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# B-mode ultrasound images of the carotid artery wall: correlation of ultrasound with histological measurements

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**Abstract**

B-mode ultrasound is being used to assess carotid atherosclerosis in epidemiological studies and clinical trials. Recently the interpretation of measurements made from ultrasound images has been questioned. This study examines the anatomical correlates of B-mode ultrasound of carotid arteries in vitro and in situ in cadavers. Twenty-seven segments of human carotid artery were collected at autopsy, pressure perfusion fixed in buffered 2.5% gluteraldehyde and 4% paraformaldehyde and imaged using an ATL UM-8 (10 MHz single crystal mechanical probe). Each artery was then frozen, sectioned and stained with van Gieson or elastin van Gieson. The thickness of the intima, media and adventitia were measured to an accuracy of 0.01 mm from histological sections using a calibrated eye graticule on a light microscope. Shrinkage artifact induced by histological preparation was determined to be 7.8%. Digitised ultrasound images of the artery wall were analysed off-line. The distance from the leading edge of the first interface ($LE_1$) to the leading edge of the second interface ($LE_2$) was measured using a dedicated programme. $LE_1$-$LE_2$ measurements were correlated against histological measurements corrected for shrinkage. Mean values for the far wall were: ultrasound $LE_1$-$LE_2$ (0.97 mm, S.D. 0.26), total wall thickness (1.05 mm, S.D. 0.37), adventitia (0.35 mm, S.D. 0.16), media (0.61 mm, S.D. 0.18), intima (0.09 mm, S.D. 0.13). Ultrasound measurements corresponded best with total wall thickness, rather than elastin or the intima-media complex. Excision of part of the intima plus media or removal of the adventitia resulted in a corresponding decrease in the $LE_1$-$LE_2$ distance of the B-mode image. Furthermore, increased wall thickness due to intimal atherosclerotic thickening correlated well with $LE_1$-$LE_2$ distance of the B-mode images. B-mode images obtained from the carotid arteries in situ in four cadavers also corresponded best with total wall thickness measured from histological sections and not with the thickness of the intima plus media. In conclusion, the $LE_1$-$LE_2$ distance measured on B-mode images of the carotid artery best represents total wall thickness of intima plus media plus adventitia and not intima plus media alone.

*Key words:* Carotid artery; B-mode ultrasound; Validation; Histology; Atherosclerosis

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## 1. Introduction

Non-invasive B-mode ultrasonography of the common carotid artery is used with increasing frequency in studies of the progression and regression of atherosclerosis. The extent and distribution of plaques in the carotid system can be scored reliably from B-mode images [1]. These scores have been shown to relate to the extent of coronary artery disease in a comparative study using coronary angiography [2,3] and to the risk of atherosclerotic events in a case control study [4] and a prospective observational study [5]. These observations suggest that ultrasound inspection of the carotid artery is relevant to the study of coronary artery disease as well as to the study of carotid atherosclerosis per se.

In addition to documenting overt carotid plaques, some groups have used ultrasound to assess earlier atherosclerotic changes manifested by lesser degrees of wall thickening. Following the initial report of Strandness [6], Bond and Ball [7] demonstrated that measurements of echo patterns, attributed to the thickness of the intima-media complex, could provide a reliable index for the assessment of early atherosclerotic changes in both an animal model and in man. Using post-mortem arterial material from 18 males Pignoli and colleagues [8] showed good agreement between ultrasound measurements and histological measurements of intima-media thickness in vitro.

From these studies, a number of different measuring protocols have been developed to assess the thickness of the carotid artery intima-media complex. The intima-media thickness of the far wall alone is reported by some groups [9,10], while others measure both near and far wall thickness [11–13]. Some groups measure the echo 'thickness' and relate this to the thickness of the adventitia [11]. One report has suggested that the echo patterns measured may be artefactual [14].

Recently a standardised approach to B-mode measurements has been suggested based on the leading edges, or upper demarcation lines of echoes generated from far wall structures alone [15,16]. This approach is based on two considerations: (1) in vivo, the jugular vein and associated periadventitia mask the echoes of the near or superficial wall thus rendering measurement of the near wall unreliable; (2) the thickness of an echo is primarily a function of gain rather than of anatomy, so that as a general principle, measurements should only be made from the leading edges of ultrasound echoes.

This study examines the anatomical correlates of the two-line echo pattern seen in B-mode images of the far wall of the common carotid artery. The data demonstrate that the B-mode image best represents total wall thickness.

## 2. Materials and methods

### *2.1. Artery collection*

Segments of human common carotid artery, proximal to the bulb, were excised from 37 individuals (age range 23–86 years) at autopsy (< 24 h after death), immersed in chilled growth medium (Gibco Medium 199) and imaged within 2 h of excision. Seven vessels were used for dissection experiments. One specimen tested positive for the HIV antibody and was appropriately discarded.

### *2.2. Artery preparation*

Twenty-seven vessels (averaging 3 cm in length) were imaged whole in vitro. Prior to imaging, the loose periadventitial layer was removed to the level of the adventitia proper. The ends of each vessel segment were tied to flanged brass tubes, submerged in a growth medium filled perspex bath maintained at room temperature (21–24°C) and perfused under pressure (120 mmHg) with growth medium. A latex diaphragm was secured over the bath, air was expelled and the artery imaged freehand through this membrane. The distance from the transducer to the dorsal (near) outer surface of the mounted vessel was 3 cm. On average a 2 cm length of vessel was imaged. Each pressure-perfused vessel was imaged twice: firstly bathed in growth medium and secondly in growth medium following 2–4 h pressure fixation at 120 mmHg in 4% paraformaldehyde and 2.5% gluteraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4. The surrounding growth medium in the bath helped support the vessels and provided acoustic attenuation similar to plasma. The orientation of the artery in the bath was preserved by passing a suture through the mid-dorsal surface of the specimen. This permitted the histological measurements to be made in exactly the same plane and position as those imaged by ultrasound.

Seven arteries were opened longitudinally, fixed for 1–2 h (4% paraformaldehyde, 2.5% gluteraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4) and parts of the intima, media and adventitia excised under a dissection microscope. The vessel walls were then bathed in growth medium and imaged both from the intimal and adventitial surfaces.

### 2.3. Ultrasonography

Vessels were imaged with an ATL UM-8 ultrasound machine with an Access 10 single crystal mechanical sector multifrequency transducer (10 MHz). In several experiments the effect of increasing overall gain on the image quality and on the relationship of the LE interface was determined. Vessel images were recorded on super high grade video tape and three longitudinal representative images were digitised (Matrox IP-8 frame grabber board, 33 MHz 386) and stored on optical disk for off-line analysis.

### 2.4. Histology

Following ultrasonography, perfusion-fixed vessels were sectioned on a cryostat. Transverse frozen sections (30-μm thick) were cut at 1-mm intervals through 1 cm of the imaged vessel and alternate sections were stained with either van Gieson or elastic van Gieson solution. For each vessel ten sections were measured and the values averaged. Vessel wall thickness was measured using an eye graticule calibrated against a standard objective micrometer. The coefficient of variation for repeated measurements of total wall thickness was 2.4%. The suture placed at the time of ultrasound allowed measurements of the intima, media and adventitia to be made in exactly the same plane and at the same position as that recorded ultrasonographically. The elastin stain permitted exact measurement of the elastic component of the vessel wall, effectively the distance from internal to external elastic lamina except where the intima was thickened and contained some elastic fibres.

### 2.5. Imaging of carotids in cadavers

Twenty-four embalmed cadavers prepared for undergraduate anatomy teaching were scanned. In four cadavers it was possible to image a carotid artery clearly. It was not possible to image the remaining cadavers either because the orientation of the head was fixed during post-mortem preparations making it impossible to position the transducer over the artery or because the carotid arteries were drained of fluid. An indelible mark was made on the skin at the site of the successful imaging to guide later excision. Some months later, after student dissection of other parts of the body, the four carotid arteries were removed, immediately placed in a buffered formalin solution and imaged. The orientation of each vessel relative to the overlying skin was maintained with a suture. Each arterial segment was then imaged again in vitro and prepared for histological measurements as above.

### 2.6. Estimation of histological shrinkage artifact

To determine the amount of shrinkage artifact associated with histological sectioning, processing and staining, two carotid arteries (57 years and 80 years) were collected at autopsy, pressure perfused and fixed as detailed above. These vessels plus two further vessels collected from cadavers (69 years and 91 years) were cryosectioned. Each vessel was removed from the cryostat and orientated with the cryostat cut surface facing upward. The cut surface was photographed with a macro camera lens. Corresponding areas from the stained sections and from photographs of whole vessels were then measured and compared for shrinkage. Twenty sites from each vessel were measured. The mean shrinkage due to histological preparation was 7.8% (S.D. 4.9) for the pressure-perfused fixed vessels and 5.8% (S.D. 9.5) for the cadaver vessels.

### 2.7. Ultrasound measurements

Digitised B-mode images were analysed off-line using a locally developed computer program. The grey scale of each image, magnified five times, was reversed to display black on white. A measuring grid comprising 10 equal vertical line segments scaled over 1 cm of artery was placed over the region of interest. A cursor was placed over the leading edges of each of the two interfaces which characterise the echoes of the near and far walls and the position recorded. $LE_1$-$LE_2$ measurements were made at each of the 10 vertical lines and the values averaged. The coefficient of variation of repeated measurements was 6.6% for the $LE_1$-$LE_2$ distance.

*2.8. Statistical analysis*

Data were analysed using the SAS statistical package [17]. Comparison of the values obtained from ultrasound and from the histological preparations was made using Spearman's rank correlation coefficient and Student's $t$-test for related groups. Coefficients of variation and reliability (95% confidence intervals) [18] and simple absolute and mean differences were determined. A sample size of 27 would provide in excess of 80% power ($\alpha = 0.05$) [19] to detect 'almost perfect' [20] agreement between pairs of measurements. Intra-class correlation coefficients were calculated from the between- and within-person mean squares for a one-way analysis of variance [21]. The standard deviation of the intrameasurer error for the repeatability studies was calculated as the standard deviation of the differences over $\sqrt{2}$.

## 3. Results

*3.1. Effect of gain on B-mode image of carotid artery*

A typical scan of a section of carotid artery in vitro, showing the two-line pattern for the near wall ($LE_1$-$LE_2$) and the two-line pattern for the far wall ($LE_1$-$LE_2$) is shown in Fig. 1a. Increasing the gain did not affect the position of these leading

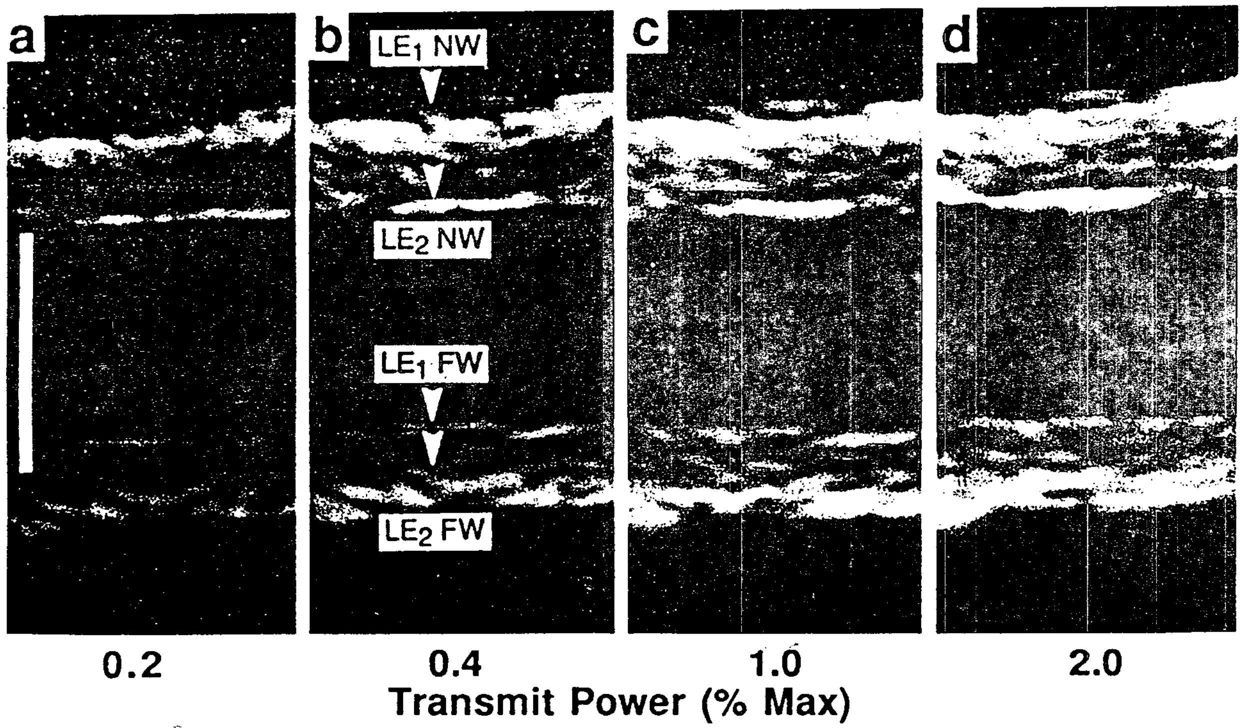


Fig. 1. Effect of increasing gain on the leading and trailing edges of a B-mode image of a segment of common carotid artery. With increasing gain the distance between the leading edges of both the near wall ($LE_1$ NW and $LE_2$ NW) and the far wall ($LE_1$ FW and $LE_2$ FW) remain the same. At the higher gain settings the trailing edges increase in width and the echo-free zones of both walls are obscured. Scale bar = 5 mm.

edges, nor the distance between them, but rather increased the width of the echoes thus altering the position of the trailing edge. An increase in gain from 0.2% to 2.0% (Figs. 1a–1d) resulted in an approximately 50% increase in the thickness of the double line echoes with a corresponding decrease in the middle echo-free zones. Since no change was made to the tissue being imaged, the trailing edges of these images can have no anatomical correlate. For this reason leading edge measurements (imaged at minimum gain) alone were used.

### *3.2. Comparison of ultrasound and histological measurements*

All of the histological measurements were significantly correlated with the ultrasound ($LE_1$-$LE_2$) measurements (all $P < 0.001$). Fig. 2. shows the histological measurements of the thickness of the intima-media complex and total wall thickness (intima plus media plus adventitia) for both the near and far walls plotted against the ultrasound measurements ($LE_1$-$LE_2$) of the near and far walls. While both plots demonstrated good agree-

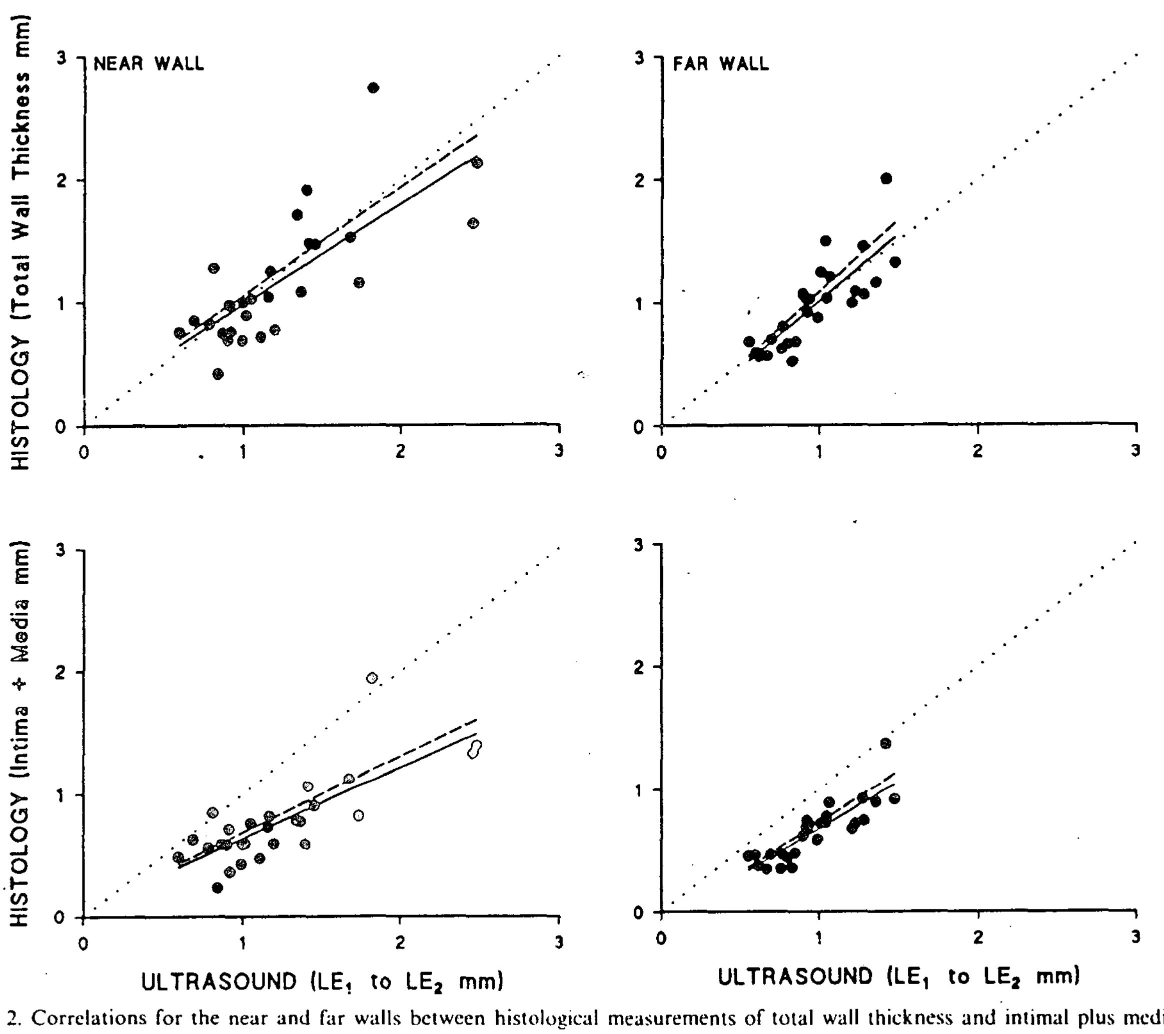


Fig. 2. Correlations for the near and far walls between histological measurements of total wall thickness and intimal plus medial thickness and $LE_1$-$LE_2$ distances measured from ultrasound images. The dotted lines represent the line of identity. The continuous regression lines are fitted to the uncorrected data points (●) which have not been corrected for shrinkage. The dashed regression lines are fitted to data points (not shown) corrected for shrinkage.

ment between ultrasound and histology for both the far and near walls ($r > 0.7$) the plots of intima-media thickness against B-mode fell below the lines of identity (dotted line). Correction of histological measurements for 7.8% shrinkage reduced the mean difference between ultrasound and total wall thickness for the far wall from −0.05 (S.D. 0.24) to 0.03 (S.D. 0.23). Neither of these differences was significantly different from 0 ($P = 0.33$ and $P = 0.45$, respectively). The mean difference between ultrasound and histological measurements of intima-medial thickness, corrected for shrinkage, changed from 0.38 (S.D. 0.20) to 0.33 (S.D. 0.19), both differences were significantly different from 0 (both $P < 0.0001$). Fig. 2. presents this correction for shrinkage as a least-squares regression line (dashed) compared with that for the uncorrected data (continuous line).

In Fig. 3 the data are presented diagrammatically (to scale) for the near and far walls. For each wall the mean ultrasound measurement ($LE_1$-$LE_2$) is shown as the right box. For comparison boxes representing average intima-media thickness and average total wall thickness are placed alongside. Correction for a 7.8% shrinkage artifact is shown by adding the starred boxes to the measurements. The thickness of the intima-media complex, even after correction for shrinkage, was approximately two-thirds the distance measured from the leading edges of the near and far ultrasound patterns, while total wall thickness corresponded almost exactly. The distribution of elastin closely matched the thickness of the media, except in thickened intimas where some elastin was present in the deeper regions and the width of the elastin zone was correspondingly wider. In no case however, did the maximum extent of the elastin approach the $LE_1$-$LE_2$ distance (data not shown).

Fig. 4a. shows a transverse section of carotid artery (59-year-old female) stained with van Gieson solution. A fibrous lesion extended for approximately 1.5 cm along the artery. The vessel was orientated within the bath so that the lesion was in the plane of the transducer and a longitudinal image was obtained (Fig. 4b). Large calcified lesions were identified in the left main coronary artery of this person. It was apparent that the dis-

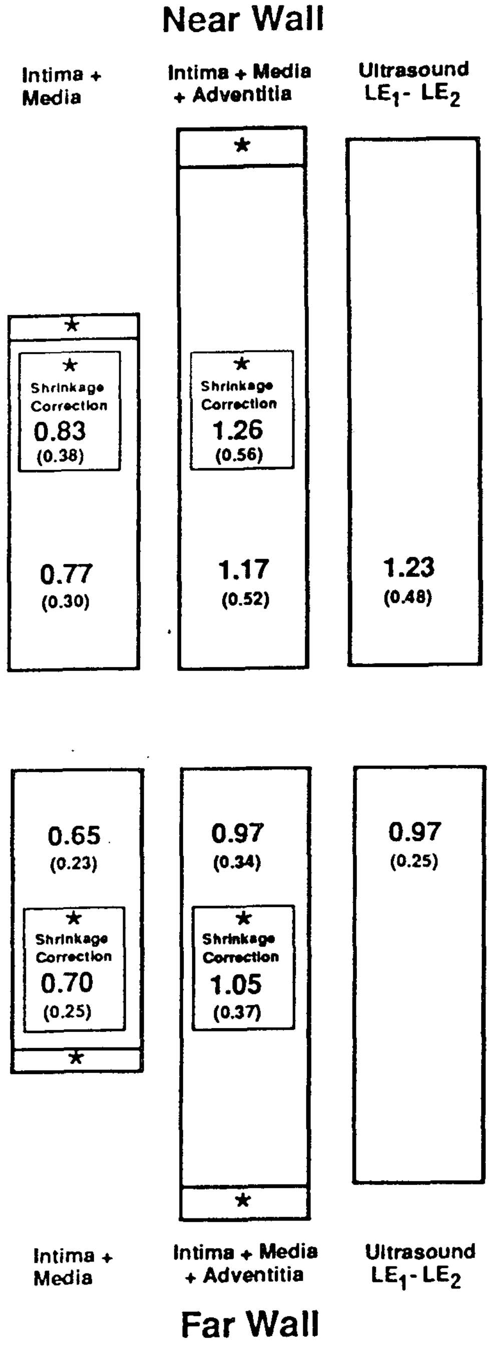


Fig. 3. Diagrammatic representation, to scale, of the mean values (S.D.) for intima plus media and total wall thickness measured from histological sections and mean values for $LE_1$-$LE_2$ distances determined from ultrasound images of the near and far walls. The starred areas and values represent correction for shrinkage artifact.

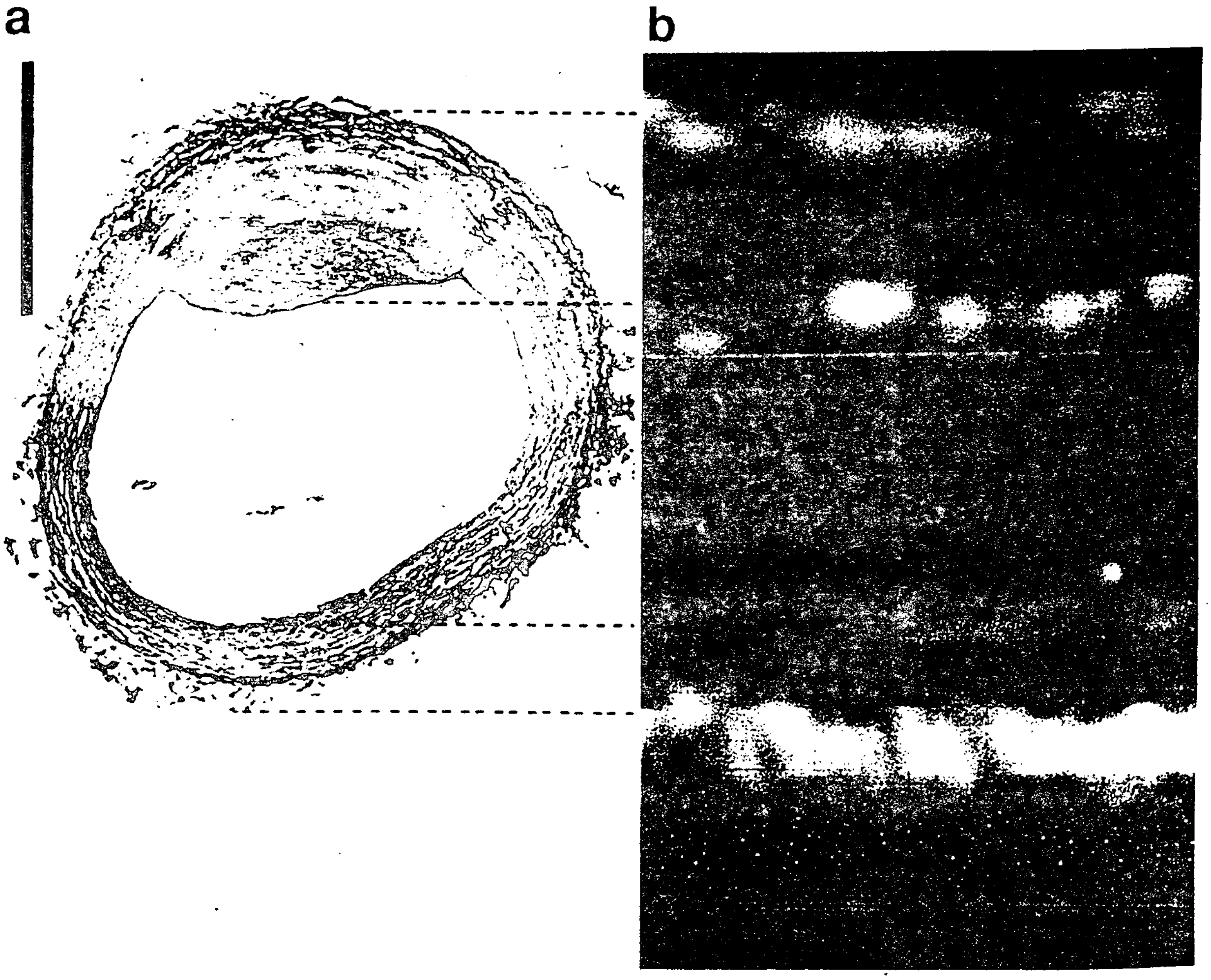


Fig. 4. (a) Transverse section of a carotid artery (59-year-old female) stained with van Gieson, showing a prominent eccentric fibrous lesion. The plane of the ultrasound beam and the full extent of total wall thickness for the near and far walls are indicated by the dashed lines. (b) B-mode ultrasound image corresponding to the same section of artery shown in (a). Both images are to scale (scale bar = 3 mm).

tance separating the leading edges for both the near and far walls corresponded to the total wall thickness of the sectioned artery. Rotation of the vessel and imaging of the lesion through the lumen did not affect this relationship.

Removal of sections of the intima-media complex resulted in a corresponding decrease in the size of the echo-free zone between the two interfaces. Fig. 5a. shows the effect of excision of approximately 53% of vessel wall on the resulting B-mode ultrasound image (Fig. 5a, insert). Despite removal of the entire intima and part of the media, the two leading edges were clearly evident. The total wall thickness of the undissected wall, determined from histology (and corrected for shrinkage) was 1442 $\mu$m and the distance $LE_1$-$LE_2$ of the ultrasound (shown in insert) was 1341 $\mu$m. In the excised region, vessel wall thickness was 768 $\mu$m from histology whilst the ultrasound $LE_1$-$LE_2$ distance in the excised region was 854 $\mu$m. The average distance $LE_1$-$LE_2$ in Fig. 5b (left) was 1.2 mm.

Removal of some of the adventitia (Fig. 5b,

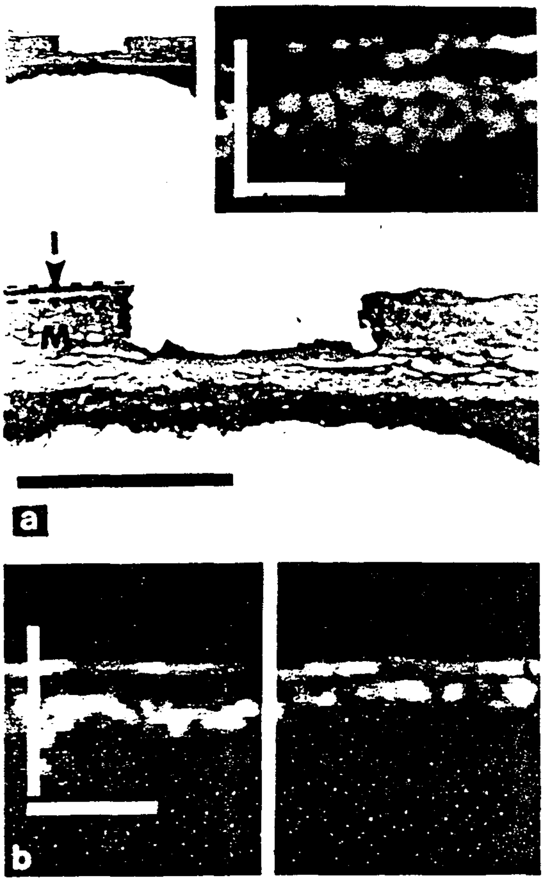


Fig. 5. (a) Section of carotid artery stained with van Gieson demonstrating histological appearance after excision of the intima (I) and part of the media (M). The adventitia (A) is left intact. The top right insert depicts the corresponding B-mode ultrasound image and the top left insert the histology to the same scale as the ultrasound. Histology scale bar = 2 mm; B-mode scale bars = 5 mm. (b) B-mode images of a section of artery before (left) and after (right) removal of the adventitia. Scale bars = 5 mm. The intimal surface was dorsal in all these frames.

right) reduced the distance $LE_1$-$LE_2$ by 0.3 mm (Fig. 5b, left). Histological examination showed that none of the media was excised. Had the $LE_2$ interface been generated by the media-periadventitia boundary then excision of the adventitia would have had no effect on the $LE_1$-$LE_2$ distance.

Results of intima-media excision experiments are tabulated in Table 1. In some experiments if the region excised exceeded approximately 75% of the vessel wall the two-line pattern disappeared, presumably because the interface generating structures were smaller than the axial resolution of the equipment.

Table 2 lists the results of measurements on four cadavers. Comparison between ultrasound measurements made in situ and again some months later in vitro yielded a strong correlation ($r = 0.96$) and an average difference of 0.017 mm (S.D. 0.099). The differences between in vitro B-mode measurements of far wall thickness and histological measurements, corrected for shrinkage, of total wall thickness and intima-media thickness were 0.108 mm (S.D. 0.29) and 0.287 mm (S.D. 0.24), respectively. Although neither of these differences were statistically significant the agreement between ultrasound and total wall thickness was much closer ($P = 0.469$) than that between ultrasound and intima plus media ($P = 0.09$). This close agreement between ultrasound and total wall thickness is consistent with the findings for isolated carotids and demonstrates that the presence of skin, muscle and fat tissue overlying the carotid artery does not affect the fundamental relationship between B-mode ultrasound and total artery wall thickness.

## 4. Discussion

These findings confirm that measurements made from B-mode ultrasound images of the carotid artery reliably indicate the extent to which arterial walls are thickened and show that the distance $LE_1$-$LE_2$ measured on B-mode images of the carotid artery corresponds most closely to total wall thickness of intima plus media plus adventitia. Importantly, this association was maintained after adjusting the histological measurements of wall thickness for shrinkage artifact. Furthermore, when imaged both in situ and in vitro, $LE_1$-$LE_2$ measurements were not significantly different indicating that the isolated artery preparation is an appropriate model.

The primary conclusion of this study was strengthened by excision studies. Excision of the intima-media complex resulted in corresponding decreases in the $LE_1$-$LE_2$ distance. Additionally,

Table 1
Comparison, in four vessels, of histologic and ultrasound measurements (μm) from adjacent regions of dissected and intact carotid artery wall

| Dissected | | | | | Intact | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Intima | Media | Adventitia | Total | Ultrasound $LE_1$-$LE_2$ | Intima | Media | Adventitia | Total | Ultrasound $LE_1$-$LE_2$ |
| 0 | 284 | 446 | 730 | 900 | 122 | 834 | 405 | 1361 | 1300 |
| 0 | 527 | 365 | 892 | 900 | 243 | 770 | 446 | 1459 | 1400 |
| 0 | 332 | 664 | 996 | 900 | 89 | 770 | 308 | 1167 | 1600 |
| 0 | 284 | 510 | 794 | 1100 | 105 | 851 | 648 | 1604 | 1900 |

lesions produced a corresponding increase in $LE_1$-$LE_2$ distance. Thus the interfaces were produced by the junctions of the intima and the fluid on the one hand and the adventitia and the fluid on the other and not by some reverberation artifact generated endogenously. These findings support the conclusion that the distance between the two leading edges is a measure of total wall thickness.

Studies validating intravascular ultrasound have suggested that the internal elastic lamina contributes to the generation of ultrasound interfaces [22]. We sought specifically to test the hypothesis that the internal and external laminae (and hence primarily media alone) are the source of the two-line pattern commonly seen in these echoes. However, comparison of histological preparations stained specifically for elastin showed that the extent of the elastin-containing regions were some 60% smaller than the $LE_1$-$LE_2$ measurements taken from ultrasound.

The results of this study differ from the findings of Pignoli et al [8]. Whereas in their studies the $LE_1$-$LE_2$ measurement of the far wall was taken to represent the width of the intima-media complex, in our study the same measurement was found to best represent, in vitro and significantly in situ in cadavers, total wall thickness of intima plus media plus adventitia. Unlike the earlier study of Pignoli and colleagues, [8] in the present study the adventitia was measured histologically and not from gross pathology. Whereas they calculated wall thickness from combined histology and gross pathology, all the measurements in the present study were made from histology. In addition we quantified the extent of the shrinkage attributable to histology preparation (7.8%) and were able, by preserving the orientation of the vessel, to compare the B-mode ultrasound image with histological measurements in the same ultrasound plane.

The implication of these findings is that assessment of growth or regression of intimal lesions by ultrasound must assume that there is no contributing bias from the adventitia. If there is no change in the width of adventitia then interpretation of the $LE_1$-$LE_2$ distance as representing media and intima would not affect conclusions

Table 2
Comparison of histologic and ultrasound measurements (mm) of the far wall of the common carotid artery in four cadavers The histologic measurements have been corrected for 5.8% shrinkage (see Materials and Methods).

| | Histology | | | Ultrasound $LE_1$-$LE_2$ | |
|---|---|---|---|---|---|
| | Intima plus media | Adventitia | Total | In situ | In vitro |
| | 0.77 | 0.43 | 1.20 | 1.35 | 1.40 |
| | 1.10 | 0.32 | 1.42 | 1.30 | 1.36 |
| | 0.84 | 0.33 | 1.17 | 1.195 | 1.03 |
| | 1.24 | 0.46 | 1.81 | 1.33 | 1.32 |
| Mean | 0.99 | 0.38 | 1.40 | 1.29 | 1.28 |
| S.D. | 0.22 | 0.07 | 0.30 | 0.07 | 0.17 |

regarding changes in vessel wall thickness. Our intimal plus medial measurements were, of course, still highly correlated with $LE_1$-$LE_2$ measurements, although below the line of identity. Previously demonstrated associations between measurements made from B-mode images and lesions and cardiovascular risk factors (smoking [10,24–26], male sex [27], hypertension [24], serum LDL cholesterol [10,25,26,28,29], HDL cholesterol [25,29], diabetes [26,27,28], familial [31] and hypercholesterolemia [9], age [32,33]), are based on the interpretation that ultrasound is measuring intima plus media. While the present study does not invalidate these studies we believe that some caution should be exercised in making conclusions about intimal progression or regression. Associated changes in the thickness of the adventitia, or indeed the media, would lead to erroneous conclusions about intimal changes. The role of the adventitia, and particularly the vasa vasorum, in atherosclerosis is currently attracting increased attention [34]. In saphenous bypass grafts there is evidence that intimal thickening is accompanied by medial atrophy and an increase in adventitial width [35].

Finally, although the association between carotid atherosclerosis, based on counts of plaque prevalence, and coronary atherosclerosis has been established [2–5] additional studies will be required to determine the strength of association between carotid total wall thickness, as determined by ultrasound and coronary artery disease and whether total wall thickness can serve as an indicator of early atherosclerotic disease.

In summary, B-mode ultrasound measurements, made from the leading edges of both near and far vessel walls (in situ and in vitro) correspond best with total wall thickness and not with elastin or with the extent of the intima-media complex alone as has previously been proposed.

## 5. Acknowledgements

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