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The Journal of Vascular Technology 26(2):000–000, 2002

Ultrasonographic Volumetry of Atherosclerotic Plaques

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ABSTRACT *Background.*—Clinical investigation of atherosclerotic plaque regression depends on imaging quantification to assess results. Conventionally used, arteriography is limited to lumen-narrowing measurements, has errors induced by projection angle, contrast density and timing, is expensive, and risks morbidity. Ultrasonographic (US) imaging with computer-assisted three-dimensional processing yields direct measurement of plaque volume, is low cost, and is risk free. We investigated US volumetry methods for accuracy and reproducibility.

Methods.—Volumes of 12 carotid endarterectomy specimens measured by three US techniques (US I, II, and III) were compared with liquid displacement (LDV) serving as control. US images were made in a fluid-filled, tissue-mimicking phantom. US-I and II obtained cross-sectional images at 0.635-mm intervals with a linear stepper. Summation of plaque cross-sectional area times 0.635 mm distance provided volume. In US-I, plaque boundaries traced by the operator in each image identified plaque area. US-II used adjustment of low-pass and high-pass filter thresholds of US echo amplitudes to identify plaque. US-III used freehand scanning with a position-sensing device to create a three-dimensional data set. Plaque boundaries were based on geometry and US echo thresholding. Volume was calculated automatically.

Results.—Plaque control volumes ranged from 233–1050 mm^3 (average, 465 mm^3). Differences between LDV control and US-I, II, and III measurements averaged −2.3% ± 5.6 (SD), −1.2% ± 3.0, and +2.7% ± 3.5, ranging from −10%–9%, 7%–3%, and −4%–10%, respectively. These differences were not statistically significant by t test (p = 0.85, 0.92, and 0.92, respectively). Linear regression between US-I, II, and III and control volume displacement resulted in correlation coefficients >0.99.

Conclusions.—In vitro measurement of atherosclerotic plaque volume with ultrasonography by three methods had an average difference from controls less than 3%, suggesting clinical usefulness. Additional studies of plaque volume by US techniques in vivo may demonstrate effectiveness of ultrasonography volumetry for serial evaluation of arterial occlusive disease.

Introduction

The health risk caused by atherosclerosis resulting in cardiac, cerebrovascular, peripheral arterial, and other diseases is well documented. Investigation of novel treatment modalities includes surgical, pharmacologic, and risk modification strategies. Most patients receive nonsurgical treatment, and investigation on methods of plaque regression is expanding.[1–4] Results are being verified by imaging quantification. Conventionally, this has been done with two-dimensional arteriography to measure lumen narrowing. This technique has errors induced by projection angle, contrast density, and timing. It is expensive and risks morbidity. Arteriography also provides false information, because the lumen enlarges with expanding atherosclerotic plaque volume.[5,6] Therefore, nonluminographic techniques that image the atherosclerotic plaque directly are desirable.

Computed tomography (CT) and magnetic resonance imaging (MRI) with three-dimensional volume rendering have been used to assess atherosclerotic disease.[7–10] These techniques, however, are still in evolution, and high-quality protocols are rare. Foremost, usefulness is hindered by their expense and lack of portability. Therefore, ultrasonography remains a viable, practical, and efficient method to evaluate peripheral and carotid atherosclerotic plaques.

Recent developments in ultrasonographic (US) technology enable noninvasive measurement of plaque volume and plaque characteristics. Volumetry is a simple, direct approach for quantifying progression or regression of the atherosclerotic plaque. Before US methods can be reliably applied in vivo, their accuracy and reproducibility must be determined in vitro against an acceptable standard for volume measurement. We studied carotid plaques in vitro and compared volume measurements obtained using three US methods with data obtained with liquid displacement volumetry.

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Methods

Liquid displacement and three US methods were used to measure the volume of 12 atherosclerotic plaques in vitro.

Atherosclerotic Plaques

Carotid endarterectomy specimens were provided by the Department of Pathology, The Toledo Hospital, Toledo, Ohio. The institutional review board approved the protocol for US data collection in vitro. The plaques were preserved in a 10% buffered formaldehyde solution. Twelve plaques were selected for volumetric measurements on the basis of size and appearance (Figure 1). F1

Liquid Displacement Control

A 10-ml cylindrical tube, graduated in 1-ml marks, was filled with 5 ml of formaldehyde solution. The plaque was immersed in the liquid. Using a 1-ml sy-

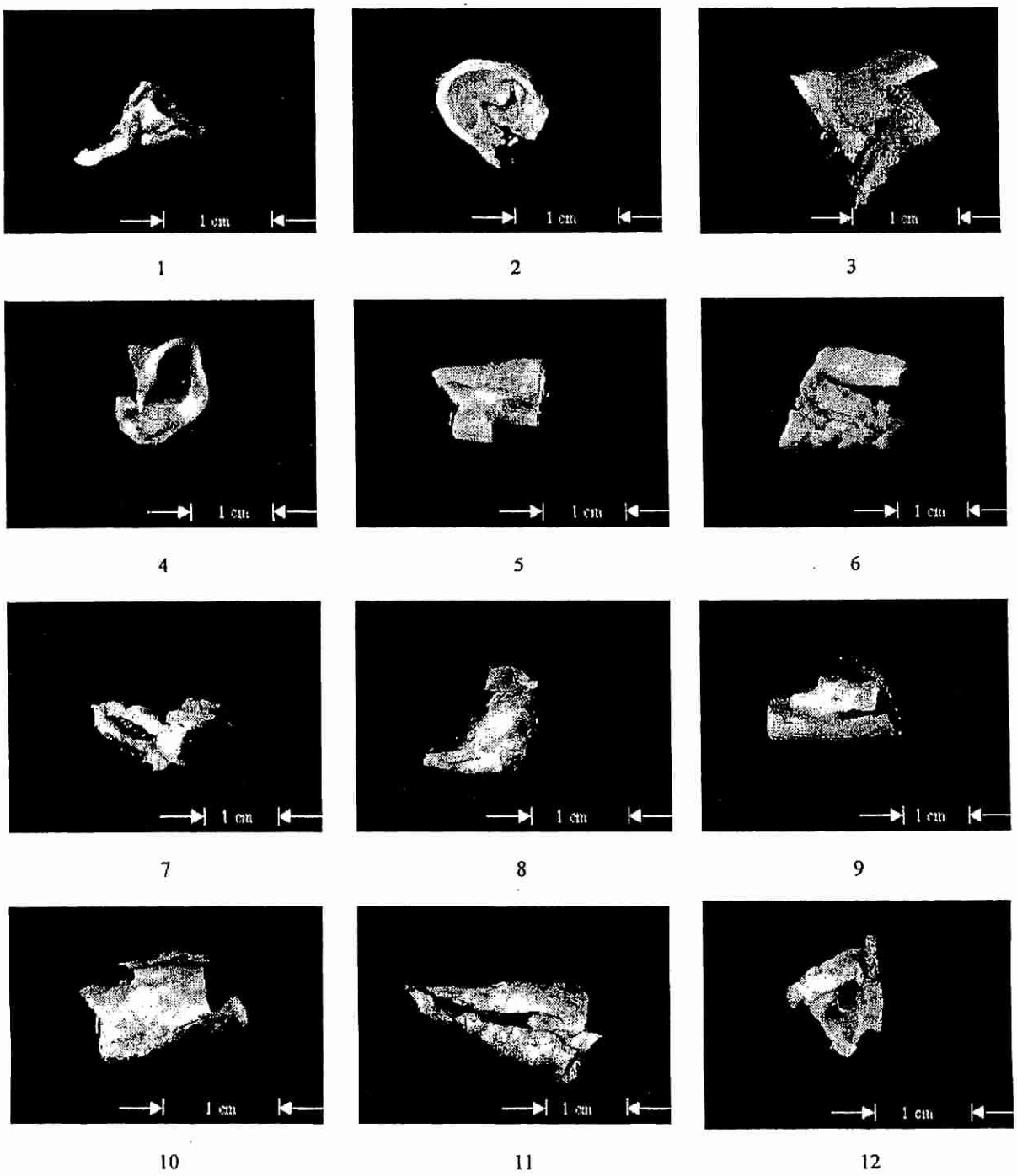

Figure 1.

Volume of each of the 12 carotid endarterectomy specimens shown in the photograph was measured with ultrasonography.

ringe connected to a 25-gauge needle, graduated in 0.1-ml, liquid was withdrawn from the tube until the level of solution returned to 5 ml. The solution withdrawn was the liquid displaced by the plaque, used as an estimate of plaque volume. This liquid displacement volume (LDV) measurement was repeated three times. The average was compared with ultrasonic estimates of plaque volume.

Ultrasound Techniques

Three US techniques were used for volumetric measurements of atherosclerotic plaques: (1) summation of incremental volumes obtained by multiplying manually traced plaque areas in cross-sectional images by the step distance between cross-sections (US-I); (2) summation of incremental volumes obtained by multiplying plaque areas selected on the basis of echo amplitude by step distance between cross-sections (US-II); and (3) volume calculation after "free-hand" scanning (US-III). These techniques are described in detail in the following.

Ultrasound Phantom and Stepper

A rectangular phantom was made with silicon to mimic US characteristics of tissue, 6-cm wide, 8-cm long, 3-cm deep. A cylindrical flow channel was drilled through the phantom material. Channel diameter was 15 mm, with its center located 12 mm from the phantom surface in contact with the US probe. The plaque to be tested was placed in the phantom channel. The phantom was placed in a larger container filled with ATS707-G Doppler fluid (ATS Laboratories, Inc., Bridgeport, CT) to mimic blood US characteristics. Doppler fluid filled the phantom channel, completely surrounding the plaque.

A clamp connected to a linear stepper held the US F2 probe (Figure 2). The stepper consisted of a microstage linear guide that transformed the rotation of a lead screw into translation of a platform (Thomson Industries, Inc., New York, NY). A full turn of the lead screw resulted in a platform linear motion equal to 0.635 mm.

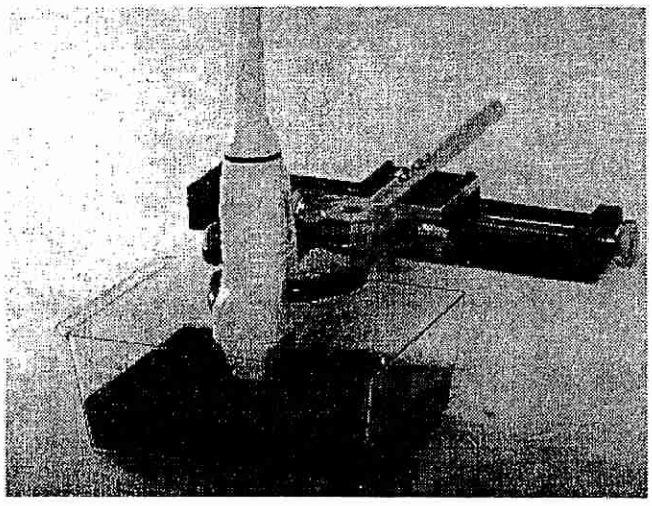

Figure 2.

Ultrasonographic data acquisition setup.

Method US-I: Summation of Traced Area Times Step Distance

Atherosclerotic plaque volume measurements were conducted on the basis of cross-sectional US images obtained with a GE LOGIQ 700 Expert Series (GE Medical Systems). A 7.5-MHz, H7039ML (Linear M12L), probe was used. US transducer gel was placed on top of the phantom. A cross-section was acquired every 0.635 mm. The US probe slid over the plaques using the stepper mentioned previously. The first and last cross-sectional image did not contain any plaque.

In each cross-sectional US image, the contour of the plaque was traced by the operator using the scanner's trackball (Figure 3). The plaque area was automati- F3 cally calculated by the US scanner software. To calculate the incremental volume represented by each cross-sectional image, this area was multiplied by the step distance, 0.635 mm. Summation of incremental volumes, or step distance times summation of areas, provided plaque volume. This process was repeated once for each of the 12 selected plaques.

Method US-II: Summation of Echo Amplitude Selected Area Times Step Distance

Atherosclerotic plaque volume measurements were also conducted based on cross-sectional US images obtained with a GE LOGIQ 700 using a 7.5-MHz transducer probe. The same US cross-sectional images collected with US-I were stored in an optical disk in a CIR format according to the scanner's specifications. CIR data were converted into TIF format using Adobe PhotoShop (Adobe Systems Inc., San Jose, CA) to comply with specifications of the data processing software MIMICS (Materialise N.V., Leuven, Belgium). Images were imported into the software as parallel cross-sections similar to CT planes separated by a constant thickness equal to the 0.635-mm step distance. This software converted TIF data into its own internal format and stored such data as an MIMICS executable project file in a unique directory. As the file execution started, images were displayed serially in the working space window. To create a three-dimensional orthogonal data set cube, X-Y axes were defined from left to right and bottom to top in cross-sectional images, and the Z axis was defined "anterior" (first image) to "posterior" (last image).

The next step was to define plaque contour by US amplitude thresholding. Initially, a region of interest was selected around the plaque. The contrast function of the "visualization properties" toolbar was then selected to closely define the plaque contour. By alternating two contrast controls, high-amplitude echoes were saturated as green, and low-amplitude echoes were blackened until best plaque visualization according to the operator's visual perception was accomplished (Figure 4). Area was calculated automatically F4

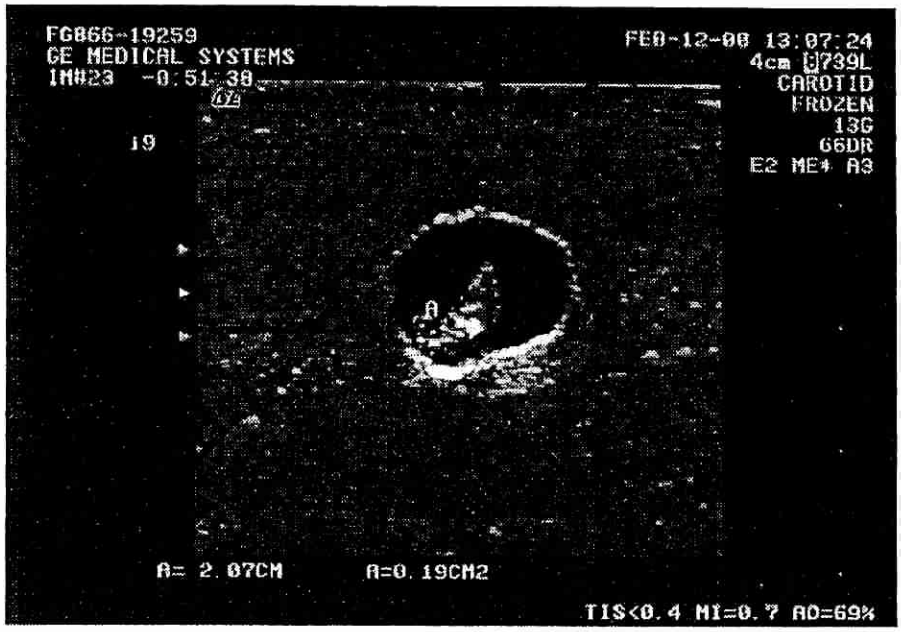

Figure 3.

Planimetric tracing of carotid plaque contour used to measure area (US-I).

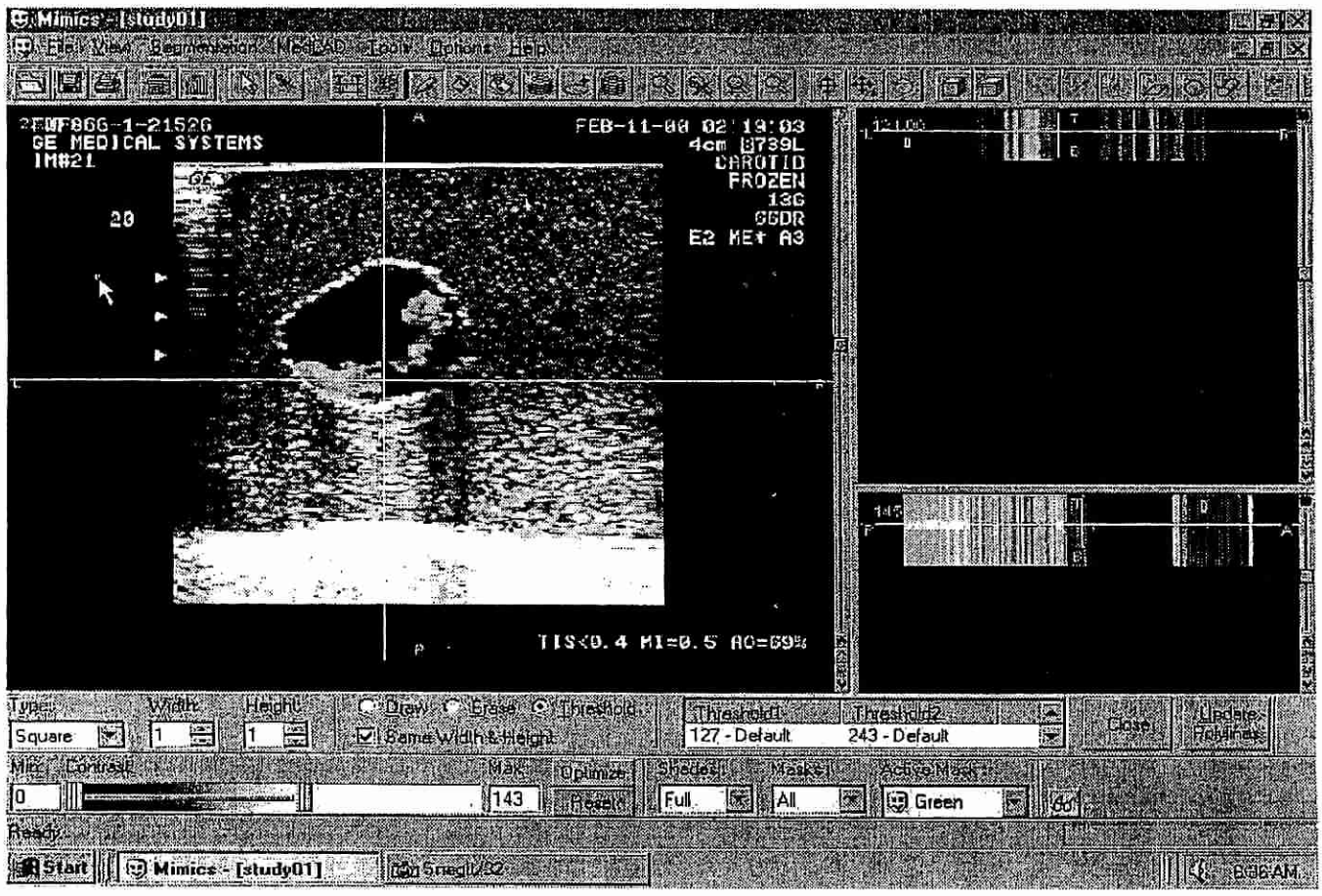

Figure 4.

Contrast selection of plaque region for area measurement using threshold 1 and 2 for low-level and high-level amplitude signals (US-II).

by the software. This area was multiplied by its defined thickness, the 0.635-mm step distance, to calculate the incremental volume for each cross-section. Summation of incremental volumes performed by the software provided plaque volume. Volume of each of the 12 selected atherosclerotic plaques was obtained once with this technique.

Method US-III: Free Hand Scanning

A freehand US scanning/data processing system, 4D Echo-Scan (Tomtec Imaging Systéms, Inc., Munich, Germany), coupled to an ATL HDI 5000 (Advanced Technology Laboratories, Bathell, WA) was used for data acquisition, data processing, and volume calculation. The freehand scanning system included three-dimensional/four-dimensional image processing software, Echo-View. A 7.5-MHz US probe (ATL L7-4) was used to image the plaques. The video signal output from the ATL HDI 5000 was connected to the video input of the Echo-Scan system.

A foot pedal was attached to the serial port of the data processing system to initiate the start and finish of data acquisition. An electromagnetic position-sensing device provided spatial reference for creation of a three-dimensional US volume data set. The sensing device was connected to the US probe. Because the physical relationship between the sensing device and the probe varies with the type of probe, each probe had to be calibrated individually. This calibration was performed for X, Y, and Z linear dimensions and orthogonal of X-Y, X-Z, and Y-Z planes in the three-dimensional cube.

Freehand scanning with the US probe moving steadily over the phantom with the plaque was performed. Multiple sequential two-dimensional images were acquired. Each two-dimensional data set together with the position sensing for motion were converted and stored to the coordinate system of a three-dimensional data cube. To identify the plaque in the three-dimensional data set, the software presented 10 cut planes to the operator. The operator defined F5a plaque boundaries as shown in Figure 5A. The operator was then presented with 10 cross-sections within the limits of the plaque and traced the contour of the plaque (Figure 5B).

Three volume measurement techniques were provided by the data processing system: Average Rotation Mode, Simpson Mode, and Disk Summation Mode. The Simpson Mode technique was selected because it provided the shortest computation time. Each of the 12 plaques was scanned three times, and volume measurements were generated. Three-dimensional image reconstruction routines were also used with the collected data sets for each plaque to generate three-dimensional image representations as shown in AU1 Figure 5C.

Statistical Analysis

Averages (means) and standard deviations were calculated for the three measurements performed for LDV and US-III. Coefficient of variance was calculated for each plaque as standard deviation divided by the mean. A variability coefficient was also calculated as (max-min) values divided by the mean. Percent differences were calculated for US-I, II, or mean III minus mean LDV, for each plaque, using the average measurement (LDV + US)/2 as the reference: 2 × (US-LDV)/(LDV + US) × 100. Similar percent differences were calculated for US-II or III minus US-I and US-III minus US-II. Paired and unpaired t tests were performed to compare mean LDV, US-I, US-II, and mean US-III using the Excel function (Microsoft Corp., Seattle, WA). The three LDV and three US-III measurements were also compared using the unpaired t test.

Linear regression was performed to relate LDV to US-I, II, and III using Excel regression function and Pearson coefficient calculation.

Results

Table 1 presents the actual volume measurements T1 obtained for each of the 12 plaques. Averages and standard deviations of liquid displacement and freehand scanning technique measurements were also included. Volumes varied from 200 mm^3 to 1,000 mm^3, covering a wide range of plaque volumes.

Coefficients of variance, calculated on the basis of the three measurements obtained with LDV and US-III for each plaque averaged 2.87% ± 1.44% (range, 1.62%–5.80%) and 1.98% ± 0.82% (range, 0.89%–4.10%) for LDV and US-III, respectively. The variability coefficients, based on maximum minus minimum for each plaque, averaged 5.55% ± 2.98% (2.83%–11.39%) and 3.85% ± 1.54% (1.78%–7.89%) for LDV and US-III, respectively.

Table 2 presents the percent differences between average LDV and single measurements (US-I and US-II) or US-III average. T2

US-I and US-II measured lower volumes than LDV in 9 of 12 plaques. By paired t test, volumes obtained with LDV and US-I or II were not statistically different ($p > 0.1$). US-III measured higher volumes than LDV in 11 of 12 plaques. Volumes obtained with US-III were significantly higher than volumes obtained with LDV, US-I, and US-II ($0.04 > p > 0.01$). By unpaired t test, however, p values were greater than 0.76 for all comparisons. Furthermore, differences between the sets of three measurements for LDV and US-III were not statistically significant for 10 of 12 plaques ($0.08 < p < 0.73$). Two of the four smallest plaques had significant differences in volume measurements (7% for plaque #9 and 10% for plaque #10).

Linear regression provided the following equations and Pearson correlation coefficients:

US-I = 42.23 + 0.8738 LDV (0.9940)
US-II = 21.63 + 0.9329 LDV (0.9980)
US-III = 10.57 + 0.9985 LDV (0.9987)

Discussion

Volumes of carotid endarterectomy specimens were successfully measured in vitro with ultrasonography.

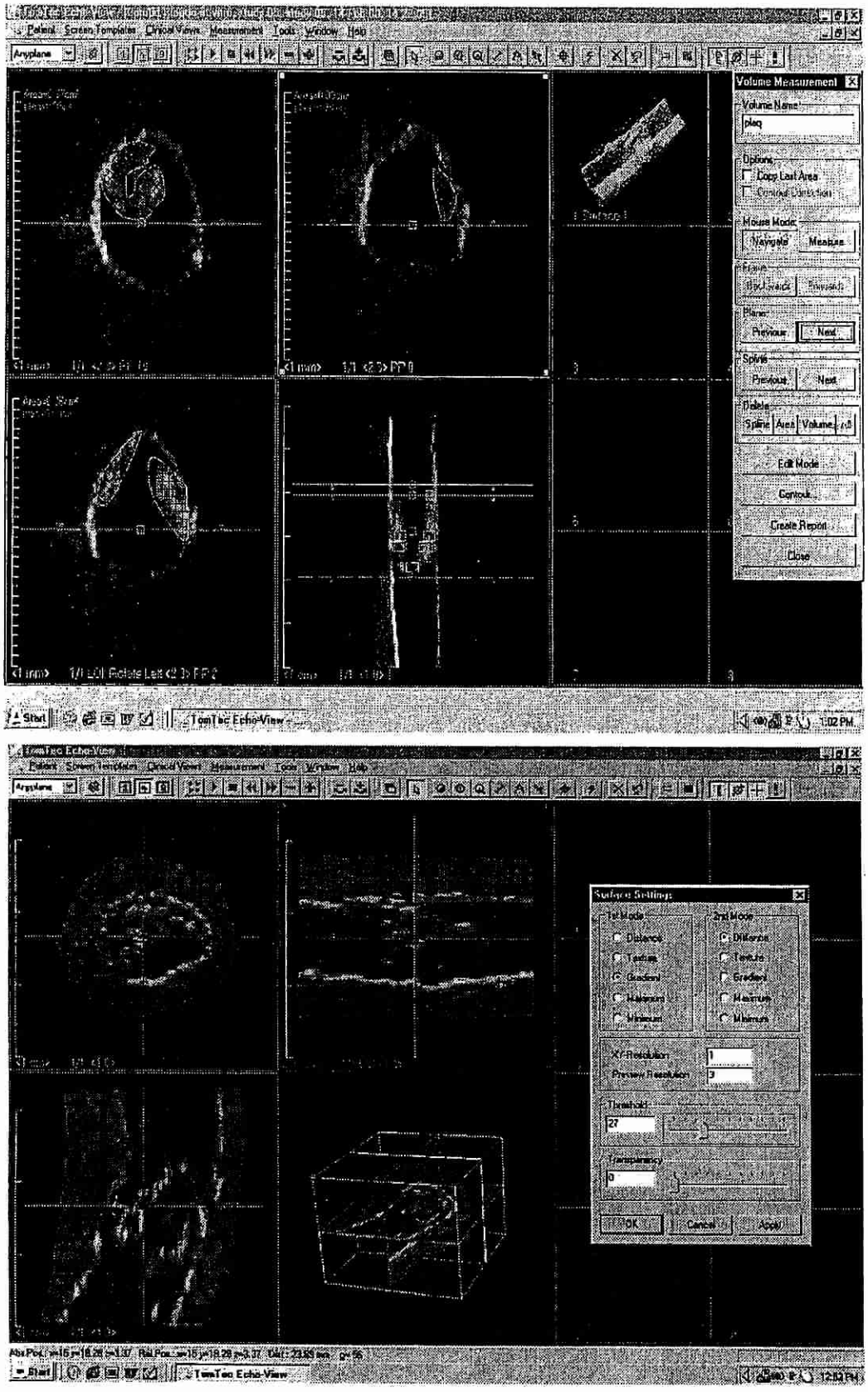

Figure 5

(A) Identification of the plaque limits from the three-dimensional data cube orthogonal axes, X, Y, and Z, were represented by the colors blue, green, and yellow. By moving the colored axes to the beginning and end of the plaque, the operator defined plaque limits (US-III). (B) Within the defined limits of the plaque (1 cm in this example), the operator traced the contour of the plaque in 10 cross-sections (1-mm thickness in this example) (US-III). (C) Three-dimensional image reconstruction routines applied to the collected data to generate three-dimensional image of each plaque inside the phantom channel for better visualization (US-III).

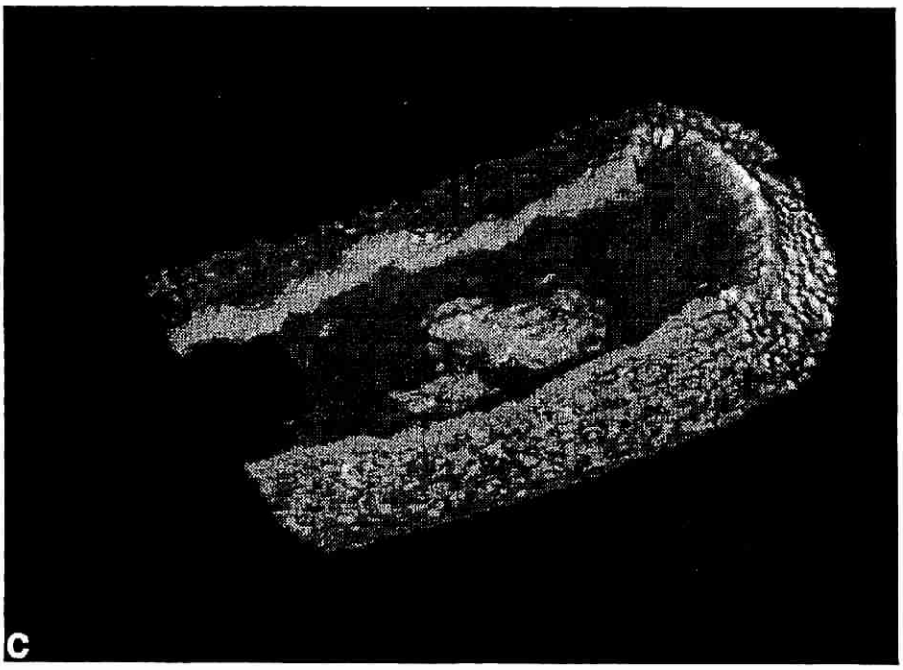

Figure 5 Continued.

Differences between US methods and volume measured by liquid displacement averaged less than 3%, suggesting clinical usefulness. Emphasis was given to the three-dimensional US freehand scanning technique over two-dimensional methods[11,12] because of the potential for practical, direct measurements with positional-space–sensing capabilities and construction of a reliable three-dimensional data cube.[13–17] Therefore, three measurements were performed for each plaque with this technique to estimate measurement variability.

For multiple measurements with US-III, the coefficients of variance were less than 4% for almost all plaques, averaging less than 2%. This finding indicates that the three-dimensional technique can measure plaque volume consistently. On the basis of these data, this technique has been selected as the standard method for in vivo measurements in our vascular laboratory. A critical review of the data revealed that the free-scanning technique consistently measured the highest volumes. Statistically, however, the difference of only two plaques, numbers 9 and 10, had signifi-

Table 1

Volume of Carotid Endarterectomy Specimens
Plaque Volume
Cubic Millimeters (mm^3)

Plaque Number	Liquid Displacement Volume (LDV)					US-I	US-II	US-III				
	Measurement #*					Measurement #		Measurement #				
	1	2	3	Mean	SD	1	1	1	2	3	Mean	SD
5	1,000	1,100	1,050	1,050	50.0	950	984	1,049	1,085	1,064	1,066	18.2
6	750	720	730	733	15.3	690	722	752	738	745	745	6.6
4	540	560	550	550	10.0	510	537	570	562	555	562	7.5
3	500	520	550	523	25.2	480	518	496	506	512	504	8.1
12	450	460	440	450	10.0	490	457	456	458	469	461	7.0
2	380	390	370	380	10.0	390	375	375	385	393	384	9.0
1	350	360	350	353	5.8	370	357	366	355	361	361	5.6
11	350	360	350	353	5.8	340	351	362	360	345	356	9.0
9	340	350	350	347	5.8	350	332	365	378	372	372	6.4
8	330	350	340	340	10.0	330	349	366	351	356	357	7.7
7	280	260	250	263	15.3	260	245	267	275	264	269	6.0
10	230	240	230	233	5.8	220	235	270	249	255	258	10.6
Mean	458	473	463	465	14.1	448	455	474	475	474	475	8.5
SD	220	239	232	230	12.6	202	215	226	234	230	230	3.4
Min	230	240	230	233	5.8	220	235	267	249	255	258	5.6
Max	1,000	1,100	1,050	1,050	50.0	950	984	1,049	1,085	1,064	1,066	18.2

*US-I and US-II had single measurements, whereas LDV and US-III had three measurements.

Table 2

Volume of Carotid Endarterectomy Specimens: Differences Between Methods in Percent Volume

Plaque Number	(US-I)-(LDV)	(US-II)-(LDV)	(US-III)-(LDV)	(US-II)-(US-I)	(US-III)-(US-I)	(US-III)-(US-II)
5	−10.0	−6.5	1.5	3.5	11.5	8.0
6	−6.1	−1.6	1.6	4.5	7.7	3.2
4	−7.5	−2.4	2.2	5.2	9.7	4.6
3	−8.6	−1.0	−3.7	7.6	5.0	−2.7
12	8.5	1.5	2.4	−7.0	−6.1	0.9
2	2.6	−1.3	1.1	−3.9	−1.5	2.5
1	4.6	1.0	2.0	−3.6	−2.6	1.0
11	−3.8	−0.7	0.7	3.2	4.5	1.3
9	1.0	−4.3	7.0	−5.3	6.0	11.3
8	−3.0	2.6	5.0	5.6	8.0	2.4
7	−1.3	−7.2	2.0	−5.9	3.3	9.2
10	−5.9	0.7	10.0	6.6	15.8	9.3
Mean	−2.5	−1.6	2.6	0.9	5.1	4.2
SD	5.7	3.1	3.4	5.5	6.2	4.3
Min	−10.0	−7.2	−3.7	−7.0	−6.1	−2.7
Max	8.5	2.6	10.0	7.6	15.8	11.3

cance. Even for these plaques, the difference between US-III and LDV was 10% or less. Therefore, for a worse case scenario, changes between measurements performed on different occasions that are less than 10% should be considered part of measurement variability, not necessarily taken as plaque regression or progression. This 10% "rule" is also suggested by noting that three times the average coefficient of variance for 95% confidence interval of US-II measurements was 8% (3 × 1.98%). This argument reinforces that measurement changes greater than 10% should be considered significant changes in volume rather than measurement variability.

The coefficient of variability was calculated to compare the findings of this study with literature data. A value of approximately 4% obtained for US-III is comparable to intraexaminer and interexaminer variabilities reported by Delcker and Diener[18] and Delcker and Tegeler.[19] Their variability in follow-up studies was about doubled. In vivo measurements benefited from electrocardiogram-triggered data acquisition, because intrareader, interreader, and the follow-up variability decreased significantly compared with non-electrocardiogram–triggered measurements.[20]

Volumes of carotid endarterectomy specimens measured in this work were greater than carotid plaque volumes previously reported. Delcker and others[17,19,21] in a series of articles, consistently measured carotid plaque volume in the 2–200 mm^3 range. Serena, Palombo, and others[3,13] measured carotid plaque volume ranging from 7–450 mm. The large range of plaque volume observed in this study allowed for appropriate linear regression analysis. This analysis favored the freehand scanning three-dimensional method that, compared with LDV, besides having a very high Pearson correlation coefficient, also had a slope coefficient mutually identical to 1. Palombo et al.[13] also obtained high correlation coefficients and slope coefficients close to 1 during intraobserver and interobserver studies of carotid plaques in vivo, using a three-dimensional system.

Some applications of carotid plaque US volumetry have already been described. Delcker and others[17,21–26] demonstrated the influence of diastolic pressure on carotid plaque progression. Volume measurement becomes an additional tool to evaluate the atherosclerotic plaque. Extensive research has been conducted in plaque characterization, plaque stabilization, and intima-media thickness.[27–31] These techniques may turn out to be complementary, with volume being a variable linking these variables together.

In summary, several articles have demonstrated the feasibility of measuring atherosclerotic plaque volume with ultrasonography.[11,12] On the basis of our evaluation of three two-dimensional and three-dimensional alternatives to calculate plaque volume of carotid endarterectomy specimens, our tendency is to use an electromagnetic position sensor to create a three-dimensional data cube for analysis. Carotid specimens with a broad range of volume were evaluated. Acceptable accuracy, reproducibility, and linear correlation in a large range of volumes suggest that atherosclerotic plaque volumetry using noninvasive ultrasonography is a viable method to evaluate regression and progression in clinical applications. Therefore, the armamentarium to evaluate the atherosclerotic plaque with ultrasonography may include, in addition to intimal plus medial thickness and plaque characterization, the measurement of plaque volume. Future US volumetry analysis of carotid artery plaques in vivo is planned using the electromagnetic position-sensing device and freehand scanning technique.

Acknowledgment. This work was supported in part by grant HL60301 from the National Institutes of Health, Bethesda, Maryland.

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