Quantitation of absolute area of a coronary arterial stenosis: experimental validation with a preparation in vivo

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ABSTRACT The absolute cross-sectional area of a coronary stenosis measured by quantitative coronary angiography correlates well with its hemodynamic significance. We evaluated a combined approach using edge detection applied to the normal segment and videodensitometry applied to the stenosis to determine the absolute cross-sectional area of the stenosis (videodensity method). The results were then compared with those with the edge detection method applied directly to the stenosis. The area of the stenosis by the edge detection method was calculated by analyzing two orthogonal projections for irregular stenoses and with use of the formula for the area of an ellipse (ellipse method). The accuracy of both these techniques was assessed by analyzing digital angiograms acquired from closed-chest dogs in which 10 plastic cylinders with precisely machined circular and irregular lumina were inserted into the coronary arteries. Angiograms of irregular stenoses were acquired in two orthogonal views. The ellipse method applied to circular stenoses was very accurate, with \( r = .97 \), average absolute difference (AAD) = 0.21 mm\(^2\), and SEE = 0.30. For the videodensity method \( r = .97 \), AAD = 0.84 mm\(^2\), and SEE = 0.40. Irregular stenoses were better quantitated by the videodensity method applied in one view (AAD = 0.50 mm\(^2\), SEE = 0.47) than by the ellipse method applied in two orthogonal projections (AAD = 1.03 mm\(^2\), SEE = 0.87). Overall, the two methods were comparable in accuracy (for videodensity, AAD = 0.65 mm\(^2\), SEE = 0.71 vs AAD = 0.54 mm\(^2\), SEE = 0.79 for ellipse). Although the ellipse method is more accurate for circular stenoses, the videodensity method has the advantage of quantitating the full range of shapes of stenosis in only one projection.

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ANGIOGRAPHIC ASSESSMENT of the percent narrowing of a coronary arterial stenosis has been traditionally accepted as the standard for the evaluation of coronary disease.\(^1\) Yet, studies have shown that the physiologic significance of a coronary arterial stenosis correlates poorly with the percent stenosis.\(^2-5\) Recently, Harrison et al.\(^6\) showed that the absolute cross-sectional area of a coronary stenosis is a better determinant of the physiologic significance of an obstruction.\(^6\)

A number of different methods have been developed to determine the absolute cross-sectional area of a coronary stenosis seen on the angiogram. Some investigators manually traced the edges of coronary arteries from projected images of angiograms.\(^7,8\) Others have digitized angiograms and used the computer to determine the vessel edges.\(^9,10\) Since most lesions are eccentric,\(^11\) Brown et al.\(^7\) suggested that the diameter of a stenosis be determined for each of two orthogonal views and the area calculated by assuming that the lesion is an ellipse. However, some investigators believe that even two views may be inadequate and they advocate the use of as many angiographic projections as possible to assess the severity of a stenosis.\(^12,13\) In contrast, videodensitometry has been shown to accurately determine the relative stenosis for irregular lesions with the use of only one projection.\(^12,14\)

In the present study we evaluated a new method combining computerized edge detection and videodensitometry for quantitating absolute cross-sectional area with the use of only one projection. The accuracy of this method and the two-view edge detection method

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as applied to the same stenoses was determined with digitized angiograms of a preparation in vivo with precisely measured stenoses.

Methods

Animal experiments. Ten mongrel dogs (weight 25 to 35 kg) were sedated with 30 mg/kg of pentobarbital given intravenously. An endotracheal tube was placed with the cuff inflated and left open to room air. A cutdown of the femoral artery was performed and a left coronary catheter was used to visualize the left coronary artery. The dogs were systemically anticoagulated with heparin.

Radiolucent Delrin cylinders were placed within the circumflex or left anterior descending arteries over a guidewire by a technique described by Gewirtz and Most. The plugs were drilled with circular, elliptical, or irregular lumina (figure 1). The circular lumina ranged in diameter from 0.8 to 2.0 mm. The external diameter of all the cylinders ranged from 2.4 to 3.2 mm, with the length ranging from 3.0 to 5.2 mm. The diameters of the circular lumina were measured directly with a machinist’s micrometer and the area was calculated with the formula for a circle. The area of the irregular lumina was calculated by planimetry of a magnified photograph of the cross section of the cylinder.

Arteriography was performed with the use of either a Judkins’ catheter (No. 5.0F or 5.7F) or a Sones’ catheter (No. 7F). The tip of each type of catheter was measured with a micrometer. Angiograms were acquired after selective intracoronary injections of 2 to 4 ml of diatrizoate meglumine (Hypaque-76) (figure 2, A). Respiration was transiently interrupted by clamping the endotracheal tube for less than 30 sec. Up to 10 different projections, including cranial and caudal angulation, were acquired for the cylinders with the irregular lumina. From among these angiograms, two orthogonal projections that showed the least foreshortening of the stenosis were chosen for analysis (figure 2, B and C). After the angiographic examination, each dog was killed with an intravenous euthanasia solution (T-61 Euthanasia Solution, American Hoechst) and the heart was examined. The location and patency of each cylinder was documented at autopsy. Animal experiments adhered to the guidelines of the American Physiological Society.

Phantom model. A half-inch thick nylon block was drilled with cylindrical holes ranging in diameter from 0.8 to 6.4 mm. The diameter of each hole was measured by use of a machinist’s micrometer and the holes were filled with undiluted contrast material. The phantom was then imaged at 75 kV, 400 mA, pulsed at 6.3 msec over a 0.8 mm thick copper plate to provide background attenuation. These x-ray settings were the same as those used for the animal studies. Digital images were acquired on a 512 × 512 matrix that was digitally magnified two times, by bilinear interpolation, before analysis. The diameter of each column of dye was calculated by placing a region of interest 2 pixels wide perpendicular to the column extending well beyond its edges. An edge detection algorithm that used three-point smoothing and a weighted average of the first and second derivatives was used to locate the edges of the column of dye. The number of pixels within the edges was determined and compared with the directly measured diameter of the phantom. The videodensity of each column of dye was calculated by placing a

FIGURE 1. Cross-sectional shape of lumina drilled into the Delrin cylinders. A, Circular; B, elliptical; C, irregular.

FIGURE 2. A, Digital angiogram of cylinder with circular lumen in the mid left anterior descending artery of a dog. Lead block, visible on left of field, was used to correct for scatter and veiling glare. B, Digital angiogram of cylinder with elliptical lumen in the proximal left anterior descending artery seen in the left anterior oblique projection. The artery appears to have a tight stenosis. C, Same cylinder as in B seen in the right anterior oblique projection. There is no narrowing of the vessel diameter seen. Only a decrease in the brightness of the column of contrast is noted at the location of the stenosis.
A rectangular region of interest 20 pixels wide perpendicular to the column and extending beyond the edges of the column of dye. Two rectangular regions of interest also 20 pixels wide were placed on either side of the column and used to determine average background density. Background-corrected videodensity was compared with the cross-sectional area of the hole determined by micrometer.

**Arteriographic equipment.** A General Electric MSI 1250 IV x-ray system with a FL300V generator with servo LADII and 0.6 focal spot was used. Images were enhanced by a TH Triple Field MX 100 image intensifier. All angiograms were acquired with the 6 inch field on the image intensifier. Angiograms were obtained at 8 frames/sec, with a pulse width of 6.3 msec at 400 mA. The kilovolt range was adjusted from 70 to 85 kV to enhance image quality. A commercially available digital imaging system (Fischer Imaging) was used to acquire images directly from the image intensifier. Each image was digitized with a 512 × 512 matrix with 256 gray levels and stored in an unsubtracted format directly on disk. A lead block was placed within the field for the initial animal studies to determine x-ray scatter and veiling glare of the image intensifier. The pixel density for the studies was determined with the 6 inch field of the image intensifier placed 20 cm above a calibration grid. The resulting pixel density for the unmagnified images was 20 pixels/mm² (horizontal density 3.9 pixels/mm, vertical density 5.2 pixels/mm).

**Method of quantification of stenosis.** All studies were acquired and analyzed with the digital imaging system and an operator-interactive program for videodensitometry and edge detection. One frame from each run was selected based on adequate opacification of the stenosis and an adjacent normal segment with the least overlap of ribs or other vessels. The 6 inch field size for the image intensifier was used for all studies. This resulted in less than a 1% error in measurement of length due to pincushion distortion at the periphery of the field. Therefore, no correction for pincushion distortion was needed. The catheter was used as a measurement standard for determining the diameter of the normal and stenotic segments. This can cause an error due to magnification if the artery and the catheter are positioned at different distances from the source of the x-ray beam. The expected error is about 1.5% for each centimeter difference. This would result in a negligible error in our preparation and therefore was not considered in the calculations.

The diameters of the contrast-filled catheter, the coronary arteries, and the stenoses were calculated with a weighted first- and second-derivative edge detection technique. The regions of interest on the angiogram were magnified twofold on the digital monitor by bilinear interpolation. This resulted in a pixel density of 80 pixels/mm². A rectangular region of interest 2 pixels wide was placed perpendicular to the long axis of the catheter or artery so that it extended beyond the edges of the column of dye (figure 3). The videodensity of each pixel was calculated by computing the logarithm of the brightness value of each pixel. The location of the maximum of the first and second derivatives was determined for both edges after applying a three-point smoothing algorithm to the videodensity values in the region of interest. A weighted average of the two derivatives was used to locate the vessel or catheter edge and this yielded the number of pixels for the diameter of the stenosis, artery, or catheter. The actual diameters of the artery and the stenosis were calculated with use of the known diameter of the catheter as a standard. Each measurement was obtained twice by repositioning the region of interest in an area adjacent to the initial measurement, thereby reducing possible errors due to background variation and quantum noise inherent in the system. The average absolute error of repeat measurements at two adjacent locations on the artery, stenosis, and catheter was 0.9 pixel.

**Videodensitometry** was used to calculate the relative area narrowing of the stenotic segment as a percent of the area of the normal segment. A method similar to the one developed by Nichols et al. was adapted to the Fischer imaging system. Rectangular regions of interest were placed across the normal segment and the stenosis as outlined above. The videodensity of each region was calculated by summing the videodensity values for each pixel within the region of interest. For each region of interest, a 2 × 2 pixel square was positioned outside of the artery on each side of the rectangular region of interest. This was used to calculate the average background videodensity per pixel, which was then subtracted from the videodensity of each pixel in the rectangular regions. Each set of measurements for videodensity was repeated at least three times while repositioning the regions of interest. Studies in our laboratory have shown low interoperator and intraoperator variability for quantitating percent area stenosis by videodensitometry. In this preparation in vivo, the interobserver and intraobserver variabilities, as reflected by the SEE, are 3.8% and 6.6%, respectively.

The background-corrected videodensitivities were then used to calculate the percent area stenosis as follows:

\[
\text{Percent stenosis} = 100 \times \left(1 - \frac{V_s}{V_n}\right)
\]

where \(V_s\) = total background-corrected videodensity of stenosis; \(V_n\) = total background-corrected videodensity of normal segment.

Lead blocks were placed within the field for the initial runs to determine the videodensity due to scatter and veiling glare of the image intensifier. There was no contribution to videodensity from these factors on any run. Therefore, the Lambert-Beer law was assumed to apply to the output of the image intensifier and the above calculations are valid.

The catheter was used as a measurement standard for each run. This allowed calculation of the diameters of the artery and stenosis for that particular run.

The minimal cross-sectional area of the stenosis was calculated in two ways. The first method, introduced by Brown et al., uses edge detection to calculate the diameter of the stenosis in two orthogonal projections. The area of the stenosis is then calculated, assuming the stenosis is elliptical, as follows (ellipse method):

\[
\text{Stenotic area} = \pi / 4 \times D_{11} \times D_{12}
\]

**FIGURE 3. Magnified angiogram showing rectangular regions of interest placed across the catheter, stenosis, and adjacent normal segment.** (Image contrast was enhanced for improved photographic effect. Saturation of image did not occur in the regions of interest on any run.)
where $D_1$ and $D_2$ are the diameters of the stenosis measured in each of the two projections. With circular stenoses this method is equivalent to the measurement of the diameter of the stenosis in only one projection and the calculation of the area with the formula for the area of a circle. Therefore, only one projection was analyzed when the circular stenoses were quantitated in the animal study.

The second method uses a combination of edge detection and videodensitometry for angiograms taken in one projection. The diameter of a normal segment adjacent to the stenosis is calculated by the edge detection method described above. The cross-sectional area of this segment is calculated with the formula for a circle. Videodensitometry is then used to calculate the percent area stenosis compared with the area of the normal segment adjacent to the lesion. The minimal cross-sectional area can be calculated as follows (videodensitometry method):

$$\text{Normal area } (A_n) = \left(\frac{\pi}{4}\right) \times D_n \times D_n$$

$$\text{Stenotic area } = A_n \times (1 - S/100)$$

where $D_n$ is the diameter of the normal segment, $A_n$ is the area of the normal segment, and $S$ is the percent area stenosis of the lesion compared with the area of the normal segment as determined by videodensitometry.

**Statistical analysis.** Correlations were calculated as the Pearson correlation coefficient in both phantom and animal studies. Regression analysis was used to determine the regression equations and the SEE for results with both methods compared with the actual stenotic areas measured in the animal studies. The average of the absolute difference (AAD) between the calculated and the actual stenotic areas for each method was computed and used as a measure of accuracy.

**Results**

**Phantom study.** Results of application of the combined first- and second-derivative edge detection algorithm in the phantom model showed a very high correlation with the measured diameters ($r = .99$; figure 4). No stenosis smaller than 0.8 mm in diameter was analyzed in this study so that no correction of the edge detection method for smaller stenoses was necessary. The background-corrected videodensity values for the phantom also showed a very high correlation to the measured cross-sectional areas of the columns of dye ($r = .99$; figure 4).

**Animal study.** Ten dogs had 17 cylinders placed in their coronary arteries. Ten cylinders were selected for analysis based on adequate visualization of the stenosis, a normal segment, and the catheter and, in the case of the cylinders with irregular lumina, the acquisition of two orthogonal views. Two cylinders were not visualized despite the fact that images were obtained in multiple projections. One of the cylinders was obscured by large overlying vessels and the other was found at a distal branch of the artery and was not visualized due to poor filling of the distal vessel with contrast. Three stenoses were excluded from analysis because of dye streaming around the cylinder that made area analysis by either method impossible. Two other cylinders were excluded due to small overlying branches that were present on all views. Thus, 10 cylinders were analyzed, including six with circular lumina, three with elliptical lumina, and one with an irregular lumen.

Actual stenotic cross-sectional area was plotted against the cross-sectional area calculated by the ellipse method and the videodensity method (figure 6). There were 14 data points for the videodensity method compared with 10 points for the ellipse method. The additional four points by the videodensity method resulted from the quantitation of four irregular stenoses independently in each orthogonal projection for a total of eight data points. The ellipse method requires both orthogonal views to be used to calculate the stenotic area, resulting in four data points for the irregular stenoses. The correlation coefficient for the ellipse method was $r = .70$ compared with $r = .76$ for the

![FIGURE 4. Edge detection algorithm applied to the phantom model. The number of pixels determined by the edge detection algorithm is compared with the actual diameter of the phantom in millimeters. There was an excellent correlation between results with this method and measured diameter, with $r = .99$.](image)

![FIGURE 5. Cross-sectional area of phantom in square millimeters compared with the normalized background-corrected videodensity values. The solid line is the best fit to the data determined by linear regression.](image)
videodensity method. The accuracy of both these methods was determined by the calculation of the AAD and the SEE. Overall, the AAD for the ellipse method was slightly less than that for the videodensity method (ellipse, 0.54; videodensity, 0.65) while the SEE was lower for the videodensity method (ellipse, 0.79; videodensity, 0.71). The regression equations were $y = 0.67x + 0.64$ for the ellipse method and $y = 0.79x + 0.81$ for the videodensity method.

For circular stenoses the ellipse method was very accurate, with a high correlation coefficient of $r = .97$ (AAD, 0.21; SEE, 0.30; regression equation, $y = 0.97x + 0.05$) (figure 7, A). The videodensity method was not as accurate as the ellipse method for quantitating circular stenoses (AAD, 0.84; SEE, 0.40; regression equation $y = 1.3x + 0.44$), although the correlation coefficient was high ($r = 0.97$) (figure 7, B). However, as shown in table 1, quantitation of irregular stenoses by the ellipse method resulted in AAD = 1.03 and SEE = 0.87, with a regression equation of $y = 0.25x + 2.7$. The videodensity method yielded AAD = 0.50, SEE = 0.47, and $y = 0.35x + 1.4$ when applied to irregular stenoses (table 1). Two values are listed for each of the irregular cylinders under the heading “Videodensity” in table 1. Each value results from one of the two orthogonal views quantitated independently for each cylinder.

**Discussion**

Experimental studies in animals have shown that more than a 45% diameter narrowing of a coronary artery limits maximal flow response to a hyperemic stimulus.\(^{17}\) However, human coronary stenoses have been considered significant by some investigators if they exceed 50% diameter narrowing, while others require at least a 70% diameter narrowing.\(^{18-20}\) This is further complicated by recent studies, using a Doppler probe or contrast medium appearance time, which have shown that many lesions producing 20% to 60% diameter narrowing result in an abnormal response to hyperemia.\(^{4, 6}\)
The discrepancy between clinical and experimental studies may be due to the presence, in some patients, of diffuse disease throughout the coronary arteries, including the segments that appear normal. This results in relatively low measured percent stenosis since a severely stenotic area is being compared to a moderately stenosed segment that appears normal. Therefore, it was not surprising when Harrison et al.,6 found that the minimal cross-sectional area of a coronary stenosis was a better predictor of the hyperemic response than the percent stenosis. Harrison and others have quantitated stenotic areas using the method of Brown et al., although its accuracy has not been determined in a preparation in vivo. Therefore, this study was designed to evaluate the accuracy of two techniques of quantitation of cross-sectional area stenosis: edge detection as represented by the method of Brown et al. (ellipse method) and a new method combining edge detection and videodensitometry (videodensity method).7

Application of the ellipse method involves certain assumptions.7 The two orthogonal views acquired on the angiogram are assumed to be parallel to the major and minor axes of the elliptical stenosis. The use of two different views also assumes that the stenoses are analyzed at the same point in the cardiac cycle since the shape of the artery may change with cardiac contractions. In addition, since the location of the most severe narrowing is used as the major and minor diameters of the ellipse, this assumes that the most severe narrowing in each view occurs at the same point on the artery. The preparation selected in the present study addresses these issues. For the known circular stenoses, there was no need to obtain images in more than one projection. Angiograms of the elliptical stenoses were acquired in as many as 10 different projections in an attempt to find the true major and minor diameters. Use of a stiff plastic cylinder that does not alter its shape with the cardiac cycle obviates the need to control for the phase of the cardiac cycle. Since the location of the stenosis was clear in both orthogonal views there was no concern about locating the most severe narrowing. Thus, the assumptions implicit in quantitation of stenoses from human angiograms by the ellipse method are more easily resolved with use of this preparation in vivo and the results of this study are probably comparable to those with human angiograms obtained under ideal conditions.

**Shape of stenoses**

*Circular stenoses.* The accuracy of the ellipse method when applied to the circular stenoses in vivo is very high (AAD, 0.21 mm²). The highest accuracy reported by Spears et al.,9 for different edge detection methods applied to digitized film of cylindrical phantoms with an inhomogeneous background was ±0.103 mm for the determination of diameter. In our study, the ellipse method applied to circular stenoses had an accuracy of ±0.069 mm for the determination of diameter. Thus, there is no loss of accuracy for quantitation of stenosis produced by circular lesions in vivo when compared with quantitation of an appropriate phantom. Our study therefore provides evidence to support the use of edge detection methods in vivo for circular stenoses.

The videodensity method, on the other hand, is less accurate for circular stenoses (AAD, 0.84 mm²). This probably results from the edge detection error added to the videodensity error.

*Irregular stenoses.* Three of the four irregular stenoses in this study were elliptical, theoretically allowing for accurate quantitation by the method of Brown et al. However, it is clear that in spite of attempts to correct for geometric irregularities by the use of two orthogonal projections, the edge detection methods cannot be applied to irregular stenoses with confidence (AAD in this study 1.03 mm²). Videodensity does not rely on any assumptions concerning the shape of the stenosis12,14 and its accuracy is not reduced when irregular lesions are being examined. In our study, use of the videodensity method resulted in more accurate quantitation of stenotic area (AAD, 0.50 mm²). This is even more significant when one considers that this accuracy can be achieved with the use of only one projection.

**Overall accuracy of quantitation of stenotic area.** One conclusion that can be drawn from this study is that neither method we tested provides accuracy comparable to that reported in cylindrical phantom studies (errors of about 0.1 mm).9, 21 The average absolute error
with the videodensity and ellipse methods is 0.65 and 0.54 mm², respectively. However, this is well within the range of error that was achieved by use of an edge detection method on digitized angiograms by Reiber et al.²¹ In that study, the short-term variability for quantitation of stenosis diameter showed a mean and SD of 1.66 ± 0.34 mm. When converted to absolute area measurements this gives a 1 SD range of 1.37 to 3.14 mm². Thus, the relatively low accuracy of the ellipse method for quantitation of stenoses in an animal preparation is not unexpected since the reproducibility of the edge detection method in man is relatively poor. This can be explained by the common occurrence of irregular lesions in human coronary arteries. Vlodaver and Edwards¹¹ found 29% of coronary stenoses in autopsies specimens to be slitlike and an additional 40% to be eccentric and polymorphous. Therefore, the number of arteries with irregular lesions encountered on human angiograms may have been more than the four of 10 in this study.

The errors encountered in this study and in the study by Reiber et al.²¹ appear to be a reflection of the current limits of quantitation of stenotic area from coronary angiograms. When applied to an individual stenosis there can be considerable inaccuracy in the calculated area and this may have clinical implications. The clinician needs to be aware of these limitations when applying quantitative methods to coronary angiograms.

**Methodologic considerations.** The videodensity method used in this study has been shown to be accurate and reproducible¹⁴ and our experience confirms these findings. The SEE for the accuracy of this method for quantitation of relative stenosis is less than 10% for stenoses in the range of 20% to 70% diameter narrowing.

The digital imaging system used in this study is a commercially available system that provides a pixel density of 20 pixels/mm² for the acquired images. This value is lower than the pixel density that can be achieved by use of film and optical magnification before digitization. However, the increased pixel density does not appear to improve quantitation since the results of the phantom study correlated highly with those from digitized film.⁹,¹⁴,²¹ In addition, the ellipse method, when applied to circular stenoses in which geometry of the lesion is not a factor, had an accuracy that appeared to be at least as good as studies using digitized film.²¹ The digital imaging system used in this study has the advantage of eliminating the need for film. Since images are acquired directly from the image intensifier there is one less step in which the signal could be degraded. In addition, there is no need to calibrate the brightness scale of each angiogram before videodensitometric analysis since the output of the digital system is relatively stable.

The method described in this study uses an operator-interactive program for the edge detection and videodensity methods. The videodensity method has been shown to be highly reproducible between different operators in our laboratory, with an SEE of less than 7%. The edge detection algorithm used in this study included a repeat analysis in which different regions of interest were used for each run. There was very close agreement between results of repeat analyses with use of the edge detection algorithm, with an average absolute error of 0.9 pixel.

In this study we used a weighted average of the first and second derivatives of the videodensity level to localize the edges of the catheter, the artery, and the stenosis. This method has been used by Reiber et al.²¹ and appears to be the best method for avoiding systematic errors in edge detection. The phantom study confirmed the linearity of this edge detection method using our digital imaging system for cylinders that ranged in size from 0.8 to 6 mm. The sizes of the stenoses, arteries, and catheters in the animal study fall within this linear range. We used cylinders the smallest luminal diameters of which were 0.8 mm, which resulted in an area of about 0.5 mm². In some patients, stenoses may be even more severe and the accuracy of the techniques used in this study have not been assessed under such conditions.

One assumption implicit in the videodensity method is that the normal segment is circular. The normal segment does not have to be free of disease, but it does have to provide a circular lumen. It is probably reasonable to assume that most segments that appear normal on angiograms will have a lumen approximating a circle. Even in segments with significant diffuse disease, the areas with less stenosis are more likely to be circular, although this has not been systematically studied. A normal segment adjacent to the stenosis is needed since the assumptions of this method require that the same concentration of dye be present in the normal and the stenotic segment to provide accurate videodensitometric quantitation.

In summary, we have used an animal preparation to assess the accuracy of two methods of quantitation of stenotic cross-sectional area: the method of Brown et al. in which it is assumed that the stenosis is elliptical, and a new method in which a combination of edge detection and videodensitometry is used. We found that the ellipse method is very accurate for circular stenoses. The videodensity method is not as accurate.
as the ellipse method for quantitation of circular stenoses, but has the advantages of improved accuracy for irregular lesions and requirement of only one angiographic projection. Thus, for quantitation of the full range of stenoses this method may have wider clinical applicability.

References
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