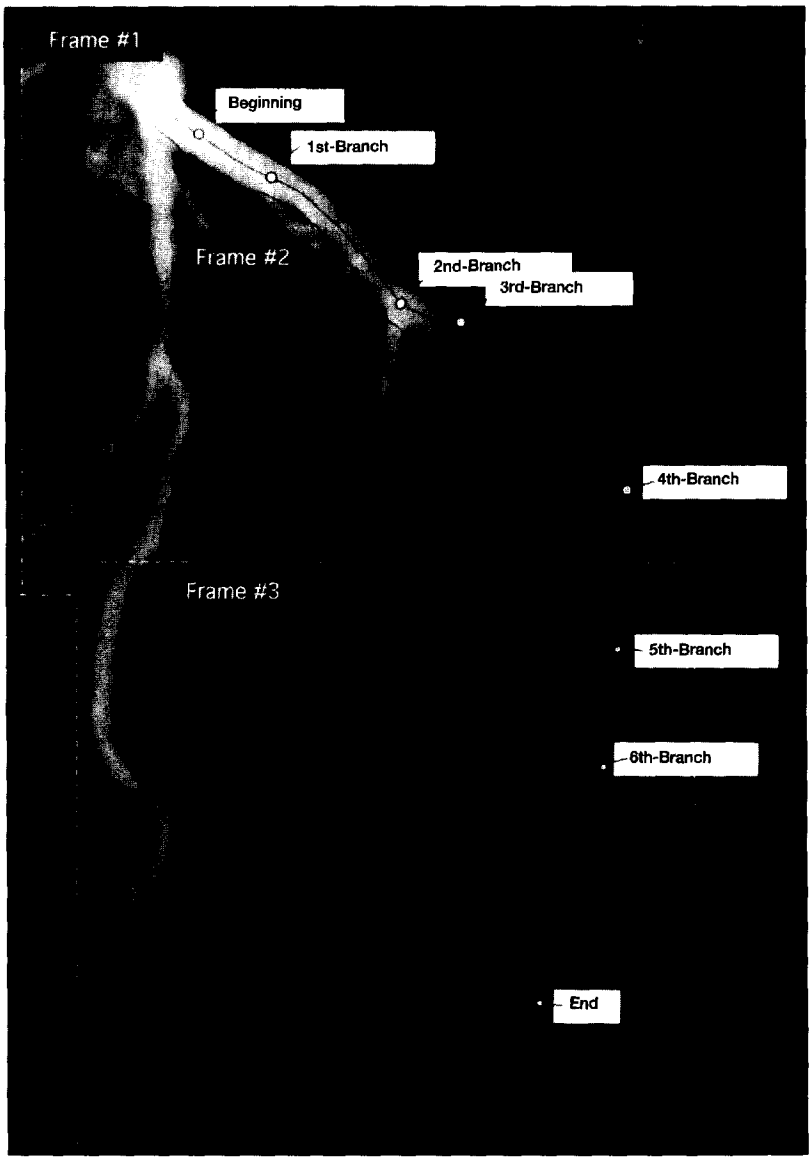


Figure 1. Spliced image of a left anterior descending coronary artery (three separate end-diastolic frames) with superimposed vessel edges determined by edge-recognition algorithm, annotated branch points and beginning and end points of diameter function. See text for details.

measured and actual diameter values correlated with an r value of 0.99.

The operator annotated all of the anatomic fiducial points on the centerline: 1) vessel branch points; 2) overlapping or foreshortened regions to be excluded from analysis; 3) common points between different images to be used to reconstruct the full composite image for the whole vessel; and 4) the beginning and end points. These annotations were subsequently used for splicing together two to four diameter arrays and image segments by autocorrelation of centerline and image segments for common parts of the vessel. All images and diameter sequences were reconstructed to yield one full image (Fig. 1) as well as one single array of diameters.

Smoothing and interpolation. Each diameter array was smoothed using a 20-point moving average filter. For each of the four diameter arrays (control and three levels of acetylcholine) in a given patient, the number of diameters between branch points was not identical. To compare data from these four diameter arrays, linear interpolation was performed on the data acquired during the infusion of the three levels of acetylcholine. All diameter arrays, between fiducial branch points, then had the same number of data points as on corresponding segments of the control angiogram (Fig. 2). After interpolation, the data were partitioned into segments of 50 diameters each that were then averaged to allow regional analysis of the vessel. Any vessel segment with marked foreshortening or overlap with other arteries was excluded.

Statistical analysis: variance of multiple control injections. To analyze each arterial segment independently of others in the same artery or in other patients, the reproducibility of sequential injections was determined (17,18). Three successive control angiograms were performed at 3-min intervals in 17 patients, 11 of whom subsequently also participated in the acetylcholine protocol. Measurements of the selected artery from these injections were then subjected to interpolation, smoothing and partitioning into segments, as described earlier

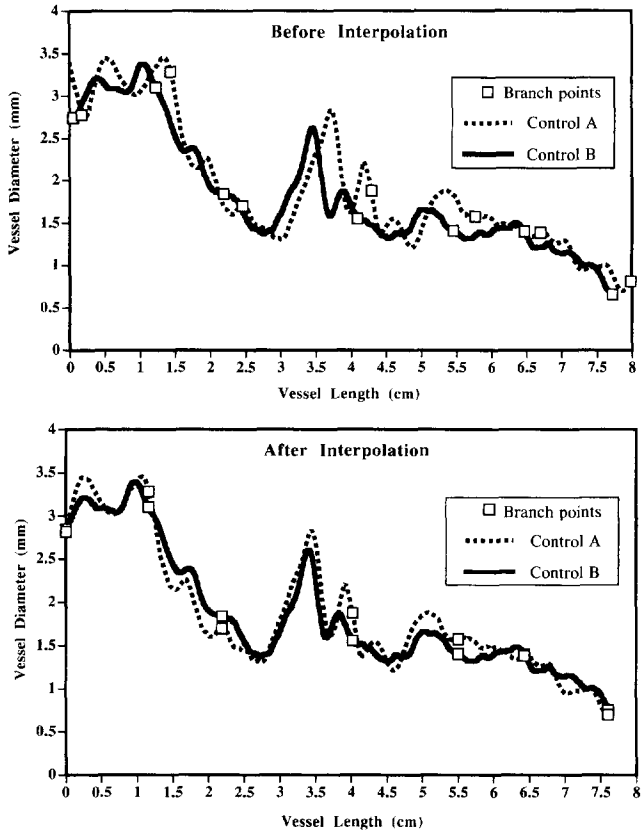


Figure 2. Top, Vessel diameter (mm) versus length (cm) functions for two successive control injections. Note slight change in overall length and lack of registration of branch points. **Bottom,** Same curves as in **top panel;** control A has now been interpolated to control B to effect anatomic registration of beginning point, end point and definable branch points.

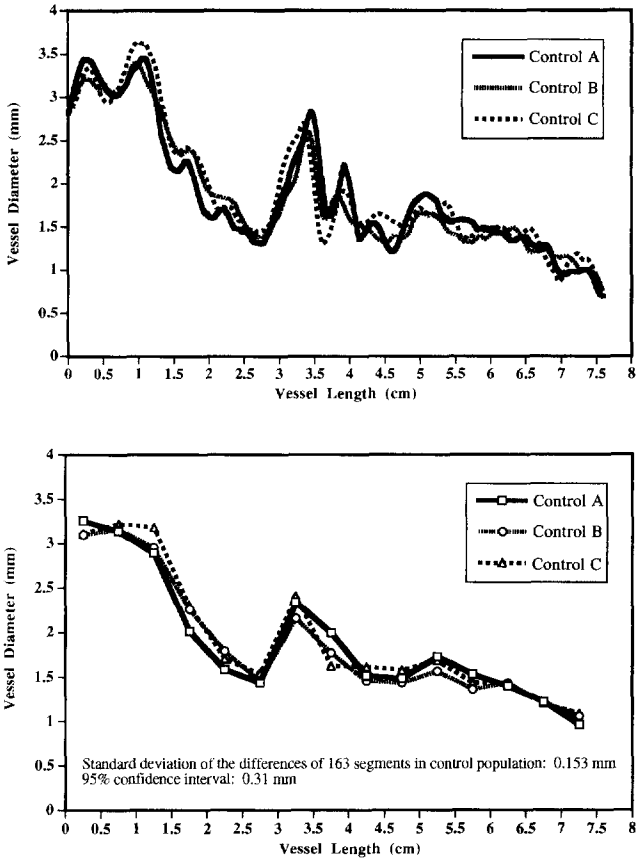


Figure 3. Top, Vessel diameter (mm) versus length (cm) functions (interpolated and smoothed) for three successive control injections. **Bottom,** Same curves as in **top panel;** sequential 50-point bins have been averaged as segments.

(Fig. 3). The averages of the diameters of corresponding 50-point segments were then subtracted from each other (i.e., A − B, B − C and C − A), and the standard deviation of these differences was found to be 0.153 mm. Thus, the limit of detection of a significant change from control diameter was established as a change in diameter of at least 0.31 mm (≥2 SD).

Results

Quantitative analysis. The length of artery analyzable in the 31 study patients ranged from 3.4 to 9.3 cm (mean [±SD] 5.7 ± 1.3), corresponding to 5 to 21 segments (mean 11.3 ± 3.5) of 5.6 ± 1.1 mm/segment per patient. Analysis of the distal vessel was terminated when background interference or vessel overlap precluded accurate edge detection; the mean diameter of the most distal segment measured in each patient was 1.34 ± 0.46 mm. After exclusion of segments for technical reasons (vessel overlap, foreshortening, poor opacification), 349 (94%) of 367 total segments were analyzed during control infusion and each concentration of acetylcholine.

Response to acetylcholine infusion. The change in mean vessel diameter versus control in all segments from each patient demonstrated a logarithmic dose–response relation (Fig. 4, top). Mean change in vessel diameter was −0.036 ± 0.011 mm on infusion of 10^{-6} mol/liter of acetylcholine; −0.074 ± 0.013 mm on infusion of 10^{-5} mol/liter; and −0.024 ± 0.018 mm on infusion of 10^{-4} mol/liter, with percent change from control of −1.39 ± 0.63%, −3.41 ± 0.73% and −5.86 ± 0.91%, respectively (all mean ± SEM). However, these mean values include individual segments with disparate responses to acetylcholine. Using the definition of a significant vasomotor response as ≥0.31-mm change from control vessel diameter, vasodilation was demonstrated on infusion of 10^{-4} mol/liter of acetylcholine in 24 (6.9%) of 349 segments, vasoconstriction in 86 (24.6%) and no response in 239 (68.5%) (Fig. 4, bottom). At lower doses of acetylcholine, fewer significant vasomotor changes were seen. With 10^{-5} mol/liter of acetylcholine vasodilation was found in 18 segments (4.9%), vasoconstriction in 49 (14.0%) and no response in 283 (81.1%). With 10^{-6} mol/liter of acetylcholine vasodilation was demonstrated in 15 segments (4.3%), vasoconstriction in 25 (7.2%) and no response in 309 (88.5%).

Vasomotor responses were demonstrated not only in proximal but also in distal segments, with significant vasoconstriction seen up to 7.3 cm distal to the infusion site in one patient

JACC Vol. 25, No. 5
April 1995:1046–55

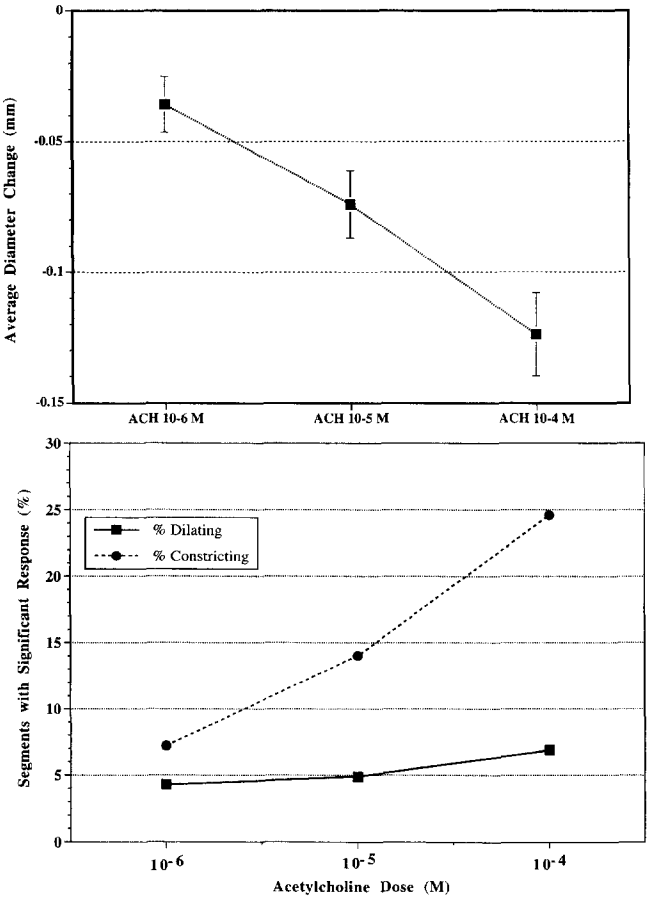


Figure 4. Top, Logarithmic dose–response relation of average segmental diameter change from control state (mm) on infusion of acetylcholine (ACH) over the dosage range of 10^{-6} to 10^{-4} mol/liter. **Bottom,** Dose–response relation of percent of all segments studied exhibiting a significant vasodilator or vasoconstrictor response to acetylcholine over the dosage range of 10^{-6} to 10^{-4} mol/liter. Note that the greatest frequency of vasodilator as well as vasoconstrictor responses is seen at the 10^{-4} mol/liter dose.

at the acetylcholine 10^{-4} mol/liter dose. Because of variation in artery length, the total number of segments cumulatively analyzed decreased with distance from the assigned beginning point along the vessel, but the proportion of segments demonstrating significant change did not (Fig. 5).

Pattern of response to acetylcholine. At the acetylcholine 10^{-4} mol/liter dose, only 4 (13%) of the 31 patients showed a homogeneous response in all segments, and in each of these patients all segments demonstrated no significant change (Fig. 6 [top], Table 1). The response to acetylcholine was heterogeneous in the other 27 patients (87%). Fourteen patients had some segments that vasoconstricted and other segments that showed no change; seven had segments that vasodilated and other segments that showed no change; and six had segments that demonstrated vasoconstriction and other segments that demonstrated vasodilation. A heterogeneous pattern of response was seen in 20 patients (65%) at the 10^{-5} mol/liter dose of acetylcholine and in 19 patients (61%) at the 10^{-6} mol/liter dose (Table 1). Again, all of the homogeneous responses seen were in patients who demonstrated no significant change in any segment. When the response to acetylcholine at all three doses was combined, 29 (94%) of the 31 patients demonstrated a heterogeneous pattern, and 14 (45%) had both significantly dilated segments and significantly constricted segments in the same artery (Fig. 6, bottom).

Dosage correlations. Comparison of the lowest with the highest doses of acetylcholine demonstrated concordant responses in 252 (73%) of 349 segments; either significant vasodilation was seen at both doses (Fig. 7, area VD_6/VD_4) or there was no change (center area) or vasoconstriction was seen at both doses (VC_6/VC_4). In no segment did acetylcholine at 10^{-6} mol/liter give rise to significant vasodilation, whereas 10^{-4} mol/liter caused vasoconstriction, or vice versa (discordant responses, areas VD_6/VC_4 and VD_4/VC_6). Of 349 segments, vasodilation at high dose in segments with no change at

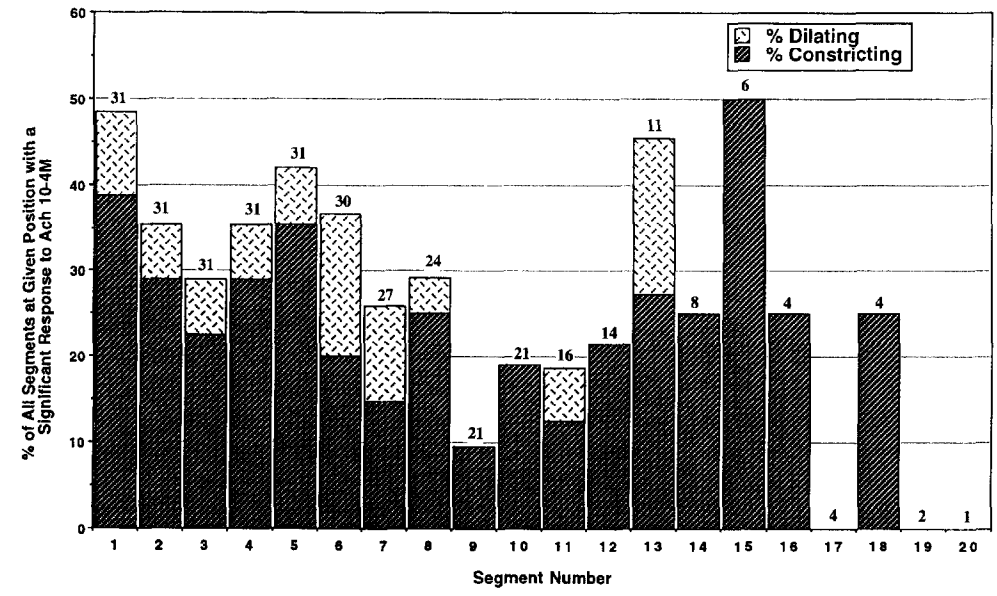


Figure 5. Percent of vessel segments at a given position along the coronary artery that exhibit significant dilation (**hatched bars**) or constriction (**shaded bars**) on infusion of acetylcholine (ACH) (10^{-4} mol/liter). **Segment 1** was closest to the tip of the infusion catheter, with larger segment numbers successively more distal. **Numbers above bars** denote total number of segments at each position. Artery lengths varied by patient. Vasomotion was not related to segment position.

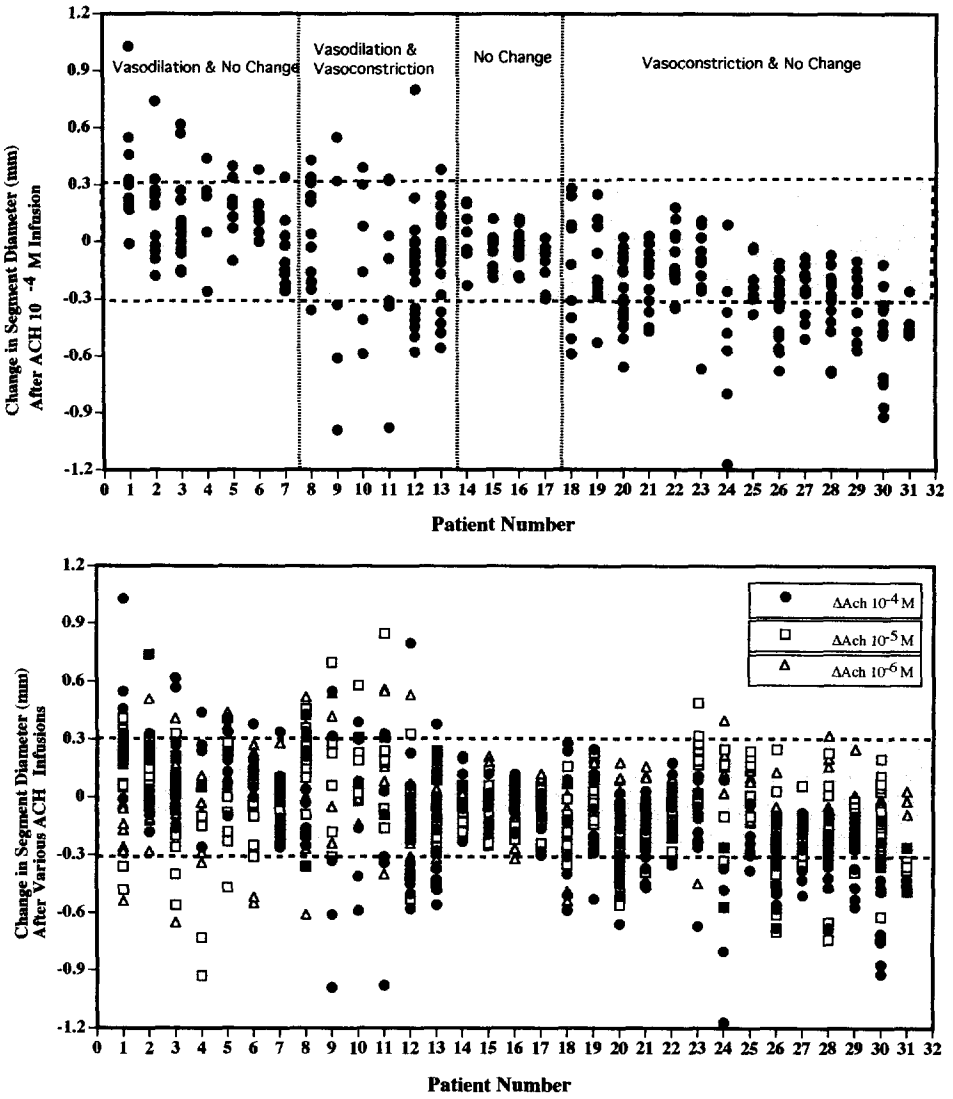


Figure 6. Top, Plot of absolute change (mm) from control diameter of vessel segments of each study patient on acetylcholine (ACH) 10^{-4} mol/liter infusion. Patients are grouped by categories of response. **Shaded area** represents control reproducibility limits. A heterogeneous response was seen in 27 of 31 patients, and all four homogeneous responses correspond to no change in any segment. **Bottom,** Plot of absolute change (mm) from control diameter of vessel segments of each study patient, with responses from acetylcholine 10^{-6}, 10^{-5} and 10^{-4} mol/liter infusions combined and superimposed. Patient numbers as in **top panel.** When all doses were combined, all but two patients exhibited a heterogeneous response to acetylcholine; dilation and significant constriction were seen in the same vessel in 14 (45%) of 31 patients.

low dose was seen in 17 segments (5%, area VD_4/NC_6), whereas high dose vasoconstriction with no change at low dose was noted in 59 segments (17%, area VC_4/NC_6). Vasodilation at low dose only, with no change at high dose, was seen in nine segments (2%, area VD_6/NC_4).

Discussion

Coronary artery dilation in response to acetylcholine implies the normal release of endothelium-derived relaxing factor (EDRF); a vasoconstrictor effect results when endothelium-derived relaxing factor action is impaired, and direct acetylcholine-induced constriction of vascular smooth muscle becomes manifest (1–14). In many studies (5,9,11,13,14), patients have been classified as demonstrating a constrictor or dilator effect of acetylcholine on the basis of a percent change in mean vessel diameter in a single segment of the vessel (2 mm to 2 cm in length) compared with that after a control coronary arteriographic injection; heterogeneity of response then reflects the variable and opposite effects seen in different patients. Previous studies (8,9,16) that correlated coronary artery disease risk factors to coronary endothelium-dependent vasomotion reported analysis of only a single, short area of the artery and excluded potentially disparate data from other arterial segments. Data presented here indicate that the response to acetylcholine is heterogeneous, with disparate responses often noted in different segments of the same vessel (Fig. 8). Vasomotor responses can occur at almost any point along the vessel and may vary with acetylcholine concentration. Thus, classification of patients as manifesting vasoconstriction or vasodilation is dependent on the amount of vessel analyzed, method of selection of segments, dose of acetylcholine delivered and degree of reproducibility in imaging and quantitating arterial dimensions.

Approach to quantitative coronary angiography. Our computer software quantitates the dimensions of multiple contiguous segments of a coronary artery or a large proportion of any given vessel (Fig. 1). A continuous diameter function, extend-

JACC Vol. 25, No. 5
April 1995:1046–55

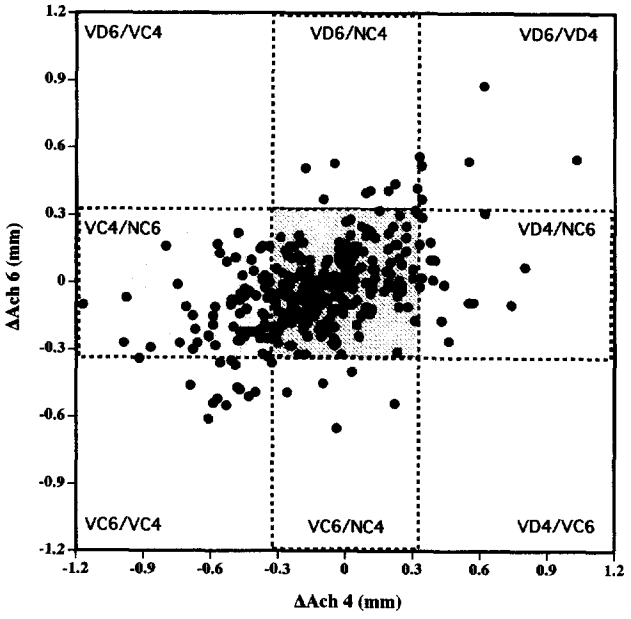
Table 1. Vascular Responses to Acetylcholine

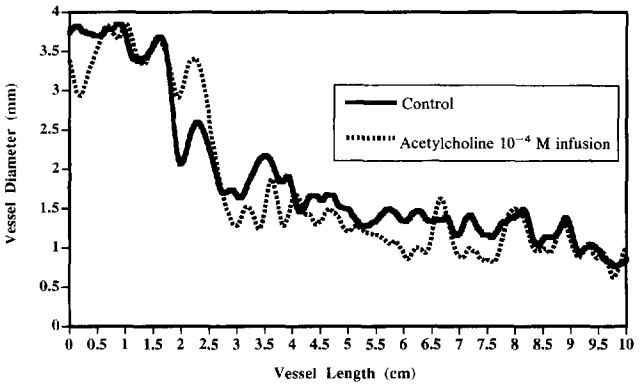
Response	Acetylcholine Dose (mol/liter)		
	10^{-6}	10^{-5}	10^{-4}
Heterogeneous			
Vasodilation and no change	5	5	7
Vasodilation and vasoconstriction	5	3	6
Vasoconstriction and no change	9	12	14
Total	19 (61%)	20 (65%)	27 (87%)
Homogeneous			
All vasodilation	0	0	0
All vasoconstriction	0	0	0
All no change	12	11	4
Total	12 (39%)	11 (35%)	4 (13%)

Data presented are number (%) of patients.

ing from a proximal beginning point to an end point (both operator determined), is obtained by splicing autocorrelated digital cine frames imaged at the same time in the cardiac cycle (usually end-diastole). To ensure that dimensional changes were analyzed in corresponding loci, an interpolation routine is applied that either contracts or expands local, successive measurements so that beginning, end and branch points correspond within the whole diameter function (Fig. 2). Because each linear interpolation is carried out between two successive diameter measurements, further smoothing of the final diameter function occurs, but the measured and calculated diameter values (noninterpolated vs. interpolated) perforce differ by less than the difference between two successive values. Thus, the algorithm changes the profile but not the accuracy of the overall diameter function. We believe that this approach ensures that differences in segment dimension brought about by pharmacologic challenge are calculated over the same and corresponding anatomic loci.

Figure 7. Change in segment dimension with 10^{-6} mol/liter of acetylcholine (ΔAch 6) versus 10^{-4} mol/liter (ΔAch 4). **Gray areas** represent reproducibility limits for detection of a significant change. Note that most responses do not represent a significant vasomotor change at both high and low dose infusions of acetylcholine. NC_4, NC_6 = no change with 10^{-4} and 10^{-6} mol/liter of acetylcholine; VC_4, VC_6 = vasoconstriction with 10^{-4} and 10^{-6} mol/liter of acetylcholine; VD_4, VD_6 = vasodilation with 10^{-4} and 10^{-6} mol/liter of acetylcholine.





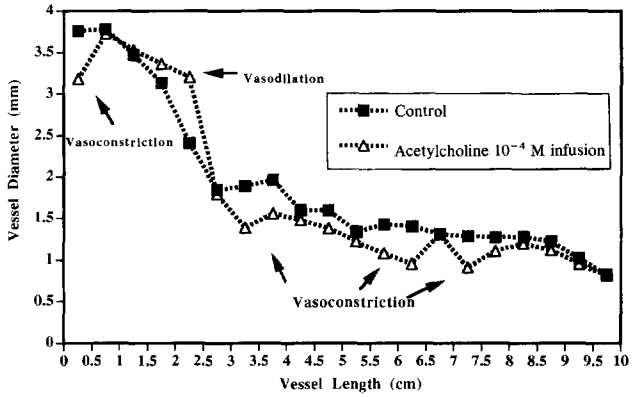


Figure 8. Top, Vessel diameter (mm) versus length (cm) functions (interpolated and smoothed) for control and acetylcholine (10^{-4} mol/liter) infusions. Functions are shown after interpolation and splicing but before 50-point bin averaging. **Bottom,** Vessel diameter (mm) versus length (cm) functions after 50-point bin averaging of curves in **top panel.** Constriction and dilation in the same artery are indicated.

Statistical analysis of dimensional data. Assessment of the effects of acetylcholine on coronary dimensions, compared with those in a control state, requires multiple, successive radiographic films exposed while the patient is held in a constant position. Simple calculation of the percent change of arterial dimension from baseline does not allow for change caused by the inherent limit of reproducibility of the quantitative angiographic method (19,20). Moreover, this variability may be different for an obstruction, as opposed to a reference, diameter. Reiber and Serruys (20) found a minimal threshold value for the occurrence of "vasomotor changes at the individual patient level (95% confidence interval)" to be 0.47 mm for

an obstruction but 0.35 mm for a reference diameter. This latter reproducibility limit is very close to the 95% confidence interval of 0.31 mm obtained from control patients in this investigation.

Methodologic variability might be overcome by comparing groups of measurements, where differences <0.31 mm may be statistically significant. However, grouping data is complicated because different arterial segments from the same patient or from different patients may respond independently, and this variability may be obscured in a group average. Thus, when single arterial segments are compared independently, we believe that the 95% confidence interval for detecting a change in diameter is ~0.31 mm; smaller changes cannot be detected using current techniques.

A potential limitation of our approach is the use of an absolute diameter change (±0.31 mm) as the definition of significant vasomotion. This means that in a segment averaging 3.0 mm in diameter, slightly more than a 10% change in diameter would be considered significant, whereas in a 1.0-mm vessel, a 31% change would be required. A given percent change in diameter thus could be classified as significant in one segment but as no change in a smaller segment. However, the absolute 95% confidence limit of ±0.31 mm represents an average reproducibility limit for vessels of all sizes; the use of this standard was necessary to distinguish physiologic heterogeneity of response from that engendered by variability in the method of detection itself (Fig. 6). Despite this, when the vasomotor effect of acetylcholine was expressed as a percent change from control vessel diameter, a heterogeneous pattern also was observed.

Acetylcholine responses: regional and dose-related considerations. Using the previously described methods and reproducibility limit, there was a heterogeneous pattern of response to acetylcholine: 27 of 31 patients showed a difference in categorization of segmental dimensional changes within the same vessel. Variable vasomotor responses could occur at almost any site along the vessel, and there was no consistent type of response in the more distal compared with the more proximal segments. The change in a given artery's dimensions was heterogeneous in response to the dose of acetylcholine administered. A heterogeneous response was seen in 29 of 31 patients when data from all three doses were included. In 45% of patients there was significant constriction of at least one segment and significant dilation of another segment in the same vessel. Although comparison of low and high dose acetylcholine showed no discordant responses (Fig. 7), many segments exhibited a significant change at one dose but no change at another. Segments that demonstrate no change at any dose of acetylcholine may be truly inert or may undergo a change too small to be detected by our method. Alternatively, segments demonstrating no change at low dose only may be understimulated by acetylcholine, and there may be an intermediate stage in which marginally dysfunctional endothelium is unable to mediate dilation but can still counterbalance the direct constrictive effect of acetylcholine on smooth muscle. Over a range of doses, this balance may be altered, with, for instance, a higher dose of acetylcholine stimulating more endothelium-derived relaxing factor release and producing dilation in place of no change where normal endothelium is present or, conversely, stimulating more direct smooth muscle effect that overcomes a limited endothelium-derived relaxing factor effect in regions of abnormal endothelium. A direct vasoconstrictor effect of acetylcholine that may override even normal endothelial function has been described at higher doses (10^{-3} mol/liter) than those used in this study (21). We suspect that this effect was not seen here because of the linear, incremental dose–response relation and the induction of significant vasoconstriction in only 25% of segments at the highest acetylcholine dose.

This dose-related and regional heterogeneity of response to acetylcholine underscores the importance of analyzing segments independently and distinguishing significant vasomotor responses from those resulting from inherent variability of the method. Ideally, the vessel should be partitioned into segments short enough so that focal diameter changes are not "averaged out" by the adjacent nonreactive vessel. As much of the vessel length as technically possible should be included for analysis, because significant changes may occur at any point along the artery, and the heterogeneous response to acetylcholine precludes assumption of the nature of the effect on any segment not specifically analyzed.

Previous studies. Horio et al. (2) described in patients with minimal diameter narrowing a variable response along the course of the coronary artery when exposed to intracoronary acetylcholine in doses of 30, 50 and 100 μg. Proximal, middle and distal portions of the coronary arteries were sampled at the site showing maximal vasoconstriction or vasodilation, and percent dimensional changes were assessed by electronic calipers. In those patients undergoing left anterior descending coronary artery analysis, the proximal and middle regions showed only constriction or no change with all doses of acetylcholine. Different responses among arterial segments in the same artery in the same patient were not reported, and intercontrol variability was not described. Similarly, Vita et al. (6) described marked change in some vessel regions and minimal change in others in response to acetylcholine, but they did not report marked constriction and dilation within the same vessel. They subsequently noted vasoconstriction at branch points with slight dilation in straight segments (13). Intercontrol variability was not reported in either study.

Yasue et al. (7) reported on the response to acetylcholine in 49 patients with a normal coronary angiogram and in 25 patients with signs of coronary atherosclerosis. In patients with disease, as well as patients of advanced age without coronary disease, vasoconstriction with acetylcholine was noted, particularly in proximal segments of the artery. However, none of their patients exhibited both vasoconstrictor and vasodilator responses in the same artery.

Recently, El-Tamimi et al. (22) described acetylcholine vasomotor responses, quantitated by a cinevideodensitometric technique, in 28 patients with coronary artery disease (one- to three-vessel disease, lesions >50% diameter stenosis). At least

six segments were analyzed in each patient and then averaged over proximal, middle and distal regions of the artery. Although a reproducibility limit was not explicitly defined, at least a ≥10% diameter change compared with control diameter was required to define segmental constriction or dilation. In the left anterior descending coronary branch, acetylcholine (10^{-4} mol/liter) produced vasoconstriction in all segments in 21 (75%) of 28 patients. In the remaining seven patients, the response was variable, with some segments vasoconstricting and some dilating in the same artery. In three of the seven patients the response was variable in the left anterior descending coronary artery, whereas in five patients undergoing study of the left circumflex coronary artery all proximal and middle segments constricted, and all five distal segments dilated with acetylcholine (10^{-4} mol/liter); individual responses to lower doses were not reported.

These previous studies support the variability of responses to acetylcholine between patients, between vessels and at selected sites along the same vessel, although these investigations have not assessed vasomotion at multiple, sequential sites along the vessel or based the analysis on a 95% confidence limit for reproducibility of control measurements. Our data fail to confirm proximal and midvessel constriction versus distal dilation as the major type of single-vessel heterogeneity (2,22).

Implications and conclusions. Previous experimental as well as clinical observations suggest that vasodilation in response to acetylcholine stimulation is mediated by endothelium-derived relaxing factor, a response that may be compromised by early endothelial involvement by atherosclerosis. In the setting of endothelial dysfunction, the direct muscarinic effect of acetylcholine becomes dominant, and vasoconstriction results. Therefore, a vasoconstrictor response may constitute an early marker of atherosclerosis not detectable by other means. Because of the nonuniform development of atherosclerosis along the course of a given artery, regional differences in endothelial dysfunction would most likely explain the heterogeneity in response noted in this investigation.

Hypothetically, the heterogeneous response in the coronary arteries of our patients may have been related to an inconstant density or distribution of the number and types of muscarinic receptors. However, this hypothesis is unproved because, to our knowledge, no studies exist that definitively quantitate the subtypes and density of muscarinic receptors, both transmurally and longitudinally, along the human coronary artery. Moreover, these receptors might vary with age, coronary risk factors or naturally among patients. Other locally vasoactive substances elaborated in the endothelium (e.g., endothelin, leukotriene, prostacyclin) also may have affected the direct influence of acetylcholine on coronary tone.

The demonstration in this patient group of a heterogeneous response to intracoronary acetylcholine confirms that this pharmacologic test of endothelial integrity is not consistent in all regions of a given coronary artery and may, in fact, be opposite in different segments of the same artery. Moreover, when a definition of segmental change based on methodologic variability is applied, most individual coronary segments do not exhibit a statistically significant change of dimension with acetylcholine challenge. Studies in which responses of a single segment (or even several segments) are examined may not provide results representative of the entire vessel. Inclusion of the entire analyzable region of a coronary artery and establishment and application of the limit of reproducibility of the quantitative angiographic system used are essential for optimal assessment of segmental coronary vasomotor response.

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References

1. Ludmer PL, Selwyn AP, Shook TI, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986;315:1046–51.
2. Horio Y, Yasue H, Rokutanda M, et al. Effects of intracoronary injection of acetylcholine on coronary arterial diameter. Am J Cardiol 1986;57:984–9.
3. Gordon JB, Ganz P, Nabel EG, et al. Atherosclerosis and endothelial function influence the coronary vasomotor response to exercise. J Clin Invest 1989;83:2046–52.
4. Werns SW, Walton JA, Hsia HH, Nabel EG, Sanz ML, Pitt B. Evidence of endothelial dysfunction in angiographically normal coronary arteries of patients with coronary artery disease. Circulation 1989;79:287–91.
5. Hodgson JMcB, Marshall JJ. Direct vasoconstriction and endothelium-dependent vasodilation. Mechanisms of acetylcholine effects on coronary flow and arterial diameter in patients with nonstenotic coronary arteries. Circulation 1989;79:1043–51.
6. Vita JA, Treasure CB, Nabel EG, et al. The coronary vasomotor response to acetylcholine related to risk factors for coronary artery disease. Circulation 1990;81:491–7.
7. Yasue H, Matsuyama K, Okumura K, Morikami Y, Ogawa H. Responses of angiographically normal human coronary arteries to intracoronary injection of acetylcholine by age and segment: possible role of early coronary atherosclerosis. Circulation 1990;81:481–90.
8. Brush JE Jr, Faxon DP, Salmon S, Jacobs AK, Ryan TJ. Abnormal endothelium-dependent vasomotion in hypertensive patients. J Am Coll Cardiol 1992;19:809–15.
9. Treasure CB, Manoukian SV, Klein JL, et al. Epicardial coronary artery responses to acetylcholine are impaired in hypertensive patients. Circ Res 1992;71:776–81.
10. Vita JA, Treasure CB, Yeung AC, et al. Patients with evidence of coronary endothelial dysfunction as assessed by acetylcholine infusion demonstrate marked increase in sensitivity to constrictor effects of catecholamines. Circulation 1992;85:1390–7.
11. Zeiher AM, Drexler H, Wollschlaeger H, Saurbier B, Just H. Coronary vasomotion in response to sympathetic stimulation in humans: importance of the functional integrity of the endothelium. J Am Coll Cardiol 1989;14:1191–200.
12. Hodgson JMcB, Ravi N, Sheehan HM, Reddy KG. Endothelial dysfunction in coronary arteries precedes ultrasonic or angiographic evidence of atherosclerosis in patients with risk factors [abstract]. J Am Coll Cardiol 1992;20 Suppl A:323A.
13. McLenachan JM, Vita J, Fish RD, et al. Early evidence of endothelial vasodilator dysfunction at coronary branch points. Circulation 1990;82: 1179–83.
14. Zeiher AM, Drexler H, Wollschlager H, Just H. Modulation of coronary vasomotor tone in humans: progressive endothelial dysfunction with different early stages of coronary atherosclerosis. Circulation 1991;83:391–401.
15. Penny W, Rockman H, Bhargava V, et al. Vasomotor response heterogeneity along the human coronary artery [abstract]. Circulation 1993;88: Suppl I:I-449.

16. Dijkstra EW. A note on two problems in connection with graphs. Numer Math 1959;1:269–72.
17. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;307–10.
18. British Standards Institution. Precision of test methods I: guide for the determination and reproducibility for a standard test method (BS 5497, part 1). London: BSI, 1979.
19. Reiber JHC, Serruys PW, Kooijman CJ, et al. Assessment of short-, medium-, and long-term variations in arterial dimensions from computer-assisted quantitation of coronary cineangiograms. Circulation 1985;71:280–8.
20. Reiber JHC, Serruys PW. Quantitative coronary angiography. In: Marcus ML, Schelbert HR, Skorton DJ, Wolf GL, editors. Cardiac Imaging. Philadelphia: Saunders, 1991:242.
21. Newman CM, Maseri A, Hackett DR, El-Tamimi HM, Davies G. Response of angiographically normal and atherosclerotic left anterior descending coronary arteries to acetylcholine. Am J Cardiol 1990;66:1070–6.
22. El-Tamimi H, Mansour M, Wargovich TJ, et al. Constrictor and dilator responses to intracoronary acetylcholine in adjacent segments of the same coronary artery in patients with coronary artery disease: endothelial function revisited. Circulation 1994;89:45–51.