

HIGH RESOLUTION ULTRASOUND AND ARTERIAL  
WALL CHANGES IN EARLY ATHEROSCLEROSIS

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## **Abstract**

### **High resolution ultrasound and arterial wall changes in early atherosclerosis.**

Non-invasive vascular testing evolved initially to meet the needs of the surgeon to identify haemodynamically significant lesions. However, with refinement of techniques and the development of high resolution ultrasound, it has become possible to detect early lesions and to measure the thickness of the arterial wall with an accuracy of 0.2mm. Such measurements have epidemiological and prognostic potential. They may allow the study of progression (or regression) of atherosclerotic disease before symptoms develop. The aim of this thesis was to assess the value of arterial wall measurements of intima-media thickness and compliance of the common carotid artery in the prediction of early atherosclerotic disease.

Four different anatomical patterns of carotid bulb morphology have been identified, according to the position of the bulb origin in relation to the flow divider. It has been demonstrated that bulb morphology influences the site of early plaque formation. The presence of plaque at the carotid and femoral bifurcations was found to be associated with increased intima-media thickening of the common carotid artery. Histological analysis of common carotid arteries taken at post-mortem showed that this diffuse intima-media thickening is the result of deposition of cholesterol crystals, medial atrophy, fatty and fibrous change, and accumulation of necrotic debris, the features characteristic of plaques, even though discrete plaques rarely occur at this site.

Subsequently, a number of clinical studies were undertaken which demonstrated that the intima-media thickness is increased in diabetics, hypopituitary patients and claudicants as compared to controls. It was found that the intima-media thickness of the common carotid artery could be used to predict the presence of bifurcation plaques and macrovascular disease.

The work of this thesis has demonstrated that high resolution ultrasound is a powerful technique for the study of the arterial wall and should be tested in prospective studies for its suitability as an epidemiological tool.

## **Statement of Originality**

At the time that this thesis was undertaken, it was obvious that high resolution ultrasound could offer more than simply identifying the vessel lumen so that the arterial flow could be interrogated by Doppler. High resolution ultrasound imaging of the artery wall revealed a hypoechogenic layer bounded by echogenic layers, and Pignoli had demonstrated that the inner two layers correlated with the intima-media layer on histology (Pignoli, 1984). Subsequently, Salonen and colleagues reported an abnormally thick intima-media layer in hypercholesterolaemics (Salonen et al, 1988). It was becoming clear that intima-media thickness measurement had the potential of being a powerful epidemiological tool. Interest at this time was also focusing on arterial compliance as an indicator of early atherosclerosis. However a number of questions remained unresolved including the nature of intima-media thickening, and the association between diffuse wall thickening and evidence of atherosclerotic disease. The aim of this thesis was to assess the value of intima-media thickness and arterial wall compliance in the prediction of early atherosclerotic disease.

To assist the identification of early bifurcation plaques, preliminary studies were undertaken to identify the sites most frequently involved with plaque. These studies revealed that four bulb types could be identified by angiography and by ultrasound. Bulb type varied according to the position of the bulb origin in relation to the flow divider, and the bulb origin was the site most frequently affected by plaque. Though geometric variations at the carotid bifurcation have previously been reported (Fisher and Fieman, 1990), this is the first time that four bulb types have been described, furthermore, this is the first time that the bulb origin has been singled out as the site of origin of early plaques detected by ultrasound.

The presence of plaque at the carotid and femoral bifurcations was associated with common carotid artery intima-media thickening. Histological analysis of common carotid arteries taken at post-mortem showed that this diffuse intima-media thickening is the result of deposition of cholesterol crystals, medial atrophy, fatty and fibrous change, and accumulation of necrotic debris. It has previously been proposed that intimal thickening is an adaptive response to altered wall shear and tensile stresses (Glagov and Zarins, 1989) and that such intimal thickenings differs from atherosclerotic lesions by the



absence of lipid accumulations, necrosis or characteristic plaque topography. Results reported in this thesis show that diffuse intimal thickening has all the characteristic features of plaque except the local plaque formation.

Several clinical studies on volunteers and patients with a high prevalence of atherosclerosis are reported, the aim of these studies was to assess the ability of the common carotid artery intima-media thickness to predict the presence of bifurcation plaques and macrovascular disease. These showed that common carotid artery intima-media thickness is a good predictor of disease as only 6% of subjects with a mean common carotid artery intima-media thickness of 0.58mm or less had bifurcation plaques, compared to 50% of subjects with an intima-media thickness of between 0.59-0.82mm, and all subjects with an intima-media thickness of 0.82mm and over. Common carotid artery intima-media thickness is also a good predictor of the presence of occult macrovascular disease (on stress testing) as less than 5% of individuals with an intima-media thickness less than 0.82mm had macrovascular disease compared with 49% of individuals with an intima-media thickness over 0.82mm. This is the first time that a relationship between intima-media thickness, bifurcation plaques and macrovascular disease has been reported.

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**PART I**  
LITERATURE REVIEW

CHAPTER 1

THE HISTORY OF ULTRASOUND

## **1.1 The development of ultrasound.**

Ultrasonics, the study of sound propagated at frequencies above the range audible to the human ear is not new. In 1883, Galton was aware of the existence of ultrasound and the dog whistle used by him in his studies of the limits of the acoustic spectrum perceived by the human ear can be regarded as one of the first man made ultrasonic devices (Galton, 1883). All currently used ultrasound techniques are variations of echo-ranging devices. A pulse is generated and travels through a medium until it strikes a barrier (interface). A portion of the pulse will be reflected back and the time elapsed from emission to reception may be used to calculate the distance from the barrier to the sound source.

The first major development in ultrasound took place during the first world war, when Langevin in France began to investigate the use of quartz transducers for transmitting and receiving ultrasonic waves of relatively low frequencies in water and in 1916 Chilowsky and Langevin (Chilowsky and Langevin, 1927) developed the first working version of a marine sonar system.

After the war, rapid developments took place in the field of electronics and by 1925, quartz and nickel transducers were being used to generate and detect ultrasound at frequencies extending into the megaHertz range. During this time, several groups began to study the physical and biological effects of ultrasound. Langevin himself observed that when high intensity ultrasound was generated in a water tank, small fish were killed rapidly. Furthermore, severe pain resulted if the human hand was exposed to this intense ultrasound. Following the early recognition of biological effects, lower intensities ultrasound were used as a therapeutic agent. The diagnostic potential of ultrasound was identified much later.

## **1.2 The development of diagnostic ultrasound**

### **1.21 Transmission ultrasound**

The use of ultrasound in diagnosis appears to have been developed independently in several countries including Germany, America and England. Dussik was experimenting with transmission ultrasound imaging of the head in 1937 and published the first report a decade later (Dussik et al,1947). His encouraging results stimulated activity in a number of centres

and in the early 1950's, similar ultrasound transmission images were produced by a number of different groups. At that time, it was thought that the images contained an outline of the cerebral ventricles within the head. However, calcium in bones acts as a deflector of ultrasound, therefore it is not surprising that some of the original workers subsequently discovered that similar images could be achieved by scanning a water filled skull in which there was neither a brain nor ventricles. Following this, the use of transmission ultrasound as a diagnostic tool fell into disrepute and many workers abandoned their research.

### **1.22 Pulse echo scanners**

At the same time as Dussik was experimenting with transmission ultrasound, the possibilities of pulse echo techniques were being investigated by several groups. Howry and Bliss in Denver, Colorado developed a water immersion B-scanner during the late 1940's and published their first clinical images in 1952 (Howry and Bliss,1947). Independently, Wild and Reid were working along the same lines (Wild and Reid,1952). At about the same time, an industrial A-scan device was being used for the detection of echoes from within the skull, and as a consequence of the discovery of a cerebral midline echo by Leskell in Sweden (Leskell, 1956; Leskell, 1958) and Turner in London (Turner, 1952), interest was renewed in cerebral imaging, using echo techniques instead of transmission. Despite these promising early results, the American Atomic Energy Commission claimed that ultrasound pulse echo techniques were unsuitable for the detection of cerebral disease (US Atomic Energy Commission, 1955). Undeterred, workers in Europe continued their research which culminated in the development of echoencephalography (Leskell, 1958; Gordon,1959; Kazner, 1965).

### **1.23 Compound B-mode scanners**

The American water tank immersion scanners could not easily be applied to routine clinical examinations. This difficulty was overcome when the world's first contact compound B-scanner was developed by Brown in the late 1950's (Brown, 1960). Surprisingly good compound images were produced by this machine which could be used on a wide range of patients by Donald during the early 1960's (Donald and Brown, 1961). Another contact compound scanner which was producing clinical images in the mid 1960's was developed in

the USA (Holmes et al, 1965). Both devices consisted of a transducer mounted upon rectilinear frames, which restricted the range of movement of the scan head to the two dimensional plane of the frame. In 1966, Wells developed a hinged arm scanner, thus allowing the scan head to be moved in all directions (Wells, 1966). The hinged arm has now been abandoned, and the scan head is now held free hand. This is the most flexible arrangement, however, the hinged arm scanner could give spatial orientation, and position in coordinates, thus allowing you to return to the same scan position, this information is lost with the hand-held probe.

In B-mode ultrasonography, each reflection is represented as a dot on a screen, the brightness of which depends on the amplitude of the returning echo. The picture is built up from the multiple reflections. Early experimental B-scanners had displays with a moderately wide dynamic range, and therefore a limited degree of grey scale display. However, for the purpose of convenience in both scanning and photography, storage oscilloscopes were later introduced and produced bistable images in which there was no significant dynamic range. Apart from a few users, who continued to prefer open shutter photography techniques, most commercial B-scanners continued to use a bistable display until the mid 1970's. The notable exception to this rule was Kossoff in Sidney, Australia, who continued with the development of a variety of water bath scanners and produced some excellent high resolution images with a good dynamic range (Kossoff and Garrett, 1972). It was perhaps his work more than any other that stimulated the ultrasound equipment manufacturers to re-introduce greyscale imaging for the display of a wide range of echo amplitudes. This development was greatly facilitated by the development of the television scan converter tube in which the image could be stored as a charge pattern on a target within a vacuum tube and could then be read off as a conventional television image. Subsequently electrical digital techniques have been developed which permit storage of echo amplitudes in 256 increments. This gives a very satisfactory grey scale, but the image is composed of pixels which give an inherent resolution of approximately half that of the analogue scale converter.

### **1.24 Real time scanners**

The second major technical development to occur during the 1970's was the development of the real time scanner. In real time ultrasonography, retrieved ultrasound information is updated at a rate that is rapid enough to allow visualization of physiological motion within the field of view (over 15 frames per second is perceived as continuous by the eye). The early research on these devices was performed during the late 1960's notably by Somer in Utrecht (Somer, 1968) and Bom in Rotterdam (Bom et al,1971). Bom pioneered the linear array transducer in which a series of adjacent transducer elements were energised in sequence and produced moving images of the intra-cardiac structures. Somer developed the phase array scanner, though his early work was hampered by a lack of adequate electronic switching devices and delay lines, which were essential to allow rapid switching between transducers and the transmission of the image to the screen.

The third major development in real time imaging was the introduction of the simple mechanical rotating probe device. Many people seem to have thought of the device simultaneously and it is difficult to be certain who should be credited for the first prototype but it was probably McDicken in Edinburgh (McDicken, 1974).

### **1.25 Ultrasonic Doppler arteriography**

While research in to ultrasound imaging continued, other workers were investigating the value of the Doppler technique for the detection and measurement of moving structures. Continuous wave Doppler ultrasound uses the phenomenon of the Doppler effect: this is the phenomenon by which the frequency of a wave received after reflection by a moving target is shifted from that of the source. The classic example of this is the change in pitch in a train's whistle as it passes a stationary listener.

In 1954, Kalmus reported the development of an electronic flowmeter system (Kalmus, 1954), and by 1959, flow patterns in peripheral arteries were being studied with an ultrasonic Doppler shift velocity meter (Satomura, 1959). Early Doppler devices used continuous wave ultrasound, and this technique is still used today. However continuous wave Doppler systems detect the movement of any object in the path of the sound beam and

for this reason cannot be used to detect the depth of the vessel being examined, its diameter or the nature of velocity distribution across the vessel. By the late 1960's the development of coded Doppler signals and pulsed Doppler devices were being considered and by the early 1970's several Doppler vessel imaging devices were described (Reid and Spencer, 1972; Fish, 1975). Hokanson and associates took advantage of the ability of pulsed Doppler to identify a locus in space to construct a flow map that depicted an image of the internal dimensions of the artery - "the ultrasonic arteriogram" (Hokanson et al, 1971; Mozersky et al, 1972). The system they used consisted of a 5MHz pulsed Doppler, a position sensing arm and a storage oscilloscope. A directional Doppler device with six sample gates was used to allow positioning at the desired depth. For each point in the tissue where flow was detected with any of the six sample gates, a spot would be illuminated on a storage oscilloscope corresponding to that point in space. Each picture took 10 minutes to build and required the patient to lie very still. The result was a picture of the lumen of the vessel. The quality of this picture was inadequate for accurate assessment of degrees of stenosis, this still required audible analysis of the Doppler signal at the centre of the lumen. The main value of the technique was in the diagnosis of occlusion where the error rate was 4% (Barnes et al, 1976). This system found its greatest application in the carotid region. One of the problems of ultrasonic arteriography was that arterial calcification either in the wall of the artery or in the plaque itself, resulted in acoustic shadowing and a sonolucent blank on the oscilloscope screen, mimicking arterial occlusion and making it impossible to determine the degree of stenosis present accurately. By 1976, it was recognised that the spectral analysis of the velocity information could be a useful adjunct in detecting stenoses that reduced the diameter by as little as 20% if the recordings were made within a few diameters of the lesion itself (Giddens et al, 1976; Reinerston and Barnes, 1976); this observation contributed to the development of a system which included real time spectral analysis to provide flow velocity information with flow visualisation.

### **1.26 Duplex Scanning**

In 1974, the duplex scanner was created by the combination of real time ultrasonic imaging and gated Doppler ultrasound (Baker, 1970; Barber et al, 1974). By altering (i.e. gating) the interval between an emitted burst of ultrasound and the time of listening for the reflected



echoes, ultrasonic sampling of the Doppler effect could be accomplished at different depths and positions defined by the gating selected by the operator. The Doppler shift signal was then presented on a real time display using fast Fourier transform analysis. A further advantage of the duplex scanner was that, by imaging the tissues, it permitted the measurement of the angle of insonation in relation to the axis of the vessel, allowing calculation of velocity in centimetres per second.

The first generation of duplex scanners were developed in the 1970's for use in the detection and grading of carotid bifurcation disease (Breslau et al, 1982). By the early 1980's, a second generation of duplex scanners was developed. These machines had a superior image quality. Also, the size of the Doppler sample could be varied so that insonation across the whole lumen of the vessel became possible (wide gate) with automatic calculation of the angle of insonation, mean Doppler shift and automatic expression of the result in cm/sec (Persson and Robichaux, 1983). In addition, low frequency probes (2.5-3MHz) enabled the study of the abdominal vasculature. These second generation instruments were more versatile and the operator was not limited to insonating a vessel to a standard angle, usually  $60^{\circ}$ . This period saw the application of duplex scanning to practically all the vessels in the body.

### **1.27 Colour duplex scans**

One of the advantages of duplex scanning, over simple ultrasonic imaging, is the ability to detect fresh thrombus and plaques with the same echogenicity as blood. These may be detected by recording the haemodynamic disturbance produced by such a thrombus, stenosis or wall irregularity. In the carotid arteries, this is relatively quick and easy, however in long vessels, the detection of a high velocity jet indicating a stenosis requires sampling at regular intervals along the vessel lumen; a process that is tedious and time consuming. This problem has been overcome by the third generation of instruments which provide real time colour flow imaging superimposed on the real time grey scale imaging. The use of phased array transducers and microchip technology provides a means of testing every pixel on the screen for Doppler shift. Thus, any movement is depicted as colour; red for flow in one direction, blue for the reverse; and the higher the frequency of the Doppler shift, the whiter the colour

depicted. Absence of colour indicates absence of flow. The colour flow map is superimposed on the real time grey scale image. Thus an artery can be seen as a pulsating red lumen, a thrombus or atherosclerotic plaque as a black area protruding into the lumen and a tight stenosis as a white jet (Persson, 1989). Visual localisation of the problem area allows the sample volume to be quickly positioned at the tightest point; velocity and diameter can be measured, and flow will be calculated automatically (ml/min). As yet, the reproducibility and clinical usefulness of the flow measurements have not been fully evaluated.

A colour duplex scanner, although approximately twice as expensive as earlier scanners, is three to four times quicker to use. Because more patients can be studied per session, the instrument has been shown to be more cost effective. Colour flow imaging allows follow up studies to be performed safely and quickly, at regular intervals, holding the promise of a better understanding of the pathophysiological changes in arterial disease (eg. flow patterns in relation to atherogenesis, and progression or regression of disease in response to therapy).

### **1.28 B-mode ultrasound and the carotid artery**

In 1969, Olinger published the first report of the visualisation of the carotid artery by a B-mode ultrasound technique (Olinger, 1969). Initial B-mode studies were performed with a handheld transducer and a compound scan was recorded on a storage oscilloscope (Olinger, 1969; Blue et al, 1972). These studies demonstrated wall abnormality and lumen information. They were limited by the technical inability to obtain satisfactory multiple-plane scans of the bifurcation and the inability to separate the external and internal carotid arteries at the bifurcation in many cases. This was primarily related to the time-consuming effort of compound scanning as well as anatomic restrictions of the anterior/posterior scanning position.

B-mode imaging allowed the carotid artery to be visualised, however the limitations were quickly recognised. Firstly, as calcium inhibits the transmission of the ultrasound, a calcified plaque produces a bright echo with an acoustic shadow behind. Therefore, in a heavily calcified vessel, visualisation of the lumen is impaired. Secondly, the acoustic reflectivity of soft fibrofatty plaque often approaches that of blood, making a sharp

delineation of the vessel lumen difficult. This problem is even greater in the case of thrombus, either in occluded vessels or in association with plaque ulceration, as the acoustic reflectivity of a thrombus is the same as that of blood. These technical problems and the poor quality pictures obtained by the early ultrasound scanning machines did not inspire a great deal of confidence in the use of the ultrasonic arteriograph in the investigation of carotid artery disease and the emphasis lay in the use of velocity waveform analysis to detect and grade internal carotid artery stenosis.

The earliest studies used probes with a 2MHz transmitting frequency. Resolution is dependent on frequency, therefore, to improve the resolution, higher frequency probes were developed. In 1978, Green reported the results obtained using a focusing probe with a 10MHz transmitting frequency (Green, 1978). These B-mode images were recorded on video tape and Polaroid pictures taken for subsequent review. The optimum focal depth of the focusing 10MHz probe is 3-4cms.

Table 3.1 lists the most notable early reports on the use of B-mode ultrasound, with the transducer frequency and the size of the study. The largest of these studies (Camerota, 1982) examined 4000 patients over a 26 month period, of these 884 had contrast arteriography. In addition pulse arrival time oculoplethysmography and carotid phonangiography were performed. Noninvasive studies were evaluated as to their quality and the quality was then correlated to accuracy. This study showed that B-mode ultrasound had a specificity of 87%, with a sensitivity of 72% for detecting a stenosis of between 40-69% and 62% for a stenosis of greater than 70%. They noted that in the majority of their 49 scan/angiogram mismatches, the scan showed a higher level of disease than the angiogram; however, 12 of these patients underwent carotid endarterectomy, and, in the opinion of the operating surgeon, the scan was more correct than the angiogram in 10 cases. They drew several important conclusions from their study which are still applicable today. Firstly, that the accuracy of the real time B-mode image is directly related to the quality of the image and the severity of the disease within the vessel ie the grade of stenosis. Secondly, that as disease increases, the quality of the scan decreases. Thirdly, a scan of good quality had a high degree of accuracy no matter what the grade of disease. Finally, an occluded internal carotid artery cannot be diagnosed by B-mode ultrasound alone.

**Table 1.1**      **Studies using B-mode ultrasound to detect carotid arterial disease**

Author	Year	No. vessels scanned	Transducer (MHz)
Olinger	1969	120	2
Blue	1972	30	2
Green	1978	15	10
Mercier	1978	21	2
Cooperberg	1979	52	5-7.5
Hobson	1980	84	6-11
Humber	1980	81	10
Fell	1981	270	5
Camerota	1982	884	8

### **1.3 Applications of duplex scanning in diagnosis**

#### **1.31 Duplex scanning of the carotid arteries**

At present, Duplex scanning is the method of choice in screening for suspected extracranial cerebrovascular disease (Roederer and Strandness 1984; Williams et al, 1989). It has replaced most of the indirect tests such as bruit analysis, oculoplethysmography and periorbital Doppler velocity studies, because both functional and anatomical information can be achieved by the one study. In addition to the detection and grading of stenoses, the high resolution imaging achieved with modern day linear transducers allows plaques to be characterised and intraplaque haemorrhage detected (Reilly et al, 1983; Bluth et al, 1986). Duplex scanning has provided information on the natural history of patients with asymptomatic carotid disease and has allowed us to identify a high risk group. As a result, we now know that stenoses of less than 80% have a 1-3% risk of producing a stroke, whereas, in the presence of a stenosis greater than 80%, the risk is 4-12% per annum (Roederer GO(iii), 1984; Chambers and Norris, 1986; and Hennerici et al, 1987.). The risk is greater still if, in the presence of a stenosis of greater than 80% as detected by the peak systolic flow, the end-diastolic flow at the stenosis is creating a Doppler shift of greater than 200 cm/sec. (Bogousslavsky et al, 1986, Moneta et al, 1989). Such a high diastolic velocity indicates the presence of a high pressure gradient as a result of poor collateral circulation.

#### **1.32 Duplex scanning of the subclavian, innominate and vertebral arteries.**

Duplex scanning may also be used for the examination of the subclavian and innominate arteries as well as the origin of the vertebral artery (Visona et al, 1986). These vessels can be identified during their course through the supraclavicular fossa. In this region, a stenosis of greater than 50% can be identified with a sensitivity of 88% and a specificity of 98% (Ackerstaff et al, 1984). Colour flow facilitates the identification of the vertebral arteries at their origin and enables them to be followed along their course between the transverse processes to the base of the skull. With colour flow imaging, the diagnosis of subclavian steal is greatly facilitated as retrograde flow in the vertebral artery will be blue i.e. the opposite to the carotid which is red; also, when the peripheral resistance of the arm is reduced by

releasing a clenched fist, the steal is increased, hence the frequency of the Doppler shift is increased. One of the drawbacks in the examination of the subclavian, innominate and vertebral arteries is that the above applies only to the portions visualised through the supraclavicular fossa.

### **1.33 Duplex scanning of the aorta and its branches**

At the present time, with the exception of the ascending aorta, the supra-diaphragmatic aorta is relatively inaccessible to duplex scanning. Below the diaphragm, the aorta is easily accessible to ultrasound and, in the last 15 years, this method of examination has become common practice in the monitoring of early aneurysmal dilatation. Duplex scanning has recently been applied to this area. The aorta has a characteristic Doppler spectral pattern with a clear "window" in systole, indicative of flow at a single velocity across the entire vessel. Pathological widening or narrowing of the aorta induces changes in the spectrum and aneurysmal dilatation will result in flow separation and turbulence. This is seen particularly well with colour flow imaging. Also, the ability to show the extent of flow makes this a suitable method with which to demonstrate the true lumen of a dissecting aneurysm or to map thrombus that may not be apparent on the B-mode image.

### **1.34 Duplex scanning of the peripheral vascular tree**

Until recently, arteriography has been considered the "gold standard" in the assessment of the anatomy of the peripheral vascular system, complimented by a variety of non-invasive tests to assess the haemodynamics. Following the improvement in the resolution of the B-mode image, duplex scanning is being used increasingly in the assessment of peripheral vascular disease. Duplex scanning has been shown to reliably differentiate between haemodynamically significant and insignificant lesions in the aorto-iliac and femoro-popliteal arteries and has the potential to replace angiography. In a study comparing duplex scanning with intra-arterial digital subtraction angiography, Legemate and his colleagues have shown a stenosis of greater than 50% in diameter could be detected with a sensitivity of 92% and a specificity 98% for the aorto-iliac segment and 88% and 98% respectively for the femoro-popliteal segments (Burns, 1990). Duplex scanning of the peripheral vasculature is slow and time-consuming. The examination is much faster with the colour flow duplex.

Colour flow imaging can accurately identify the presence and extent of occlusions in 94% of cases when compared with angiographic and operative findings. In the diagnosis of stenoses of greater than 50%, a specificity of 87% as compared to the 99% for arteriography has been obtained. This means that a decision to refer a patient for angioplasty can be made without an angiogram (Legemate et al, 1989). Duplex scanning may also be used before operation to assess the adequacy of the long saphenous vein for in situ by-pass grafting with an accuracy of 90% (Cossman et al, 1989)

### **1.35 Intraoperative scanning**

A duplex scanner, due to its relative mobility, lends itself well to use in the operating theatre. Intraoperative duplex scanning is now replacing intraoperative angiography after carotid endarterectomy (Bandyk et al, 1988; Cata et al, 1989), infrainguinal bypass grafting and mesenteric or renal reconstructive procedures. Wall defects that warrant immediate surgical correction have been demonstrated in 5% of internal carotid arteries following endarterectomy and 7% of renal and visceral reconstructions. Retained valves and anastomotic defects occur in 5% of in-situ saphenous vein by-pass grafts, and prompt action as a result of intraoperative diagnosis can reduce the incidence of early graft failure. Using colour flow imaging, a carotid endarterectomy site can be scanned in 5 minutes and an in situ saphenous vein arterial by-pass in 10 minutes. This technique is not only safer than angiography but also far quicker, and can be repeated as necessary.

### **1.36 Postoperative surveillance**

Stenotic narrowing develops in 25% of infrainguinal vein grafts; unchecked, this may lead to premature graft occlusion. The post-operative monitoring of infrainguinal grafts allows early detection of stenosis and therefore early intervention with a consequent improvement in graft patency rates; and, whereas graft failure could result in amputation, a stenosis may be treated by angioplasty or a minor operation. In the absence of a stenosis, a low peak velocity (<45cm/sec) in the graft indicates proximal or distal disease progression and impending graft failure (Grigg et al,1988; Bandyk et al,1988). In 1986, our radiology department performed 406 angiograms in patients with infrainguinal grafts. In 1987, all grafts were examined by duplex scanning as well as angiography. As a result, duplex scanning was shown to have a

sensitivity of 100% and a specificity of 96% (Grigg et al, 1988). Since then, duplex scanning has been our method of choice for routine graft surveillance. In 1988, only 22 graft angiograms were performed; all were performed to confirm the duplex scan finding of a stenosis of greater than 50%. This practice has kept the waiting list for angiography relatively short.

### **1.37 The detection of deep venous thrombosis**

The use of duplex scanning is not limited to arterial disease. In the detection of deep venous thrombosis, the accuracy of duplex scanning is high. Comparison to venography has revealed both a sensitivity and a specificity close to 100% above the calf (Hannan et al, 1986; Semrow et al, 1987) with a sensitivity of 70% below the knee. In hospitals with a duplex scanning service, this is now the investigation of choice and venography is rarely performed in patients with suspected deep venous thrombosis.

### **1.38 The detection of venous insufficiency**

In varicose vein surgery, the anatomy of the popliteal fossa has been shown to be very variable, with only 56% of sapheno-popliteal junctions arising within 2-5cm of the knee joint. In 30% the short saphenous vein joins the deep system in the thigh or continues to the proximal long saphenous vein. The accuracy of a clinical examination at identifying the sapheno-popliteal junction to within 2cm is only 56% whereas the accuracy of duplex scanning to identify this junction to within 2cm is 96%; thus a duplex scan to mark the site of the junction on the day of operation would eliminate the need for on table venography practiced by some and would provide every surgeon with the ability to accurately position the skin incision and do the correct operation.

In the investigation of chronic venous disease, not only can duplex scanning be used to detect reflux in both superficial and deep veins, but it also makes quantification of reflux practical. Abnormal retrograde flow at peak reflux can vary from 2 to 40ml/sec. Reflux greater than 10ml/sec is associated with a high incidence of venous ulceration irrespective of whether such reflux is in the deep or superficial system (Vasdekis, 1989). Colour flow imaging can provide a quick and accurate method of demonstrating and localising sites of



reflux from the deep to the superficial system (saphenofemoral and saphenopopliteal junctions) and is particularly useful in the localisation of incompetent thigh and calf perforating veins (Nicolaidis et al,1989).

### **1.39 Duplex scanning in abdominal ultrasonography**

The duplex scan is not only a powerful diagnostic tool for the vascular surgeon, but also provides a versatile imaging technique with a multitude of clinical applications. The recent introduction of probes that combine high resolution ultrasound with Doppler at frequencies suitable for abdominal scanning has opened exciting new avenues for the abdominal ultrasonographer. With duplex scanning, the intra-abdominal vessels can be identified both visually and by their "flow signatures", Characteristic deviations from these appearances are seen with stenoses. In addition, the ability to indicate a direction of flow using Doppler techniques is helpful in evaluating portal vein haemodynamics in disease. In portal hypertension, there is loss of the normal respiratory variation (Bolondi, 1990) and reversal of flow may be seen in the portal or splenic vein. Spontaneous portosystemic shunts may also be identified (Taylor, 1988) and, at present, duplex scanning is the method of choice for checking portocaval shunt patency following surgery.

The mesenteric vasculature is also amenable to examination by duplex scanning. Each vessel has its own characteristic blood velocity pattern and change in flow can be detected following physiological stress (standard meal). This response is altered by pathological changes in the vessels. For instance, in health, blood flow in the superior mesenteric artery doubles following a standard meal; in the dumping syndrome, this response is greatly exaggerated (Qamar et al, 1986). Many investigators now use duplex scanning as their first line investigation for superior mesenteric and coeliac trunk stenosis in patients with suspected mesenteric angina.

The detection of renal artery stenosis is difficult and time consuming, and requires a skilled operator; however, in the right hands, a sensitivity of 91% and specificity of 95% can be achieved (Norris et al, 1984; Kohler et al, 1986 ). Only 1% of patients with hypertension have renal artery stenosis and a quick non-invasive screening technique is

needed. Colour flow imaging will improve the ease with which this condition can be diagnosed.

In the field of transplantation medicine, duplex scanning has been used to monitor renal transplant parenchymal blood flow and renal artery velocity patterns. In acute rejection, the renal vascular resistance increases, therefore diastolic blood flow velocity decreases and sometimes even reverses (Rigsby et al, 1987). This is the first indication of the onset of rejection. Improved diastolic flow can be detected in patients who respond to therapy. In hepatic transplantation, duplex scanning allows pre-operative identification of the anatomy. After operation, regular monitoring by duplex excludes thrombosis of the major vessels (Wozney, 1986; Letourneau et al, 1987).

An exciting area of promise is in the identification of neoplastic growths. Neovascularization occurs in malignant tumours and its detection may aid diagnosis, characterise the tumour as slow growing or aggressive and allow assessment of response to therapy. Liver tumours may sometimes be differentiated into haemangiomas, hepatomas and metastases on the basis of flow characteristics (Taylor et al, 1987).

#### **1.4 High Resolution Ultrasound**

The development of real time B-mode imaging greatly improved the capability of the instrument to determine lumen and arterial wall characteristics and dynamics. The resolution of the ultrasound probe is dependent on frequency, transducer design, depth of field, and echogenicity of the tissue. Early studies used low resolution probes with a long focal depth, originally developed for intraabdominal work. At that time, it was felt that the main use of ultrasound in arterial disease was to guide the operator in the placement of the sample volume in duplex ultrasonography. In the late 1970's, advances in the miniaturisation of electronics meant that a greater number of transmitting and receiving crystals could be incorporated on to the scan head. Also, dedicated linear ultrasound probes with higher transmitting frequencies (7.5-10 MHz) and short focal lengths were developed specifically for study of superficial vessels. This led to improvement in the image quality and a renewed interest in B-mode ultrasonography in the diagnosis of arterial disease.

In 1981, the National Heart, Lung and Blood Institute awarded contracts to five centres in the United States to establish the capabilities of high resolution B-mode ultrasound imaging for detection and quantification of atherosclerotic disease of the carotid and iliofemoral arteries of human subjects. Comparison of ultrasound, angiography and pathology in the evaluation of atherosclerosis of the carotid bifurcation was performed on a cohort of 892 patients (Ricotta et al, 1990). The ability of B-mode ultrasound to identify angiographically apparent atherosclerotic disease was found to be good with a sensitivity of 88% and a specificity of 79%. In addition, B-mode ultrasound was more sensitive than angiography in detecting minor degrees of atherosclerosis, since 50% of normal angiograms were found to have disease on ultrasound examination. Quantitative comparisons between ultrasound angiography and pathology were disappointing. Lesion width, minimal residual lumen and standard lumen were all larger when measured sonographically than by angiographic techniques. Furthermore the predictive value of ultrasound with respect to angiographic findings was modest. The authors concluded that functional studies, specifically Doppler frequency analysis, improved correlation of maximal residual lumen, % stenosis and diagnosis of occlusion. Both diagnostic techniques underestimated lesion width and overestimated maximal residual lumen when compared to data obtained from endarterectomy specimens, although ultrasound correlated better with lesion width and angiography correlated better for maximal residual lumen.

## CHAPTER 2

### ASYMPTOMATIC CAROTID ARTERY DISEASE

## **2.1 Introduction**

Stroke is the third commonest cause of death in the Western world. It is also a major cause of disability in middle aged and elderly people. More than 68,000 people died of cerebrovascular disease in England and Wales in 1991, and the majority of these died following strokes. It is estimated that in the United States there are approximately 450,000 new strokes a year, and approximately 75% of these are due to thromboembolic disease. Carotid atherosclerosis is most important single aetiological factor responsible for the development of focal cerebral ischaemia (Gelabert and Moore, 1991). Unfortunately, the majority of strokes occur without warning. To reduce the incidence of strokes, we need to identify patients at risk of developing carotid artery disease and to understand the natural history of the disease along with the benefit and risks of medical and surgical intervention.

## **2.2 Anatomy of the carotid bifurcation**

The common carotid artery arises from the innominate artery on the right and the aortic arch on the left. It is a conduction vessel with no branches. The common carotid artery bifurcates within the carotid sheath, usually at the level of the upper border of the thyroid cartilage. The carotid bulb is found at the origin of the internal carotid artery and may extend to the terminal portion of the common carotid artery (Last, 1978). Studies of the carotid bifurcation have recognised variations in carotid bulb morphology, in particular variations in the angle between the internal and external carotid artery (Bharadvaj et al, 1982; Fisher and Fieman, 1990), but the frequency with which variations occurs is unknown. The carotid bulb is richly innervated by the glossopharyngeal nerve and has a baroreceptor function. It is also the point of transition from the elastic (CCA) to the muscular type of artery (ICA). In the carotid bulb, the inner zone of the media has the structure of a conducting artery (Heath et al, 1973). In the outer zone the elastic fibrils are often packed closely together with no muscle fibres interspersed between them. According to the studies reported by Burring and Hort, the absolute thickness of the media is 260-300  $\mu\text{m}$ , at the bulb origin, and there is a short segment of medial thinning (Burring and Hort, 1988). This corresponds with a transitional area of endothelial organization. Opposite the flow divider, this forms an

eccentric intimal fibrosis and anatomical studies have confirmed that this is the position in which fatty streaks and plaques develop (Burrig and Hort, 1988).

## **2.3 Pathology of the carotid artery**

### **2.31 Atherogenesis**

Three theories exist which explain the development of atherosclerosis. These are the thrombotic mechanism (initially proposed by Rokitansky in 1853), the endothelial damage/platelet deposition theory (Ross and Glomset, 1976) and the insudation of plasma theory (Kritchevsky, 1986).

The thrombogenic theory proposes that blood components are repeatedly deposited in the arterial wall lumen, these subsequently become incorporated in to the vessel wall with fibrous tissue formation. This theory was initially proposed by Rokitansky in 1853, and subsequently reintroduced by Duguid in 1946 as a result of his observations on coronary arteries (Duguid, 1946). By this theory, microemboli become adherent to the vessel intima and become incorporated in to the wall at sites of altered endothelial function. This theory was supported Lusby and colleagues who showed that thrombi formed on the luminal surface of plaques a few hours after sudden mural distension (Lusby et al, 1982). Similar thrombi were also seen on carotid endarterectomy specimens in association with plaque ulceration (Lusby et al 1982). At sites of increased endothelial turnover, such as at the bifurcations, the function of the endothelium is disturbed and platelets aggregate as microthrombi. These can release a number of growth factors or mitogens including platelet derived growth factor (PDGF) and transforming growth factor-B (TGF-B) which promote connective tissue synthesis. PDGF is also secreted by endothelial cells on exposure to activated factor X or thrombin. The thrombotic theory can account for the presence of blood components within plaques, but not lipid. (Born, 1992).

The endothelial damage/platelet aggregation theory was proposed by Ross and Glomset in 1976. According to this theory, atherosclerosis develops as a consequence of endothelial cell denudation or retraction which allows an influx of lipoproteins across the basement membrane (Ross and Glomset, 1976). Lipoproteins can either be taken up by

macrophages, or remain free in the extracellular space. Subsequently, it was observed that monocytes and leucocytes can adhere to the endothelial surface and migrate between the cells (Ross and Wight, 1984; Ross, 1986; Ross et al, 1986). Some of the biochemical signals attracting monocytes to the vessel wall and mediating their augmented adherence to endothelial cells are beta-VLDL, modified LDL, interleukin-1, eicosanoids, complement activation products, and polyanions (Hartung and Hennerici, 1988). In hypercholesterolaemic animals, these cells take up lipids and, once in the subendothelial space, become foam cells. Endothelial denudation also results in platelet aggregation, these release PDGF and stimulate smooth muscle cells to migrate in from the media. Experimentally, the smooth muscle cells regress if a sustaining factor such as elevated LDL is not present. Both smooth muscle cells and macrophages are capable of secreting growth factors and initiating atherosclerosis. The basis of this theory is that endothelial injury induces atherogenesis, but there is no clinical or pathological evidence that this occurs in humans (Born, 1992).

Lipid accumulation is now believed to occur due to insudation of plasma lipoproteins through the intima. This was first proposed by Anitschkow in 1933, as a result of studies on hypercholesterolaemic animals (Anitschkow, 1933), but lipid insudation is probably a normal physiological event (Kritchevsky, 1986). The predominant lipid in developing plaques is low density lipoprotein (LDL) (Stenders and Silversmit, 1981). These large plasma proteins can pass through an unbroken layer of viable cells by transcytosis, by receptor mediated and receptor independent mechanisms (Brown and Goldstein, 1983; Brown and Goldstein, 1986). Once through the vessel wall, the lipid is taken up by macrophages which have a number of receptors for this purpose (Weisgraber et al, 1985). Lipids may be chemically altered by endothelial cells and macrophages creating acetylated and oxidised derivatives (Steinberg et al, 1985). These modified lipids accumulate in macrophages where they are chemotactic to monocytes and toxic to endothelial cells (Hartung et al, 1986). They also have antigenic effects which contribute to the inflammatory reaction and may stimulate T-cells to secrete cytokines which promote cellular recruitment and proliferation (Fogelman et al, 1984). Thus the insudation of lipid may contribute to atherogenesis in a number of ways (Born, 1992). Macrophages have a number of functions which contribute to the development of atherosclerosis: they are phagocytic scavengers which produces intimal debris, they process antigens activate lymphocytes and secrete complement; they

catabolise lipoproteins and synthesise biologically active lipids (PGE<sub>2</sub>, PGI<sub>2</sub>, TXB, PAF), they secrete mitogens (eg PDGF) which promote smooth muscle proliferation, they also secrete a number of other compounds which cause pronounced inflammatory responses including the enhancement of endothelial permeability, recruitment of leucocytes and platelets, induction of release reactions as well as promoting tissue damage such as the endothelial injury caused by oxygen radicals (Hartung and Hennerici, 1988).

### **2.32 Natural history of carotid plaques**

Atherosclerosis is the principal pathological lesion which affects the carotid bifurcation. Three lesions have been described, the fatty streak, the gelatinous plaque and the fibrous plaque (Solberg and Eggen, 1971). Mature atheromatous lesions are characterised by four histological features, namely cellular proliferation, predominantly of the smooth muscle cell, increased lipids, particularly cholesterol esters, increased connective tissue elements, such as elastins and glycosaminoglycans, and foam cells, predominantly representing lipid laden monocyte derived macrophages.

#### **2.321 The fatty streak**

The fatty streak used to be considered the precursor to atherosclerotic plaques, but this was debated by Haust (Haust, 1971) and it is now generally believed that only a few fatty streaks progress to form fibrous plaques, the majority either regress or do not change with time (Millan and Lusby, 1993). Both the fatty streak and the gelatinous plaque are characterised by the accumulation of lipids. The lipid responsible for the fatty streak is derived from circulating lipid which infiltrates the vessel endothelium, this is then taken up by macrophages derived from circulating monocytes. The macrophages migrate subendothelially and become foam cells, the characteristic feature of fatty streaks (Fagiotto et al, 1984). More advanced lesions may also contain media derived smooth muscle cells, which are capable of collagen production (Moss et al, 1968).

#### **2.322 The gelatinous plaque**

The gelatinous plaque is composed of extra-cellular matrix and fluid, with a variable degree of cellularity and a relatively low lipid content (Smith and Staples, 1982; Smith, 1988). Gelatinous plaques may be the precursor to fibrous plaques.

#### **2.323 The fibrous plaque**



The fibrous plaque is the lesion responsible for the atherosclerotic disease process. The fibrous plaque is made up of two distinct entities, the fibrous cap overlying a lipid core. The fibrous cap is composed of a collagen rich matrix containing smooth muscle cells and macrophages. The lipid core is largely made up of lipid deposits and foam cells. Extracellular lipids are found close to the internal elastic lamina (Hata et al, 1974) and in this position provoke an intense inflammatory reaction (Modrak and Langer, 1980; Baranowski et al, 1982). Angiogenesis develops within the plaque in response to this inflammatory reaction, and these fine vessels may haemorrhage within the plaque. Intraplaque haemorrhage, either due to blood arising from the rupture of angiogenetic vessels or from blood entering through cracks, results in sudden changes in plaque size, (Lusby et al, 1982). Areas of necrosis may also develop within the plaque, creating a heterogeneous core containing extracellular lipid, cholesterol crystals and tissue debris. Finally calcification may occur.

The complications of fibrous plaques which appear to result in clinically significant sequelae are ulceration, plaque fracture, the development of luminal thrombosis overlying the plaque and haemorrhage within the plaque (Lusby et al, 1982; Imparato et al, 1983). As part of the healing process after plaque haemorrhage, calcium may be deposited. This results in the formation of a complex plaque which may release emboli that are incapable of disaggregation if released in to the blood stream (Matalanis and Lusby, 1988).

### **2.33 Sites of atheroma formation**

The development of the fatty streak is not a uniform process, certain vessels, namely the carotid bifurcation, the superficial femoral arteries, the infra-renal aorta and the coronary arteries are most affected, whereas the subclavian, profunda femoris and brachial arteries are relatively spared. Atherosclerotic lesions also tends to occur predominantly in certain vessels, namely vessels of conduction, or at points of origin of their side branches or bifurcations rather than in vessels of supply. Atherosclerotic lesions of the intracranial vessels are predominantly fibrous while extracranial arteries, such as the carotid bifurcation are both fibrous and complex.

There are racial and sex differences in the distribution of vascular lesions, for instance white men have a predilection<sup>for</sup> atherosclerotic lesions at the carotid bifurcation, and also

have a high incidence of coronary and peripheral vascular disease. In contrast, women, blacks and orientals have a higher incidence of intracranial stenosis, and a lower incidence of severe carotid bifurcation, coronary, and peripheral vascular disease (Gorelick, 1986). The incidence of disease at the carotid siphon is less than that at carotid bifurcation, and occurs equally in both sexes and in blacks and whites. Main stem middle cerebral artery disease is much less common than carotid bifurcation disease, and is more common in black and individuals of oriental origin, but unlike carotid bifurcation disease, does not correlate with male predominance, or associated coronary or peripheral vascular disease.

### **2.38 Site of origin of atherosclerotic lesions in the carotid bifurcation**

The site of origin of plaques at the carotid bifurcation has been questioned by a number of researchers. Solberg and Eggen examined cross-sections of the carotid bifurcation and found that early lesions start at the bifurcation itself and from that point progress proximally along the common carotid and distally only to the proximal portion of the internal carotid artery (Solberg and Eggen, 1971). In contrast, Meyer and Noll examined fatty streaks in the carotids of children and young adults and found that lipid deposits appeared to occur first in the peripheral portion of the carotid bulb and extend centrally (Meyer and Noll, 1974). Burrig and Hort report that fatty streaks and fibrous plaques were found predominantly on the outer walls in a U shaped pattern, while the flow divider remains relatively free of plaque (Burrig and Hort, 1988). They also noted that in over a half of vessels they studied a small fibrous ridge could be seen at the origin of the ICA, if fatty streaks or fibrous plaques were present.

### **2.4 Haemodynamics and carotid bifurcation disease**

The predilection sites for spontaneously occurring atheromatous lesions are the sites opposite the flow divider at the bifurcation of large arteries. These sites are characterised by endothelial cells which exhibit increased permeability to macromolecules and lack the normal longitudinal axial orientation to the vessel axis (Schwartz et al, 1983; Burrig and Hort, 1988). It is generally believed that the haemodynamics at <sup>the</sup> bifurcation are the reason the atherosclerotic lesions develop preferentially at these sites, and a number of studies have been reported which address this issue. At the carotid bifurcation, velocity patterns have been examined with the use of experimental and numerical models

(Bharadvaj et al, 1982; Ku and Giddens, 1983; Rindt et al, 1987). Measurements have been performed in vitro (Motomiya and Karino, 1984) and in man (Reneman et al, 1985) and flow patterns have been recorded using a multigate pulsed Doppler system (Hoeks, 1982).

Historically, attempts to explain arterial flow abnormalities were guided by the misconception that flow in arteries was laminar, and abnormalities of flow produce local stasis or local turbulence. However, studies on fluid dynamics have revealed that in pulsatile flow, true turbulence does not occur, rather, regular yet complex separation phenomena occur during the acceleration of systole and the deceleration of diastole. These produce vortices in which laminar shearing of fluid elements occur despite the fact that they are travelling on trajectories deviating from the direction of the average pressure gradient (Naumann, 1969; Schmid-Schonbein and Wurzinger, 1988). Flow in systole has a high kinetic energy, and fluid elements tend to pursue the shortest course, therefore, in a Y shaped bifurcation, the velocity of fluid elements closest to the flow divider is consistently higher than the average velocity across the vascular lumen, and in areas where the flow is predominantly axial and unidirectional, as at the flow divider, the shear stress is high (Reneman et al, 1987). On the opposite wall, the shear stress is low, and there is a continuously changing region of flow separation from which evolve vortices of variable size and location. It used to be argued that shear stress was the most important factor in the development of atherosclerotic plaques (Fry, 1968; Fry 1969; Caro et al, 1971; Flaherty et al, 1972; Cornhill and Roach, 1976) but it is now thought that the continually changing regions of flow separation and recirculation are the most important determinants of the development of plaques. Flow separation and recirculation are most pronounced in the young (Reneman et al, 1988) and the path of the vortical flow depends on a number of factors including the angle of divergence, the sharpness of flow divider, the change in total cross-sectional area, the rate of acceleration and deceleration, and the blood viscosity. These vortices grow and diminish with each cardiac cycle and have been demonstrated by filming pulsatile particle flow through a stenosis (Karino and Goldsmith, 1985) and in models of the carotid bifurcation exposed to pulsatile flow (Ku and Giddens, 1983; Rindt et al, 1987, Reneman et al, 1988). Similar flow separation phenomena are observed across stenoses exposed to pulsatile flow and fluid elements originating in the immediate vicinity of the wall of a stenosis are first led through the boundary layer between laminar flow and vortical flow, these carry damaged

and thereby activated platelets toward the wall where they are deposited. The boundary layer migrates with the cardiac cycle and migrating stagnation causes a repetitive subtle injury associated with platelet adherence (Karino and Goldsmith, 1979; Schmid-Schonbein and Wurzinger, 1988). In the absence of a stenosis, similar changes may occur in relation to the wall opposite the flow divider. This is supported by the findings of Murphy and colleagues that in Y shaped bifurcation models, platelets are almost exclusively deposited on the outside regions (Murphy et al, 1962). The boundary can be considered as an area of stagnant flow. Stagnant flow also develops at the flow divider, but this is better adapted to withstand potentially injurious influences as well as being better equipped to clear endothelial deposits as it is drained by a dense network of lymphatics and fed by a prominent vasa vasorum (Winternitz et al., 1938). Naumann and colleagues have proposed that during peak systole in the post-stenotic area and in areas of maximum flow separation during early systole, suction occurs due to a local reduction in pressure (Naumann et al, 1983), while Blackshear and colleagues have shown that high shear exposure when associated locally with a steep pressure gradient causes welling up of fluid from the media and intima into the subendothelial space (Blackshear et al, 1983). Thus, in post-stenotic areas with high velocity gradients, not only shear stresses acting on the endothelium, but also the transmural pressures acting on them, oscillate more than elsewhere in the arterial tree.

The diameter and wall thickness of most arteries increases with age. Caro and colleagues have reported a constant ratio between these two parameters (Caro et al., 1978). These age related changes have also been observed in the CCA and ICA, but within the carotid bulb, the diameter increases but the wall thickness remains constant (Burrig and Hort, 1988). Arteries adapt to chronic changes in flow or pressure by alterations in dimension, configuration and wall composition. It has been proposed that an increased flow velocity causes the artery to enlarge until the lumen radius results in restitution of normal base line wall shear stress (Glagov et al, 1990). As wall shear stress is inversely proportional to a third power of the radius, a small change is usually sufficient to reestablish baseline values of wall shear stress. A common response to lowered wall shear stress is intimal thickening (Glagov and Zarins, 1989) and it has been proposed that the pattern of intimal thickening in the region of the carotid bifurcation is the response to relatively low shear stresses in this region (Zarins et al, 1983)

## **2.5 Arterial wall movement**

Intima-media thickness and plaque formation are structural changes of the artery which may alter or be altered by arterial wall movement. A number of factors determine the stiffness of the arterial wall, these include the elastin and collagen content, the elastin-collagen ratio, the number of smooth muscle cells present and the sum of the humoral and neural activation mechanism.

Compliance, distensibility, the elastic modulus and the stiffness index are all parameters which have been used to assess arterial wall movement. Arterial distensibility is the fractional volume increase occurring within the artery during the cardiac cycle divided by the arterial pulse pressure. For a simple model of the artery consisting of a thin walled cylindrical tube, the arterial distensibility can be defined as the relative change in volume per unit pressure change, whereas compliance is the absolute change in volume per unit of pressure change. This is the reverse of the elastic modulus (stress over strain). In calculating distensibility and compliance, three assumptions have to be made: a) There is no lengthening of the artery and all increase in volume is reflected as an increase in cross-sectional area; b) The artery is circular in cross-section. With careful placing of the probe, this is likely to be true for the common carotid arteries and the common femoral arteries, but less likely to be so in the bulb due to the different morphological types of bulb (see part II, 5.2) and the difficulty in aligning the probe with the artery; c) No change in wall thickness occurs with the increase in cross-sectional area.

Both distensibility and compliance are blood pressure dependent. The stiffness Index  $\beta$  has been proposed by Hayashi and colleagues (Hayashi et al, 1974; Hayashi et al, 1980) to exclude the effect of changes in blood pressure on arterial wall stiffness. On the assumption that an artery consists of homogeneous incompressible material, Hayashi and colleagues analysed the stress-strain relation from pressure-diameter data using a finite deformation theory. From this analysis and their experimental data, they assumed that a simple exponential relation existed between relative pressure and the distension ratio. They defined the distension ratio as the arterial diameter ( $D_x$ ) at a given pressure ( $P_x$ ), normalised by the diameter ( $D_o$ ) at a standard pressure. When the distension ratio was plotted against the logarithmic value of the relative pressure ( $P_x/P_o$ , a given pressure

normalised by a standard pressure of 100mmHg) a linear relation was observed in the physiological range of pressures. This relation was expressed as:

$$\beta = (\ln(P_x - P_o)) / (D_s - D_o) / D_o$$

and  $\beta_1 = (\ln(P_s - P_d)) / (D_s - D_d) / D_d$

However in the normotensive subject, the following are observed:

$$D_o - D_d / D_o < D_s - D_d / D_d \ll 1$$

They therefore propose that  $\beta$  approximately equal to  $\beta_1$  and can be calculated from the above equation. Arterial compliance provides a measure of the elastic distensibility of arteries. It can be assessed non invasively by using Doppler ultrasound.

Initial interest in arterial wall compliance arose from studies in animals. These demonstrated that the early phases of experimentally induced atherosclerosis were associated with an increase in compliance and distensibility, whereas in more advanced disease, when focal lesions are formed, the compliance and distensibility decreased (Gosling et al, 1969; Newman et al, 1971), and the arterial wall became stiffer (Farrar et al, 1978). Furthermore, Farrar and colleagues demonstrated a reduction in arterial wall stiffness in regression of atherosclerosis in Rhesus monkeys (Farrar et al, 1980).

### **2.51 Compliance and age**

Using Doppler shifted ultrasound to measure pulse wave velocities, Laogun and Gosling have demonstrated a clear relationship between aortic compliance and age (Laogun and Gosling, 1982). They have shown that both sexes have approximately the same compliance at birth, which rises at the same rate to a peak around 10 years of age. Thereafter both male and female compliance decreases steadily with age reaching the birth value at around 18 years of life. At this point, a difference between the sexes becomes obvious with the compliance of female arteries staying similar to the birth level while the compliance in male arteries plateaus out below the birth value. The sex differences disappear after the menopause, the value becoming equal to that of males after the fifth decade. when there appears to be no observed difference in aortic compliance between the two sexes.

Changes in the arterial wall movement of the carotid artery with age have also been examined using wall tracking devices (Riley et al, 1986). Changes observed with age are a significant reduction in lumen diameter change during the cardiac cycle and a significant damping of the pulse wave contour. This is associated with a fall in arterial wall compliance and distensibility, with an associated increase in the elastic modulus. Baskett and colleagues have also demonstrated a fall in carotid compliance with age (Baskett et al, 1990). Another group of workers have examined the relationship between compliance of the common carotid artery and age using a multigate pulsed Doppler system (Van Merode (i) et al, 1989). This study found that with increasing age, the carotid artery diameter increased, and the arterial wall distensibility decreased, but there was no overall change in the cross-sectional compliance. The distensibility of the carotid bulb is greater than that of the common carotid artery in young normotensive individuals, but this diminishes with age. The diminishment in the relative magnitude of the arterial wall displacement is most pronounced in the carotid bulb, thus in young subjects, blood flows from a distensible CCA to a more distensible carotid bulb, but in older individuals, blood flows from a relatively stiff CCA to an even stiffer bulb. This may account for the differences in flow separation discussed in 2.4. In the presence of plaque, a maldistribution of vessel wall tension may be a factor responsible for acute changes such as plaque rupture and intraplaque haemorrhage (Lusby et al, 1983; Woodcock, 1983).

### **2.52 Arterial wall movement and risk factors**

The relationship between arterial wall elasticity and risk factors for cardiovascular disease has been examined in the Bogalusa Heart Study (Riley, 1990). This study examined the elasticity of the carotid artery in children in the upper and lower tertiles for both serum total cholesterol and systolic BP and found that the elastic modulus was significantly higher in the high risk groups after correction for age, race and sex. Increases in the elastic modulus independent of race, sex, age, systolic BP and total cholesterol were observed in children with reported parental histories of myocardial infarct. In addition to parental myocardial infarct, a number of other cardiovascular risk factors have shown significant associations with the elastic modulus ( $E(p)$ ) including age, gender (male arteries stiffer than female arteries), race (blacks stiffer than whites), and a combination of elevated total serum cholesterol and systolic blood pressure. A number of other factors

have been reported to affect arterial wall compliance including body weight (Toto-Moukono et al, 1986), blood pressure (Messerli et al, 1982; Randall 1982; Safar and London, 1987; van Merode et al, 1988), familial hypercholesterolaemia (Lehmann et al, 1992) and diabetes mellitus (Lo et al, 1986; Lehmann et al 1992). Using pulse wave velocities over peripheral arterial pathways, insulin dependent diabetic children have been reported to have stiffer arteries normal children (Pilsbury et al,1974), and this increased stiffness persists in to adulthood (Scarpello et al,1980; Lo et al, 1986, Walkqvist et al, 1984). However Lehmann and colleagues dispute this and report an increased compliance in diabetic children (Lehmann (ii) et al, 1992), in particular in the first year of the disease. They suggest that the disparity between their study and the above reports is due to a damping effect of the pulse wave in the peripheral vessels, as well as the failure of these studies to take into account the duration of disease and the age of the patient.

## **2.6 The prevalence and natural history of asymptomatic carotid artery disease**

The detection of carotid artery disease with duplex scanning was discussed in Chapter 1. The increasing availability of duplex scanning has resulted asymptomatic carotid artery disease being increasingly identified. This review will look at the prevalence and natural history of asymptomatic carotid artery disease and the risk factors associated with progression of the disease and the development of symptoms. Asymptomatic carotid artery disease is defined as individuals or patients with extracranial cerebrovascular disease who have never experienced focal hemispheric or vertebrobasilar symptoms. Patients who have previously undergone a carotid endarterectomy for focal hemispheric symptoms and are now asymptomatic despite a carotid artery stenosis on the unoperated side will be considered separately.

### **2.61 The prevalence of asymptomatic carotid artery disease in the community.**

Information on the prevalence of carotid artery disease is scanty. No large population studies exist. The only data available is from studies of a small number of volunteers, or patients with known coronary artery or peripheral vascular disease. The studies that have examined the prevalence of asymptomatic carotid artery disease in the community have drawn their sample from individuals at health fairs, and groups of civil servants, veterans and, in one study, members of a congregation (Ramsey et al,1987; Colgan et al, 1988;



Walsh et al, 1988; Niederkorn et al, 1990; Fowl et al, 1991; Jungquist et al 1991; Pujia et al 1992). Most examine men over 50, and use duplex scanning or Doppler as the screening tools. Few of these studies give the criteria used to classify the carotid artery disease, and the overall prevalence of a lesion producing a stenosis of greater than 50% of the lumen varied from 0.1% - 6.5%. The prevalence of asymptomatic carotid artery disease increases with age, as demonstrated by Ramsey and colleagues, who found a 4% prevalence in individuals under 60, compared to 11% in individuals over 70.

The prevalence of a tight stenosis is low. Colgan and colleagues found 3 individuals in their sample with a stenosis greater than 80%, however, in one of these the internal carotid artery was occluded, therefore the prevalence of severe carotid artery stenosis was only 0.6% (2:284) (Colgan et al, 1988). Surprisingly, none of the other studies identified a single individual with a tight stenosis, not even the study reported by Walsh et al, which examined over 2000 men over 40 years old at a health fair, though the wide age range of this study may account for the low prevalence of severe asymptomatic carotid artery disease.

#### **2.62 The prevalence of asymptomatic carotid artery disease in patients with coronary artery disease.**

It has long been recognised that patients with coronary artery disease have a high prevalence of carotid artery disease. In a study of over 4,000 male patients, Brenner and colleagues reported a 3% prevalence of carotid artery stenosis over 50% (Brenner et al, 1987). As in the general population, the prevalence increases with age, and in one study, the prevalence of stenoses over 75% was 11.3% for patients over 60, compared to 3.8% in patients under 60 (Faggioli et al, 1990). Similar results were reported by Berens and colleagues; their study examined 1087 patients over 65 years old with cardiac disease, and reported a 17% prevalence of lesions producing a stenosis of greater than 50% and a 5.9% prevalence of lesions producing a stenosis greater than 75% (Berens et al, 1992).

#### **2.63 The prevalence of asymptomatic carotid artery disease in patients with peripheral vascular disease.**

Four studies have used duplex or Doppler to screen for asymptomatic carotid artery disease in patients with peripheral vascular disease (Ahn et al, 1991; Fowl et al, 1991; Klop et al, 1991; Ellis et al, 1992). The largest of these examined 1198 patients and

found that 13.7% of them had a carotid artery stenosis of greater than 50% and 2.8% had a stenosis greater than 80% (Ellis et al, 1992). In a similar study of 416 patients with peripheral vascular disease, a quarter had a stenoses of greater than 50%, 7.7% had a stenosis greater than 75% and a further 7% had an occluded internal carotid artery (Klop et al, 1991). These results are similar to those reported in patients with coronary artery disease. A much higher prevalence of carotid artery disease is reported by Hertzler and colleagues. This study examined 139 peripheral vascular patients who also had carotid bruits. Using cerebral and coronary angiography as the diagnostic procedures, they found a very high prevalence of carotid disease. Two thirds had a stenosis greater than 50%, and in half of these the stenosis was greater than 75%.

Thus, the prevalence of asymptomatic carotid artery disease is low in the general population, but increases in the presence of coronary artery and peripheral vascular disease. Asymptomatic carotid artery disease may also indicate the presence atherosclerotic disease elsewhere in the body.

## **2.7 Natural history studies**

This part of the review deals with the significance of clinical findings such as a cervical bruit, carotid bifurcation bruit and internal carotid artery stenosis in terms of risk of developing symptoms. particularly stroke and death.

A number of natural history studies have followed up a large number of asymptomatic patients to determine the event rate, the stroke rate and in particular, the unheralded stroke rate (Humphries et al,1976; Podore et al,1980; Roederer(ii) et al,1984; Roederer(iii) et al,1984; Johnson et al,1985; Moore et al,1985; Hertzler et al,1986; Hennerici et al,1987; Moneta et al, 1987; Schroeder et al, 1987; Caracci et al,1989; Langsfeld et al, 1989; Bogousslavsky et al,1990). Three groups of patients have been studied: patients with a cervical bruit, patients who, despite a documented internal carotid artery stenosis, have never had any symptoms related to their carotid territories and patients with ipsilateral disease who have previously had a carotid endarterectomy for disease of the contralateral carotid artery. The methods for documentation of the carotid artery stenosis vary, early studies relied on angiography (Humphries et al,1976; Johnson et al,1985) or ocular plethysmography (Kartchner and McRae, 1977), more recently, the

majority of studies have used duplex ultrasonography to assess the stenosis of the carotid artery (Roederer et al(i), 1984; Roederer et al(ii), 1984; Moore et al,1985; Moneta et al, 1987; Caracci et al,1989; Bogousslavsky et al,1990) while a few have relied on digital subtraction angiography (Hertzer et al, 1986; Schroeder et al; 1987).

### **2.71 Asymptomatic bruit**

The finding of a cervical bruit by auscultation by no means always indicates carotid bifurcation disease. Many such bruits may arise from the heart (eg aortic valve) or great vessels at the base of the neck (eg subclavian). A bruit usually appears when there is a diameter reduction of 50% or more. If a mid-cervical bruit is audible, the likelihood of a stenosis being present is 64-85% (Allen, 1965; Javid et al, 1971; David et al, 1973; Gautier et al, 1975; Humphries et al, 1976; Fields et al, 1977; Thompson et al, 1978; Chambers and Norris, 1985). However, only 60% of patients with a significant stenosis (>50% in diameter) of the internal carotid artery will have a bruit and 31% of individuals with a bruit will have silent disease on the contralateral side (Chambers and Norris, 1985). Furthermore, a tight internal carotid stenosis greater than 80% may not produce a bruit as there is not enough blood flowing through the lesion. Two prospective epidemiological studies of the residents over 45 years old, in Evans County, Georgia (Heyman et al, 1980) and Framingham, Massachusetts (Wolf et al, 1981) have found a carotid (mid-cervical) bruit prevalence of 4.4% and 4.8% respectively. This figure rose to 7% in people over the age of 65. Although there was a four fold increase in the incidence of death from cardiovascular disease in the population with bruit, the actual incidence of stroke was low: 2.3% and 3% per year. Also, only 30% of the strokes were due to infarction of the hemisphere ipsilateral to the bruit (Kartchner and McRae, 1977; Chambers and Norris, 1985). These population studies indicate that bruit per se is not a good indicator of infarction in the territory of the affected carotid artery. Studies of the natural history based on the presence of bruit suggest that a bifurcation bruit as opposed to cervical bruit doubles the risk of developing symptoms (Kartchner and McRae, 1977) and in one study the risk of stroke in the population with a carotid bruit was triple that of an age and sex-matched population sample known not to have a bruit (Wiebers et al, 1990). Nevertheless, the risk of hemispheric TIA's (1.6-7% per year) and stroke (0.7-4.6% per year) is low (Thompson et al, 1978; Dorazio et al, 1980; Busuttill et al, 1981; Cullen, 1983; Colgan et al, 1985). The ratio of TIA's/stroke was on average 1.3/1

indicating that approximately 65% of patients who developed cardiovascular symptoms presented with a TIA and had a carotid endarterectomy in most centres. Those who developed a stroke did so in most instances without any 'warning' TIA's. The incidence of death from myocardial infarction (1.9-6.8%) was up to 4.5 times higher than that of stroke. Thus, studies on the natural history of patients with asymptomatic carotid bruit demonstrate that the presence of a bruit should be viewed as a risk factor for, or an indicator of, increased risk of systemic atherosclerosis.

### **2.72 Documented internal carotid artery stenosis**

The natural history studies on asymptomatic patients have shown that over a mean follow-up periods ranging from 48 months to 20 years, the neurological event rate is 7-11%, with a stroke rate of 1.5-2.4% and an unheralded stroke rate of only 0.8-1.5% (Chambers and Norris, 1985; Langsfeld et al, 1989). If the risk of developing symptoms is analysed according to the degree of stenosis present at diagnosis, then, for a stenosis of less than 50%, the annual percentage rate of TIA and stroke is low (1% and 1.3% respectively) (Norris<sup>et al</sup>, 1991), however for carotid stenosis greater than 50%, the event rate is 10-15% with a stroke rate of 3-8% and an unheralded stroke rate of 2-8% (Chambers and Norris, 1985; Moore et al, 1985; Hertzler et al, 1986; Hennerici et al, 1987; Langsfeld et al, 1989; AbuRhama and Robinson, 1990; Norris, 1990).

### **2.73 Tight stenosis**

Recent emphasis has been focused on the subgroup with a stenosis of >80%. Four studies have selectively followed up these patients (Roederer et al, 1984; Bogousslavsky et al, 1986; Moneta et al, 1987; Caracci et al, 1989). Despite short follow-up periods, these studies have all had very high event rates (33-52%) and the unheralded stroke rate has ranged from 5-18%. Moneta et al have subsequently reported that, using duplex ultrasonography, they are able to identify a further high risk subgroup according to the end-diastolic frequency (EDF) of the Doppler recording at the stenosis. On follow up, 30% of patients with an EDF > 6.5 KHz (as compared to 3.5% with an EDF <6.5 KHz) occluded their internal carotid artery, and the majority of the occlusions were symptomatic (Moneta et al, 1987). Similar high event rates were observed in the medical arm of the NASCET, where the ipsilateral stroke rate at 18 months was 19% in the

patients with 70-79% stenosis, but rose to 28% in the 80-89% stenosis group and 33% in the 90-99% stenosis group.

Patients with tight asymptomatic carotid artery stenosis are not only at increased risk of transient ischaemic attacks and stroke but also of cardiac and vascular deaths; in the population survey reported by Norris and colleagues, in patients with an symptomatic carotid artery stenosis of greater than 75%, the incidence of TIA and stroke were 7.2% and 3.3% per annum respectively, and the incidence of cardiac events was 8.3% p.a. and the incidence of vascular deaths was 6.5% p.a.

#### **2.74 Contralateral disease following carotid endarterectomy**

A further subgroup of interest are the patients who have previously had a carotid endarterectomy for contralateral disease. Follow-up studies of the ipsilateral diseased carotid artery have revealed that these patients have an overall event rate of 11-21% with an unheralded stroke rate of 0-6% over a follow-up of 22 months to 20 years (Levin et al, 1980; Podore et al, 1980; Roederer et al, 1984; Hennerici et al, 1987; Schroeder et al, 1987; Langsfeld et al, 1989). If only tight stenoses (>50%) are considered then the event rate varies from 11-33% and the unheralded stroke rate from 0-18%. (Humphries et al, 1976; Johnson et al, 1978; Levin et al, 1980; Roederer et al 1984; Langsfeld et al, 1989)

#### **2.75 Progression of disease**

Symptoms of cerebral ischaemia may develop as a result of acute changes with intraplaque haemorrhage or intraluminal thrombosis. This may produce a rapid progression of the carotid artery stenosis which may then regress. Regression of long-standing plaque is less credible but has been demonstrated (Norris and Bornstein, 1986) There is angiographic evidence of carotid stenosis progression occurring over a number of months (Ross and Lye, 1984) and chronic progressive changes have been observed over a number of years (Javid et al, 1970). Arteriographic regression of arterial stenosis is well documented in the coronary vessels (Malinow, 1984) and in the femoral arteries (Duffield et al, 1983; Lewis, 1985), usually during treatment for hyperlipidaemia, but sometimes spontaneously (Malinow, 1984). Experimental atherosclerosis in animals also regresses when dietary cholesterol is eliminated (Malinow, 1983).

Reported rates of progression vary. In the angiographic follow-up study reported by Javid and colleagues, serial studies were performed at random intervals over 1-9 years and the rate of progression was found to be 25% per annum (Javid et al, 1970). Roederer and colleagues prospectively followed up 162 patients with neck bruits, with duplex scanning, they categorized the stenoses into six grades of severity between 0-99%, they found that 60% of the lesions progressed over 3 years, and noted that the progression of disease to >80% stenosis correlated well with either the development of an internal carotid artery occlusion or new symptoms. (Roederer et al, 1984). Chambers and Norris followed up 242 patients with carotid artery stenoses over 50% and observed progression in 28% and regression in 4% over 2 years, and they also observed that progression and high grade stenosis related closely to the appearance of ischaemic cerebral events (Chambers and Norris, 1986. Plaque progression correlated with the presence of ischaemic heart disease and peripheral vascular disease but not with known risk factors. The high rates of progression observed by Roederer and colleagues and Chambers and Norris are corroborated by Langsfeld and colleagues who reported a progression rate 23% over a mean follow-up of 18 months (Langsfeld et al, 1989), however, their symptomatic progression rate was only 2%. Using B-mode imaging to observe small carotid plaques (< 30% stenosis) over 18 months, Hennerici and colleagues reported that 30% progressed, 19% regressed and 51% remained apparently unchanged. Regression included shrinkage of soft plaques and healing of ulcerated lesions. Hard or fibrous plaques apparently did not change (Hennerici et al,1984). These same workers subsequently reported a 36% progression rate over a mean follow-up of 29 months, and a strong association between progression and symptoms (22%) (Hennerici et al,1987).

### **2.76 Plaque morphology on ultrasound**

Several histological studies of carotid plaque morphology have suggested that intraplaque haemorrhage is more common in patients with focal cerebral symptoms than those without such symptoms (Lusby et al, 1982; Imparato et al, 1983; Ammar et al, 1986; Fryer et al, 1987). Attempts have therefore been made to identify these features by non-invasive methods.

Improvement in the ultrasound probes commercially available has permitted the identification of plaque morphological characteristics. Reilly and colleagues described

two distinct ultrasound echo patterns, homogeneous and heterogeneous plaques (Reilly et al, 1983). They found a good correlation between heterogeneous plaques and the presence of intraplaque haemorrhage, with 91% of all intraplaque haemorrhage and all ulcers occurring in the heterogeneous group. 78% of the symptomatic patients had heterogeneous plaques. Johnson and colleagues found that 93% of patients with "soft" lesions and a greater than 75% stenosis on duplex became symptomatic over 5 years, whereas only 62% of patients with "dense" lesions and a greater than 75% stenosis became symptomatic (under 75% stenosis only 22% of patients with "soft" lesions and 6% of "dense" lesions became symptomatic) (Johnson et al, 1985). Grey-Weale and colleagues expanded the classification according to the B-mode appearance and described four ultrasound types (1-4) (Gray-Weale et al, 1988). Of 179 lesions classified as type 1 or 2, 97.2% contained intraplaque haemorrhage or were ulcerated, whereas intraplaque haemorrhage occurred in only 29% and 25% respectively of types 3 and 4. Their group has subsequently reported that heterogeneous plaques (type 1+2) occur significantly more frequently ( $p < 0.001$ ) in symptomatic pre-operative arteries than in asymptomatic arteries (Langsfeld et al, 1989). Goes and colleagues have proposed that echogenicity, heterogeneity and irregularity of plaques on ultrasound are features relating to the age of the plaque, rather than stability (Goes et al, 1990). As a result of these studies, it has been argued that the noninvasive detection of intraplaque haemorrhage may result in improved selection of patients for surgical intervention (Gray-Weale et al, 1988; Langsfeld et al, 1989).

### **2.77 The role of carotid endarterectomy in asymptomatic carotid artery stenosis.**

The studies discussed in section 2.6 confirm that patients with a tight asymptomatic stenosis are at high risk of developing symptoms. There is now ample data on the value of carotid endarterectomy in the treatment of symptomatic patients with a carotid artery stenosis of greater than 70% in diameter (Barnett, 1990; NASCET, 1991; ECSTCG, 1991). The role of carotid endarterectomy in asymptomatic carotid artery disease is more controversial. Two trials have been conducted in the USA designed to evaluate the efficacy of carotid endarterectomy as a means of preventing stroke in asymptomatic patients (Veterans Administration Cooperative Study Group, 1986; Asymptomatic Carotid Atherosclerosis Study Group, 1989). In the VA cooperative trial, 444 male veterans with an asymptomatic carotid artery stenosis of greater than 50% were entered

in to a randomized trial of optimal medical management alone including antiplatelet therapy versus optimal medical management plus carotid endarterectomy (Hobson, 1993). The mean follow-up for this study was 47.9 months and the 30-day post randomisation permanent stroke or death rate was 4.7% for the surgical group. Two neurological events (0.9%), one transient and one permanent, occurred in the medical group in the 30-days post randomisation. Overall, 84 neurological events were observed, 27 (12.9%) in the surgical group and 57 (24.5%) in the medical group, which represents a relative risk of 0.51. Even better results were observed for the risk reduction in ipsilateral events as only 17 ipsilateral events occurred in the surgical group, compared to 48 in the medical group, with a relative risk of 0.38. No overall difference was observed in the incidence of stroke and death between the two groups. The results of this clinical trial indicate that carotid endarterectomy, when combined with optimal medical management, can reduce the incidence of ipsilateral neurological events in high risk male patients with arteriographically confirmed internal carotid artery stenosis. The asymptomatic carotid artery stenosis study is still underway. This study is addressing the same issues as the VA trial, but in a broader cross-section of patients, including both sexes. This study will also examine the incidence of re-stenosis following carotid endarterectomy, the rate of progression and regression of carotid atherosclerosis in the medically treated group, and incidence of cardiac events during follow-up (Moore WS, 1993)

## **2.8 Risk factors for carotid atherosclerosis**

This section will review the major risk factors associated with extracranial carotid atherosclerosis. Several studies have looked at the relation between risk factors and the risk of developing a stroke, and more recently interest has focused on the role of risk factors in the development of asymptomatic or pre-symptomatic disease, using ultrasound to detect the presence of carotid atherosclerosis. One of the problems in assessing the role of risk factors in the development of asymptomatic or pre-symptomatic disease is that discordant methods have been used to assess the degree of atherosclerosis, some studies have used angiography, others have used B-mode ultrasound.



## **2.81 Cigarette smoking**

A number of studies have looked at relation between cigarette smoking and the risk of stroke (Nomura et al, 1974; Salonen et al, 1982; Abbott et al, 1986; Welin et al 1987, Wolf et al, 1988). A metaanalysis of the relation between cigarette smoking and stroke has estimated the relative risk of stroke in smokers to be 1.5 (Shinton and Beevers, 1989).

Results from several population based studies have also confirmed a positive association between smoking and asymptomatic atherosclerosis. In the Framingham study, the presence of extracranial carotid artery disease measured by duplex scanning was significantly correlated with a history of cigarette smoking (Wolf et al, 1988). In the MONICA study (monitoring of trends in mortality and morbidity and their determinants) in Augsburg, Germany, which included almost 1,400 randomly selected men and women, a positive (although weak) association was found between cigarette smoking and the presence of plaques, as assessed by ultrasonography, in men (Gostomzyk et al, 1988). Two studies of patients with hypercholesterolaemia have also identified cigarette smoking as a strong risk factor for extracranial carotid artery atherosclerosis assessed by ultrasonography (Schuster ET AL, 1987; Poli et al, 1988).

In a study in Finland, 49 pairs of identical twins discordant in their life-long smoking habit were examined with duplex ultrasound (Haapanen ET AL, 1989). The twins had similar blood pressures, total plasma cholesterol, body mass index and psychological factors but on duplex, carotid artery stenosis was found in significantly more smoking twins than their non-smoking sibling, the age adjusted odds ratio among smokers for carotid stenosis was 5.99 (stenosis defined as at least 15% luminal stenosis measured by pulsed Doppler ultrasonography). As measured by B-mode ultrasonography, the mean area of all carotid plaques was 3.2 times greater in the smoking twins than in the non-smoking twin partners.

The degree of carotid atherosclerosis in asymptomatic smoking individuals has also been studied. In a study of elderly patients with isolated systolic hypertension, cigarette smoking was positively related to the prevalence of carotid atherosclerosis (Sullon et al, 1987), and Tell and colleagues report that the extent of atherosclerosis in cigarette smokers is significantly greater at all areas compared, furthermore, the effect of current cigarette smoking was almost equivalent to an additional two decades of aging (Tell et al,

1989). Both this study and the studies of Whisnant, Homer and colleagues found a positive association between the duration of smoking and carotid disease; Whisnant and colleagues reported that the risk of a smoker of 40 years having a carotid artery stenosis was approximately 3.5 times that for a non smoker of the same age (Tell et al, 1990; Whisnant et al, 1990; Homer et al, 1991). This excess risk persisted despite stopping smoking.

### **2.82 Lipids and lipoproteins**

The relationship between lipids, lipoproteins and extracranial carotid artery atherosclerosis is as yet less clear. In a review of more than 26 publications in which a relation was sought between plasma lipid and lipoprotein and carotid atherosclerosis, Tell and colleagues found a relation between blood lipids and /or lipoproteins in all but three studies, but most of these studies were based on case series or hospital based patient registries, and therefore, the findings cannot be extrapolated to the general population (Tell et al, 1988). Several population based studies have addressed the issue. In the Framingham population based study (Wolf et al, 1988), the presence of extracranial carotid artery disease measured by duplex scanning was significantly correlated with total serum cholesterol (measured 8 years prior to scanning). In the cross-sectional study of Finnish men, a strong and graded relationship between serum LDL cholesterol and the frequency of carotid artery atherosclerosis was observed, whereas HDL cholesterol was negatively related to carotid artery atherosclerosis only in men free of ischaemic heart disease (Salonen et al, 1982). In the 2 year follow up, the group reported that the atherosclerosis progressed faster in men who had a high serum cholesterol than in others and that LDL cholesterol was one of the strongest predictors of progression (Salonen and Salonen, 1990). In the MONICA study, a significant negative relationship between the presence of plaque and the HDL: total cholesterol ratio, and a borderline significant positive relationship with total cholesterol was reported (Gostomzyck et al, 1988). In the ARIC study, cases with carotid artery atherosclerosis had significantly higher mean levels of plasma total cholesterol, LDL cholesterol and total triglyceride and lower mean levels of HDL cholesterol than did control subjects, the multi-variable-adjusted odds ratios for total cholesterol (>240 vs <200 dl), LDL cholesterol (>160 vs <100 mg/dl), HDL cholesterol (<35 vs >35 mg/dl) and tryglyceride (>170 vs <170 mg/dl) were 2.9, 2.0, 1.7, and 1.7 respectively (Heiss et al, 1991).

In studies of patients with hypercholesterolaemia, Giral and associates found carotid artery plaques to be associated with an increased total and LDL cholesterol in hypercholesterolaemic individuals (Giral et al, 1991), while Schuster and associates reported that carotid artery atherosclerosis was associated with the extent of the increase in the total cholesterol level in patients with familial hypercholesterolaemia (Schuster et al, 1987).

Serum lipoprotein (a) is a low-density lipoprotein like particle which contains apoprotein B-100 and a glycoprotein apolipoprotein (a) (MBewu et al, 1990). A striking structural similarity has been observed between apolipoprotein (a) and human plasminogen, and it has been proposed that this may indicate a link between atherosclerosis and thrombosis. There is in vitro evidence to suggest that Lp (a) can interfere with the normal fibrinolytic mechanism and therefore it is proposed that Lp (a) promotes thrombosis (Miles and Plow, 1990). Native Lp (a) and recombinant apolipoprotein (a) can bind to macrophages through a specific high-affinity receptor (Zioncheck<sup>1991</sup>); the result is intracellular accumulation of cholesterol.

Lipoprotein (a) has been established as an independent risk factor in extracranial carotid atherosclerosis and cerebral infarction (Koltringer and Jurgens, 1985; Zenker et al, 1986; Murai et al, 1986; Jurgens and Koltringer, 1987; Woo et al, 1991; and Koltringer reported that Lp (a) was a more powerful predictor of the presence extent and thrombotic progression of extracranial carotid artery atherosclerosis as assessed by Doppler and B-mode ultrasonography, than either hypertension or smoking.

There is considerable diversity<sup>among</sup> the various studies reported in the pattern of findings that relate lipids and lipoproteins to extracranial carotid artery atherosclerosis. This may reflect the diversity of study design, the methods of measurement, and the procedures used for selection of patients and participants. Nonetheless, even though the magnitude of the association between lipids or lipoproteins and extracranial carotid artery atherosclerosis is consistently less than that of smoking, the atherogenic role of lipids and lipoproteins - most notably LDL cholesterol seems to be supported by the majority.

### **2.83 Hypertension**

Arterial hypertension has been found to be a risk factor for carotid artery atherosclerosis in patients examined by angiography (Duncan et al, 1977; Harrison and Wilson, 1983;

Ford et al, 1985; Inzitari et al, 1986; Passero et al, 1987; Schneidau et al, 1989; Whisnant et al, 1990) and the relative risk of developing severe carotid artery atherosclerosis in the presence of hypertension is reported to be 2.33 times that of normotensive individuals (Homer et al, 1991). Using B-mode ultrasound, hypertension has also been found to be a major risk factor for the presence of carotid artery atherosclerosis in asymptomatic patients (Crouse et al, 1987; Lusiani et al, 1987; Rubens et al, 1988; Tell et al., 1989) and in randomly selected population sample of Finnish men with a wide range of blood pressures, Salonen and associates report the systolic pressure to have a strong independent relationship with the prevalence of plaques (Salonen and Salonen, 1990); in the presence of a sitting systolic blood pressure over 175, the relative risk of having a hard plaque was 2.6 times that of an age matched normotensive individual. This study was restricted to men, but similar findings have been reported by Bonithon-Kopp and colleagues in a study of French women (Bonithon-Kopp et al, 1991). It has also been reported that high systolic and diastolic blood pressures were significantly associated with the progression of angiographic atherosclerotic lesions in the carotid arteries over an 18 month period (Schneidau et al, 1989). In the ARIC study, hypertensive status was associated with a multivariable adjusted odds-ratio of 2.9 relative to normotensive individuals (the ARIC investigators, 1989; Heiss et al, 1991).

However, a number of cross-sectional studies of ultrasonographically assessed carotid atherosclerosis have failed to find any association between blood pressure and the degree of carotid atherosclerosis (Sutton et al, 1989; Lusiani et al, 1990). These negative findings may, in part, be explained by the small variation in blood pressure in hypertensive populations and the effect of treatment.

## **2.84 Diabetes**

### **a) Non-insulin dependent diabetes**

Diabetes is associated with a decreased life expectancy (Kannel and McGee, 1979). This has been attributed to accelerated atherosclerotic cardiovascular and cerebrovascular disease in diabetics. Prospective studies have shown that for the male non-insulin <sup>dependent</sup> diabetic (NIDDM), the mortality rate from ischaemic heart disease is 2-4 times that for the non diabetic, and the increase is even greater for the female NIDDM (Pyorala and Laakso, 1983; Panzram, 1987). In the age group 15-44, the difference between diabetics and

non-diabetics is particularly prominent and in one study the mortality ratio for all cardiovascular disease for diabetic males was 12.2 times that of the general population and for females, 19.5 times that for the general population in this age group (Marks et al, 1971). Cerebrovascular disease is an important cause of death in diabetics and accounted for approximately 15% of all deaths in NIDDM in the World Health Organization Study group Survey (WHO Study Group, 1985), this is a two to four fold increase in the risk of death as compared to the general population. In the Framingham study (Kannel and McGee, 1979), the age adjusted incidence of cerebral infarction, including fatal and non fatal cases was 4.7 per 1000 for diabetic men (NIDDM and IDDM) and 1.9 per 1000 for non diabetic men. For women, the figures were 6.2 per 1000 in diabetic women as compared to 1.7 per 1000 for non-diabetic women. The Framingham study also reported an increase in the age adjusted incidence of intermittent claudication in diabetic men and women (NIDDM and IDDM) (12.6 per 1000 for compared with 3.3 per 1000 for non-diabetic men and 8.4 per 1000 compared with 1.3 per 1000 for non-diabetic women).

Using B-mode ultrasound, Tell and colleagues have reported an increased incidence in the mean plaque score in the carotid arteries of diabetic patients compared to non-diabetic patients, after adjustment for age, sex, hypertension, and cigarette smoking (Tell et al, 1989). In a prospective study of 135 controls and 286 NIDDM patients, the prevalence of carotid artery disease was 8.2% in the NIDDM patients as compared to 0.7% in the controls (Chan et al, 1983). In contrast, a history of diabetes mellitus was not significantly correlated to the extracranial carotid atherosclerosis score in an Italian multicentre study (Passero et al, 1987). A finding supported by an earlier study in which diabetes mellitus did not appear to influence the rate of angiographically determined stenotic changes in the carotid bifurcation (Javid et al, 1970).

The risk factors for cardiovascular disease in the general population (cigarette smoking, hypertension, elevated LDL (low density lipoprotein) cholesterol, lowered HDL (high density lipoprotein) cholesterol and possibly raised serum triglycerides are also thought to operate in the genesis of diabetic macrovascular disease but the degree to which each is operative is open to debate. Abnormalities of plasma lipid and lipoprotein metabolism are common in both NIDDM and IDDM.

The most common abnormality in the lipid profile of people with NIDDM is an elevated VLDL, either as total triglyceride or as VLDL-triglyceride (Howard et al, 1978), (Simpson et al, 1979; Lisch and Sailer, 1981; Barrett-Connor et al 1982; Uusitupa et al, 1986; Laakso et al, 1987). The other common lipoprotein abnormality is a reduction in the level of HDL-cholesterol (Barrett-Connor et al 1983; Uusitupa et al, 1986; Laakso et al, 1987), these changes are only partially related to glycaemia control. Existing evidence suggests that raised triglycerides and low-HDL-cholesterol are important associations of macrovascular disease in NIDDM (Santen et al, 1972; Reckless et al, 1978; Beach et al, 1979; Aro et al, 1981; West et al. 1986 ; Seviour et al, 1988). The roles of insulin resistance, obesity, and independently inherited abnormalities of lipoprotein metabolism in the aetiology of dyslipidaemia of NIDDM are complex and under investigation.

b) Insulin dependent diabetes.

Like NIDDM patients, insulin dependent diabetics (IDDM) have a very high incidence of macrovascular disease, however much of the mortality data for IDDM has to be derived from large studies in which the type of diabetes has to be inferred from use of insulin, or the age of onset of diabetes. In a cross-sectional study (Reckless et al, 1978) the prevalence of all types of vascular disease was found to be increased in insulin treated men and women and to be most marked in individuals with an elevated LDL-cholesterol. In a case controlled study, Krolewski and colleagues found a cumulative mortality rate due to IHD at the age of 55 years of 35% (Krolewski et al, 1987). This was far higher than the corresponding rate for non-diabetic individuals in the Framingham Heart study (8% for males and 4% for females). Mortality in the study group was, at least in part, related to antecedent nephropathy. It was calculated that the mortality rate without nephropathy would be halved but would still be much higher than the Framingham population. In another study, patients with nephropathy were compared to those without evidence of renal disease and followed for a period of ten years. Within 6 years on the onset of proteinuria, the cumulative incidence of IHD (as adjudged by mortality and Minnesota coded ECG criteria) was 40% in the nephropathic group as compared with 5% in the non-nephropathic groups. Blood pressure and serum cholesterol concentrations were significantly higher in the nephropathic group. It is known that the presence of nephropathy has an adverse effect on lipid profiles and this may be related to the high incidence of IHD. Despite normal or above normal HDL-cholesterol

concentrations in IDDM patients, macrovascular disease is very common and there is some evidence that HDL-cholesterol as a protective factor may not be operational in this disease.

The incidence of peripheral vascular disease (PVD) in diabetics has been studied by Beach and Strandness (Beach and Strandness, 1980). In a study of 141 IDDM, non-invasive tests that included post exercise ankle pressures and continuous wave Doppler velocitometry, they found a prevalence of occlusive PVD of 18%. Of these patients, 36.6% were hypertensive compared with 11% of the group with no evidence of PVD. They also studied the lipid profiles of these patients (Beach et al, 1979) and found that the presence of overt and covert PVD was significantly related to both raised LDL-cholesterol and VLDL-triglyceride but there was no relationship with HDL-cholesterol. In an older group of 87 patients in whom a history of intermittent claudication was elicited by cardiovascular questionnaire, men with a positive history had significantly elevated total and VLDL-triglycerides. This has been confirmed by two other studies (Santen et al, 1972; Zimmerman et al, 1981).

### **2.85 Hypopituitarism**

A premature mortality has been observed in patients with hypopituitarism despite routine replacement therapy. In one study, 333 patients with hypopituitarism were followed up and it was found that, not only was the overall mortality higher than in an age and sex-matched population, but also that twice as many deaths from vascular disorders were observed than expected (Rosen and Bengtsson, 1990). The cause of deaths from vascular disorders is uncertain, although growth hormone deficiency or endocrine replacement therapy may play a part (Merimee et al, 1973; Libber et al, 1990)

### **2.86 Sex**

Carotid atherosclerosis has been shown to be more prevalent in men than in women in autopsy studies although the differences are smaller and not as consistent as in the coronary arteries (Solberg and Egen, 1971). Similar results are reported by Tell and co-workers using B-mode ultrasound, though only for white subjects (Tell et al, 1989). Women have a lesser degree of atherosclerosis than men, a protective effect which is lost in diabetes mellitus. No difference in the incidence of atherosclerosis between pre and

post menopausal women was observed by Bonithon and associates after adjustment for age (Bonithon-Kopp et al, 1991).

### **2.87 Alcohol**

Although the issue is controversial, there is some evidence that alcohol consumption at moderate levels may be an independent factor protecting against heart disease. Several studies have examined the relationship between alcohol consumption and carotid atherosclerosis. One case controlled study of 230 patients with stroke admitted to a district based hospital suggested that the relative risk for stroke may decrease to 0.5 in light drinkers (10-90g/wk) compared with non-drinkers, whereas heavy drinkers (>300 g/wk) may have a fourfold increase in risk (Gill et al, 1986). Another study examined risk factors in patients over 50 admitted with their first ischaemic stroke, and found an inverse linear relation between light-to-moderate (up to 4 units/week) alcohol consumption and severity of internal carotid stenosis (Bogousslavsky et al, 1990). A logistic regression showed that hypertension, cigarette smoking, and age in men and diabetes mellitus and cigarette smoking in women strongly counterbalanced the potential benefit of alcohol consumption. On the other hand, acute alcohol ingestion may precipitate ischaemic stroke, although it is possible that acute alcohol consumption does not act as an independent factor (Hillbom and Kaste, 1983; Gorelick et al 1987).

In a study of carotid artery stenosis, Whisnant and colleagues showed an increased frequency of severe carotid artery stenosis in daily alcoholic drinkers, but alcohol was not a significant factor on multiple regression, presumably because two thirds of the drinkers smoked (Whisnant et al, 1990).

### **2.9 Conclusion**

The literature relating to the anatomy, pathology and natural history of carotid bifurcation disease has been reviewed in this chapter. By understanding the development of carotid bifurcation disease at its early stages, not only may we develop methods of identifying patients at risk, but also, methods to slow the progression of the disease or even produce regression. Both early identification of disease and interventions that alter the natural history of carotid bifurcation disease will ultimately reduce the incidence of stroke.



CHAPTER 3

THE INTIMA-MEDIA THICKNESS OF ARTERIES

DETECTED BY HIGH RESOLUTION

ULTRASOUND

### **3.1 Introduction**

Using high resolution ultrasound, normal arteries have a characteristic B-mode image composed of two parallel echogenic layers separated by a hypoechogenic space. This appearance is dependent on the incident angle of the ultrasound beam and was first reported by James and colleagues (James et al, 1982). The nature of these echogenic lines was investigated by Pignoli. In an in-vitro study on human cadaver aortas, he compared ultrasonic measurements at selected sites with histology measurements and demonstrated a good correlation ( $r=0.75$ ) between the ultrasonic wall thickness measurements and the thickness of the intima and media measured on the pathological specimen (Pignoli, 1984). This first study was carried out on aortas of an elderly population with a high degree of disease. In a subsequent in-vitro study, he used relatively normal cadaveric aortas and carotid arteries (Pignoli et al, 1988), the aortas were divided into those with no gross pathology (group A) and those with disease (group B). Comparison of the B-mode measurements of the distance between the inner surface of each echogenic line and the intimal plus medial thickness on gross pathology demonstrated a good correlation for both groups of aortas ( $r=0.87$  and  $0.93$  respectively). The correlations with histological measurements were less good ( $r=0.76$  and  $r=0.82$  respectively) and the differences were statistically different. The authors concluded that this discrepancy was due to artifacts introduced during histological processing. The authors also report a study to identify the structures generating the double line pattern, to do this, they excised a small section of the intima of the luminal surface of the (diseased) aortic wall prior to examination with ultrasound. This was reported to eliminate the inner reflective layer. A similar experiment was carried out by stripping the adventitia which successfully removed the outer echogenic layer. All the above experiments were carried out on cadaver aortas, however the authors did also examine the carotid arteries of healthy individuals (age range 20-30) in vitro and in vivo and found no significant difference in the measurement of the B-mode intima-media thickness and the gross pathology intima-media thickness. The authors concluded that the first echogenic layer corresponded to the luminal surface of the intima, and the second echogenic layer with the transition from media to adventitia.

The double line pattern can be identified at the far wall level of most arterial walls even in the presence of atherosclerotic plaque, but the ultrasonic pattern becomes complex in

24% and the lines disappear in 20% (Pignoli et al, 1986). A number of factors may affect the accuracy and reproducibility of measurement of intima-media thickness: firstly, the angle of the insonating beam as the acoustic interfaces between the different layers of the arterial wall are best distinguished if the beam is perpendicular to the wall, secondly, the order in which the ultrasound crosses the different interfaces as the transition from a high acoustic impedance region to a low-impedance zone tends to cause a gain-dependent reverberation and blurring of the interface, thus the periadventitial/adventitial interfaces are detected more accurately on the near wall, whereas the lumen/intima and media/adventia interfaces are best seen on the far wall (O'Leary and Polak, 1991). The double-line pattern can be recognised over a wide range of time-gain settings and a time-gain compensation in the usual range does not appreciably influence the distance between the two echogenic lines. The earliest changes that can be seen in the arterial wall indicating the onset of the atherosclerotic process are fatty streaks. The relationship between fatty streaks and atherosclerosis is unknown, nor is it clear if fatty streaks can be detected by ultrasound. Fat is only weakly echogenic and would not be expected to generate a signal. Picano and colleagues (Picano et al, 1988) pointed out that gaps can be seen with ultrasound in the lumen/intima interface, he examined fresh human aortic specimens and proposed that these defects in the lumen/intima interface could reflect the presence of fatty streaks. Hennerici and colleagues (Hennerici et al, 1987) proposed that the earliest lesion visible on ultrasound is a flat plaque composed of an eccentric intraluminal thickening with a fibrous zone adjacent to the arterial lumen and a hypocellular friable centre. In this study, 5 specimens had lipoidosis, intimal swelling and diffuse fibrocellular thickening, features thought to represent the earliest stage of atherogenesis (Glagov and Zarins, 1989) but these were not detected on ultrasound, however this study did not examine differences in intima-media thickness and its association with early stages of atherogenesis. Diffuse thickening of the intima-media layer is now recognised and a study by Weber and colleagues (Weber et al, 1988) examined the relationship between intima-media thickening and age. Interestingly, they found that lesions stabilised in individuals over 60 in all areas except the carotid and cerebral arteries, where the intima-media increased with age.

The composition of the double-line pattern of the normal arterial wall as seen with high resolution ultrasound has been questioned by Nolsoe et al. (Nolsoe et al, 1990). In an

in-vitro study of macroscopically normal cadaver aortas, they found a poor correlation between measurement of the ultrasonic picture and the histological specimen. Furthermore, stripping of the intima did not alter the ultrasonic appearance and a needle placed on the intima demonstrated that the inner echogenic line and the adjacent hypoechoic line lay in front of the true fluid-tissue interface. They also demonstrated that the lines could be seen if other materials (metal, plexiglass etc) were scanned and therefore concluded that the lines were artefacts.

### **3.2 Intima-media thickness and risk factors for atherosclerosis**

Using high resolution ultrasound, arterial wall abnormalities have been identified and several studies have examined the role of risk factors in the production of arterial wall thickening. In a study by Poli and colleagues (Poli et al 1988), computer assisted measurements of the intima-media thickness derived from the examination 36 hypercholesterolaemic patients were compared with 31 controls. This study found a significant increase in wall thickness in the hypercholesterolaemic patients compared to the controls. They also demonstrated a significant correlation between wall thickness and age in their control group. Arterial wall thickness has also been reported to be significantly increased in familial hypercholesterolaemia (Wendelhag et al, 1992). In a study of 412 Finnish men who were assessed with high resolution ultrasound of the common carotid artery for arterial wall thickening, plaques or stenosis, Salonen and colleagues observed a strong graded relationship between serum LDL cholesterol concentration and the prevalence of carotid atherosclerosis (Salonen et al, 1982). Of those studied, 49% were found to have abnormalities on ultrasound, and in 75% of these, the abnormal finding consisted of intima-media thickening of the artery wall (defined as a distance of greater than 1.2mm between the lumen/intima interface and the media/adventitia interface). For the remaining 25%, the abnormal ultrasound finding was the presence of plaque or actual stenosis with a 20% or more reduction in the lumen diameter. This group has now studied 1224 Finnish men and have found that systolic blood pressure, smoking and serum LDL are the major risk factors for an increase in carotid intima-media thickness, while a history of ischaemic heart disease and diabetes are less powerful factors. Cigarette smoking was the strongest determinant of carotid intima-media thickness and after adjusting for age, the IM thickness in smokers was thicker by 14% compared to non smokers; men with serum cholesterol levels in the

upper 50% of the LDL cholesterol distribution showed had an IM thickness 10% greater than those with a low serum LDL cholesterol; and men with systolic hypertension had an IM thickness 9% greater than normotensive men (Salonen and Salonen, 1991).

Furthermore, men with a history of ischaemic heart disease had an IM thickness 16% greater than those without. In a 2 year follow-up study (Salonen and Salonen, 1990), the group reported that maximum progression was observed in men who were current smokers and that pack years of smoking was one of the strongest predictors (after age) of the progression of common carotid intima-medial thickening. This finding is supported by the ARIC study (The atherosclerosis risk in communities study) which reports that "cases" (individuals with an intima-media thickness in the upper tenth centile) had a mean of 29 pack years of smoking in comparison to 14 pack years in controls (The ARIC investigators). The odds ratio for current versus never or former smokers in this study was 3.9.

The above mentioned studies are all quantitative studies of intima-media thickness. The non-invasive ultrasonic biopsy was developed by Belcaro and colleagues using high resolution ultrasound (Belcaro et al, 1990; Belcaro et al 1991). Their aim was to classify qualitative changes in the arterial wall that could be detected by high resolution ultrasound and study the correlation between these changes and the development of symptomatic cardiovascular disease. They performed their ultrasonic biopsy on 2000 asymptomatic individuals and 150 vascular patients. A subgroup of individuals were also tested by bicycle ergometry. They found that the ultrasonic biopsy score increased with age, and with increasing score, there was an increased risk of ischaemic changes on cardiac stress testing. Follow up of 350 asymptomatic subjects and 150 vascular patients over four years showed that the ultrasonic biopsy score correlated well with the development of cardiovascular events. The authors propose that ultrasonic biopsy could be used to predict the likelihood of ischaemic changes on cardiac stress testing, and the risk of developing symptoms on follow-up but the numbers in their higher categories were few and this method is highly operator dependent.

### **3.3 Intima-media thickness and progression of disease**

Attempts have been made to study progression of plaques with high resolution ultrasound (HRU). A number of factors make this difficult. Shadowing by calcified

plaque remains a significant problem and in irregular complex plaques, boundary definition can be difficult. Furthermore, the majority of atherosclerotic plaques are eccentric and variation in orientation at the time of interrogation can compound measurement differences. Ricotta and colleagues propose that to be confident of disease change, the minimum change in lumen diameter of 3.2mm is required, this the result of the accumulative effect of between sonographer and between reader variability, the effect of severe disease and irregular plaques on the ultrasound picture, these effects are doubled with repeated examinations; however if the same reader and sonographer are involved in longitudinal follow-up of patients, smaller changes can be detected (O'Leary et al, 1991). If intima-media thickness rather than plaque are examined, the absolute difference in blinded replicate measurements of arterial wallthickness at the site of the lesion is less than 0.2mm if standardised protocols for scanning and interpretation are used (Insull et al, 1989).

Despite these problems, attempts have been made to monitor the progression and regression of arterial wall changes and plaques. Salonen and colleagues have followed a cohort of Finnish men over 24 months, and report age, smoking habit and serum LDL to be the predictors of disease progression. Belcaro and colleagues have reported progression and regression of plaques related to the treatment of hyperlipidaemia (Belcaro et al, 1992) while Rudofsky and Hirche have reported an increase (of approximately 20 $\mu$ l) in the volume of small plaques over a 20 month period, and a relative reduction in plaque growth rate with serotonin inhibitors (Rudofsky and Hirche, 1989.)

Regression of atherosclerosis is still a matter of discussion. Although several studies carried out in animals have given encouraging results with regard to experimental atherosclerosis after return to a normal or hypocaloric diet (Armstrong, 1976; Stary, 1984), few studies in humans have shown conclusive results. Using high resolution ultrasound, spontaneous regression has been reported by two groups of workers (Hennerici et al, 1985; Norris and Bornstein, 1986) and, using three dimensional ultrasound, Hennerici and colleagues have reported a significant plaque volume reduction during heparin-induced extracorporeal LDL elimination on precipitation from plasma (Hennerici et al, 1991).

### **3.4 Intima-media thickness measurement in epidemiology studies**

High resolution ultrasound has been used in studies in Finland as part of the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD). This is a cross-sectional study which aims to establish risk factors for ischaemic heart disease and extra-coronary atherosclerosis. By studying the intima-media thickness of the common carotid artery over 2 years, they have shown a significant increase in thickness associated with age, serum LDL cholesterol concentration, pack-years of smoking, blood leucocyte count, platelet aggregation (Salonen and Salonen, 1990); and evidence of interactions between serum copper, selenium and low density lipoprotein cholesterol in atherogenesis (Salonen et al, 1991).

At present, high resolution B-mode ultrasonography is being used in several US multicenter trials aimed at evaluating atherosclerosis evolution. These studies include the Atherosclerosis Risk in Communities Project (a four field centre epidemiological study evaluating popliteal and carotid arteries in 16,000 randomly selected subjects aged 45-64) (The ARIC investigators, 1989); the Cardiovascular Health study (a four field centre epidemiological study evaluating and carotid arteries in 5,000 subjects over 64) (O'Leary et al, 1991); the Asymptomatic Carotid Artery Plaque Study (a five field centre clinical intervention study evaluating the effect of the cholesterol reducing agent Lovastatin on asymptomatic carotid artery atherosclerosis); the PLAQ II study (a single centre clinical intervention study evaluating the effect of the cholesterol reducing agent SQ 31,000 on asymptomatic carotid artery atherosclerosis); and the Multicenter Isradipine Diuretic Atherosclerosis Study (an eight field clinical intervention study designed to compare the efficacy of isradipine with hydrochlorthiazide in retarding the progression of early carotid atherosclerosis) (Borhani et al, 1991). The choice of method in these studies derives from the ability of B-mode imaging, when used with standardized protocols, to replicate measurements of arterial wall thickness with a total absolute error below 0.2mm (Insull et al, 1989). Similar methodology has demonstrated the rate of progression of atherosclerosis to be 0.3mm/year (Bond et al, 1990).

### **3.5 Aims of the thesis**

Noninvasive testing has focused on the detection and grading of high grade stenosing lesions amenable to surgery. Yet the prevalence of lesions producing a stenosis of greater than 50% of the vessel diameter is low. High resolution ultrasound allows small plaques to be detected, and early disease to be monitored. Furthermore, the intima media thickness of the artery wall can be measured with an accuracy of 0.2mm (Insull et al, 1989). Such measurements have epidemiological and prognostic potential. They may allow the study of progression (or regression) of atherosclerotic disease before symptoms develop. As yet, no studies have proven that intima media thickness is correlated with plaque development, or that the intima media thickness of the carotid arteries reflects disease elsewhere in the body. The aims of this thesis are:

- i) To examine the carotid bifurcation to detect the sites of early plaques, and to determine factors determining the distribution of such plaques.
- ii) To compare the intima media thickness at different sites, and to determine factors associated with intima media thickening in a normal group of volunteers.
- iii) To assess the value of the common carotid artery intima-media thickness, compliance and distensibility in the prediction of the presence of bifurcation plaques and macrovascular disease.



## CHAPTER 4

# THE NON-INVASIVE DETECTION OF CORONARY AND PERIPHERAL VASCULAR ATHEROSCLEROSIS

## **4.1 Introduction**

The aim of this thesis is to assess the value of common carotid artery intima-media thickness in the prediction atherosclerotic disease. The presence of plaque at the carotid and femoral bifurcations can be detected by ultrasound, but the presence of plaques is not an indicator of the severity of disease, and other methods of detecting occult atherosclerotic disease are necessary. This chapter will discuss methods of detection of occult atherosclerosis in the coronary and peripheral vasculature and our reasons for choosing ECG stress-testing and the one-minute treadmill to detect macrovascular disease.

Standardised questionnaires such as the Rose cardiovascular questionnaire have been designed to detect early symptoms of disease but will miss individuals with occult disease (Rose and Blackburn, 1968). Clinical examination, for example, the disappearance of pedal pulses after exercise, a bruit or a palpable aortic aneurysm, may suggest the presence of underlying disease, but this alone is unreliable (Greenhalgh et al, 1986; Collin et al, 1988). The majority of non-ultrasound methods of detecting occult atherosclerosis rely on tests of function, for example, methods for the detection of occult coronary artery disease include resting and exercise ECG recordings, and measurement of exercise induced stroke volume changes by transcutaneous aortography. The detection rate will depend on the method used, as a stress test will yield a greater number of subjects with occult disease than a resting test (Laing and Greenhalgh, 1983).

## **4.2 Non-invasive methods of detection of occult atherosclerosis in the coronary arteries**

### **4.21 The resting ECG**

Changes in the resting ECG may be detected in an asymptomatic individual. The presence of ST abnormalities may suggest the presence of underlying coronary artery disease. Such a sign is detected in a relatively small number of individuals, and the predictive value may increase from 8% to 44% when combined with a positive ECG stress test (Joy and Trump, 1981). Coronary artery disease is also found in one-fifth of individuals with an asymptomatic left or right bundle branch block (Frolicher et al, 1977), and an isolated inverted U wave has been reported to be confined to individuals with ischaemic heart disease (Kishida et al, 1982).

#### **4.22 Electrocardiographic chest wall mapping (ECG-CWM) stress test**

The technique of chest wall mapping was originally described by Fox and coworkers (Fox et al; 1979), it has subsequently been modified and validated in our department (Salmasi et al, 1983). The principle of this test is based on the observation that ECG changes reflect changes in the underlying myocardium. By positioning the leads it is possible to monitor changes in the distribution of all three major coronary arteries, providing spatial information.

Comparisons with angiography have shown that the first row of electrodes reflects the right coronary artery territory, the second and third rows, the left anterior descending and diagonal artery territories; and the posterior row, the circumflex coronary artery territory. Using multiple criteria (ST segment depression, Q waves and inverted U waves), the presence or absence of significant (50% stenosis) coronary artery disease could be detected by chest wall mapping with exercise stress testing with a sensitivity of 90% and a specificity of 88% compared with coronary angiography. The absence of significant coronary artery disease and the presence of single, double or triple vessel disease was correctly predicted in 70% of patients (Salmasi et al, 1983).

#### **4.23 Transcutaneous aortovelocityography.**

This technique uses a Doppler probe at the suprasternal notch to assess the aortic blood velocity signals (Light, 1976). From these signals a systolic velocity time integral is derived which represents a measure of stroke volume: the stroke distance. Studies have shown that the stroke distance correlates well with the left ventricular ejection fraction in patients with coronary artery disease, and in normal individuals the stroke distance increased by more than 6% at peak exercise while in patients with coronary artery disease and left ventricular dysfunction the stroke distance decreased at maximal exercise (Salmasi et al, 1987).

#### **4.24 Holter monitoring**

The 24 hour ECG monitor has the potential to detect abnormalities in the heart rate and rhythm within a 24 hour period and ambulatory monitoring of the ECG during everyday activities can detect silent ischaemic heart disease (Stern and Tzivoni, 1974; Schang and Pepine, 1977). A number of studies have attempted to assess the prognostic significance of ischaemic changes during daily activity, as recorded by ambulatory ECG monitoring. Hedblad and colleagues found that men with ST depression over 0.1mV and no history of coronary artery

disease, had a 4.4 times greater relative risk of fatal and non-fatal myocardial infarction on follow-up than those without. A similar poor prognosis was reported by Erikson and colleagues (Erikson and Thaulow, 1984). Rocco and colleagues found that, in patients with coronary artery disease, the frequency of future cardiac events was 51% among patients with ST depression on ambulatory ECG compared to 12% in those without ischaemic changes (Rocco et al, 1988). The frequency of silent ischaemic episodes was found to be as high as 90% in the patients with coronary artery disease studied by Mody and colleagues, however this study also showed that, though the specificity of silent ischaemic episodes was over 90%, the absence of S-T segment changes on Holter ECG recording has little predictive value, and the test has a low sensitivity (Mody et al, 1988). Therefore to identify or diagnose the presence of ischaemic heart disease a test with a higher sensitivity, such as exercise ECG testing, should be used.

### **4.3 The non-invasive detection of peripheral vascular disease**

In asymptomatic individuals, the presence of significant occult atherosclerotic disease in the lower limbs can be assessed by palpation of the peripheral pulses, measurement of the ankle brachial systolic pressure ratio and stress tests such as the measurement of the ankle pressure after exercise or during reactive hyperaemia. Each of these techniques has limitations in the assessment of occult disease.

#### **4.31 Palpation of peripheral pulses**

In around 20% of the adult population, at least one pulse is not detected when the foot pulses are palpated (Schroll and Munck, 1981; Criqui et al, 1985), but a number of factors may hinder the palpation of pulses such as obesity, deformity and congenital absence of pulses. Though relatively specific, palpation of peripheral pulses is relatively insensitive. When evaluated against other measures of peripheral arterial disease, such as ankle-brachial pressure ratios, an absent pulse in one or more arteries was found in one study to be 77% sensitive and 86% specific (Criqui et al, 1985). There is also considerable inter and intra-observer variation in the detection of peripheral pulses (Lulbrook et al, 1962, Meade et al, 1968).

#### **4.32 Ankle brachial systolic pressure ratio**

The sensitivity resting ankle brachial systolic pressure ratio in detecting angiogram positive disease has been reported to 95% in subjects with a pressure ratio of less than 0.9 (Bernstein and Fronck , 1982; Laing and Greenhalgh, 1983). These studies were performed on a small number

of hospital patients. The variability of ankle pressure measurement has been examined by Fowkes and colleagues. The 95% confidence limits of one measurement was found to be  $\pm 16\%$ . The variability in measurement related to observer, timing and repeat measurement was found to be considerably less than 'biological' variability between subjects and legs (Fowkes et al, 1988).

#### **4.33 Reactive hyperaemia**

In the reactive hyperaemia test, blood flow is occluded at the upper thigh and the ankle systolic pressure is measured on releasing the obstruction. In the presence of peripheral arterial disease, the systolic pressure two to three times further subjects and takes longer to return to pre-occlusion levels than in healthy individuals (Johnston, 1975; Hummel et al, 1978; Baker, 1978). In a study comparing reactive hyperaemia with resting ankle brachial pressure ratios, a further 16% of individuals were detected with reactive hyperaemia (Criqui et al, 1985). The variability of reactive hyperaemia is similar to that of at rest ankle brachial Doppler pressure ratios (Fowkes et al, 1988).

#### **4.34 One minute treadmill**

Intermittent claudication, usually the first sign of vascular disease of the limbs, is brought on by exercise when the metabolic requirements of the limb increase. In the normal leg the ankle pressure is the same or slightly higher than the arm pressure at rest and does not fall by more than 30 mmHg following exercise (Laing, 1991). In patients with peripheral vascular disease demonstrated by angiography, a standard amount of exercise (1 minute at 4km/h, 10% slope) will produce a significantly greater fall in ankle pressure and a longer recovery time than in normal subjects (Carter, 1972; Thuselius, 1978; Osmundson et al, 1981; Laing and Greenhalgh, 1983). Mild disease may be detected by a fall in ankle pressure following exercise in the presence of a normal resting pressure (Laing and Greenhalgh, 1983). The leg at this stage may be normal on examination and free from symptoms. In patients with severe peripheral vascular disease, the ankle pressure is reduced at rest, and will fall still further following exercise, the fall in ankle pressure is related to the severity of the disease (Yao, 1970). When the one-minute treadmill was compared with the resting ankle brachial pressure in a population of asymptomatic patients, the resting ankle brachial pressure was abnormal in 1/100 hernia patients, 3/100 diabetics and 1/50 CABG patients but the exercise test detected disease in a further 9/100 hernia patients, 27/100 diabetics and 18/50 CABG patients. Therefore this test is much more sensitive in the detection of occult peripheral arterial disease than resting ankle brachial pressure alone.

#### **4.35 Comparison between the reactive hyperaemia test and the one minute treadmill test.**

The ankle pressure response to the reactive hyperaemia test and the one minute treadmill test are similar, although the recovery period is extended after treadmill exercise (Fox et al., 1977; Hummel et al, 1978). Both are comparatively cheap and the subject tolerance of the two tests depends on age and disease status, the discomfort of the reactive hyperaemic test against the fitness for a one minute treadmill test.

#### **4.4 Conclusion**

From the above review, it is clear that stress testing is more sensitive in the detection of occult coronary and peripheral arterial disease. Furthermore, these tests are cheap, and relatively well tolerated by patients, and are suitable for detection of macrovascular disease in asymptomatic individuals.

**PART II**  
PATHOLOGY STUDIES

CHAPTER 5  
THE VARIATION IN CAROTID BULB  
MORPHOLOGY



## **5.1 Introduction**

Although classical anatomical drawings suggest that the carotid bulb lies at the origin of the internal carotid artery, there is considerable variation in carotid bulb morphology and this relates primarily to the position of the bulb in respect to the flow divider. The origin of the bulb is reported to be an area of low shear stress, hence the site of early plaque formation, yet the flow divider has been advocated as the anatomical marker at the carotid bifurcation. If several different carotid bulb morphologies exist, then the position of the flow divider will vary in relation to the origin of the bulb, and this needs to be taken in to consideration in the assessment of early plaque formation.

The aim of the study reported in this part of the thesis was to identify the major morphological types of carotid bulb anatomy and to determine the frequency with which they occur in adults.

Thus the following questions were posed:

- i) How many morphological types can be identified?*
- ii) What is the inter-observer variation in classifying the bulb types*

## **5.2 Material and method**

### **5.21 Material**

All selective carotid angiograms available from our hospital archives were examined. Studies were excluded if the carotid bifurcation or the origin of the internal carotid artery was obviously diseased. 89 studies were included, and in 104 sides the images were of adequate quality to provide at least two plane views of the carotid bifurcation. The average age of the patients was 46 and, in the majority, angiography was performed to investigate intracranial abnormalities rather than carotid bifurcation disease.

### **5.22 Method**

The carotid bulb could be classified into four morphological types:

Type A. No bulb. The internal carotid artery arises as a direct extension of the common carotid artery and the long axis of the vessel lies in line with the long axis of the

common carotid artery. No dilatation of the internal or external carotid arteries is seen (Fig 5.1)

Type B. The origin of the bulb is restricted to the origin of the internal carotid artery. (Fig 5.2).

Type C. The origin of the bulb lies proximal to the origin of the external carotid artery. (Fig 5.3)

Type D. The carotid bulb involves the origin of the internal and external carotid arteries and the angle between the internal and external carotid arteries is at least 90° (Fig 5.4).

The 104 carotid bifurcations seen on the angiograms were classified according to bulb type by two observers independently.

### **5.3 Results**

The distribution of bulb types reported by the two observers is shown in table 5.1 and the interobserver variation in table 5.2.

### **5.4 Discussion**

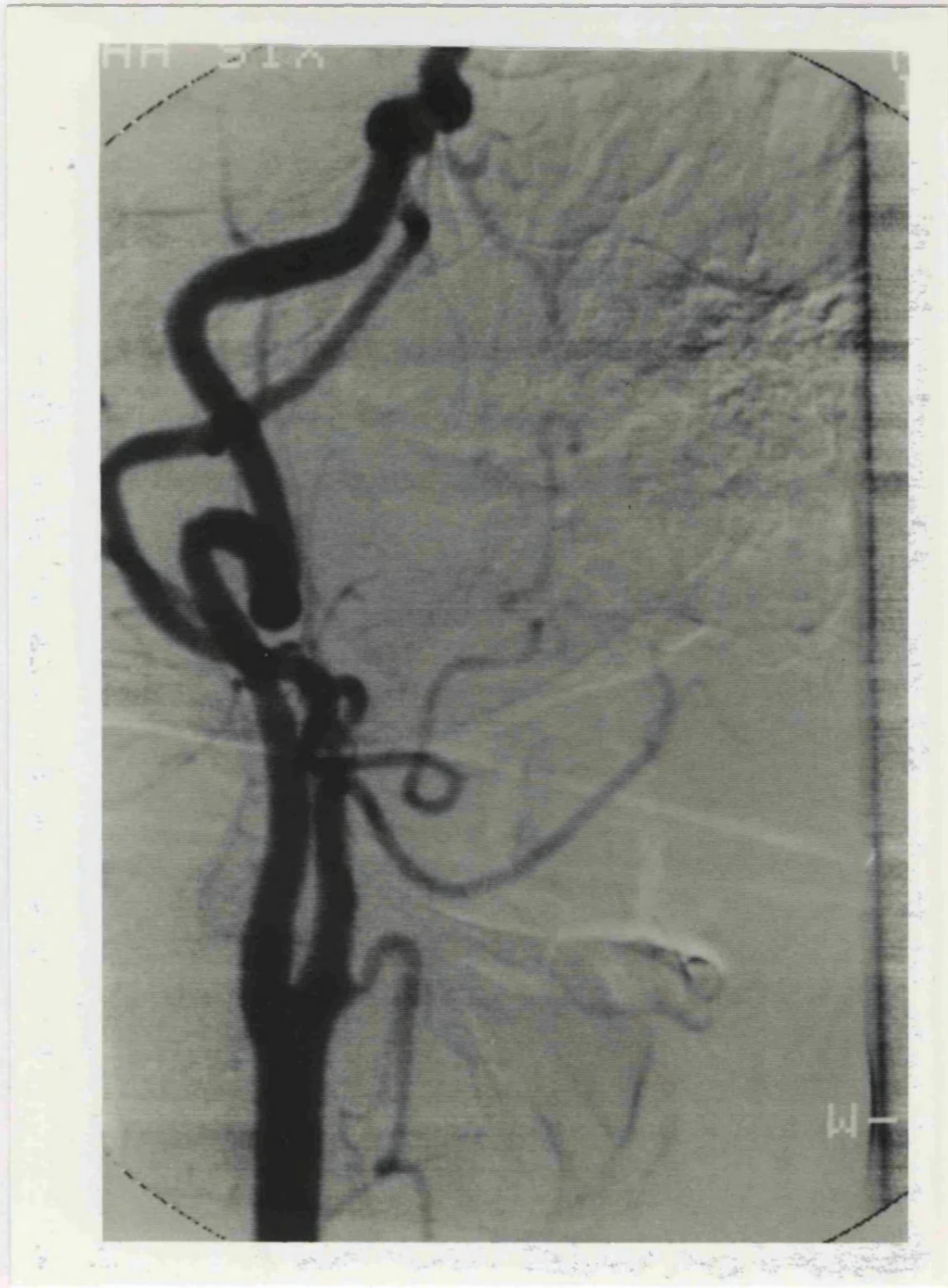
#### ***i) How many bulb types can be identified?***

Four different morphological types can be identified. The commonest, type B was found in 40%. The remaining (A, C, and D) had an incidence of approximately 20% each. As angiography can only demonstrate the lumen of the artery, A type bulbs may represent a bulb filled in by early atherosclerosis.

An assessment of the relationship between left and right sides could not be undertaken in this study as in only 15 patients had adequate views of both carotids. This issue will be addressed in the ultrasound study discussed in the next chapter

#### ***ii) What is the inter-observer variation in classifying the bulb types?***

The exact correlation between the two observers was 79%, and the overall correlation using kappa statistic was 0.7.



**Fig 5.1**      **Angiogram of a carotid bifurcation with no bulb (type A)**

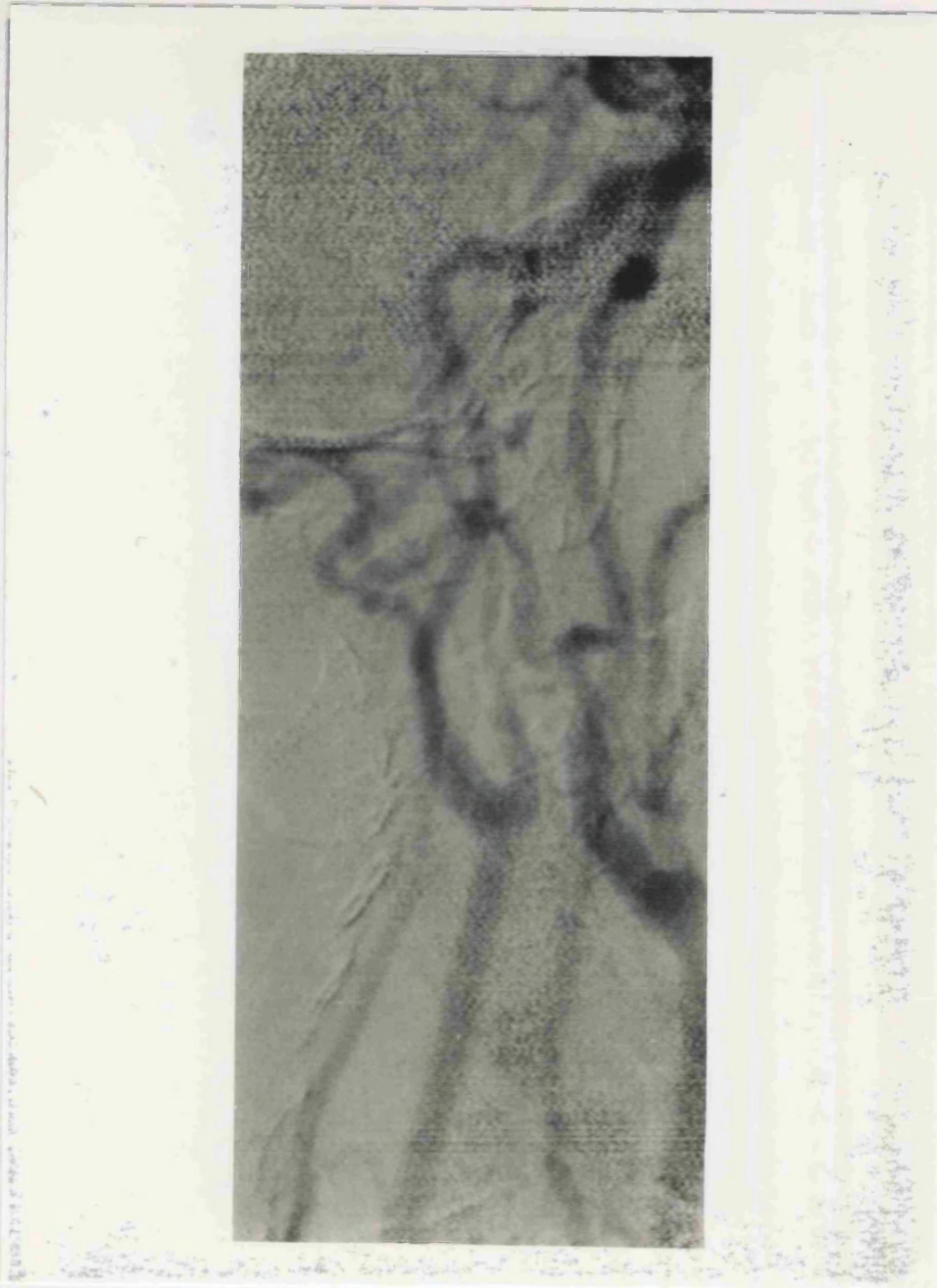


**Fig 5.2**      **Angiogram of a carotid bifurcation in which the origin of the bulb is restricted to the origin of the internal carotid artery (type B)**





**Fig 5.3** Angiogram of a carotid bifurcation in which the origin of the bulb lies proximal to the origin of the external carotid artery (type C)



**Fig 5.4** Angiogram of a carotid bifurcation in which the bulb involves the origin of both the internal and external carotid arteries (type D).

**Table 5.1 The distribution of bulb types reported by the two observers.**

Bulb type	Observer 1	Observer 2
A	18 (17.3%)	17 (16.3%)
B	43 (41.3%)	42 (40.4%)
C	22 (21.2%)	24 (23.1%)
D	21 (20.2%)	21 (20.2%)

**Table 5.2**     The interobserver variation in the reporting of bulb types

		<u>Observer 1</u>				
<u>Observer 2</u>	Bulb types	A	B	C	D	TOTAL
	A	13	0	2	2	17
	B	2	36	2	2	42
	C	2	4	17	1	24
	D	1	3	1	16	21
	TOTAL	18	43	22	21	104

kappa = 0.7



## CHAPTER 6

### THE DETECTION OF DIFFERENT BULB TYPES USING HIGH RESOLUTION ULTRASOUND

## **6.1 Introduction**

As demonstrated in chapter 4, four different bulb types can be identified on angiography. High resolution ultrasound, by virtue of its ability to delineate clearly the arterial wall and lumen, is particularly suited to the examination of bulb morphology. The aim of this study was to establish the incidence of the different bulb types in adults, using high resolution ultrasound, and to determine the relationship between the bulb type of the left and right carotid arteries. Thus the questions posed were:

- i) What is the incidence of the different types of bulb (A-D), as detected by high resolution ultrasound?*
- ii) How often is the left bulb type the same as the right?*

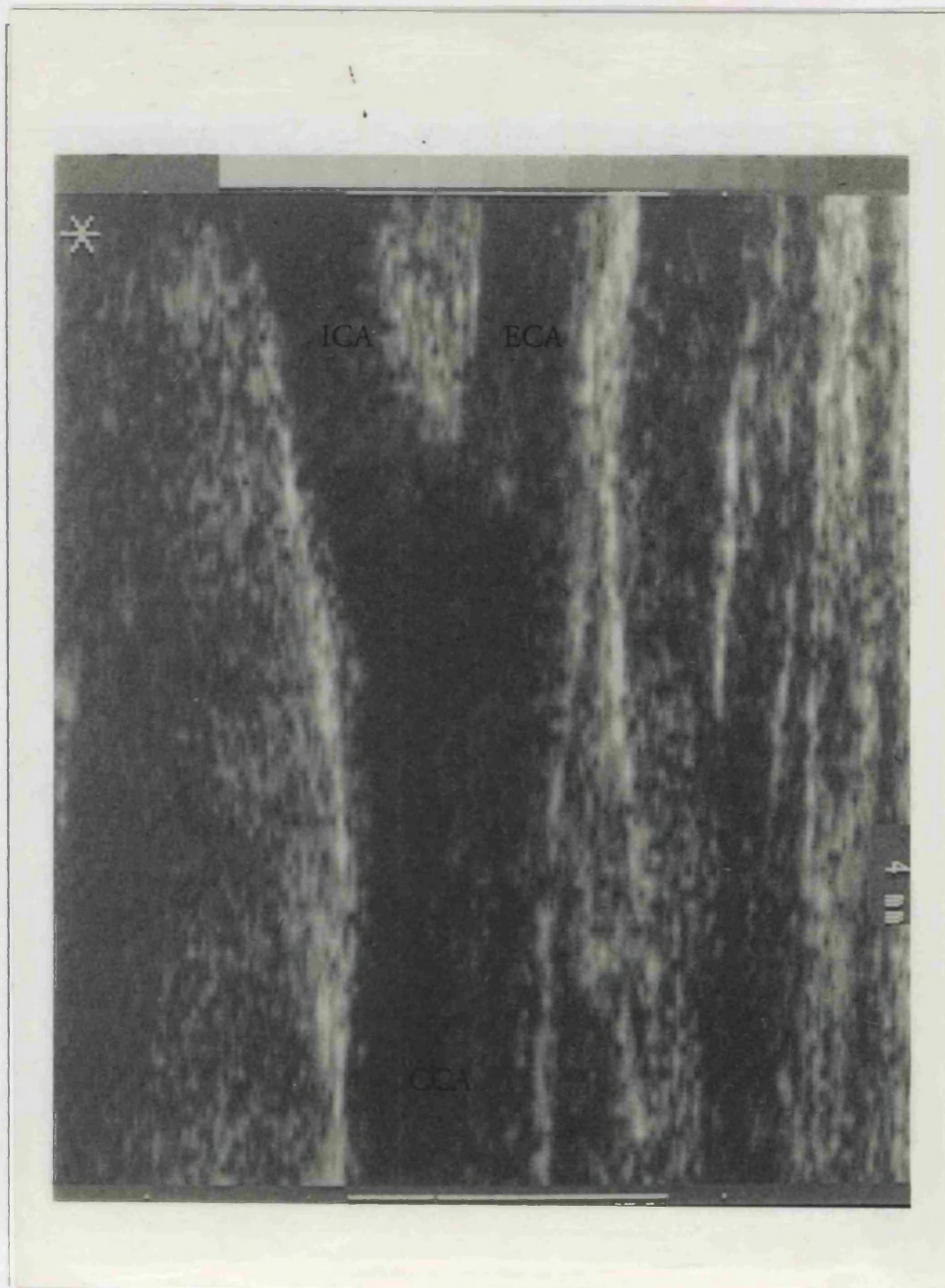
## **6.2 Material and method**

### **6.21 Material**

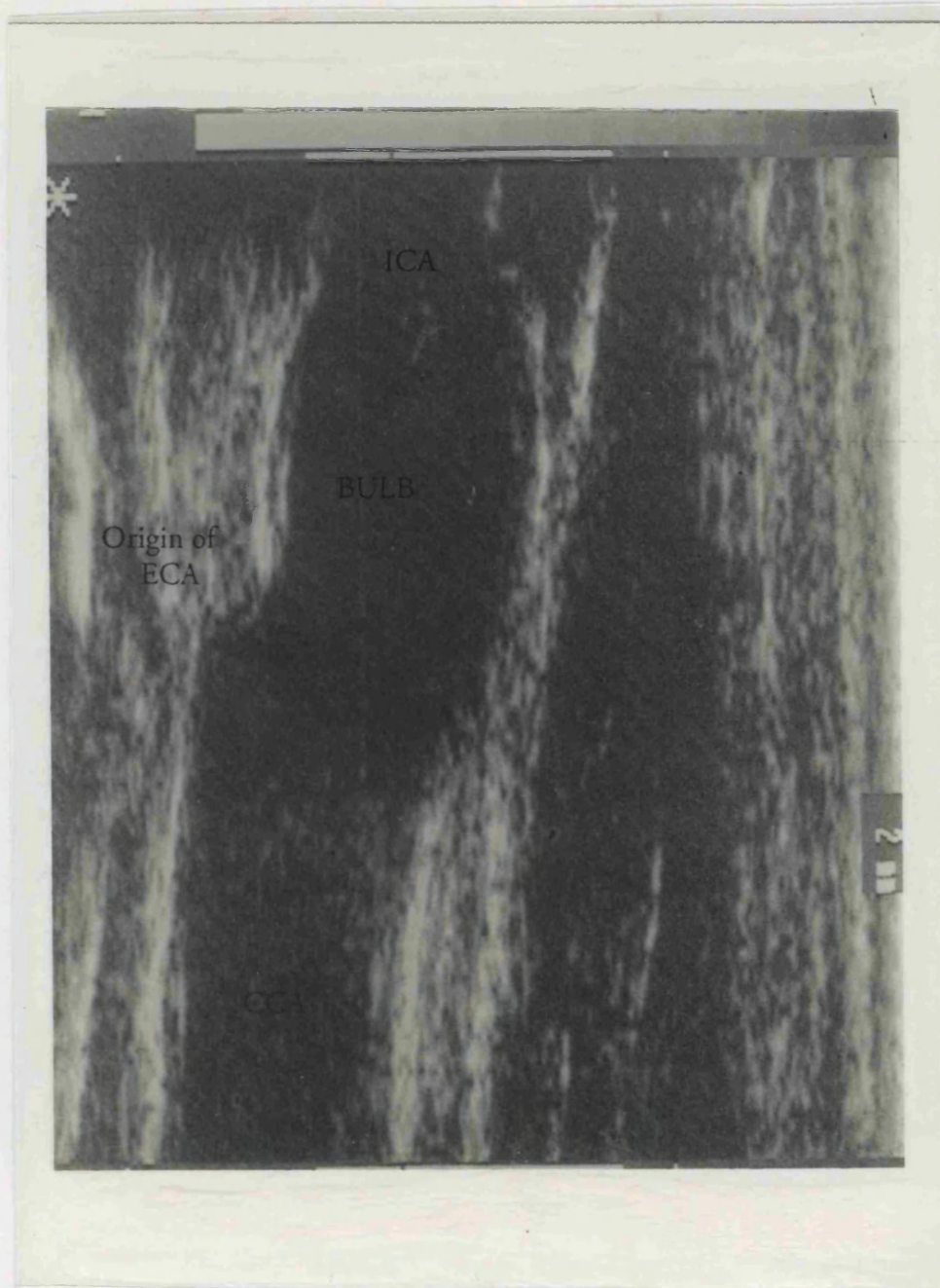
288 patients attending the vascular laboratory for high resolution ultrasound examinations were included in this study. These patients were being scanned for the studies discussed in chapters 6, 11 and 13 and included 148 volunteers, 96 patients with non-insulin dependent diabetes and 44 patients early carotid plaques.

### **6.22 Method**

Imaging was performed using an ATL Ultramark 4 with a dedicated 7.5 MHz high resolution linear ultrasound probe. The machine was preset on the following settings: Dynamic range 47db, power 50%, FPS 28, Reject 1, Edge 2, grey scale 4, and smooth F3. With the patient supine on a couch, and the head rotated 45° away from the side to be examined, the carotid bifurcation was examined with the linear probe parallel to the artery. The artery was first examined in the antero-posterior plane, by placing the probe anterior to sternomastoid, as high as possible behind the angle of the mandible. The probe was then rotated through 90° to lie over the posterior border of sternomastoid, and the carotid bifurcation was assessed in the lateral plane. Bulb type was determined according to the criteria discussed in chapter 4. Examples of each bulb type detected by ultrasound are shown in figures 6.1-6.4.



**Fig 6.1**      **Ultrasound image of a carotid bifurcation without a bulb (type A)**

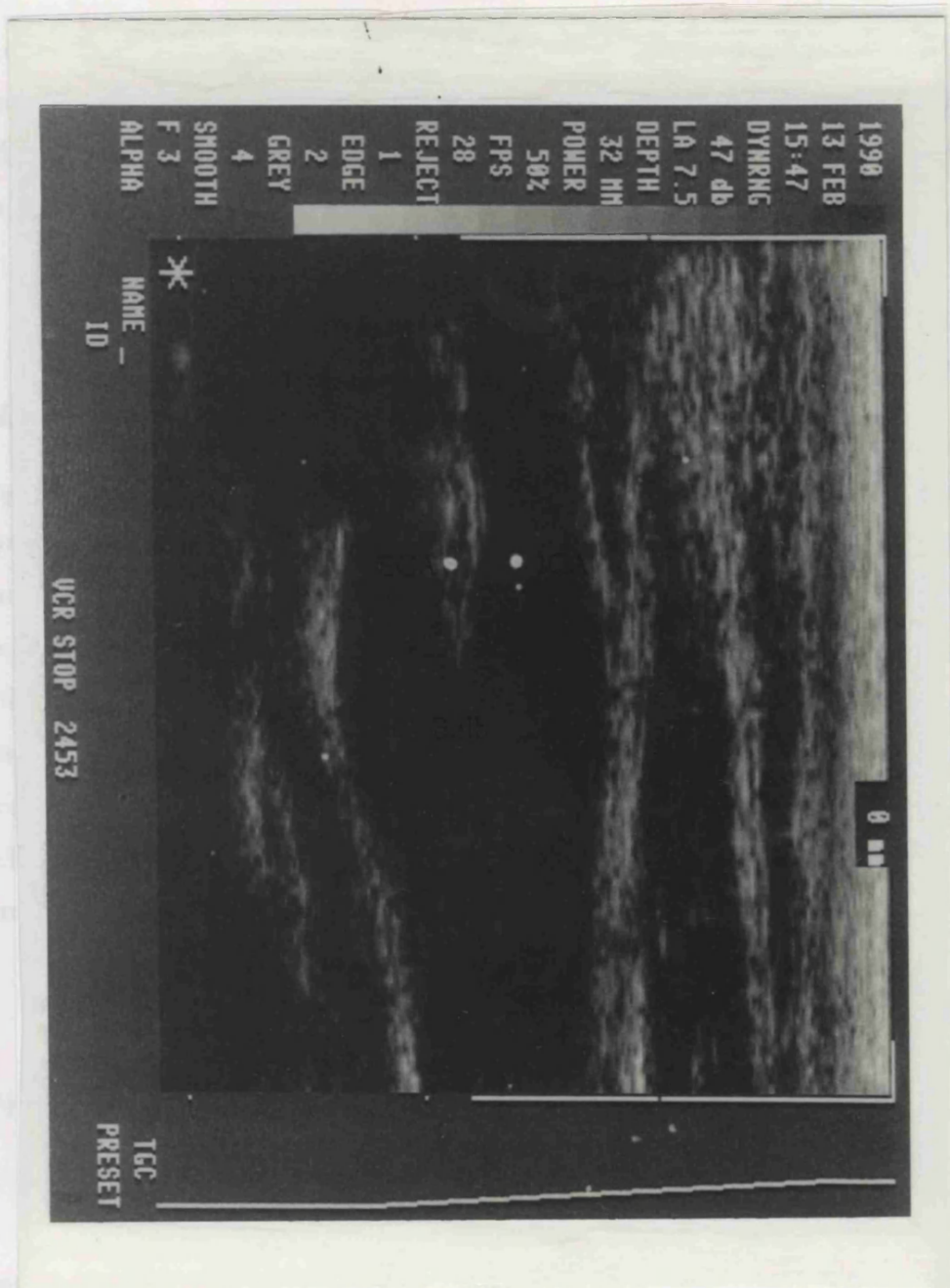


**Fig 6.2** Ultrasound image of a carotid bifurcation in which the bulb is confined to the origin of the internal carotid artery (type B)





**Fig 6.3** Ultrasound image of a carotid bifurcation in which the origin of the bulb lies proximal to the origin of the external carotid artery (type C)



**Fig 6.4** Ultrasound image of a carotid bifurcation in which the bulb involves the origin of both the internal and external carotid arteries (type D).

### **6.3 Results**

The distribution of bulb types in 545 carotid bifurcations is shown in table 6.1. In 31 patients, only one bulb could be assessed for bulb type, the second side either had too much plaque or was occluded. Comparison between bulb type between the left and right sides is shown in table 6.2.

### **6.4 Discussion**

#### ***i) What is the incidence of the different types of bulb (A-D), as detected by high resolution ultrasound***

Using high resolution ultrasound, we found that bulb types B and C are commonest, and occur with an incidence of 42% and 32% respectively. Bulb types A and D are rarer and occur with a frequency of 16% and 10% respectively. The difference in the incidence of bulb types in this study as compared to the previous study using selective arteriography may be due to the fact that, whereas, in angiographic studies, only the lumen can be assessed, high resolution ultrasound allows the full thickness of the wall to be examined, thus a small plaque at the origin a C type bulb on ultrasound may be interpreted as a B type bulb on angiography (Fig 6.5), and smooth filling in of the bulb may appear to be an artery with no bulb on angiography (Fig 6.6).

#### ***ii) How often is the left bulb type the same as the right?***

In 74% of individuals studied, the left and right carotid bulbs were similar (Table 6.2).

### **6.5 Conclusion**

In conclusion, four different bulb types can be detected by angiography and ultrasound. In view of the relationship of flow patterns and the development of plaque at the carotid bifurcation (see literature review), developing criteria for identifying the different types of bulb was an essential prerequisite for subsequent studies on the site of origin of early atheroma (see chapter 7).

**Table 6.1**      **Distribution of bulb types in 545 carotid bifurcations examined with high resolution ultrasound.**

Bulb type	n	%
A	85	16%
B	232	42%
C	175	32%
D	53	10%
Total	545	

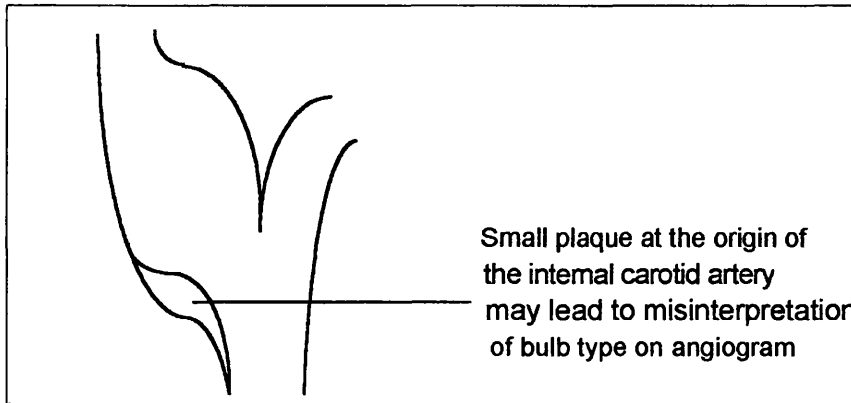


**Table 6.2      The difference in the incidence of bulb types between left and right sides.**

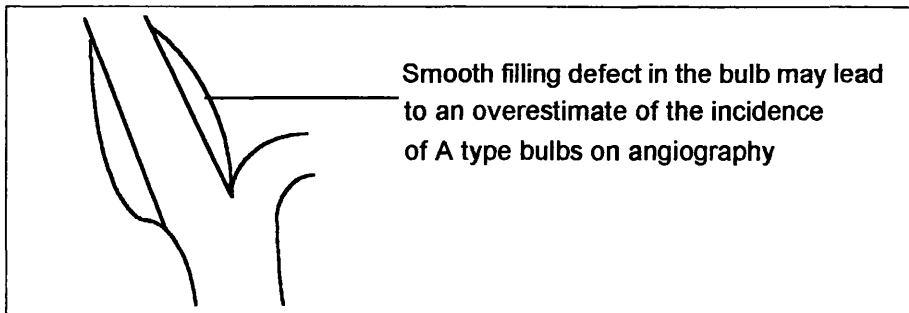
		Left side					
Bulb type		A	B	C	D	Total	
Right side	A	24	7	5	1	37	(14%)
	B	10	89	7	7	113	(44%)
	C	5	8	64	10	87	(34%)
	D	0	4	4	12	20	(8%)
	Total	39	108	80	30	257	
		(15%)	(42%)	(31%)	(12%)		

In 189 out of 257 individuals (74%) the bulb type was the same on the left as the right.

Kappa = 0.74



**Fig 6.5**      **A possible explanation for the difference in frequency of bulb types B and C with different imaging modalities**



**Fig 6.6**      **A possible explanation for the difference between imaging modalities in frequency of bifurcations with no bulb.**

CHAPTER 7

THE DETECTION OF EARLY ATHEROSCLEROTIC  
PLAQUES IN THE CAROTID BIFURCATION  
USING HIGH RESOLUTION ULTRASOUND

## **7.1 Introduction**

By the time that an atherosclerotic plaque produces a symptomatic stenosis, the whole carotid bulb is usually involved. However, post-mortem studies of fatty streaks in children and young adults have suggested that these early lesions first develop at the edges (proximally and distally) of the bulb and spread towards the centre of the bulb (Meyer and Noll, 1974)(Fig 7.1) and that the wall opposite the origin of the external carotid artery is most frequently affected (Grottum et al, 1983 ). If fatty streaks or plaques are present, a small fibrous ridge is frequently observed at the origin of the bulb (Burrig and Hort, 1988). However none of the above have been shown in vivo. The availability of high resolution ultrasound has now made this possible.

The aim of this study was to identify the site of early plaque formation using high resolution ultrasound and to determine the variability of such a site (or sites) in the bulb types described in the previous chapter.

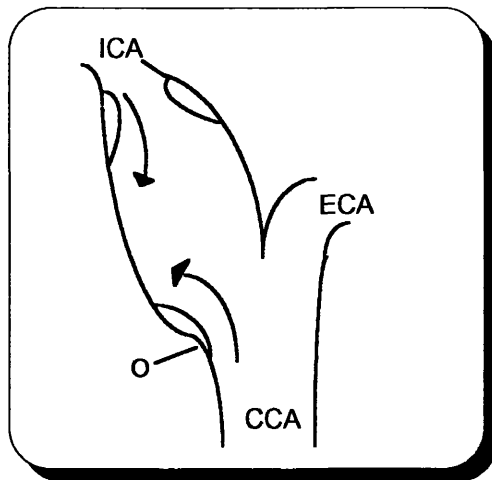
Thus the questions posed were:

- i) Which is the site (or sites) of early plaque formation at the carotid bifurcation, identifiable with high resolution ultrasound?*
- ii) How does this site (or sites) vary with the different bulb morphologies*
- iii) What is the best landmark for identifying early plaques?*

## **7.2 Material and Method**

### **7.2.1 Material**

63 carotid artery bifurcations from 45 patients were studied. These were selected from 89 consecutive routine examinations of patients referred to the vascular laboratory for carotid arterial investigations. The reasons for referral are shown in table 7.1. All patients selected for inclusion in the study had carotid plaques, defined as localised areas of arterial wall thickening of more than 1.2mm (ie the distance between the echogenic outer layer of the artery and the innermost echogenic layer >1.2mm). It should be pointed out that according to the Strandness criteria (Blackshear et al, 1979)) based on



Fatty streaks first develop at the edges of the bulb and spread towards the centre (arrows). Plaque development may proceed in a similar fashion

- O = Bulb origin
- CCA = Common carotid artery
- ICA = Internal carotid artery
- ECA = External carotid artery

**Fig 7.1**      **Proposed direction of growth of plaques at the carotid bifurcation**

**Table 7.1**     **Reason for referral for duplex scanning**

Reason for referral	Number of patients	Number of bifurcations
Contralateral carotid disease	20	20
Following contralateral endarterectomy	7	7
Amaurosis fugax or transient ischaemic attacks	8	16
Carotid bruit	4	8
As part of the assessment for peripheral vascular disease or coronary artery disease	6	12
Total	45	63

Doppler velocity changes, these carotid bifurcations would be classified as having either no stenosis (A) or a stenosis of <15% of the distal internal carotid artery (B). These criteria limited the size of the plaques to those that did not produce a significant stenosis.

## **7.22 Method**

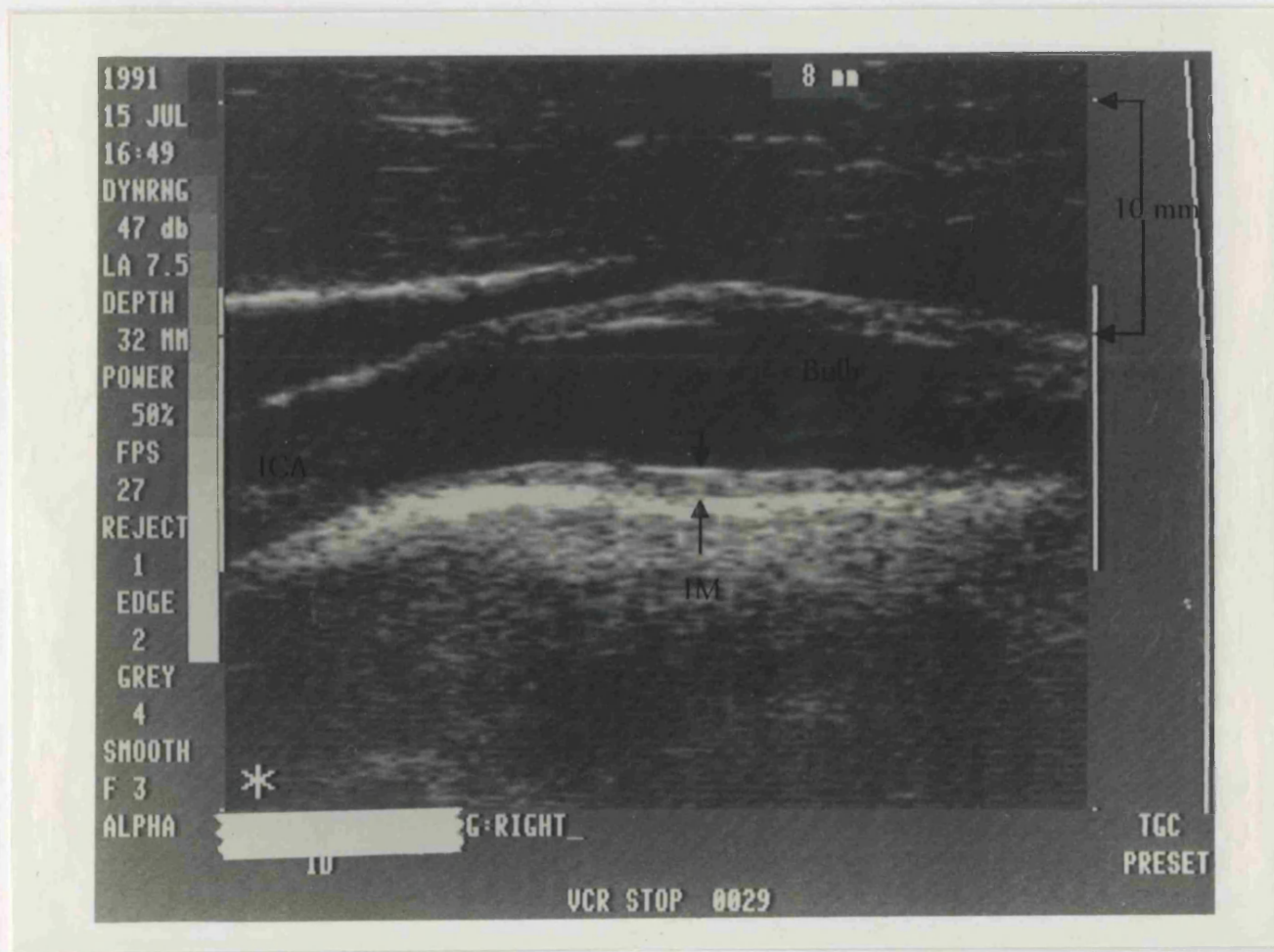
### **7.221 Imaging**

Imaging was performed using an ATL Ultramark 4 with a dedicated 7.5 MHz high resolution ultrasound probe. The machine was preset on the following settings: Dynamic range 47db, power 50%, FPS 28, Reject 1, Edge 2, grey scale 4, and smooth F3. The following longitudinal and transverse views were taken.

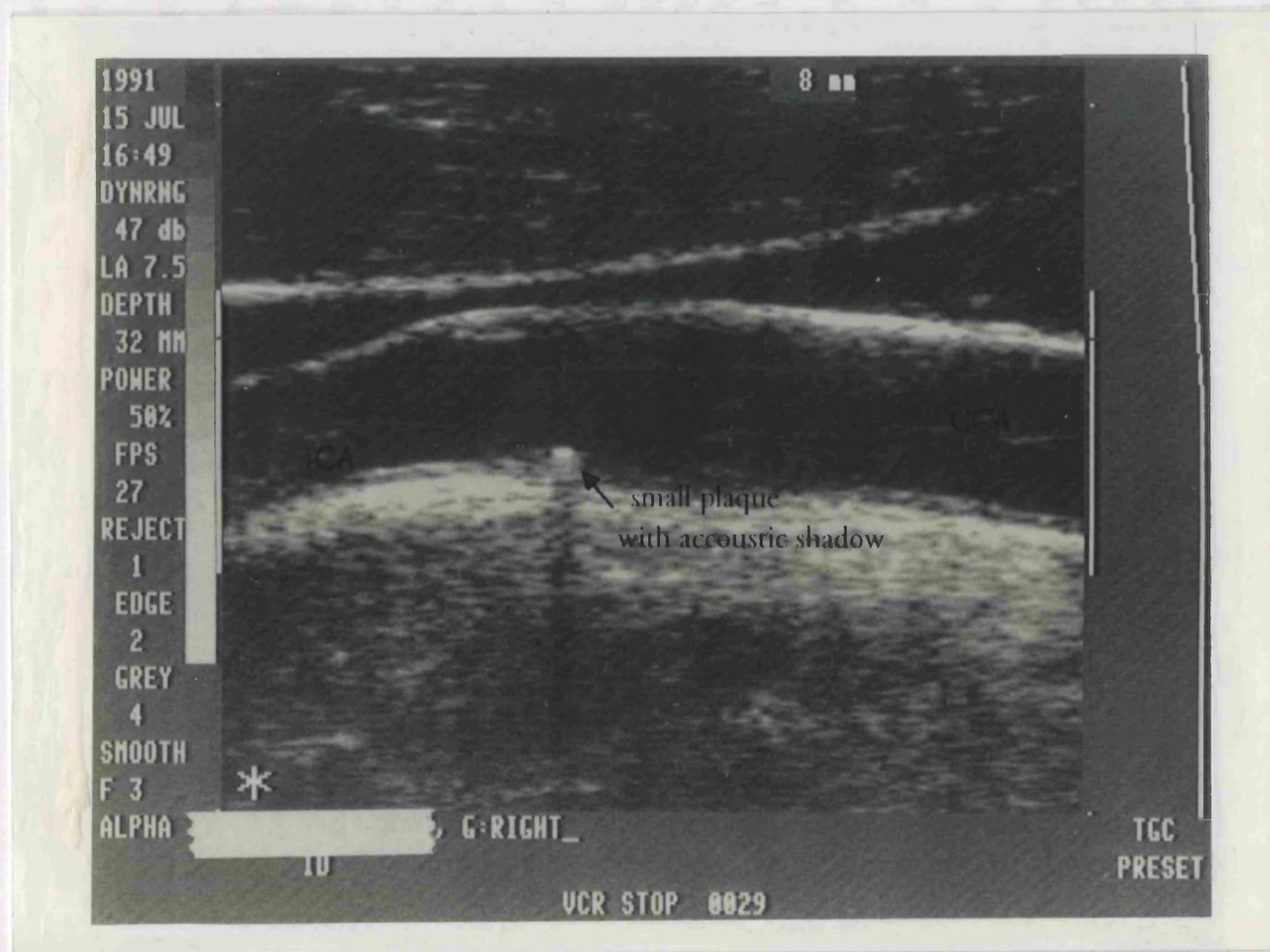
#### **a) Longitudinal views**

With the patient supine on a couch, and the head rotated 45° away from the side to be examined, the common carotid artery and carotid bifurcation were examined with the linear probe. Longitudinal views were taken in the antero-posterior plane, by placing the probe anterior to sternomastoid, as high as possible behind the angle of the mandible. The position of the probe was adjusted until the near and far walls of the carotid artery could be defined. The ultrasound picture was frozen and the frozen picture (screen capture) was printed on to thermographic paper using a Sony videographic printer. Screen captures were taken of the anterior view (Fig 7.2). The probe was then rotated through 90° to lie over the posterior border of sternomastoid, in line with the artery. The position of the probe was again adjusted until the artery walls could be defined, and further screen captures were taken of the lateral view of the artery (Fig. 7.3). As demonstrated in figures 7.2 and 7.3, the external carotid artery origin may not be visualised in which case further views would be taken to identify the bulb origin and the position of the flow divider.





**Fig 7.2** Ultrasound image of the anterior view of the carotid bifurcation in the longitudinal plane.



**Fig 7.3** Ultrasound image of the lateral view of the carotid bifurcation viewed in the longitudinal plane

### b) Transverse views

With the patient in the same position, and the probe at right angles to the line of the artery, the course of the common carotid artery was followed up the neck, following the line of sternomastoid with the probe. The position of the origin of the bifurcation and the flow divider were identified. Transverse views were then taken at the following sites :

View 1. Common carotid artery approximately 0.5cm proximal to the bulb origin

View 2. The bulb origin

View 3. At the bifurcation, just proximal to the flow divider

View 4. 0-0.5cm distal to the flow divider

View 5. 0.5-1.0cm distal to the flow divider

(Fig 7.4 and Fig 7.5)

### 7.222 Measurements

#### a) Longitudinal views

The carotid bifurcation was divided into four regions (Fig 7.6) for analysis :

Region I - 0.5-1.0cm proximal to the flow divider

Region II - 0-0.5cm proximal to the flow divider

Region III - 0-0.5cm distal to the flow divider

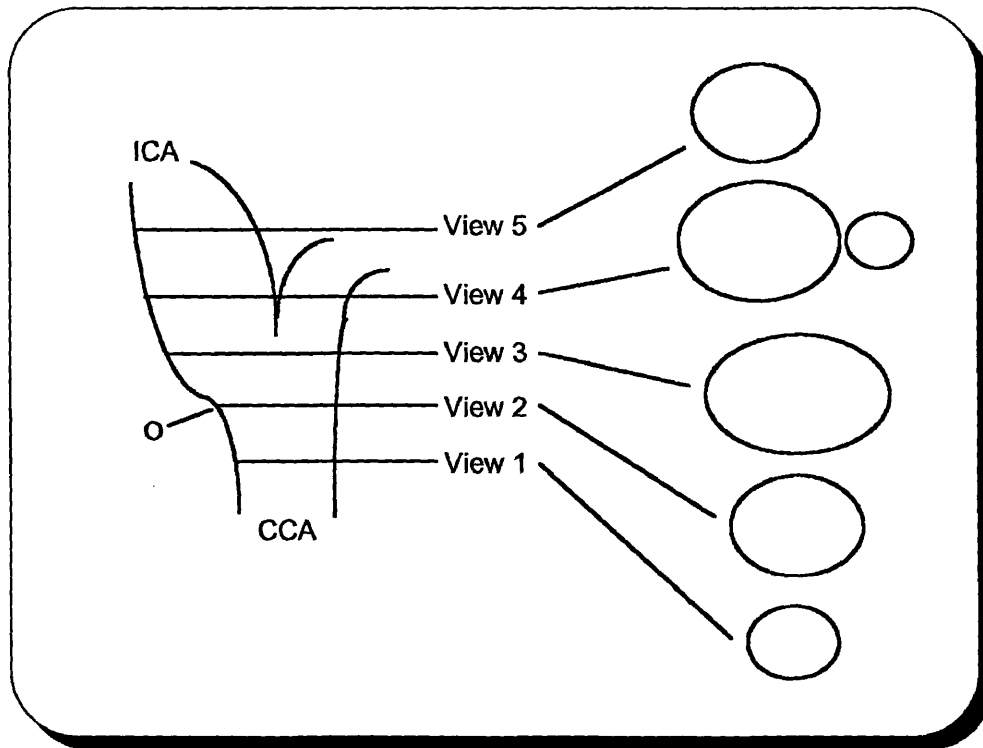
Region IV - 0.5-1.0cm distal to the flow divider

The near and far walls (in relation to the probe) of the bifurcation were assessed in each of these four regions and at a fifth site: the site of origin of the bulb "O" which lies at a variable distance from the flow divider (as discussed in chapters 5 and 6). If the wall could be clearly delineated and assessed, the distance between the echogenic outer wall of the artery and the innermost echogenic layer was measured. The maximum wall thickness for each region, both near and far walls and the maximum wall thickness at the origin of the bulb were measured.

#### b) Transverse views

In the transverse view, the echogenic layers can only be delineated when they lie at right angles to the incident beam as ultrasound is reflected away from the probe by the curvature of the vessel wall (Fig 7.7).

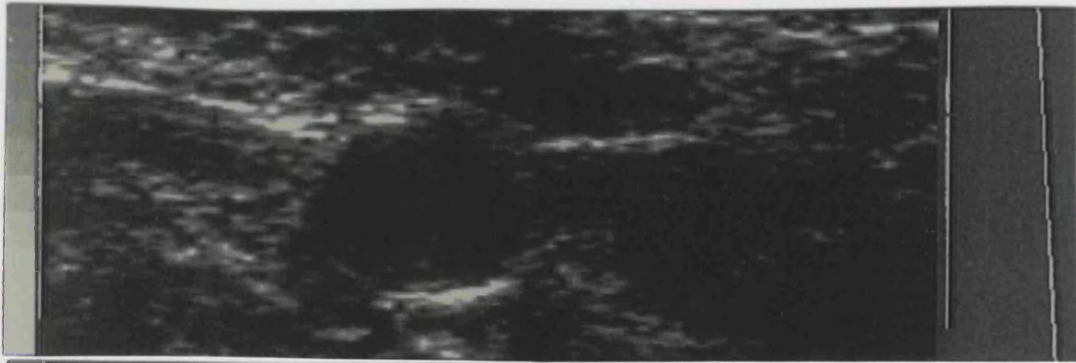
The distance between the outer echogenic and inner echogenic layers was measured for the near and far walls for each transverse view.



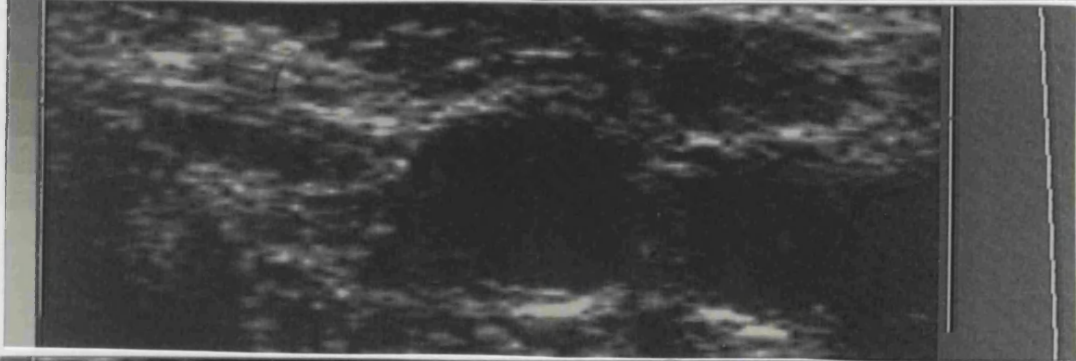
**Fig 7.4**      **The position of the transverse views in relation to the carotid bifurcation**



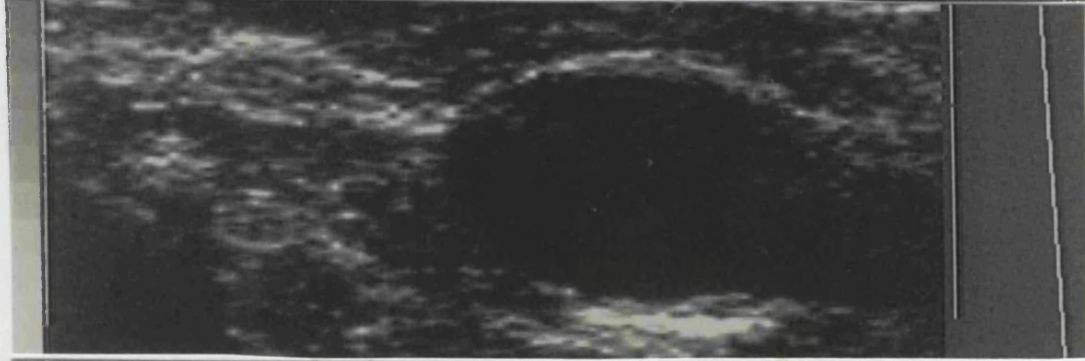
View 5



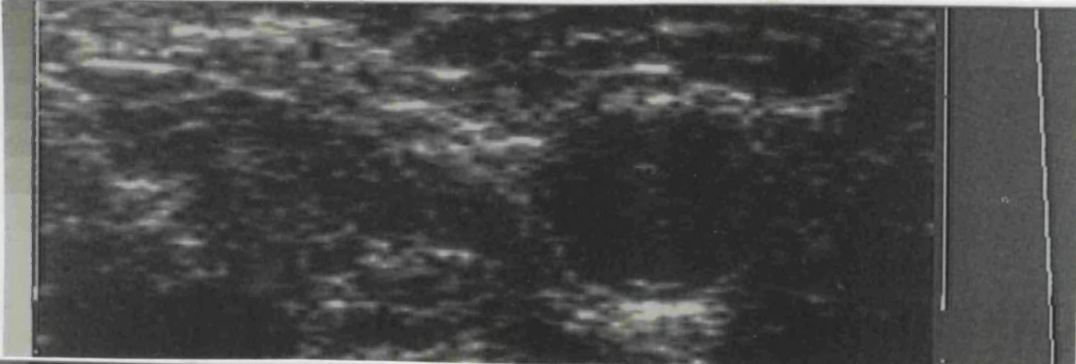
View 4



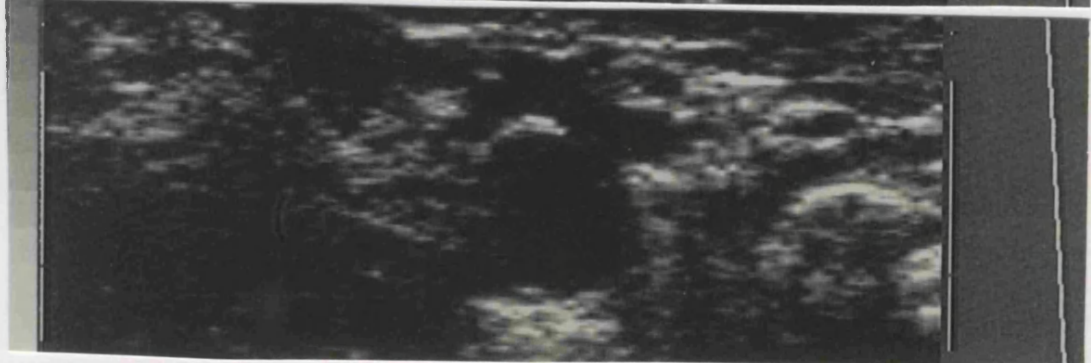
View 3



View 2

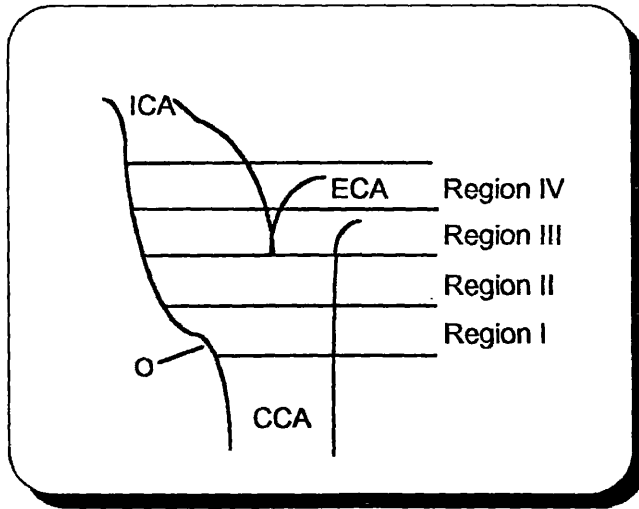


View 1

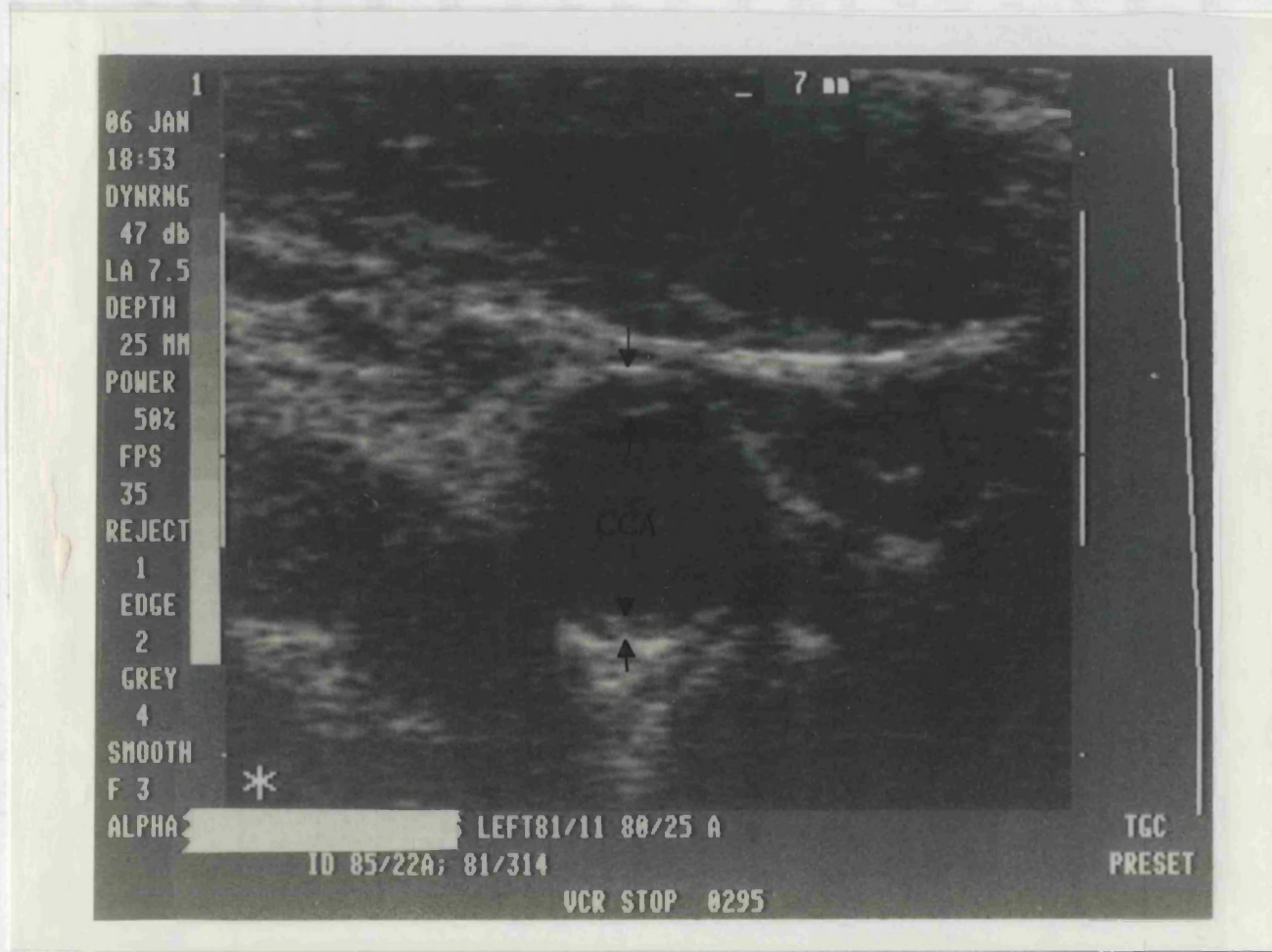


**Fig 7.5**

**An example of the five transverse views of the carotid bifurcation**



**Fig 7.6**      **The four regions of the carotid bifurcation**



**Fig 7.7** Ultrasound image of the common carotid artery viewed transversely

All measurements were made with calipers calibrated against the calibration line at the side of the ultrasound picture.

### **7.3 Results :**

#### **7.31 Wall thickness**

##### **a) In the longitudinal plane**

Basic descriptive statistics for the wall thickness measurements taken in the longitudinal view are shown in table A.1 and table A.2 of the appendix.

The median wall thickness (with upper and lower quartiles)(mm) for each region (I,II,III,IV) and at the site of origin O, for both near and far walls is shown in table 7.2. There was no significant difference between views but significant differences were observed between regions before and after the flow divider. The inter-regional difference for the combined views is shown in Fig 7.8. The mean wall thickness proximal and distal to the flow divider are compared in table 7.3

In the longitudinal views, the wall of the artery was poorly visualised at 15 sites, 5 of these were at the origin of the external carotid artery and 1 was due to an acoustic shadow; the remaining 9 sites could not be measured due to poor quality of the ultrasound image. Sites that could not be measured were excluded from the calculations.

##### **b) In the transverse plane:**

Basic descriptive statistics for the wall thickness measurements taken in the transverse views are shown in table A.3 of the appendix.

The median wall thickness ( with upper and lower quartiles)(mm) of the near and far walls for each view in the transverse plane is shown in table 7.4 and graphically in figure 7.9.

The transverse view was inadequate to allow an assessment of the arterial wall thickness in 20 bifurcations (52 measurements) (Table 7.5).The majority of poor views were restricted to view 5, the most distal view.

The median wall thickness of each view in the transverse plane is compared in table 6.6. View 3 is significantly thicker than all other views.



**Table 7.2 Median wall thickness (with upper and lower quartile) of each region and at the origin of the bulb, for near and far walls, in the anterior and lateral views.**

	Anterior view				Lateral view				All views combined
	n	Near wall	n	Far wall	n	Near wall	n	Far wall	
Origin	46	1.4mm (1.2-1.7)	46	1.3mm (1.2-1.4)	46	1.5mm (1.4-1.8)	46	1.3mm (1.1-1.5)	1.5mm (1.2 - 1.8)
Region I	63	1.2mm (1.1-1.4)	63	1.3mm (1.0-1.4)	63	1.2mm (1.3-1.0)	63	1.1mm (0.9-1.5)	1.33mm (1 - 1.6)
Region II	60	1.2mm * (1.0-1.6)	61	1.1mm ** (1.0-1.5)	61	1.3mm (1.0-1.8)	60	1.3mm # (1.0-1.6)	1.4mm - (1.1 - 1.8)
Region III	62	0.9mm * (0.8-1.0)	61	0.9mm ** (0.8-1.0)	63	1.0mm (0.9-1.3)	62	0.9mm # (0.8-1.0)	0.97mm - (0.9 - 1.3)
Region IV	61	0.9mm (0.8-0.9)	60	0.8mm (0.7-0.9)	62	0.9mm (0.8-1.0)	62	0.8mm (0.7-0.9)	0.89mm (0.8 - 1)

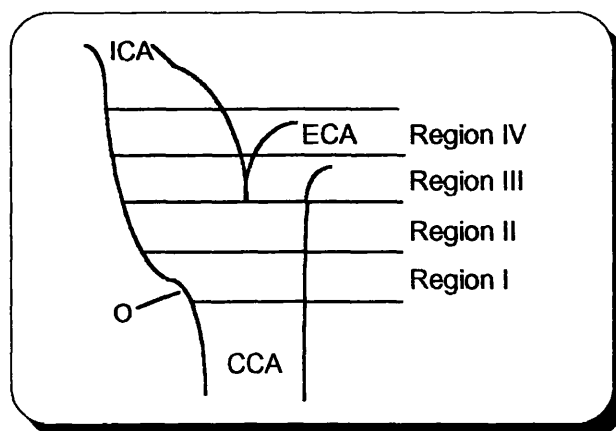
Using the Mann-Whitney U test for significance:

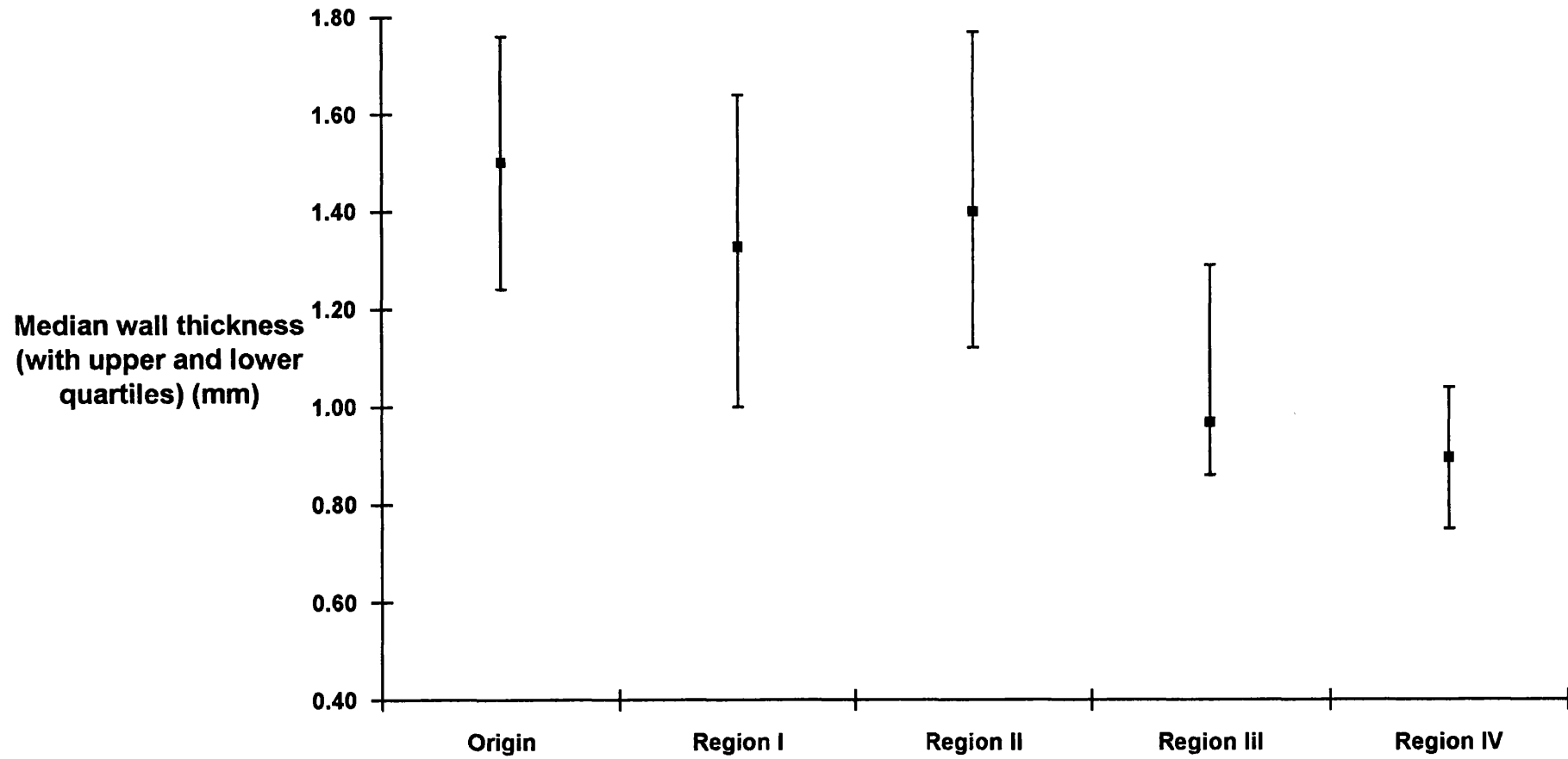
\* comparing II and III, the median difference (MD) = 0.3, 95% confidence interval (CI) = 0.14-0.48, normalised statistic (NS) = 3.67, one tailed p = 0.001.

\*\* comparing II and III, MD = 0.23, 95% CI = 0.06-0.42, NS = 2.79, one tailed p = 0.002.

# comparing II and III, MD = 0.28, 95% CI = 0.11-0.49, NS = 3.12, one tailed p = 0.009

- comparing II and III, MD = 0.34, 95% CI = 0.2-0.49, NS = 4.5, one tailed p < 0.0001





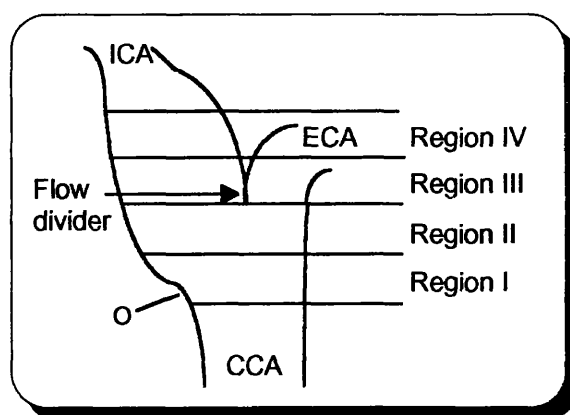
**Fig 7.8**

**Median wall thickness at the bulb origin and in each region of the bifurcation**

**Table 7.3 Comparison between the median wall thickness before the flow divider (Region I + Region II /2) and the median wall thickness after the flow divider (Region III + Region IV /2)**

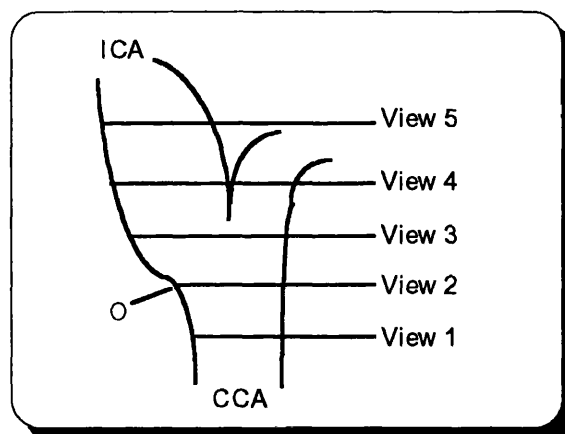
	Before flow divider		After flow divider		Median difference ( 95% CI)	Mann Whitney U	
	Median wall thickness (LQ-UQ)	n	Median wall thickness (LQ-UQ)	n		Normalised statistic	p value
Near wall, anterior view	1.3 (1-1.7)	60	0.9 (0.8-1.1)	61	0.36 (0.22 - 0.51)	4.78	<0.0001
Far wall, anterior view	1.3 (1-1.6)	61	0.9 (0.7-1.3)	59	0.37 (0.2 - 0.54)	3.97	<0.0001
Near wall, lateral view	1.4 (1 - 2)	61	1 (0.7-1.4)	62	0.33 (0.15 - 0.54)	3.4	<0.0001
Far wall, lateral view	1.2 (1-1.8)	60	0.9 (0.7-1.1)	61	0.37 (0.22 - 0.54)	4.45	<0.0001
Mean for all four views	1.3 (1.1-1.6)	63	1.0 (0.9-1.2)	63	0.27 (0.16 - 0.4)	4.75	<0.0001

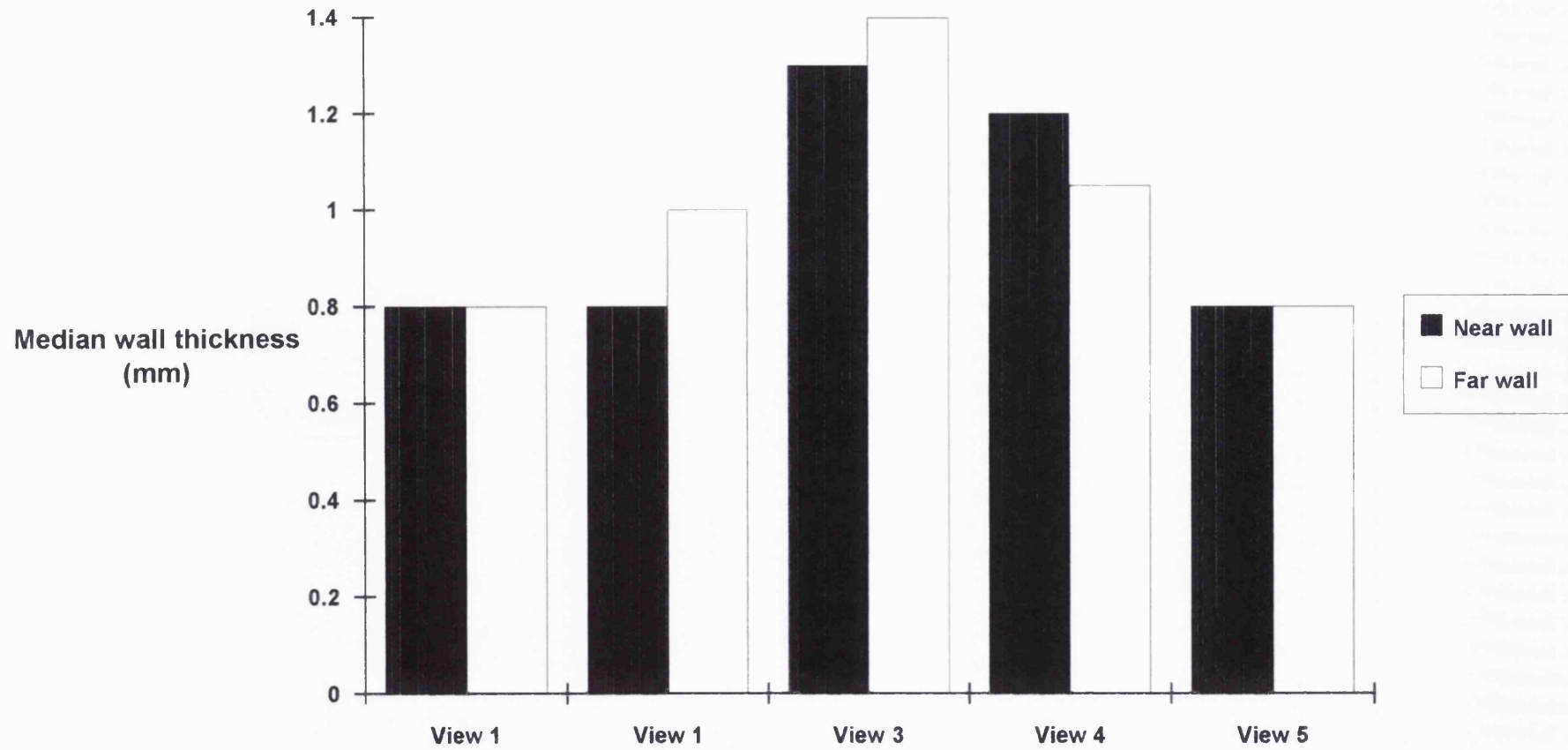
LQ = Lower quartile, UQ = upper quartile



**Table 7.4**     **The median wall thickness (and the upper and lower quartiles)(mm)of the near and far walls for each view in the transverse plane**

Transverse	n	Near wall	LQ - UQ	n	Far wall	LQ - UQ
View 1	61	0.8mm	0.8 - 1	63	0.8mm	0.8 - 1
View 2	62	0.8mm	0.8 - 1.2	63	1mm	0.8 - 1.5
View 3	61	1.3mm	0.8 - 2.2	63	1.4mm	1 - 2.3
View 4	58	1.2mm	0.8 - 1.6	58	1.05mm	0.8 - 1.7
View 5	43	0.8mm	0.7 - 1.5	46	0.8mm	0.8 - 1





**Fig 7.9**

**Median wall thickness of the near and far walls for each view in the transverse plane**

**Table 7.5 Comparison of the median wall thickness between views in the transverse plane**

Transverse views	First view		Second view		Median difference (95% CI)	Mann Whitney U	
	n	Median wall thickness (LQ - UQ)	n	Median wall thickness (LQ - UQ)		NS	p value
View 1 to view 2	63	0.8 (0.8 - 1)	63	1 (0.8 - 1.4)	0.1 (0 - 0.2)	2.91	< 0.002
View 1 to view 3	63	0.8 (0.8 - 1)	63	1.5 (1.1-2.3)	0.6 (0.4 - 0.9)	6.69	< 0.0001
View 1 to view 4	63	0.8 (0.8 - 1)	59	1.2 (0.9 - 1.6)	0.25 (0.15 - 0.45)	5.01	< 0.0001
View 1 to view 5	63	0.8 (0.8 - 1)	47	0.8 (0.6 - 1.1)	0 (-0.05 - 0.05)	0.01	NS
View 2 to view 3	63	1 (0.8 - 1.4)	63	1.5 (1.1 - 2.3)	0.45 (0.25 - 0.7)	4.34	< 0.0001
View 2 to view 4	63	1 (0.8 - 1.4)	59	1.2 (0.6 - 1.6)	0.1 (0 - 0.25)	1.84	< 0.05
View 2 to view 5	63	1 (0.8 - 1.4)	47	0.8 (0.6 - 1.1)	0.1 (0 - 0.3)	2.17	< 0.02
View 3 to view 4	63	1.5 (1.1 - 2.3)	59	1.2 (0.9 - 1.6)	0.3 (0.1 - 0.55)	2.93	< 0.002
View 3 to view 5	63	1.5 (1.1 - 2.3)	47	0.8 (0.6 - 1.1)	0.6 (0.35 - 0.9)	5.25	< 0.0001
View 4 to view 5	59	1.2 (0.9 - 1.6)	47	0.8 (0.6 - 1.1)	0.3 (0.1 - 0.5)	3.64	< 0.0002

**Table 7.6      Distribution of sites at which the artery wall was inadequately visualised in the transverse view.**

Transverse view	Number poorly visualised	
	Near wall	Far wall
View 1	2	0
View 2	1	0
View 3	2	0
View 4	5	5
View 5	20	17

### **7.32 Distribution of plaques.**

#### **a) Longitudinal plane**

The number of plaques was analysed for each wall (near and far) of each view (anterior and lateral) in the longitudinal plane. The plaque distribution for each view is shown in the appendix (A.4 a-d). There was no significant difference in the number of plaques seen when walls, views and regions were compared, except in regions III and IV where fewer plaques were seen on the near wall in the anterior view (for region III  $\chi^2=7.6$ , to 3 DF  $p<0.05$ , for region IV  $\chi^2=9.6$ , to 3 DF  $p<0.05$ ). Therefore, region by region analysis was carried out using the total number of sites involved with plaque per region, maximum score per region being 4 (1 for each wall in the anterior and lateral views).

The distribution of plaques across the regions is shown in table 7.7. Regions I and II had significantly more plaques than regions III and IV; over half of the sites examined in regions I and II were affected plaque and these accounted for 73% of all the plaques detected. The incidence of plaques in adjacent regions is compared in table 7.8. Regions I and II did not differ significantly, whereas region II had significantly more plaque than region III, and region III in turn had significantly more plaques than region IV.

The number and size of plaques per region in the longitudinal plane is shown in figure 7.10. This shows that not only were there more plaques in regions I and II, but also that the larger plaques (>2mm) occurred predominantly in these regions. Figure 7.11 compares the incidence of small and large plaques in each region.

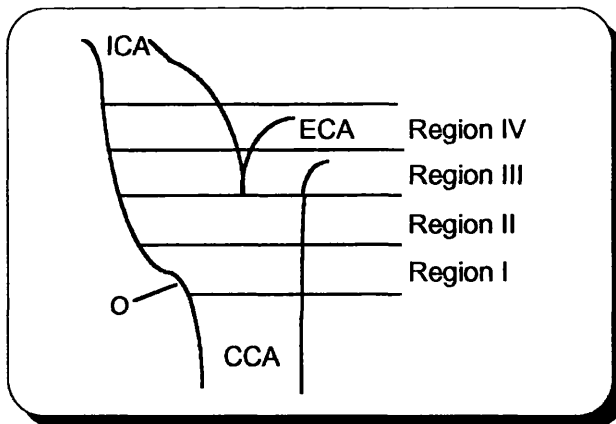
The bulb origin lies either in region I or region II depending on the bulb type. It is absent in A type bulbs. When present, the bulb origin was found to be involved in plaque in 63% of cases. The number of plaques at the bulb origin is compared to the number found in regions I and II in Table 7.9 a+b. The bulb origin had significantly more plaques than region II but not region I.



**Table 7.7 The distribution of plaques across the four regions in the longitudinal plane**

Regions	Plaques	No plaques	Total
I	134 (37%)	115	249
II	123 (34%)	117	240
III	70 (19%)	175	245
IV	38 (10%)	206	244

$\chi^2 = 106$        $p < 0.001$



**Table 7.8. Comparison of the number of plaques per region in the longitudinal plane**

**a) Regions I and II**

Regions compared	Plaque	No plaque	Total
I	134 (54%)	115	249
II	123 (51%)	117	240

$\chi^2$  0.322      NS

**b) Regions II and III**

Regions compared	Plaque	No plaque	Total
II	123 (51%)	117	240
III	70 (29%)	175	245

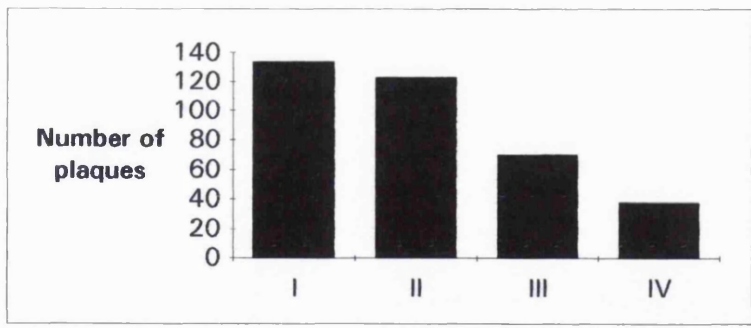
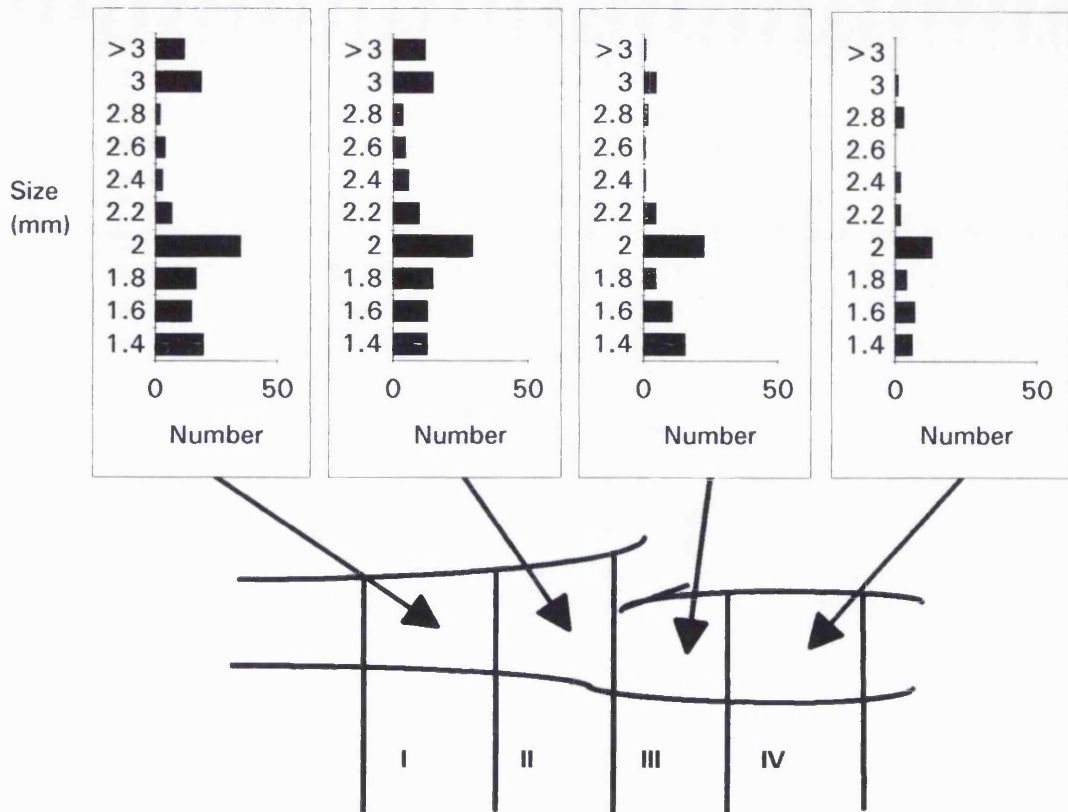
$\chi^2$  =26.03      p<0.001

**c) Regions III and IV**

Regions compared	Plaque	No plaque	Total
III	70 (29%)	175	245
IV	38 (16%)	206	244

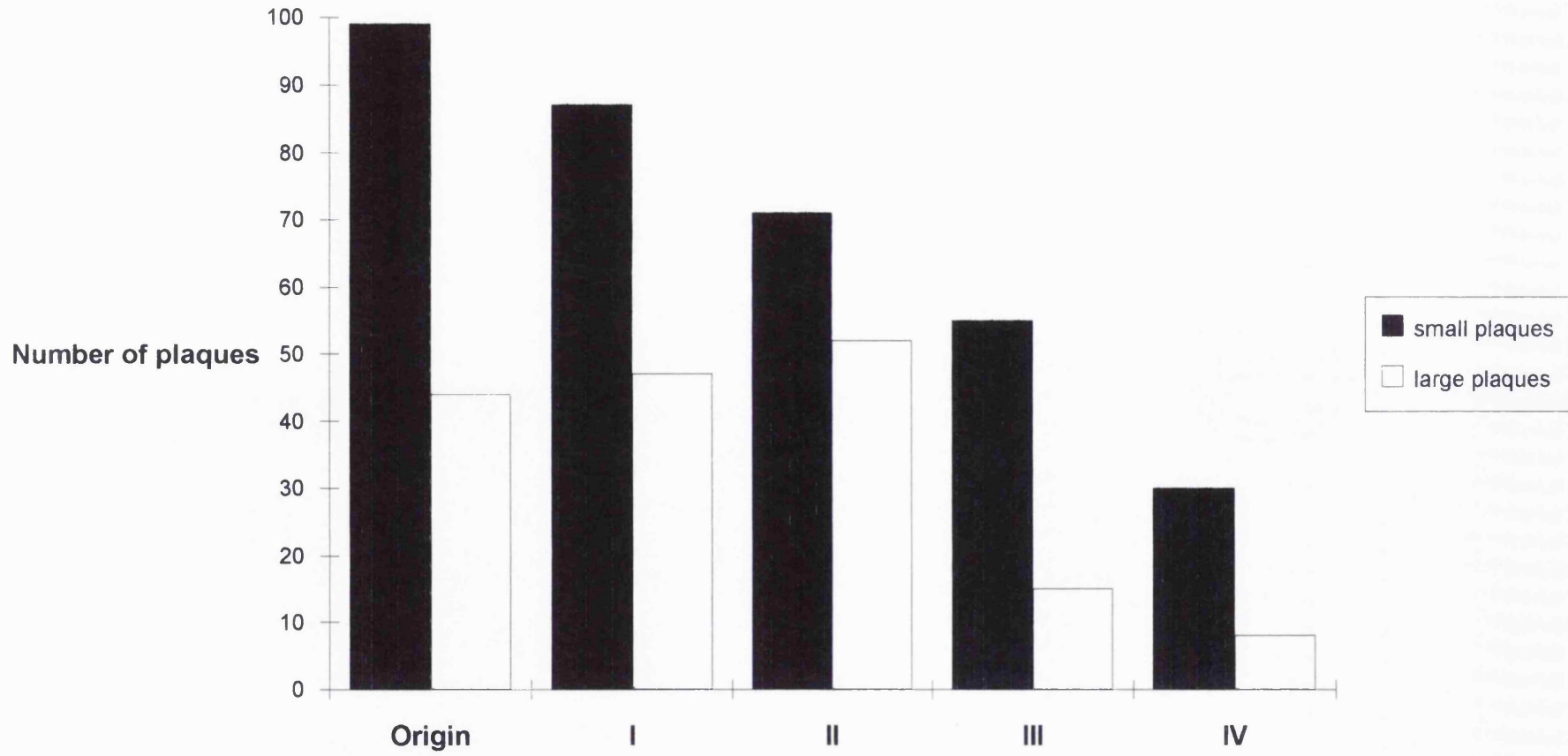
$\chi^2$  =12.002      p<0.001

The size and number of plaques per region are shown in the histograms below



The total number of plaques in each region is plotted in the bar chart above

**Fig 7.10** Plaque distribution in the longitudinal view, all walls combined



**Fig 7.11**

**Distribution of small and large plaques across the regions of the bifurcation**

**Table 7.9 Comparison between the number of plaques at the origin of the bulb and the number of plaques in region I and region II**

**a) Region I**

	Plaques	No plaques	Total number of sites
Origin	117 (65%)	64	186
Region I	134 (54%)	115	249

$\chi^2=3.60$  NS

**b) Region II.**

	Plaques	No plaques	Total number of sites
Origin	117 (65%)	64	186
Region II	123 (51%)	117	240

$\chi^2=5.78$   $p<0.02$

## b) Transverse plane

The number of plaques was analysed for each wall (near and far) for each of the 5 views in the transverse plane. The plaque distribution for each wall is shown in the appendix A.5. There was no significant difference in the number of plaques seen on each wall, therefore, the analysis of the transverse views was carried out using the total number of plaques per view, the maximum total per view being 2 (1 for each wall).

The distribution of plaques across the five views in the transverse plane is shown in table 7.10 and individual views are compared in table 7.11. Plaques were found to occur most frequently in view 3, immediately before the flow divider. The second most common site for plaques was view 4, immediately beyond the flow divider and plaques were found here significantly more often than in view 2 (Table 7.11 (e))

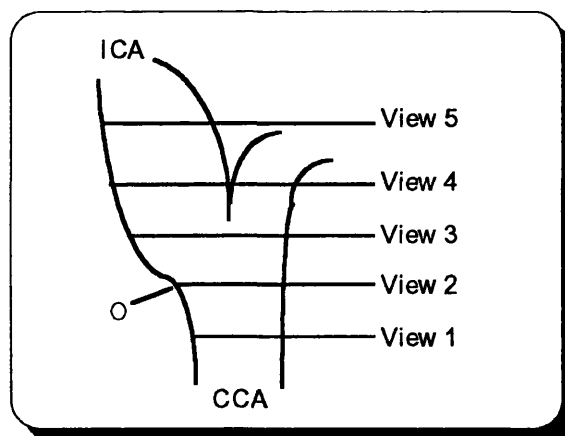
The distribution of plaques across the five transverse views is shown in figure 7.12. This shows that not only were most of the plaques found in view 3, but also that the majority of larger plaques (>2mm) are also found in this view. Few large plaques were found in either view 2 or view 4 and the main difference in these two regions was due to a greater number of small plaques in view 2 compared to view 4.

**Table 7.10 The distribution of plaques across the 5 views in the transverse plane**

	Number of sites with plaque	Number of sites without plaque	Total number of sites
View1	14 (8%)	110	124
View2	34 (18%)	91	125
View3	72 (39%)	52	124
View4	52 (28%)	64	116
View5	13 (7%)	76	89

$\chi^2 = 86$

$p < 0.001$  to 4DF



**Table 7.11 Comparison between individual views in the transverse plane of the number of plaques seen.**

**a) View 1 and view 2**

Views compared	Plaque	No plaque	Total
View 1	14 (11%)	110	124
View 2	34 (27%)	91	125

$$x^2 = 10.13 \quad p < 0.005$$

**e) View 2 and view 3**

Views compared	Plaque	No plaque	Total
View 2	34 (27%)	91	125
View 3	72 (58%)	52	124

$$x^2 = 24.25 \quad p < 0.001$$

**h) View 3 and view 4**

Views compared	Plaque	No plaque	Total
View 3	72 (58%)	52	124
View 4	52 (45%)	64	116

$$x^2 = 4.205 \quad p < 0.02$$

**j) View 4 and View 5**

Views compared	Plaque	No plaque	Total
View 4	52 (45%)	64	116
View 5	13 (15%)	76	89

$$x^2 = 21.24 \quad p < 0.001$$

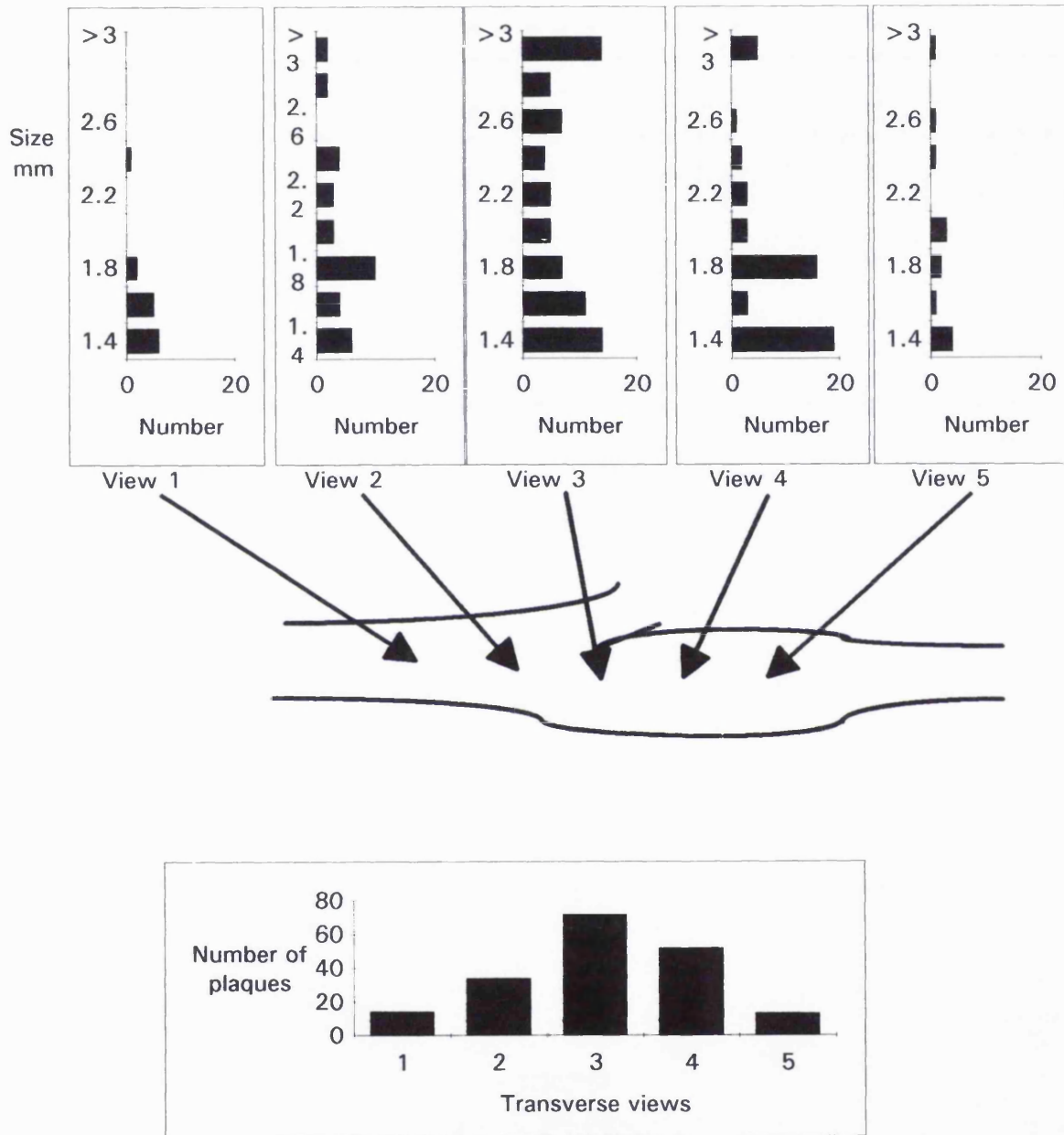
**e) View 2 and view 4**

Views compared	Plaque	No plaque	Total
View 2	34 (27%)	91	125
View 4	52 (45%)	64	116

$$x^2 = 8.14 \quad p < 0.005$$



The size and number of plaques per view are shown in the histograms below



**Fig 7.2** Plaque distribution in the transverse view, all walls combined

### **7.33 Plaque distribution and bulb morphology**

Five bulb morphologies are recognised (Part II, Chapter 5 and 6). In this study, the distribution of bulb types for the 63 carotid artery bifurcations examined was:

Bulb type	Number
A	17
B	20
C	23
D	3
Total	63

Bulb morphological type D was omitted from the subsequent analysis as the number sampled was too small to be representative of this type.

#### **a) Longitudinal views**

Plaque distribution for each bulb type is shown in figures 7.13, 7.14 and 7.15, with a diagrammatic representation of each of the bulbs.

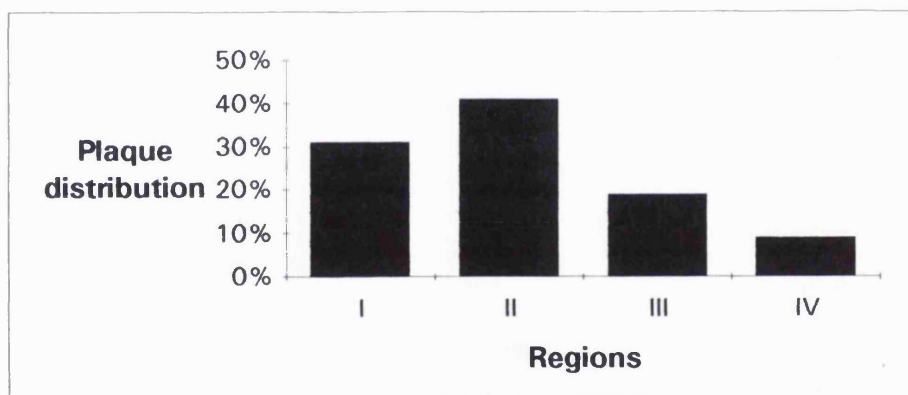
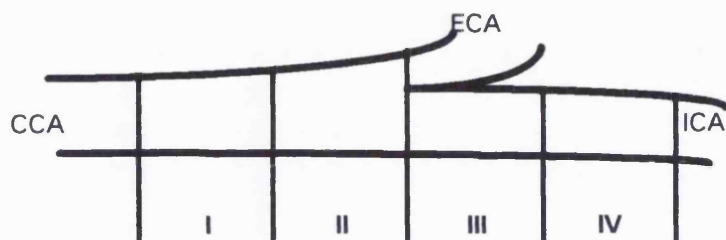
Bulb types were compared for the distribution of plaques across the regions of the bifurcation (table 7.12 and Fig 7.16) and bulb types B and C were found to be significantly different in the overall distribution of plaques.

Comparing bulbs region by region (Tables 7.13-7.16), A and B bulbs had significantly more plaque in region II than C bulbs, and beyond the flow divider, B bulbs had more plaques in region III and less plaques in region IV than C bulbs. A and B bulbs are similar in the distribution of plaques in all regions except IV where more plaques are found in B bulbs.

Bulb types differ by the position of the bulb origin in relation to the flow divider (Fig 7.17, therefore the number of plaques at the bulb origin was compared to the number of plaques in region I and II. Table 7.17 shows that the bulb origin of B bulbs had significantly more plaques than region I but not region II. In table 7.18 , the converse is true, with the bulb origin of C bulbs having an identical number of plaques as region I but significantly more plaques than region II.

### Diagrammatic representation of an "A" type bulb

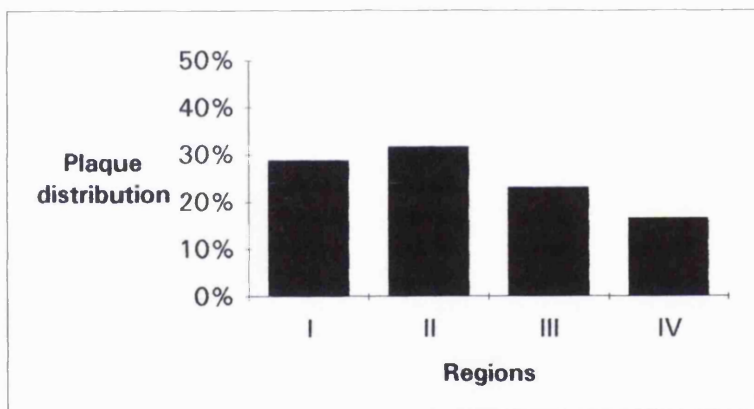
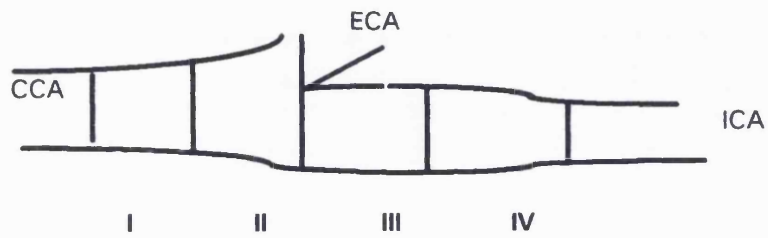
"A" bulbs have no bulb



**Fig 7.13** Plaque distribution in the A type bulbs in the longitudinal plane

### Diagrammatic representation of a "B" type bulb

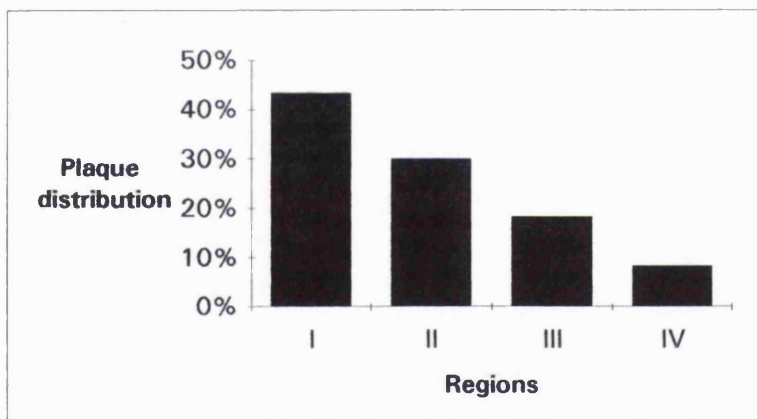
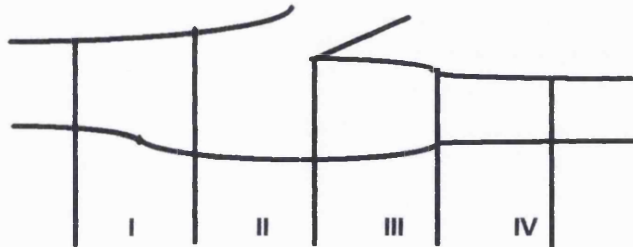
In the "B" type bulb, the bulb lies at the proximal end of the ICA



**Fig 7.14** Plaque distribution in the B type bulb in the longitudinal plane

### Diagrammatic representation of a "C" type bulb

In the "C" type bulbs, the bulb straddles the bifurcation



**Fig 7.15** Plaque distribution in the C type bulb in the longitudinal plane

**Table 7.12 Comparison between bulb types in the distribution of plaques across the regions in the longitudinal plane**

	A	B	C
I	28 (31%)	40 (29%)	52 (43%)
II	37 (41%)	44 (32%)	36 (30%)
III	17 (19%)	32 (23%)	22 (18%)
IV	8 (9%)	23 (16%)	10 (9%)

Comparing B and C  $\chi^2=7.98$ , to 3 degrees of freedom  $p<0.05$ .

Comparing A and B:  $\chi^2=4.28$ , and for A and C:  $\chi^2=3.87$ , neither are significant to 3 degrees of freedom.

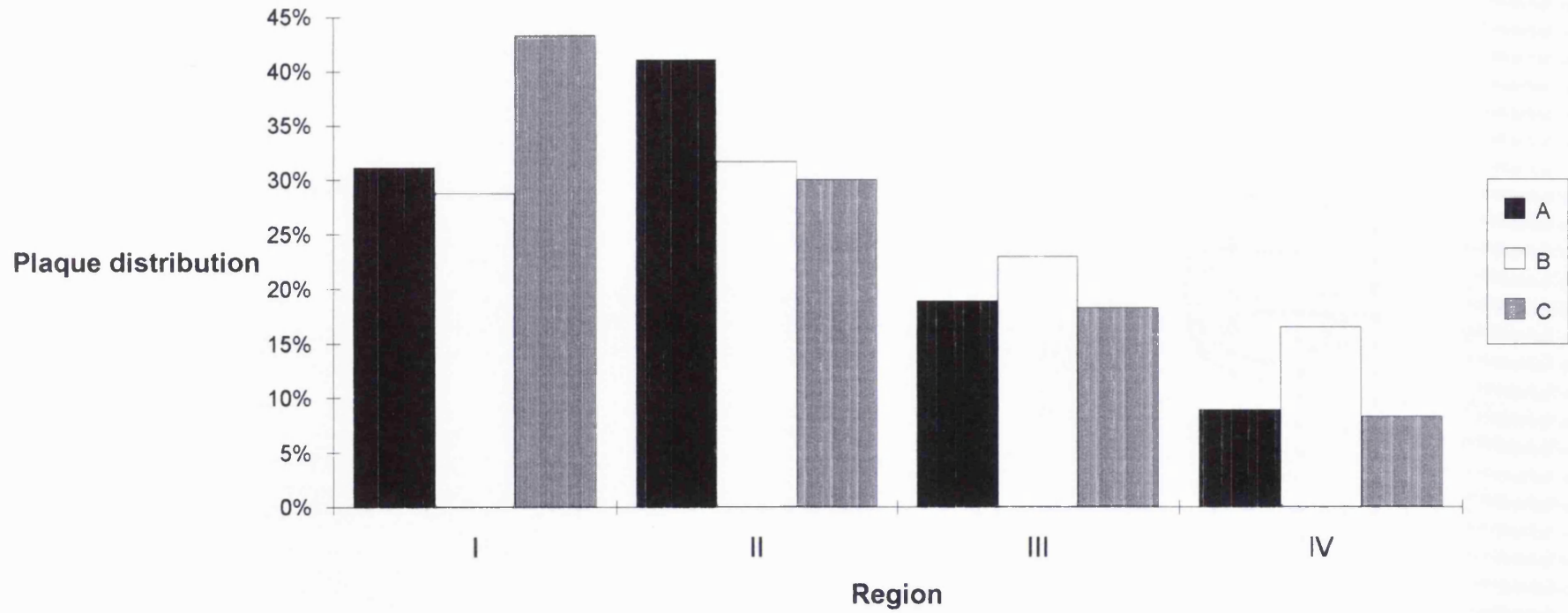


Fig 7.16

Comparison between bulb types in the distribution of plaques

**Table 7.13 Comparison between bulb types as to the number of plaques in Region I in the longitudinal view**

**a) Bulb types A and B**

	Plaque	No plaque	
A	28 (41%)	40	68
B	40 (50%)	40	80

$x^2=1.15$  NS

**b) Bulb types A and C**

	Plaque	No plaque	
A	28 (41%)	40	68
C	52 (57%)	40	92

$x^2=3.68$  NS

**c) Bulb types B and C**

	Plaque	No plaque	
B	40 (50%)	40	80
C	52 (57%)	40	92

$x^2=0.73$  NS



**Table 7.14 Comparison between bulb types as to the number of plaques in Region II of the longitudinal view**

**a) Bulb types A and B**

	Plaque	No plaque	
A	37 (56%)	29	66
B	44 (58%)	32	76

$\chi^2=0.05$  NS

**b) Bulb types A and C**

	Plaque	No plaque	
A	37 (56%)	29	66
C	36 (40%)	54	90

$\chi^2=3.94$   $p<0.05$

**c) Bulb types B and C**

	Plaque	No plaque	
B	44 (58%)	32	76
C	36 (40%)	54	90

$\chi^2=5.28$   $p<0.05$

**Table 7.15 Comparison between bulb types as to the number of plaques in Region III of the longitudinal view**

a)

**Bulb types A and B**

	Plaque	No plaque	
A	17 (26%)	49	66
B	32 (41%)	47	79

$\chi^2=3.496$  NS

**b) Bulb types A and C**

	Plaque	No plaque	
A	17 (26%)	49	66
C	22 (24%)	69	91

$\chi^2=0.05$  NS

**c) Bulb types B and C**

	Plaque	No plaque	
B	32 (41%)	47	79
C	22 (24%)	69	91

$\chi^2=5.2$   $p<0.05$

**Table 7.16 Comparison between bulb types as to the number of plaques in Region IV of the longitudinal view**

**a) Bulb types A and B**

	Plaque	No plaque	
A	8 (12%)	59	67
B	23 (29%)	55	78

$\chi^2=6.6$   $p<0.02$

**b) Bulb types A and C**

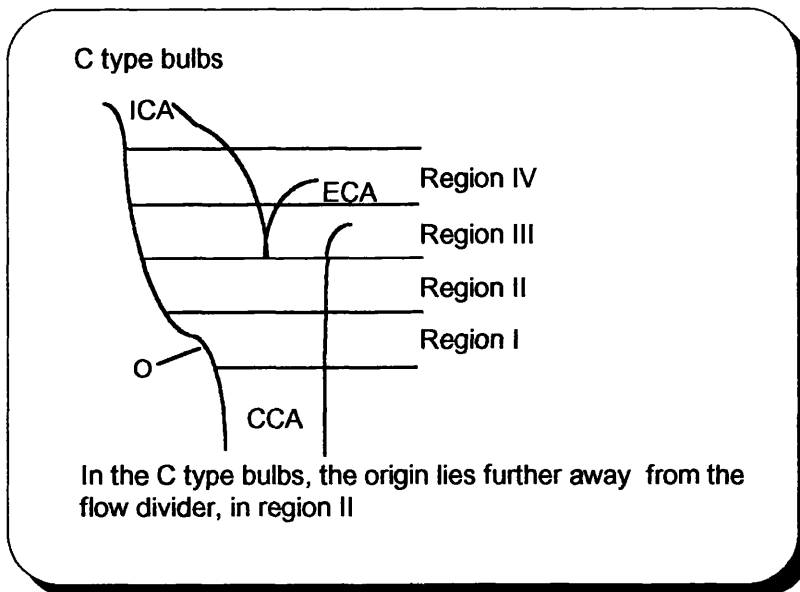
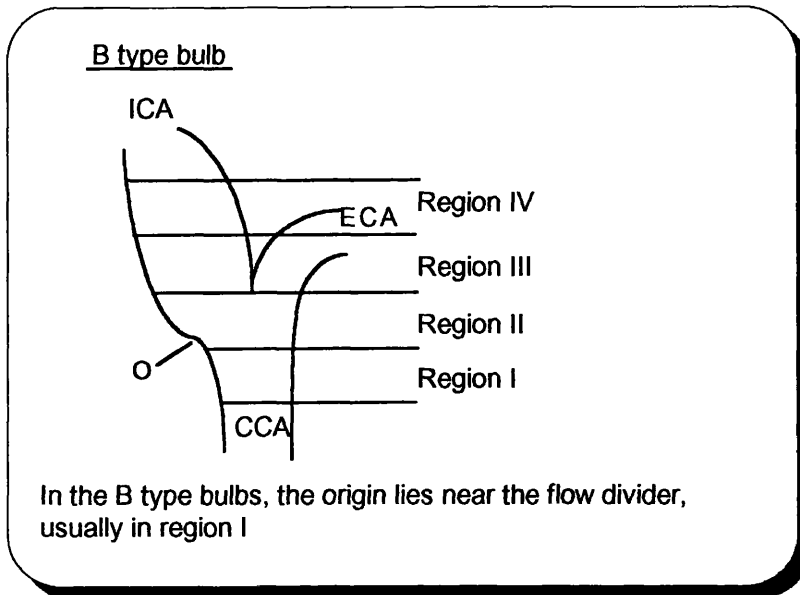
	Plaque	No plaque	
A	8 (12%)	59	66
C	10 (11%)	79	89

$\chi^2=0.02$  NS

**c) Bulb types B and C**

	Plaque	No plaque	
B	23 (29%)	55	78
C	10 (11%)	79	89

$\chi^2=8.73$   $p<0.005$



**Fig 7.17      The position of the bulb origin in relation to the flow divider is shown for B and C bulbs, (in A, there is no bulb)**

**Table 7.17 Comparison between the number of plaques at the bulb origin and the number of plaques at regions I and II of the B bulbs in the longitudinal view**

**a) Origin compared to region I**

	Plaque	No plaque	Total
Origin	58 ((73%))	22	80
I	40 (50%)	40	80

$\chi^2=8.53$        $p<0.005$

**b) Origin compared to region II**

	Plaque	No plaque	Total
Origin	58 (73%)	22	80
II	44 (58%)	32	76

$\chi^2=3.67$       NS

**Table 7.18 Comparison between the number of plaques at the bulb origin and the number of plaques at regions I and II of the C bulbs in the longitudinal view**

**a) Origin compared to region I**

	Plaque	No plaque	Total
Origin	52 (57%)	40	80
I	52 (57%)	40	80

$\chi^2=0$  NS

**b) Origin compared to region II**

	Plaque	No plaque	Total
Origin	52 (57%)	40	80
II	36 (40%)	54	79

$\chi^2=4.97$   $p<0.05$

### b) Transverse views

Bulb types were compared for the distribution of plaques across the transverse views (Table 7.19 and Fig 7.18) and there was no significant difference in the overall distribution of plaques.

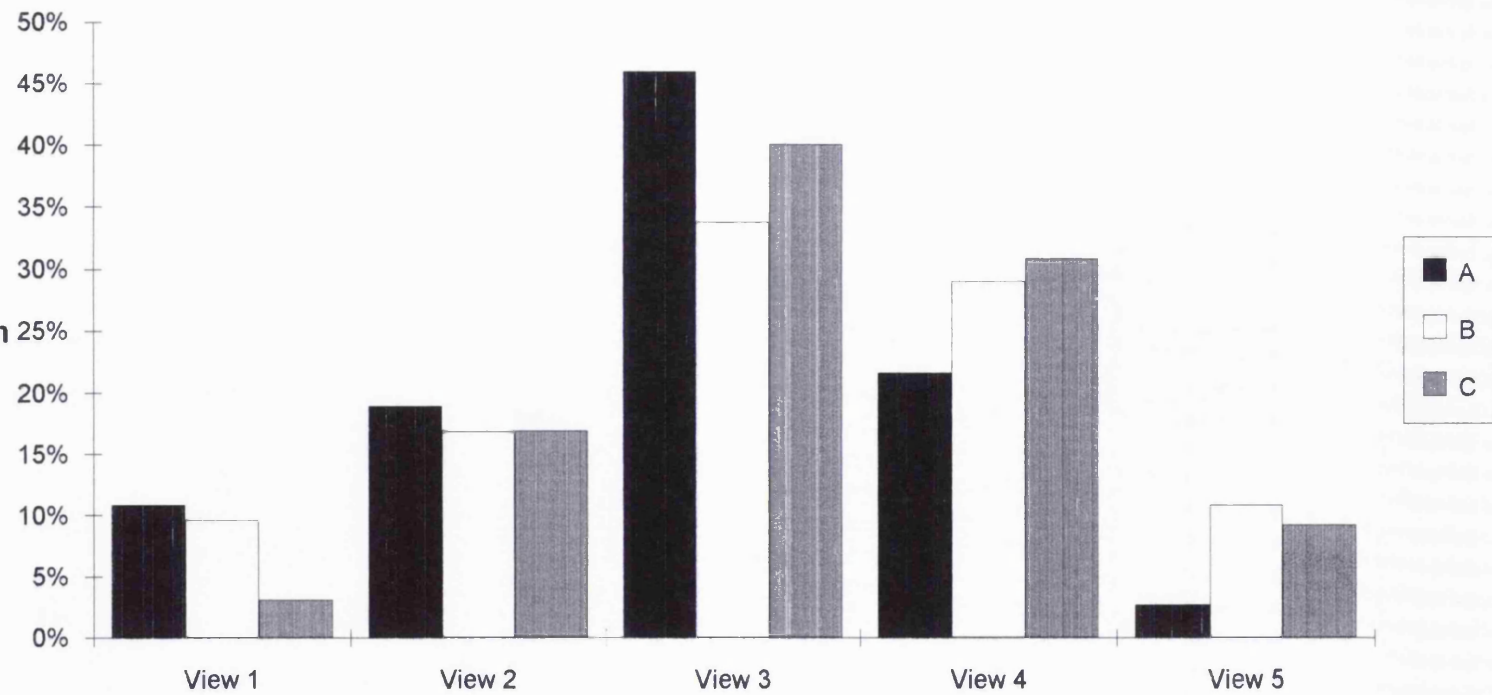
In a view by view comparison of the plaque distribution (Tables 7.20-7.23), the only difference between bulb types proximal to the flow divider (views 1-3) was an increased number of plaques in B compared to C in view 1. Distal to the flow divider, there was a significant difference between A and B bulbs in the incidence of plaques in view 4 and view 5, and between A and C bulbs in view 4.

**Table 7.19 Comparison between bulb types in the plaque distribution across the transverse views**

	A		B		C	
	Plaque	No plaque	Plaque	No plaque	Plaque	No plaque
View 1	4 (11%)	30	8 (10%)	31	2 (3%)	43
View 2	7 (19%)	27	14 (17%)	26	11 (17%)	34
View 3	17 (46%)	16	28 (34%)	11	26 (40%)	20
View 4	8 (22%)	22	24 (29%)	16	20 (31%)	22
View 5	1 (3%)	25	9 (11%)	17	6 (9%)	27



## Plaque distribution

**Fig 7.19****Comparison between bulb types in the distribution of plaques in the transverse views**

**Table 7.20 Comparison between bulb types as to the number of plaques in transverse view 1**

	Plaque		No plaque
A	4	(12%)	30
B	8	(21%)	31

$\chi^2 = 1.01$  NS

	Plaque		No plaque
A	4	(12%)	30
C	2	(4%)	43

$\chi^2 = 1.47$  NS

	Plaque		No plaque
B	8	(21%)	31
C	2	(4%)	43

$\chi^2 = 5.14$   $p < 0.05$

**Table 7.21 Comparison between bulb types as to the number of plaques in transverse view 2**

	Plaque	No plaque
A	7 (21%)	27
B	14 (35%)	26

$\chi^2 = 1.87$  NS

	Plaque	No plaque
A	7 (21%)	27
C	11 (24%)	34

$\chi^2 = 0.16$  NS

	Plaque	No plaque
B	14 (35%)	26
C	11 (24%)	34

$\chi^2 = 0.13$  NS

**Table 7.22 Comparison between bulb types as to the number of plaques in transverse view 3**

	Plaque	No plaque
A	17 (52%)	16
B	28 (72%)	11

$\chi^2 = 3.13$  NS

	Plaque	No plaque
A	17 (52%)	16
C	26 (57%)	20

$\chi^2 = 0.19$  NS

	Plaque	No plaque
B	28 (72%)	11
C	26 (57%)	20

$\chi^2 = 2.12$  NS

**Table 7.23 Comparison between bulb types as to the number of plaques in transverse view 4**

	Plaque		No plaque
A	8	(27%)	22
B	24	(60%)	16

$\chi^2 = 7.67$  p<0.01

	Plaque		No plaque
A	8	(27%)	22
C	20	(45%)	22

$\chi^2 = 3.13$  NS

	Plaque		No plaque
B	24	(60%)	16
C	20	(45%)	22

$\chi^2 = 1.26$  NS

**Table 7.24 Comparison between bulb types as to the number of plaques in transverse view 5**

	Plaque	No plaque
A	1 (4%)	25
B	9 (35%)	17

$\chi^2 = 7.92$  p<0.01

	Plaque	No plaque
A	1 (4%)	25
C	6 (18%)	27

$\chi^2 = 2.85$  NS

	Plaque	No plaque
B	9 (35%)	17
C	6 (18%)	27

$\chi^2 = 2.07$  NS

## **7.4 Discussion**

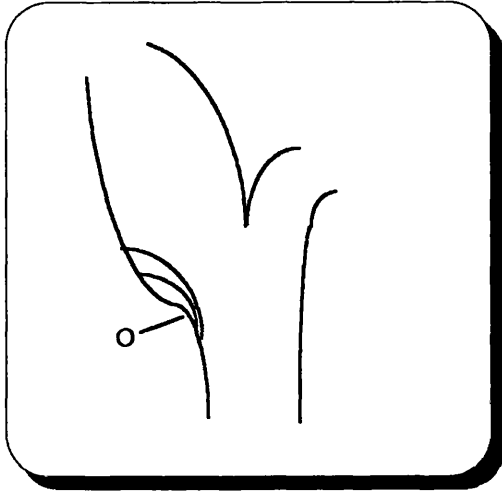
### ***i) Which is the site (or sites) of early plaque formation at the carotid bifurcation, identifiable with high resolution ultrasound?***

In the longitudinal plane, the majority of plaques (73%), both small and large, were distributed over regions I and II, proximal to the flow divider, and though plaques were found distal to the flow divider these were smaller plaques than those in regions I and II.

In 63% of the bifurcations with a bulb origin present, plaques were found at the origin. This site is the site most frequently affected by plaque and supports the hypothesis that plaques originate here. The majority of plaques at the origin are small plaques, with a greater proportion of larger plaques being found in region II.

If plaque growth is from the edge of the bulb inwards, then small plaques would be found at the origin of the bulb and larger plaques adjacent to the origin (Fig. 7.19). Also, if plaque grows in from the edges, the older and therefore larger plaques would be expected to occur at the edge of the bulb, while the centre of the bulb would be relatively devoid of plaques, and the few plaques present would be small. This is in fact what we found in the longitudinal views in this study.

In the transverse views, a different pattern is observed to the longitudinal views, with the maximum number of plaques (both large and small) being found in View 3. The second most common site is not view 2 but view 4. In choosing the position of the five views, the first view was intended to look at the distal CCA, the second the bulb origin, the third the bulb immediately proximal to the flow divider, the fourth the bulb immediately distal to the flow divider, and the fifth the distal bulb. However, the bulb origin cannot be clearly identified in the transverse plane, therefore view 2 was taken as the place where the CCA begins to widen, and this may occur before the true origin of the bulb, yielding rather less plaques than would be expected if the bulb origin had been identified. There was a high yield of plaques in view 4 immediately distal to the flow divider, and, as in the longitudinal studies, these tended to be small plaques. In the transverse view it is easier to identify exactly where the flow divider begins and therefore view 4 may be proximal to region III of the longitudinal views.



A small plaque at the bulb origin grows towards the centre of the bulb, therefore the larger plaques tend to be adjacent to the bulb origin, rather than at the origin.

**Fig 7.20**      **Plaque growth at the bulb origin**



***ii) How does this site (or sites) vary with the different bulb morphologies***

When no bulb was detected (bulb type A), the greatest number of plaques were found immediately proximal to the flow divider in region II (41%). Only 20% of plaques were found distal to the flow divider.

In bulb type B, the distribution of plaque around the flow divider was more spread out. Proximal to the flow divider, the plaques were equally spread between regions I and II (29% and 32%), though the majority of the larger plaques (>2mm) are found in region II. Comparing the incidence of plaques at the bulb origin with the incidence in regions I and II, we found significantly more plaques at the bulb origin than in region I. There was no significant difference in the incidence of plaques found at the origin and in Region II. More plaques were found distal to the flow divider in type B bulbs (23% and 16%) than the other two types of bulb (19% and 9% for A type bulbs and 18% and 9% for C type bulbs).

In bulb type C, the majority (43%) of plaques are found in region I with a further 30% at region II. Large plaques are equally divided between the two regions and the difference in the incidence of plaques between region I and II is accounted for by small plaques (1.3-2mm) in region I. Only 21% of plaques were found distal to the flow divider and the majority of these were small.

The differences in the distribution of plaque in regions I and II can be explained by the position of the origin of the bulb. In B type bulbs, the origin of the bulb lies close to the flow divider, in C type bulbs the origin of the bulb lies in region I. If the origin of the bulb is the site of origin of a plaque, then, as in this study, more plaques would be found at this site than anywhere else in the bulb. If, as has previously been proposed, plaques start at the edge of the bulb and grow towards the centre of the bulb (Fig 6.20), we would expect to find small and large plaques at the periphery of the bulb depending on the stage of development, but only small plaques nearer the centre of the bulb. This is in fact what we have found in bulb types B and C.

***iii) What is the best landmark for identifying the sites of early plaques?***

When the origin of the bulb can be identified, it is more frequently involved with plaque than any other site around the bulb, however it is absent in 10% of bifurcations.

The majority of plaques lie proximal to the flow divider in all bulb types. Furthermore, no plaque was found distal to the flow divider without a plaque proximal to the flow divider.

In the detection of early plaques, the origin of the bulb is more important as a landmark than the flow divider. When there is no bulb, the flow divider should be used to determine the position of early plaques.

***iv) Which view gives the most information?***

The longitudinal view.

Advantages of the longitudinal views over the transverse views are: firstly, the bulb origin is better identified in the longitudinal view, secondly bulb types can only be identified in this view and thirdly, fewer studies are inadequate for the assessment for the presence of plaque.

In conclusion, early plaques develop in the regions proximal to the flow divider, and when a bulb is present, the bulb origin determines the distribution of early plaques.

CHAPTER 8  
POST-MORTEM STUDY

## **8.1 Introduction**

When examined with high resolution ultrasound, the artery wall consists of two echogenic layers separated by a hypoechoic space (Fig 8.1). The first line corresponds to the lumen/intima interface and the second line with the media/adventitia interface and intima-media thickness changes have been shown to occur in patients with risk factors for atherosclerotic disease (see literature review). Several studies have attempted to predict the histological appearance of plaques by ultrasound (Reilly et al,1983; Johnson et al,1985; Gray-Weale et al, 1988; Langsfeld, 1989) but no attempt has been made to examine the relationship between intima-media thickening and plaque formation.

The aims of this study were threefold: firstly, to confirm the findings of Pignoli that the ultrasonic image correlates with the histology; secondly, to determine which histological layer or layers are responsible for ultrasonic IM thickening; and thirdly, to determine the histological changes responsible for IM thickening.

Thus the questions posed were:

- i) Which histological layer or layers correlate best with the ultrasound IM layer?*
- ii) Which layer is responsible for ultrasonic IM thickening?*
- iii) Is there any correlation between the thickness of the first echogenic line and the thickness of the intima on histology?*
- iv) Is measurement of the ultrasonic IM thickness valid for both the near and far walls of the vessel?*
- iv) What histological changes are observed with increasing ultrasonic IM thickness?*

## 8.2. Histological Method

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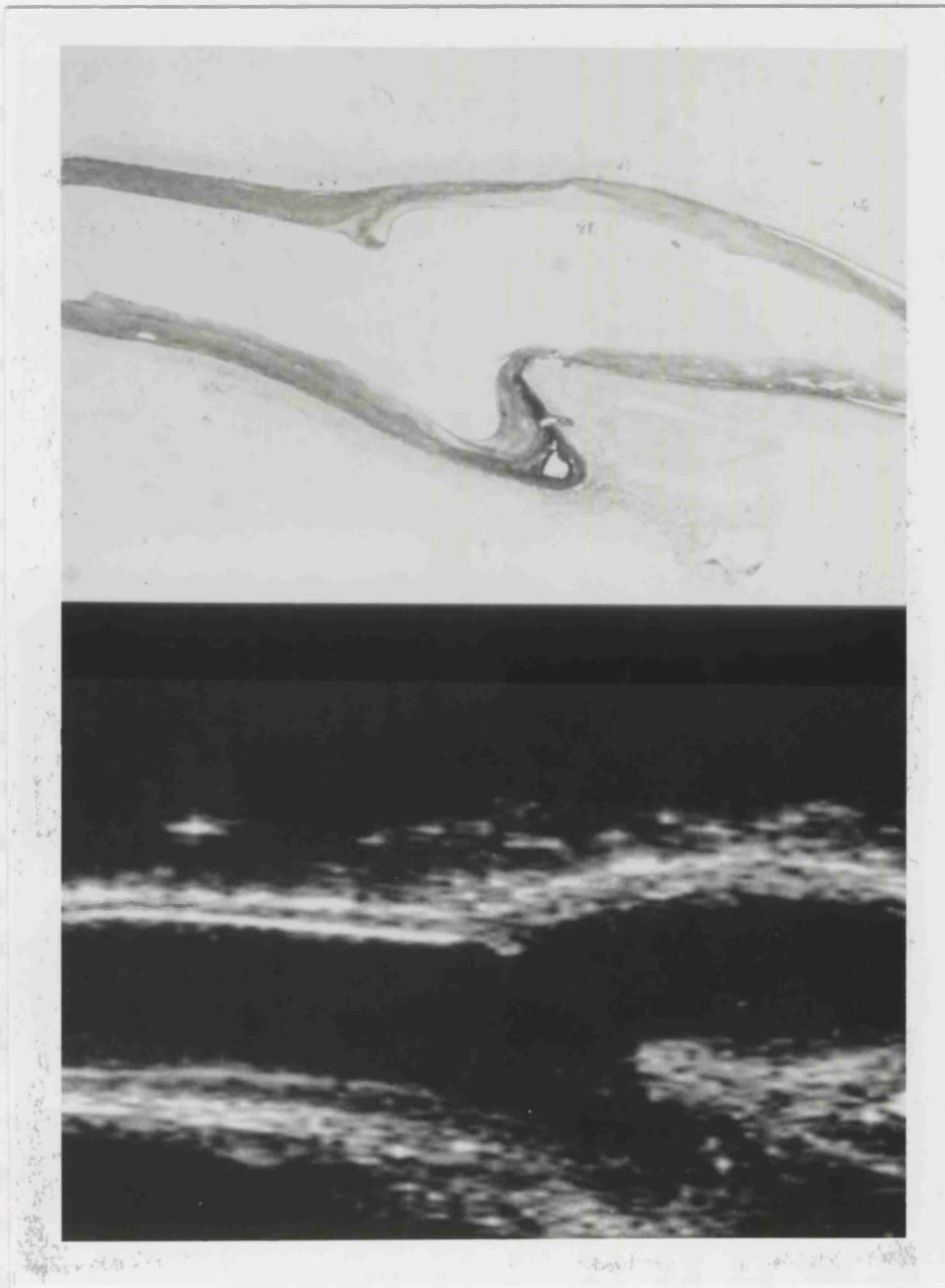
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**Fig 8.1** Comparison between the histological appearance (top) and the ultrasound appearance (bottom) of the carotid bifurcation.

(The ultrasound picture was taken with the artery suspended in a water bath.)

Ultrasound images of the artery were captured on disc and printed on a Sony videographic printer.

## **8.2 Material and Method**

### **8.21 Material**

Carotid arteries taken at post mortem from patients who had died in the hospital

### **8.22 Method**

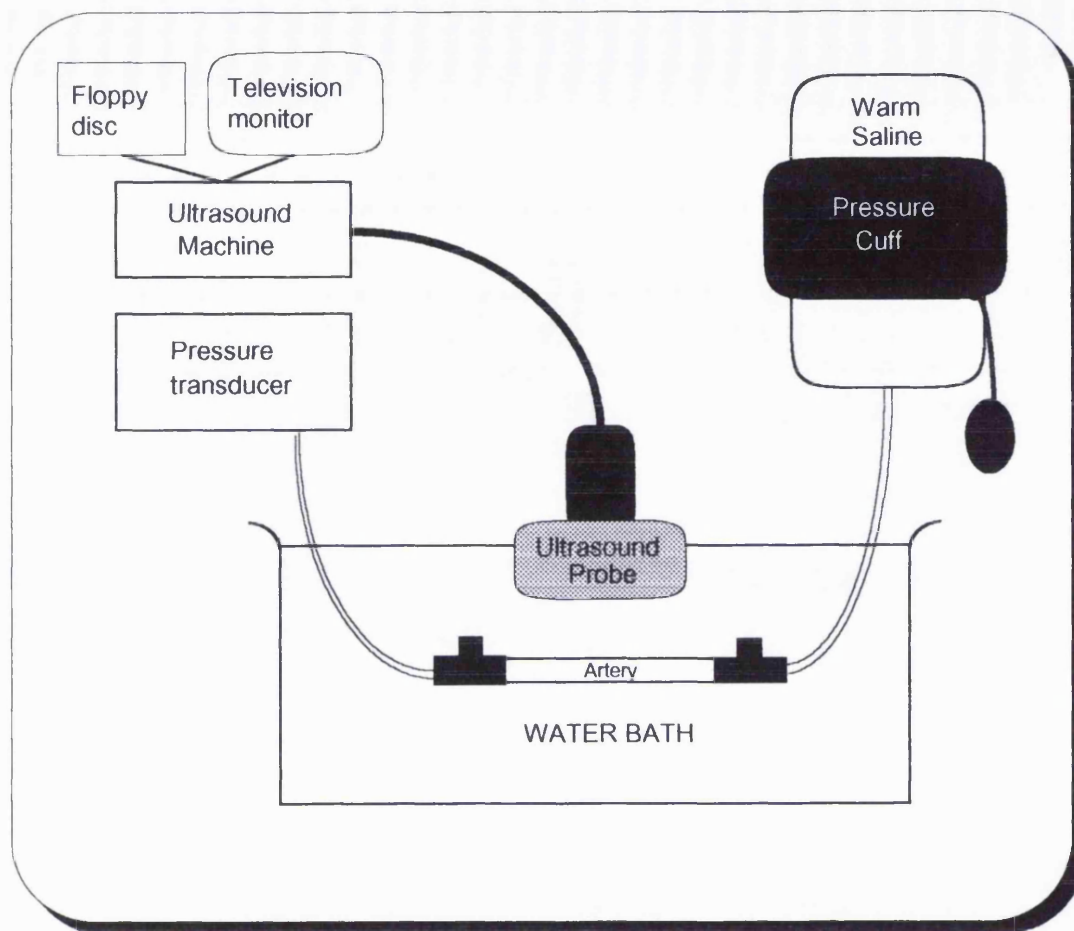
#### **8.221 Preparation**

On receipt, the specimen was gently washed in saline. The proximal end was cannulated with the hub of a three way tap and securely held by a silk ligature. One end of the three way tap was connected to a 1 litre bag of normal saline via a giving set. The distal end was also cannulated over the hub of a three way tap and held by a silk ligature. This end was connected to a pressure transducer (Fig8.2). Once set up, the system was flushed with normal saline, then with the distal tap closed, the pressure in the artery was raised to 140mmHg by pressurising the fluid in the giving set. The pressure was monitored by the pressure transducer at the distal end of the artery. The pressurised artery was suspended in a water bath at 37°C and held in position by two clamps. A 5.0 proline suture was sewn in to the adventitia, parallel to the artery wall, to orientate the vessel. The 7.5MHz linear ultrasound probe of the ATL ultramark 4 was held suspended in the water over the carotid artery.

#### **8.222 Imaging**

The artery was pressurised to 140 mmHg. With the proline suture uppermost, a linear ultrasound picture of the artery were taken in the longitudinal plane. The artery was then rotated and the picture repeated at 45°, 90°, 135°, 180°, 225°, 270°, and 315°. With the proline suture uppermost, the probe was then rotated through 90° and the artery imaged in transverse section. Using a metal protractor to guide the placement of the probe and the distal hub (as marked by the silk ligature) as the reference point, transverse pictures were taken at 5mm intervals along the length of the vessel.

Ultrasound images of the artery were captured on disc and printed on a Sony videographic printer.



**Fig 8.2** Diagrammatic representation of the method used to examine cadaveric arteries by ultrasound

### **8.223 Fixation.**

Prior to fixation the artery was marked in 5mm increments with superficial sutures. The artery was drained, and refilled with 10% formalin. The intraluminal pressure was raised to 140mmHg and immersed in formalin. The pressure was monitored overnight by means of a pressure transducer. The artery was left in formalin for 24 hours.

### **8.224 Post-fixation imaging.**

The carotid artery specimen was flushed, filled with saline and repressurised. The artery was then resuspended in the water bath and imaged in cross-section at 10 mm intervals at the sites of the sutures.

### **8.225 Sections for histological studies.**

Once imaging was complete, the artery was drained and transverse slices taken at the sites marked with sutures. The slice for histology was embedded in wax. Sections for histological examination were cut from the wax block. One section from each cut was stained with haematoxylin and eosin and a second section was stained with an elastochrome stain.

### **8.226 Measurements**

#### **8.226a Ultrasound images.**

All measurements were made using a Calcomp<sup>®</sup> drawing board and calibrated against the scanner generated calibration bar at the side of the ultrasound picture. Measurements were taken from the luminal aspect of the echogenic lines (Fig 8.3):

I: luminal aspect of the first echogenic line to the opposite side of the same line

IM: luminal aspect of the first echogenic line to the luminal aspect of the second echogenic line

Measurements were taken of both the near and far walls.



### 8.226b Histological images

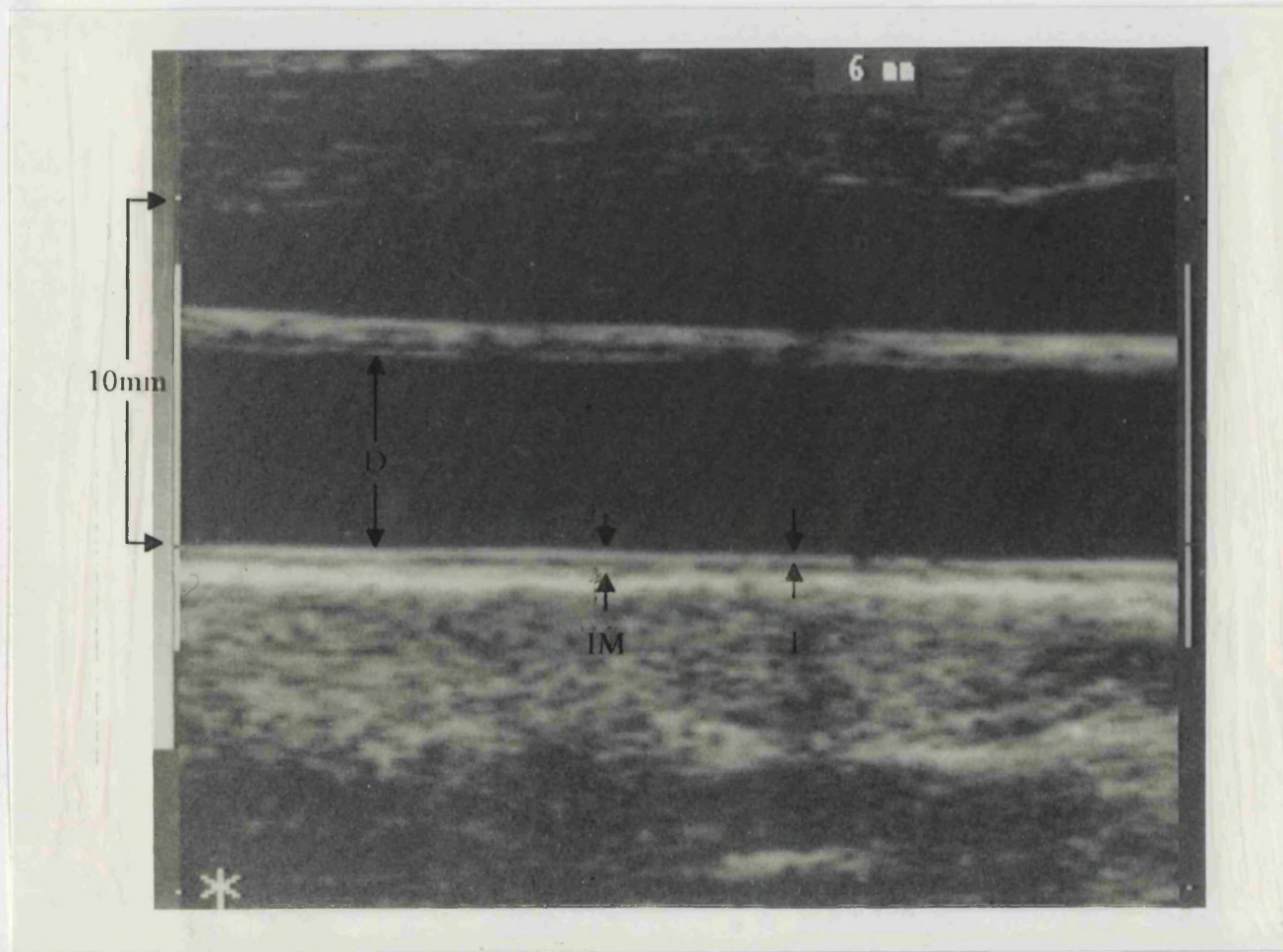
The histological appearance of a normal carotid artery is shown in figure 8.4. The intimal layer is very thin (I) compared to the medial layer (M). In figure 8.5, there is diffuse thickening of the intimal layer and in figure 8.6, a small plaque can be seen.

The thickness of the intimal and medial layers was measured using a graticule in the eyepiece of a microscope. Specimens were also examined by a histologist and the presence and extent of plaque, and any associated inflammation, calcification, cholesterol crystals, medial atrophy, fatty change and necrotic debris were recorded.

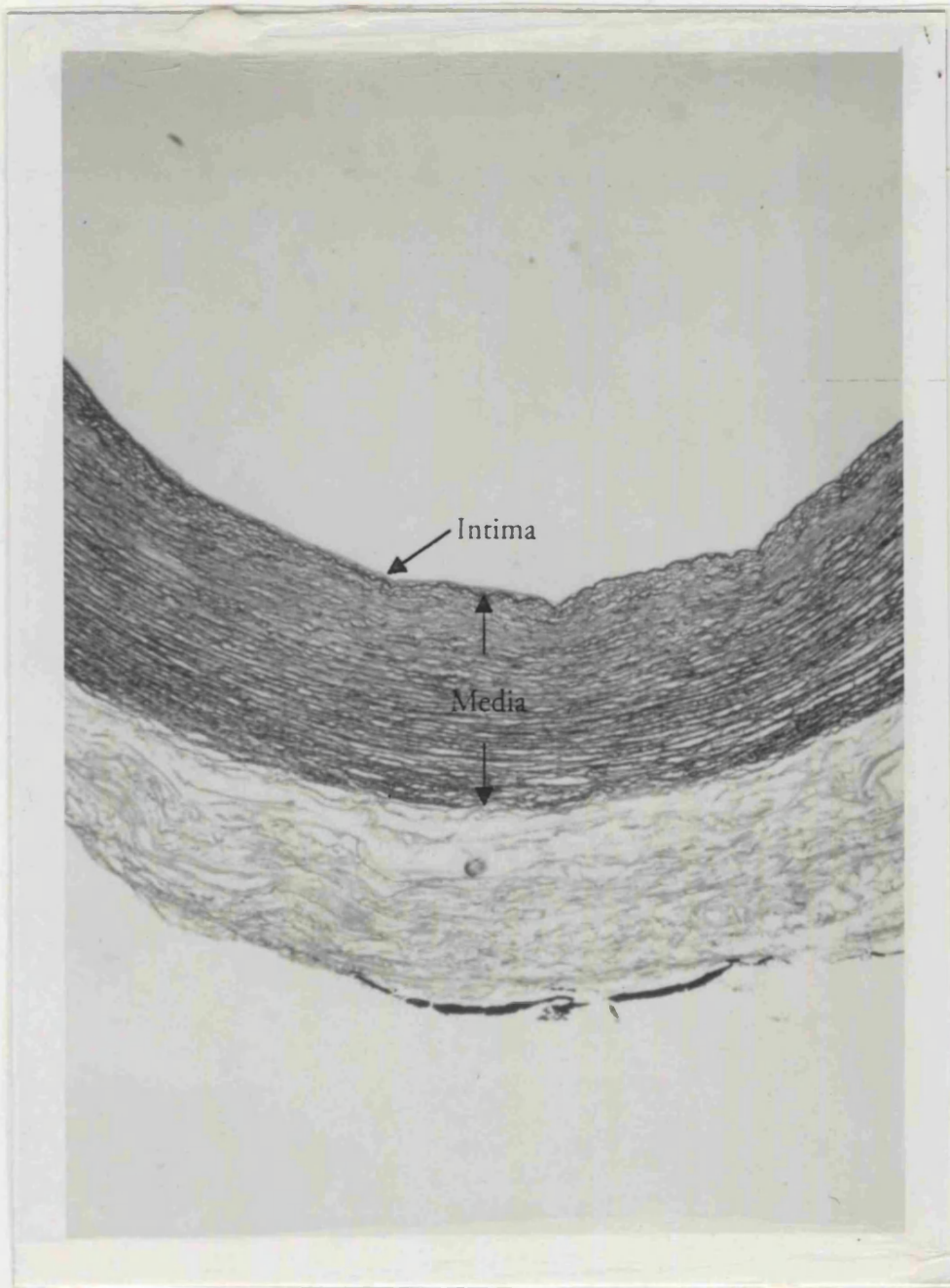
### **8.27 Statistics**

Statistical analysis was performed using standard statistical software. Pearson's product moment correlation ( $r$ ) was calculated and the confidence limits were constructed for the correlation coefficient using Fisher's  $Z$  transformation. The null hypothesis that  $r=0$  was evaluated using a modified  $t$  test (Armitage and Berry, 1987). The limits of agreement between the two methods of measurement were assessed using the technique of Bland and Altman (Bland and Altman, 1986).

The Mann Whitney U test was used to compare the IM thickness in the presence of the histological features of plaque formation.

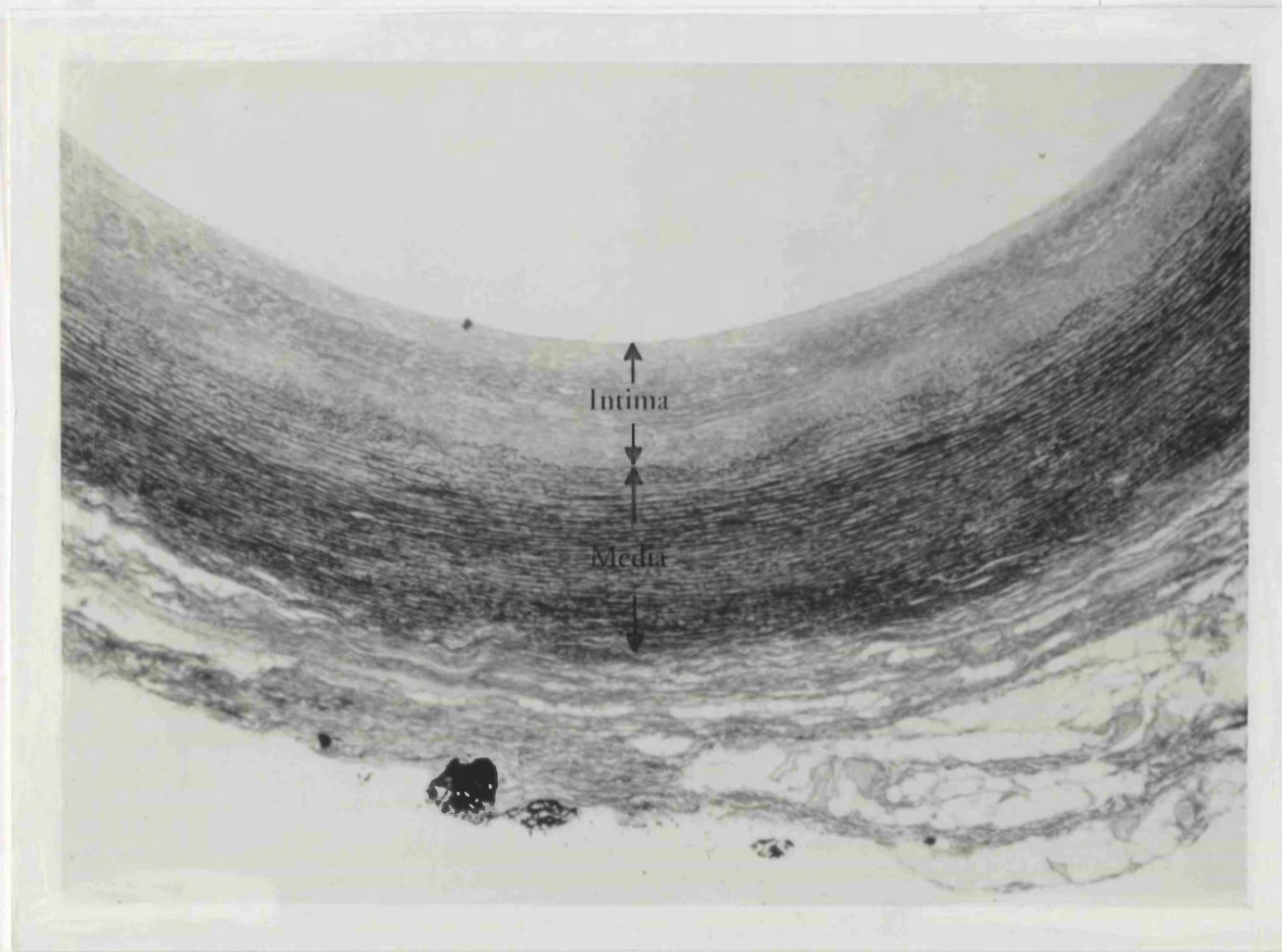


**Fig 8.3** Longitudinal view of the common carotid artery showing the points of measurement.



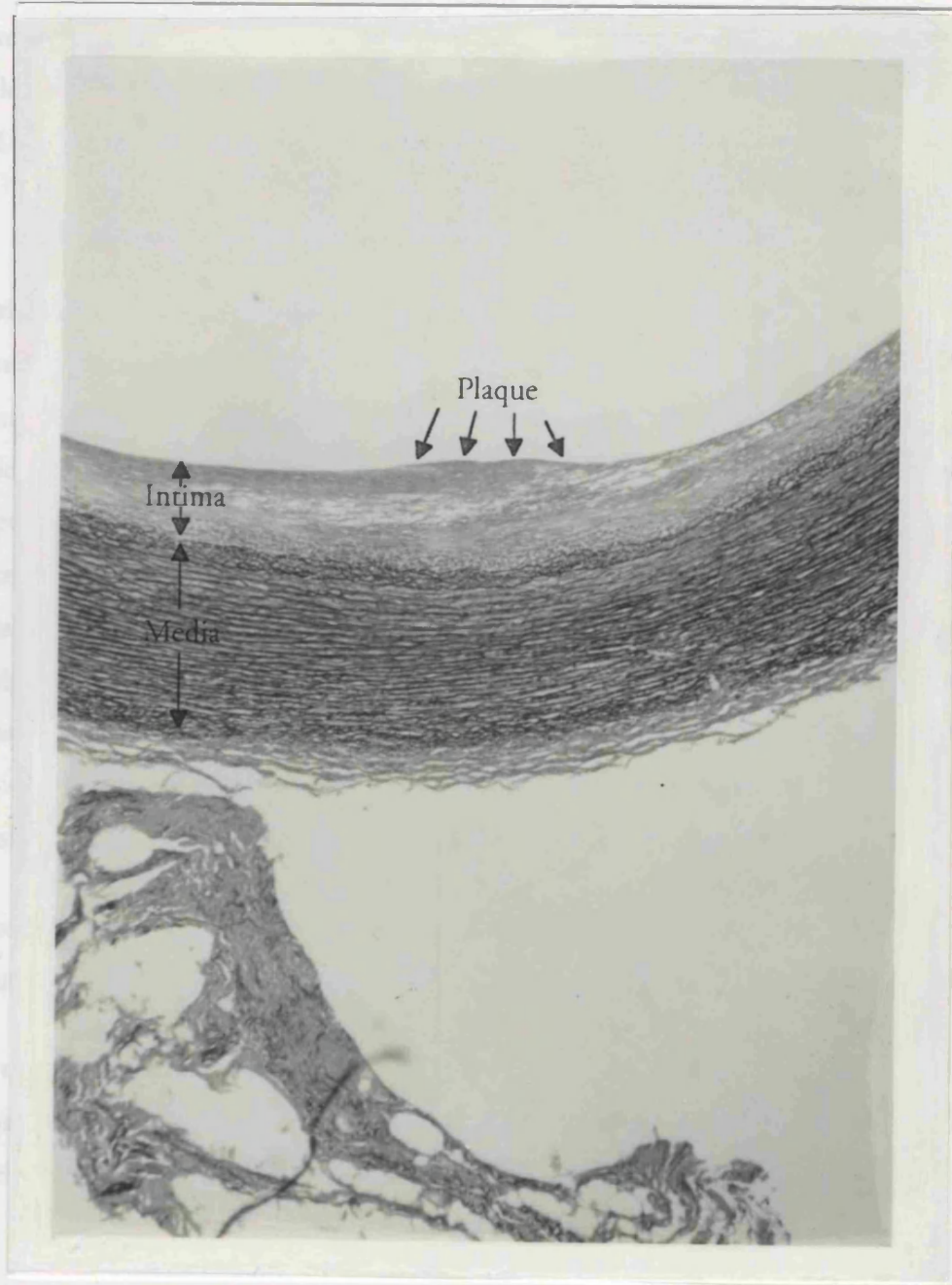
**Fig 8.4**      **Histological appearance of a healthy carotid artery**





**Fig 8.5**      **Histological appearance of a carotid artery showing diffuse intimal thickening.**

8.3 Results



**Fig 8.6** Histological appearance of a carotid artery with a small plaque

### **8.3 Results**

Thirteen cadaveric carotid arteries were examined, six from male patients and seven from female patients. The median ultrasonic IM thickness was 0.82 mm with a range of 0.5-2.4. The median thickness of the histological layers are shown in table 8.1

#### **8.31 Correlation between ultrasonic IM thickness and histological layers**

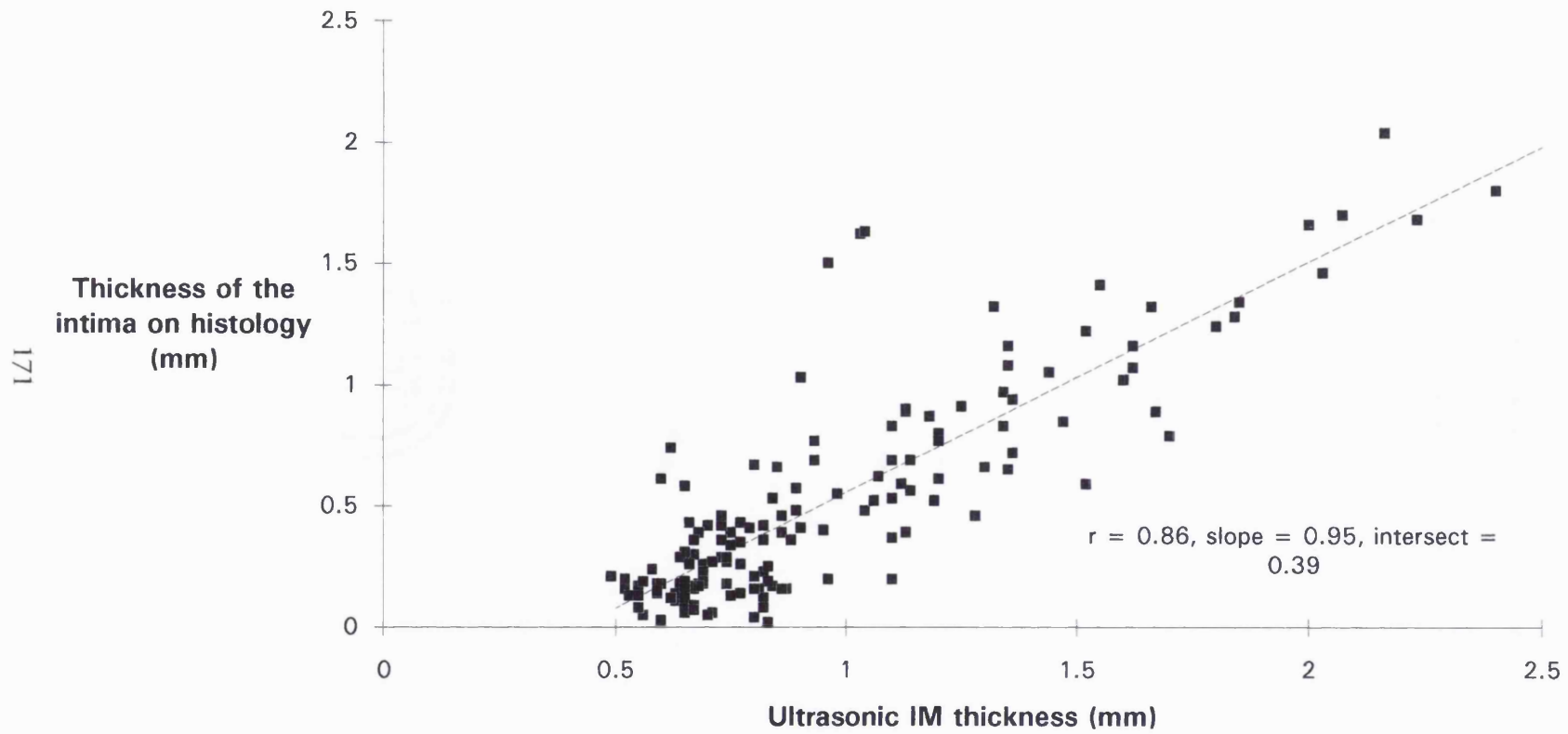
The thickness of the intima and the media are compared with the ultrasonic IM thickness measurements at the same site in the transverse plane in Figs 8.7 and 8.8. The intimal thickness correlates well with the ultrasonic IM thickness ( $r=0.86$ , 95% confidence interval for  $r = 0.81-0.89$ ,  $t = 20.28$ , one tailed and two tailed  $p < 0.0001$ ), however, the line intersects at 0.39 mm indicating a constant disparity between the thickness of the intimal layer and the ultrasonic IM thickness. The histological medial thickness remains constant despite increasing IM thickness (the correlation coefficient is not significantly different from zero). If these two layers are measured together (intima + media), the correlation with IM thickness is excellent ( $r=0.86$ , 95% confidence interval for  $r = 0.81-0.9$ ,  $t = 20.6$ , one tailed and two tailed  $p<0.0001$ ), with a slope that is close to unity (Fig 8.9). The limits of agreement are shown in figure 8.10.

No correlation is observed between the thickness of the inner echogenic layer on ultrasound compared to the intima thickness on the histological specimens (Fig 8.11).

The ultrasonic IM thickness in the longitudinal plane was compared with the intima+media thickness on histology (taken in the transverse plane) in figure 8.12. The correlation remains good ( $r=0.72$ , ) and the limits of agreement are shown in figure 8.13.

**Table 8.1     The median thickness of the histological layers of the common carotid artery**

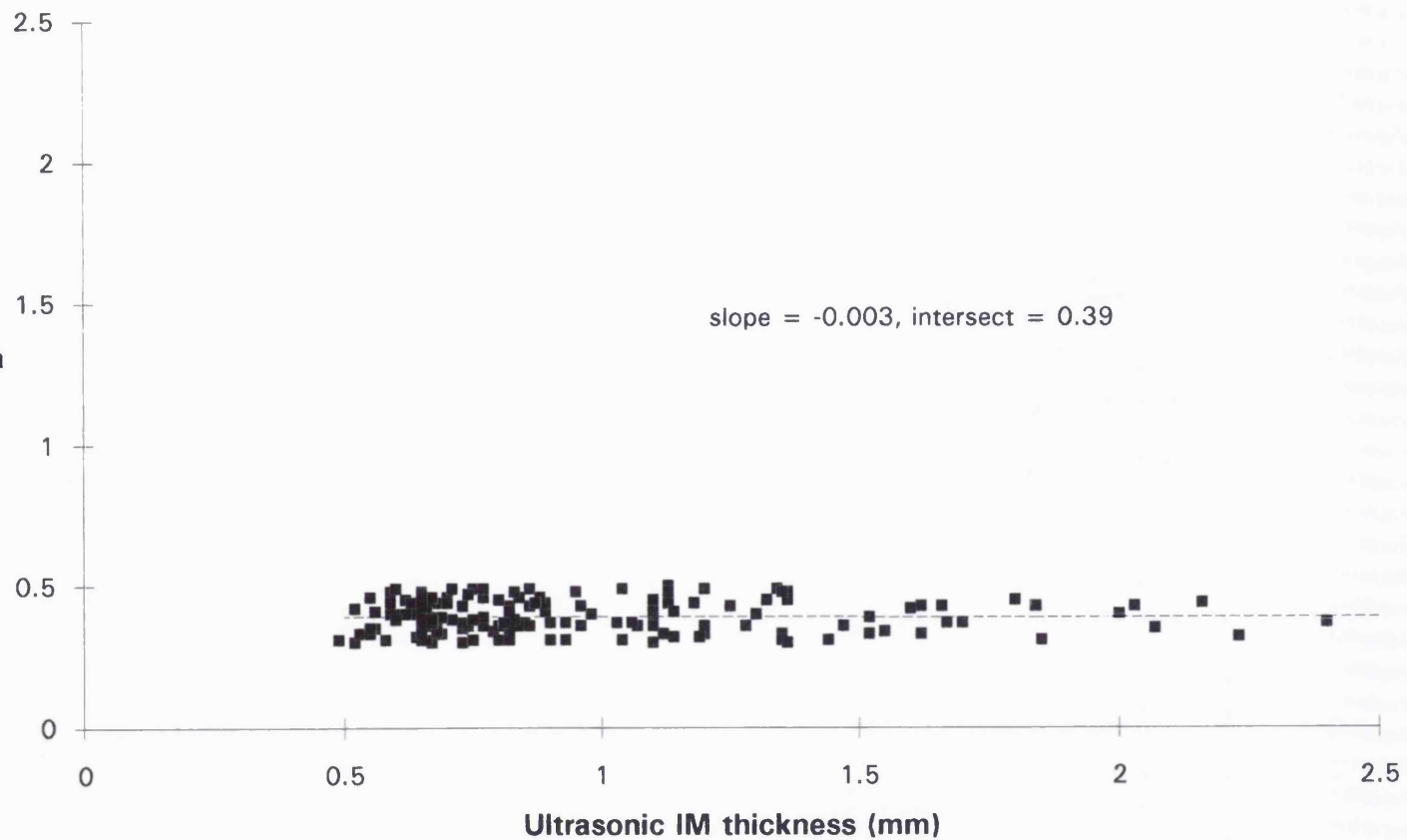
		Median thickness (mm)	Range (mm)	Number
Histological measurements:	Intima	0.39	0.02-2.04	155
	Media	0.38	0.3-0.5	155
	Intima + media	0.77	0.39-2.48	155



**Fig 8.7** Correlation between the thickness of the intima on histology and the ultrasonic IM thickness



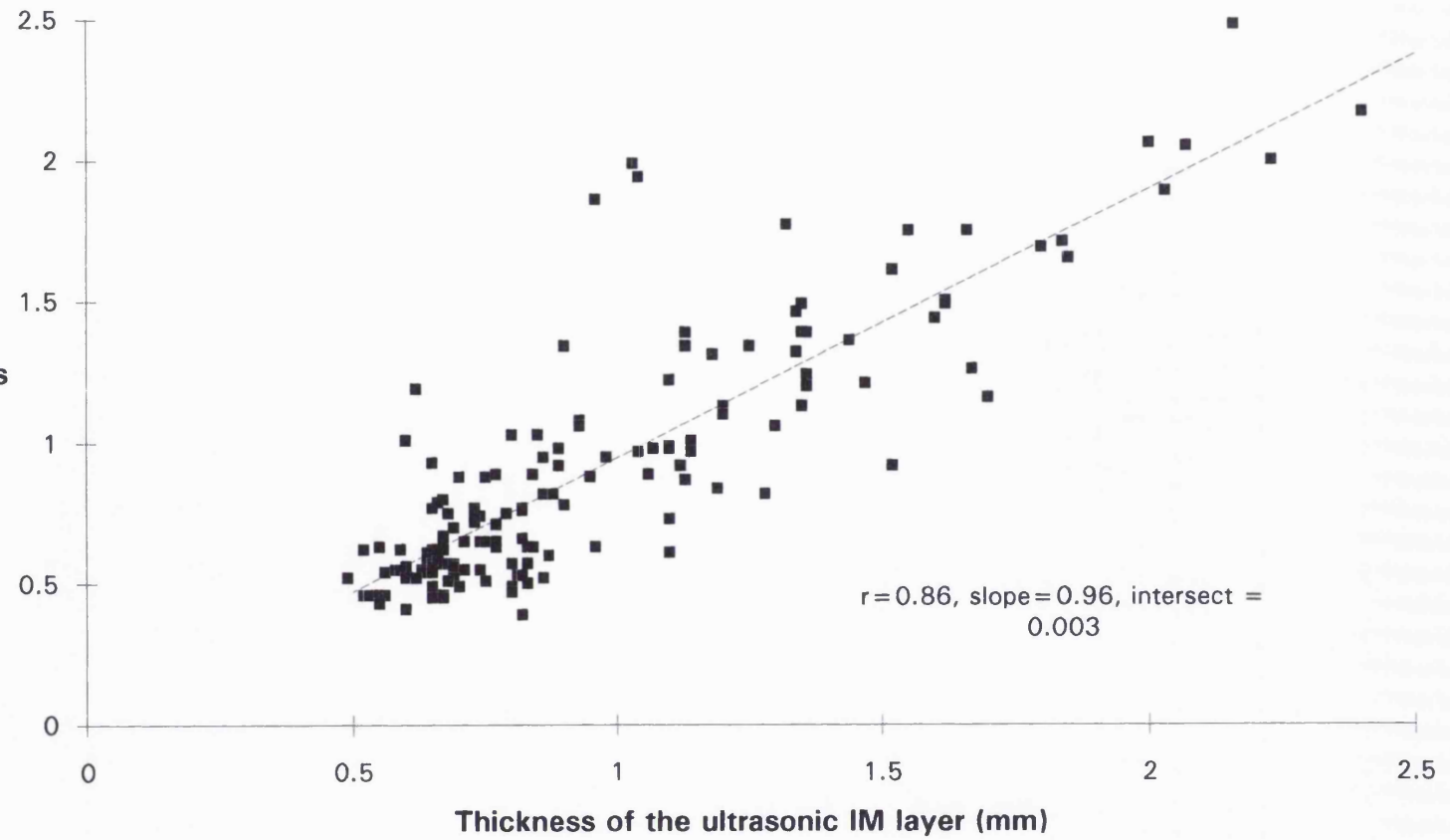
Thickness of the media  
on histology (mm)



**Fig 8.8**

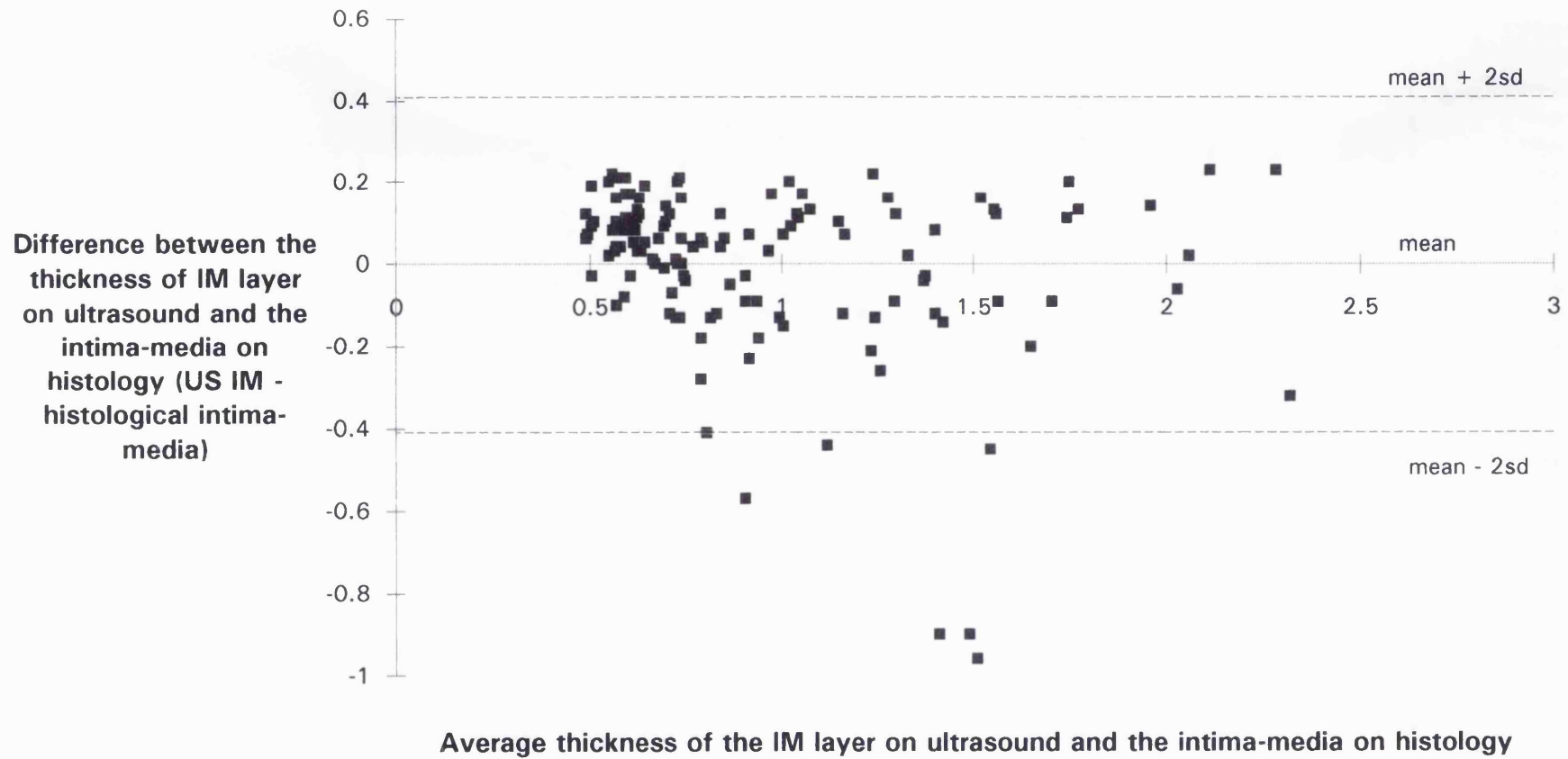
**Correlation between the thickness of the media on histology and the ultrasonic IM thickness**

Thickness of the  
intima + media layers  
on histology (mm)

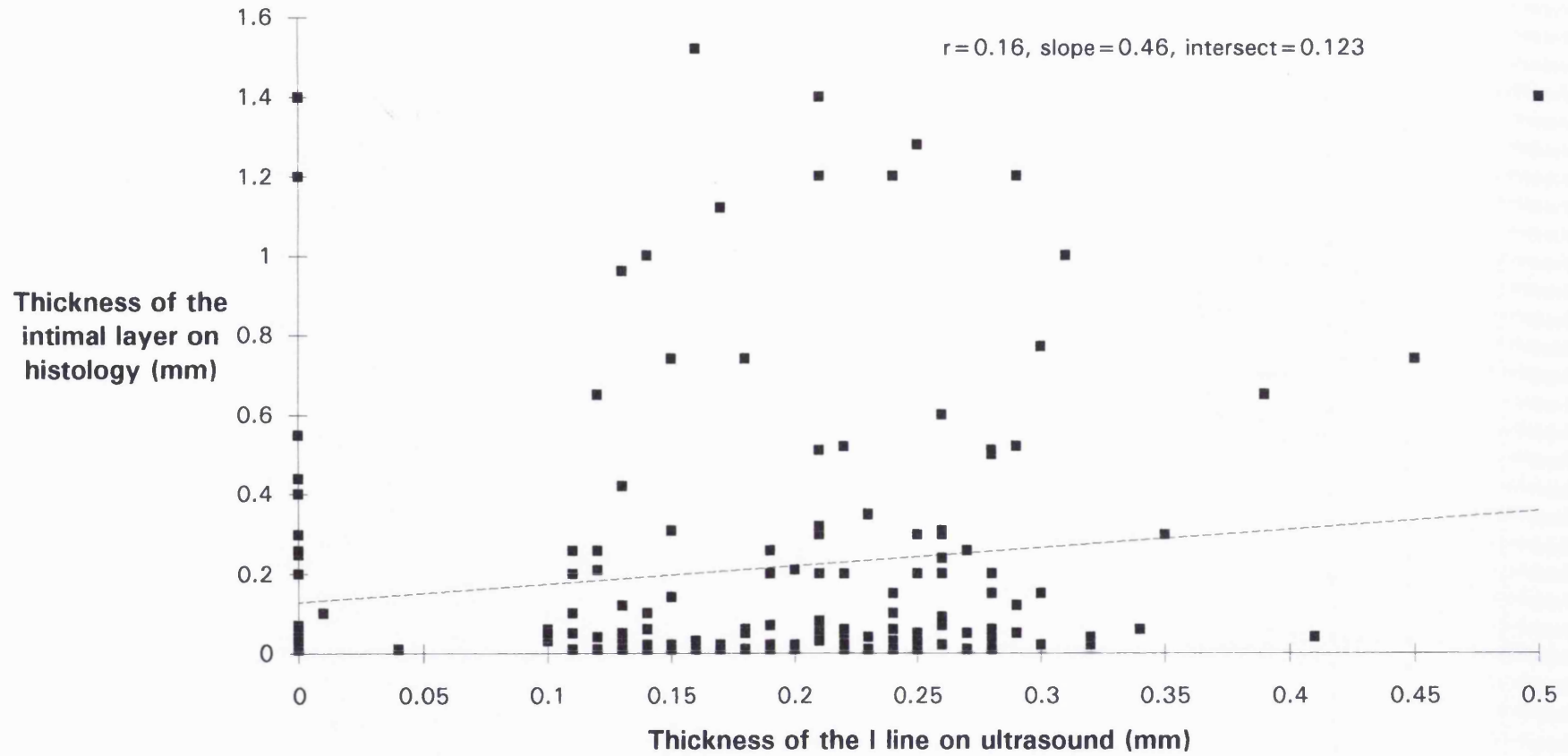


**Fig 8.9**

**Correlation between the histological intima + media thickness and the ultrasonic IM thickness**



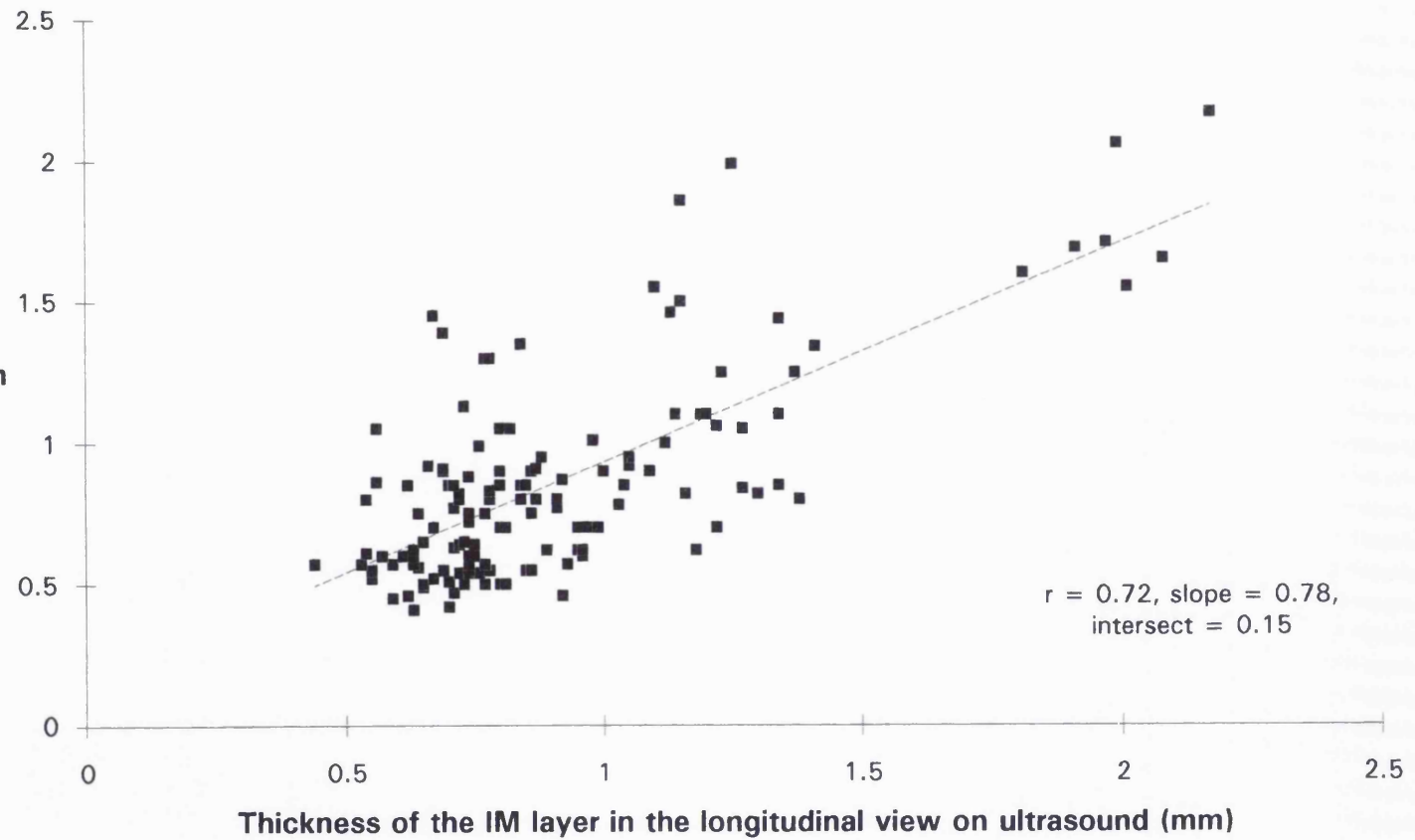
**Fig 8.10** The difference against the mean for the measurement of IM thickness by ultrasound and by histology



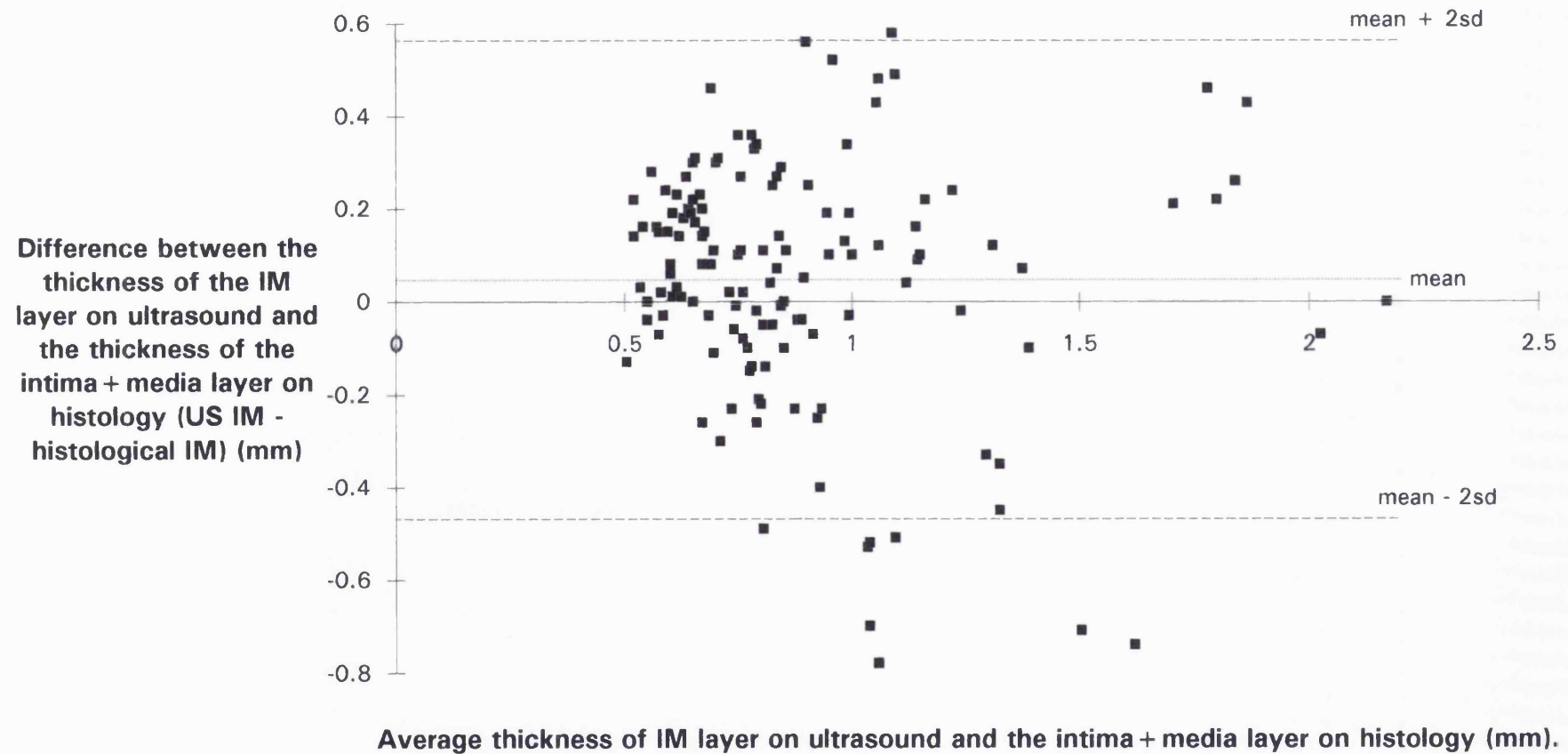
**Fig 8.11**

**The correlation between the intima thickness on histology and the I line on ultrasound**

Thickness of the  
intima + media layer on  
histology (mm)



**Fig 8.12 Correlation between the thickness of the IM layer of the far wall in the longitudinal view and the IM thickness on ultrasound**



**Fig 8.13 The limits of agreement between measurement of the IM thickness of the far wall in the longitudinal view on ultrasound and the thickness of the intima+media layer on histology.**

### **8.32 Comparison between near and far wall measurements**

The ultrasonic IM thickness of the near and far walls were compared after the vessel had been turned through 180°. The correlation between these measurements is shown in figure 8.14. The limits of agreement are shown in figure 8.15. This shows that not only is there a discrepancy between the measurements (the mean difference between measurement of the near and far walls after rotation of the vessel through 180° is 0.36mm) but also that this discrepancy increases with increasing thickness of the IM layer.

### **8.33 Correlation between ultrasonic IM thickness and histological changes.**

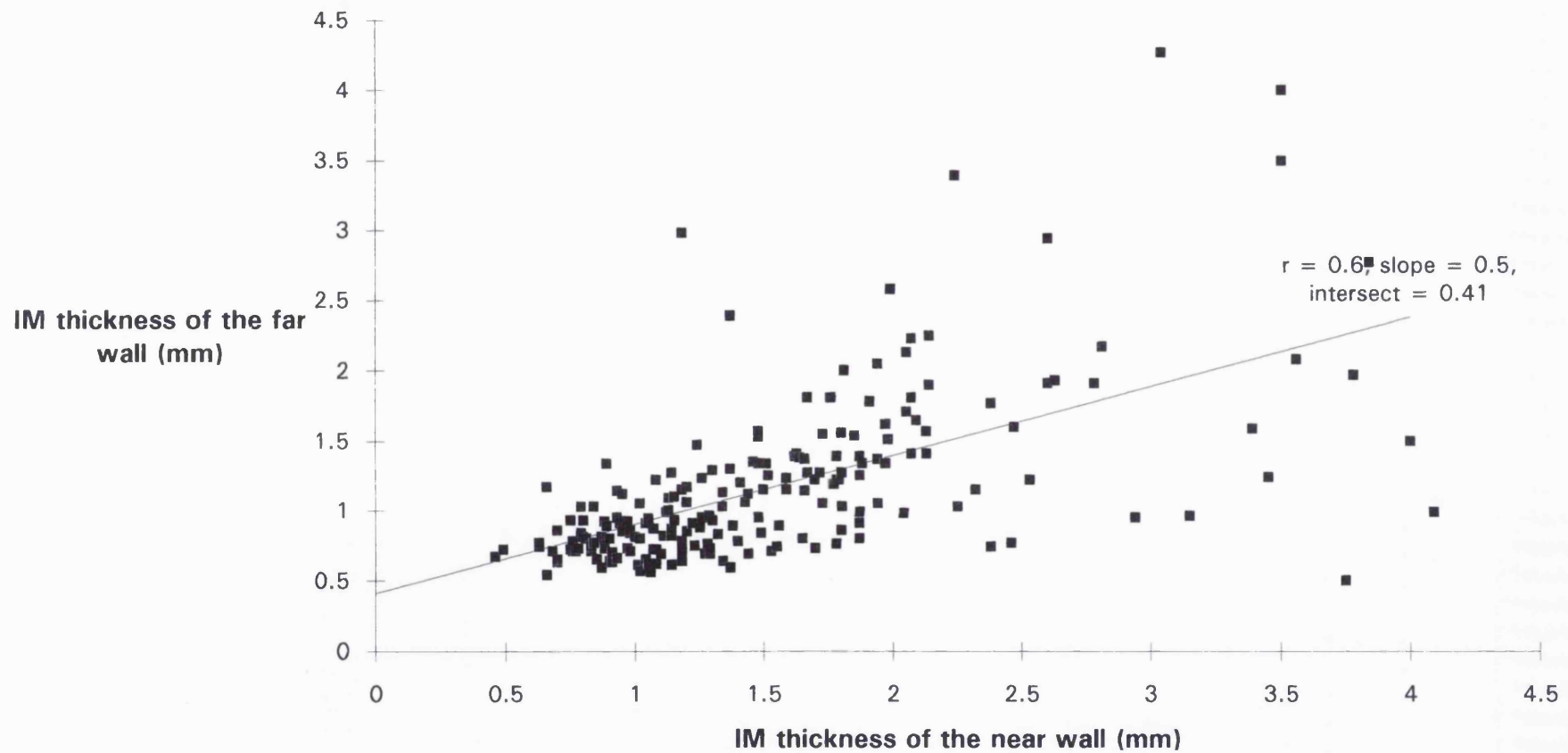
The transverse sections were examined for the histological features of plaque formation: cholesterol crystals, medial atrophy, fatty change, necrotic debris, calcification and inflammation. The relationship between these histological features and the intima media thickness is examined below.

#### **8.331 Cholesterol crystals**

The IM thickness in the absence of plaque crystals was significantly thinner than if crystals were present (median difference = 0.49mm, 95% confidence interval for the difference between population means = 0.07-0.27mm, normalised statistic = 3.69, one and two tailed  $p < 0.001$ ). The IM thickness in the presence of a few cholesterol crystals was not significantly different to the thickness when many crystals were present (median difference = 0.23, 95% confidence interval for the difference between population means = -0.11 - 0.51mm). In figure 8.16, the relationship between IM thickness and the presence of cholesterol crystals is examined, over half of the arteries with an IM thickness of less than 0.8mm have cholesterol crystals in the wall and the proportion of vessels without cholesterol crystals decreases with increasing IM thickness. All arteries with an IM thickness greater than 1.4 mm contain cholesterol crystals.

#### **8.332 Medial atrophy**

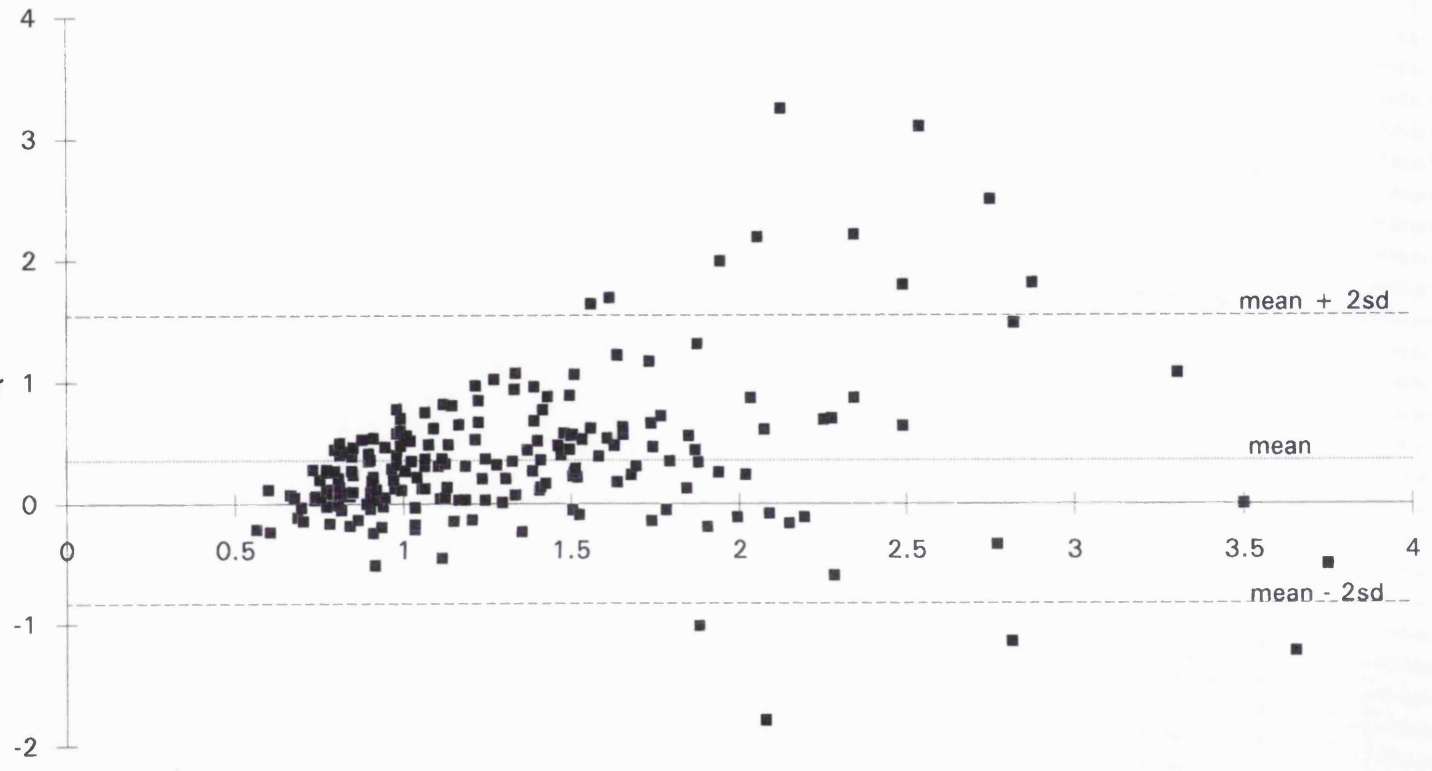
The IM thickness in the absence of medial atrophy was significantly thinner than if atrophy was present (median difference = 0.33mm, 95% confidence interval for the



**Fig 8.14 Correlation between the ultrasonic IM thickness of the near wall and the ultrasonic IM thickness of the far wall after rotating the vessel through 180 degrees**



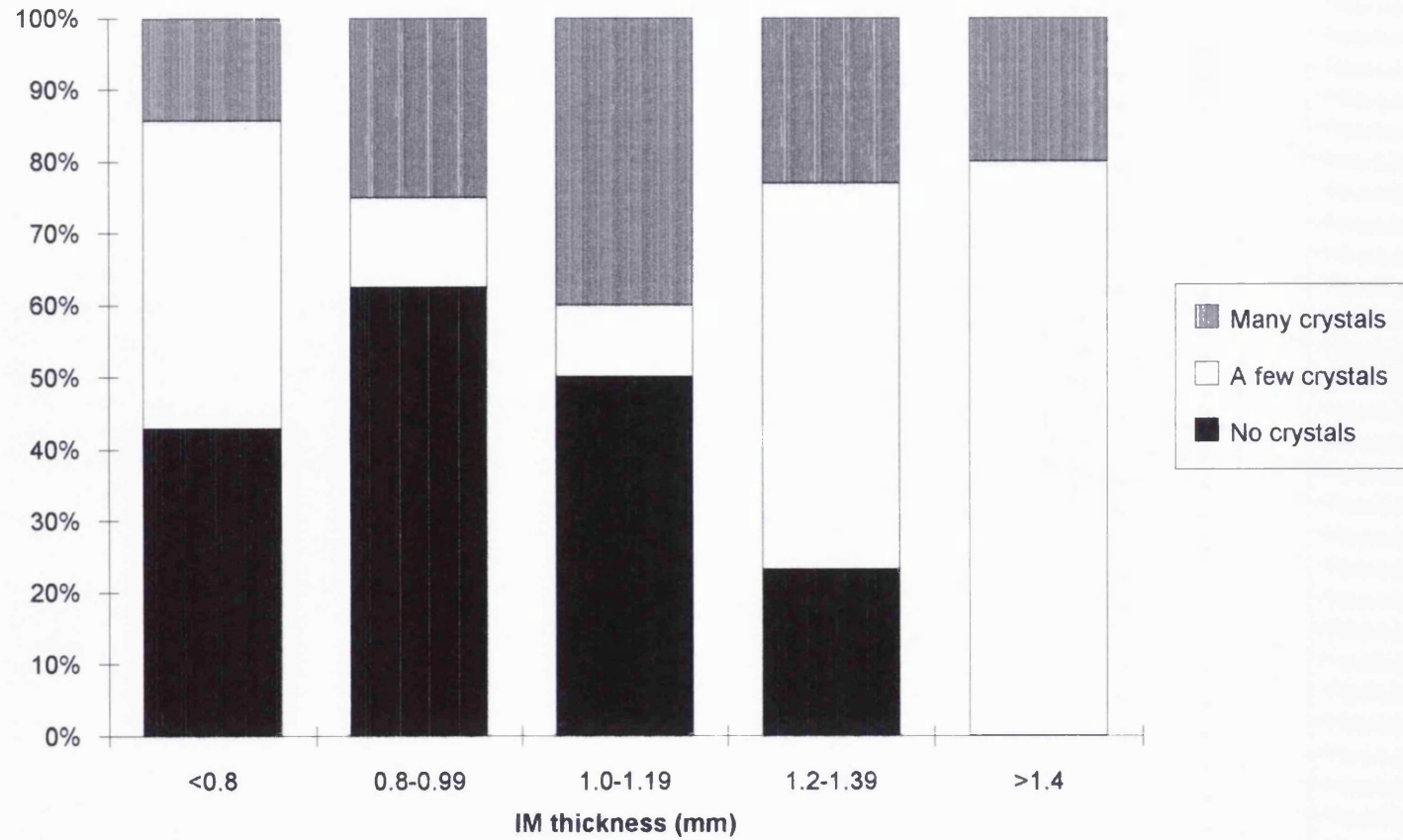
Difference in the IM thickness of the near and far walls (mm)



Average IM thickness for the near and far walls (mm)

**Fig 8.15 Limits of agreement in the measurement of the IM thickness of the near and far walls on ultrasound**

Percentage of histological sections containing cholesterol crystals



**Fig 8.16**

**The relationship between the presence of cholesterol crystals and IM thickness (mm)**

difference between population means = 0.12-0.54mm, NS = 3.46, one and two tailed  $p < 0.001$ ). There was no significant difference in the IM thickness with different degrees of medial atrophy (median difference = 0.08, 95% confidence interval for the difference between population means = -0.25 - 0.48mm). In figure 8.17, the relationship between the degree of atrophy and IM thickness is examined, and a degree of medial atrophy is found in half of the vessels with an IM thickness under 1mm.

#### 8.333 Fatty change

There was no significant difference in the median IM thickness when specimens showing fatty change were compared with those without, furthermore, the median IM thickness did not correlate with the degree of fatty change present and the proportion of vessels showing fatty change is the same irrespective of the IM thickness (fig 8.18).

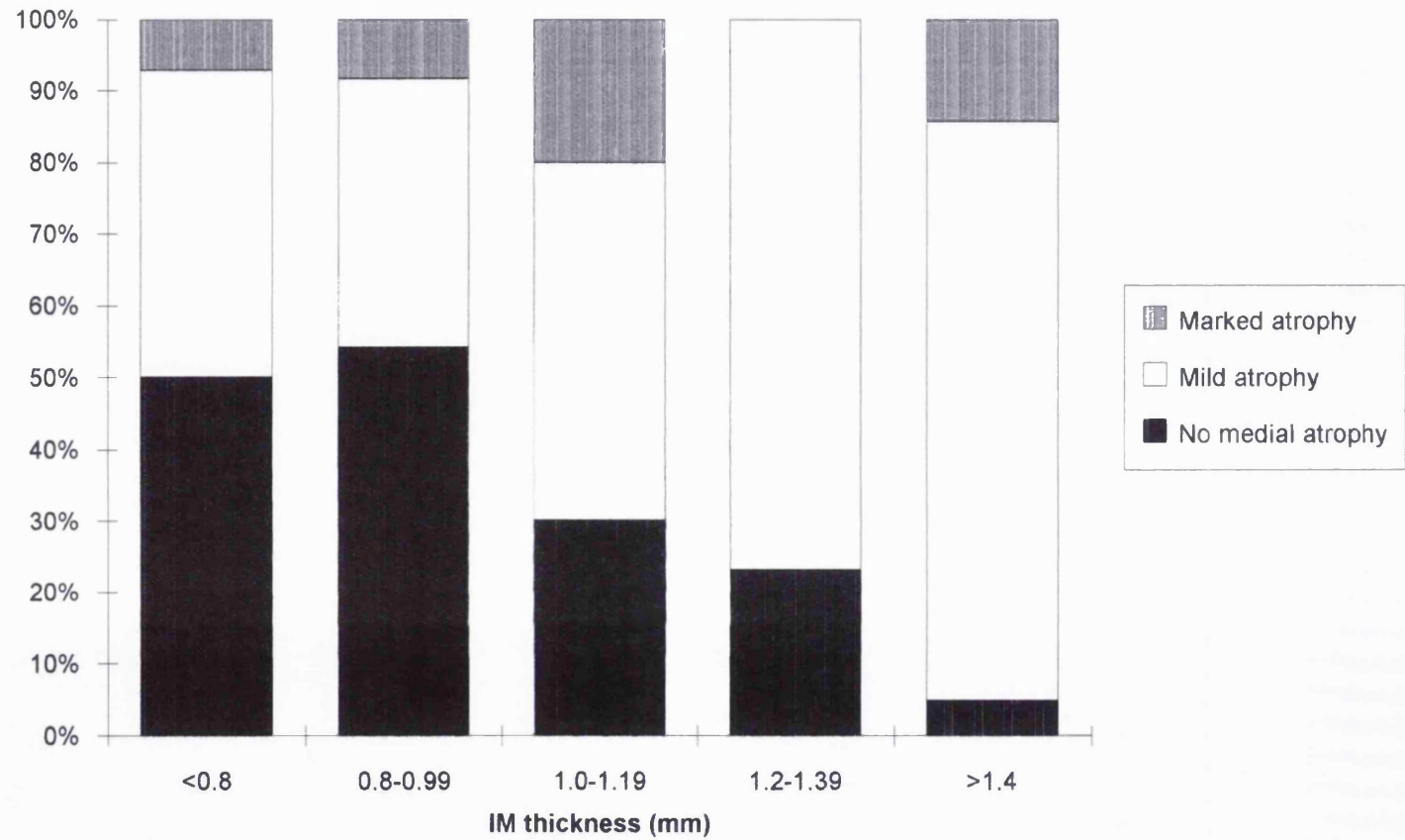
#### 8.334 Fibrous change

All but one specimen showed some degree of fibrous change. Specimens with a mild degree of fibrous change were significantly thinner than those with a marked fibrous change (median difference = 0.15mm, 95% confidence interval for the difference between population means = 0- 0.36, NS = 1.89 one tailed  $p = 0.029$ , two tailed  $p = 0.058$ ). In figure 8.19, the relationship between fibrous change and IM thickness is examined, approximately half of the arteries with an IM thickness of less than 1.4mm show mild degrees of fibrous change, but over 1.4mm the majority show a marked degree of fibrosis.

#### 8.335 Necrotic debris

Specimens that did not contain necrotic debris were significantly thinner than those with a mild degree of necrotic debris present (median difference =0.48mm, 95% confidence interval for the difference between population means = 0.19 - 0.73mm, NS = 3.37, one and two tailed  $p < 0.001$ ). There was no significant difference in the IM thickness with increasing amounts of necrotic debris (median difference = 0.27, 95% confidence interval for the difference between population means = -0.14 - 0.63mm). In figure 8.20, the relationship between IM thickness and the presence of necrotic debris is examined, some necrotic debris is found in vessels with an IM thickness of less than 0.8mm and

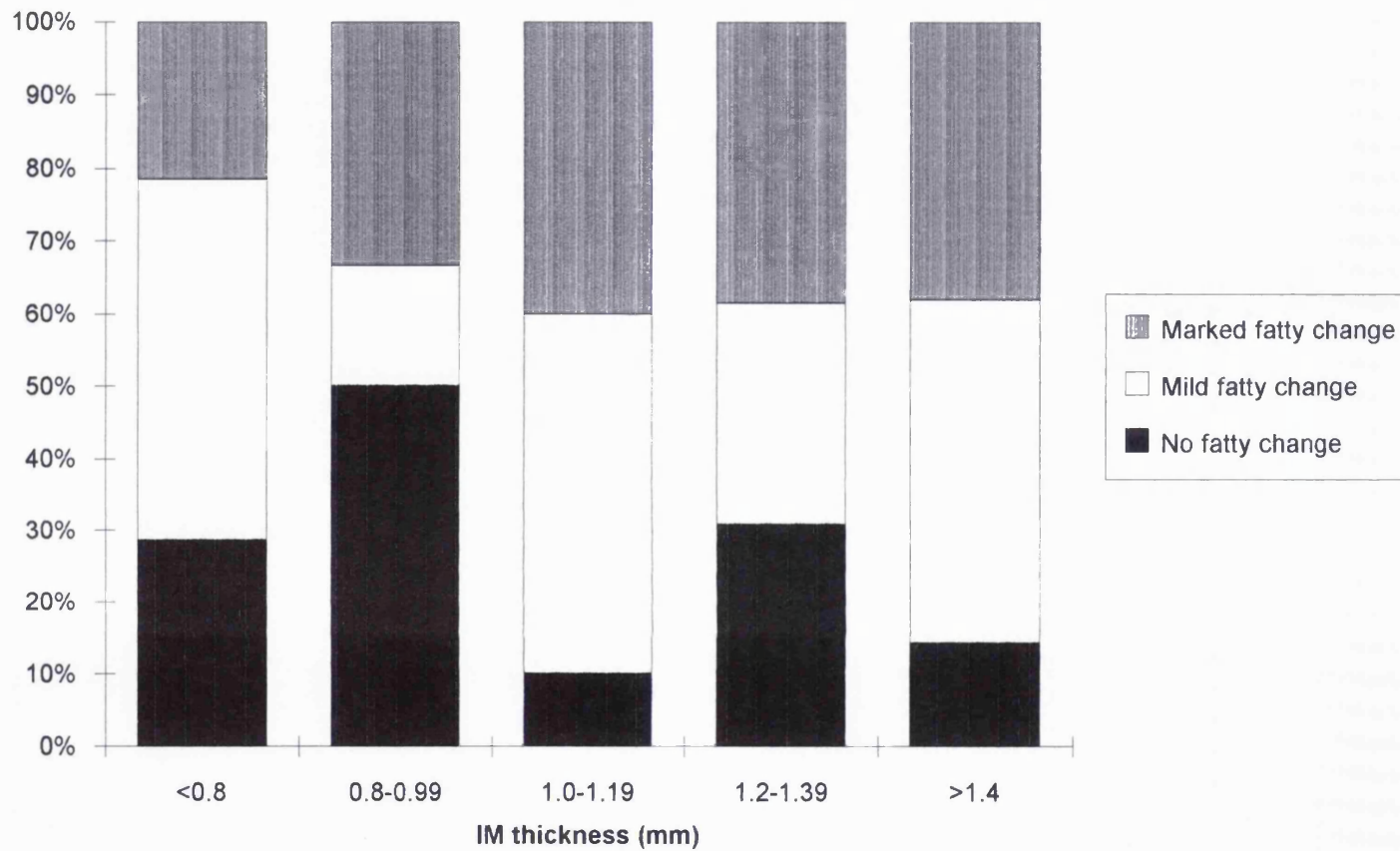
Percentage of histological sections showing medial atrophy



**Fig 8.17**

**The relationship between the presence of medial atrophy and IM thickness (mm)**

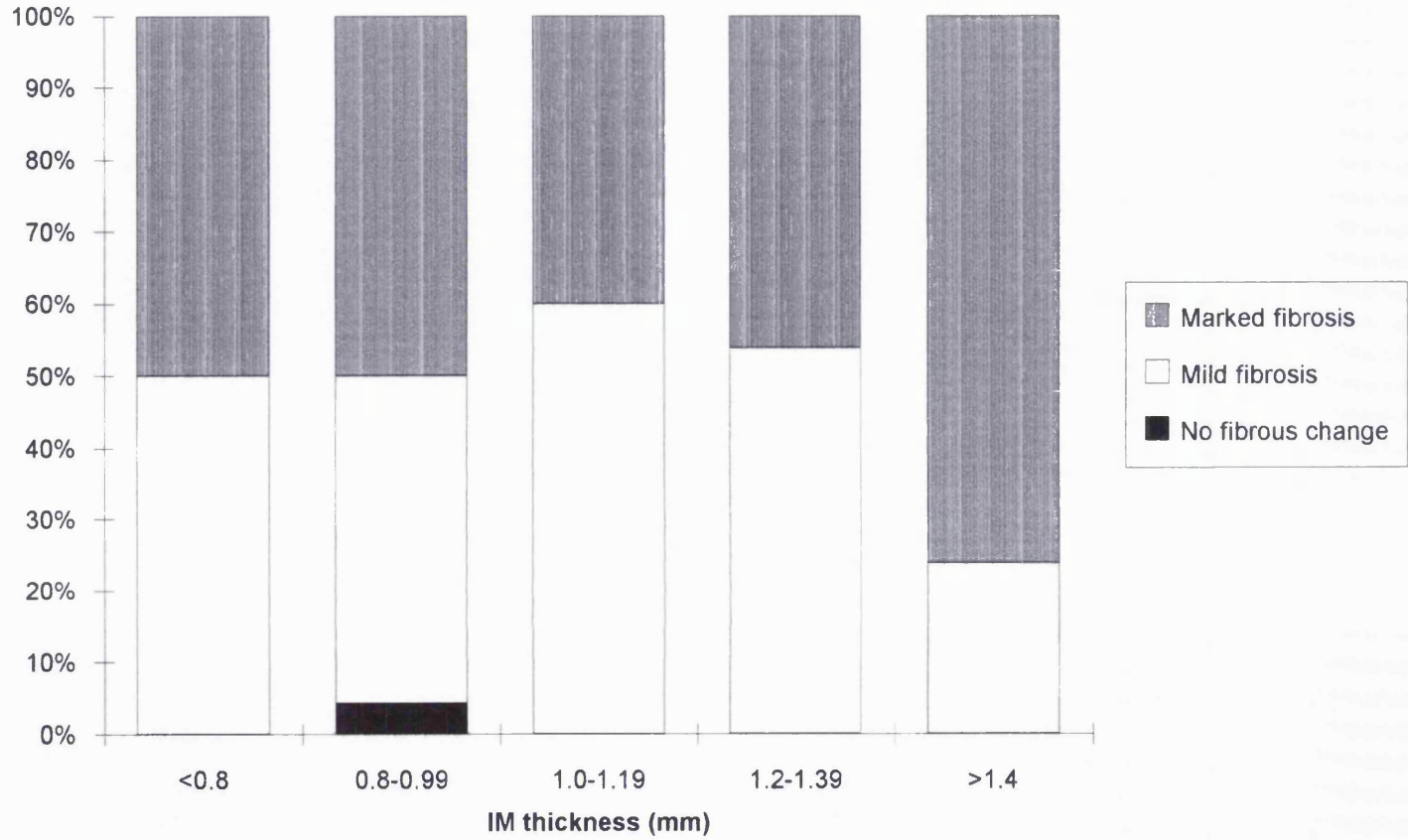
Percentage of histological sections showing fatty change



**Fig 8.18**

**The relationship between fatty change and the IM thickness (mm)**

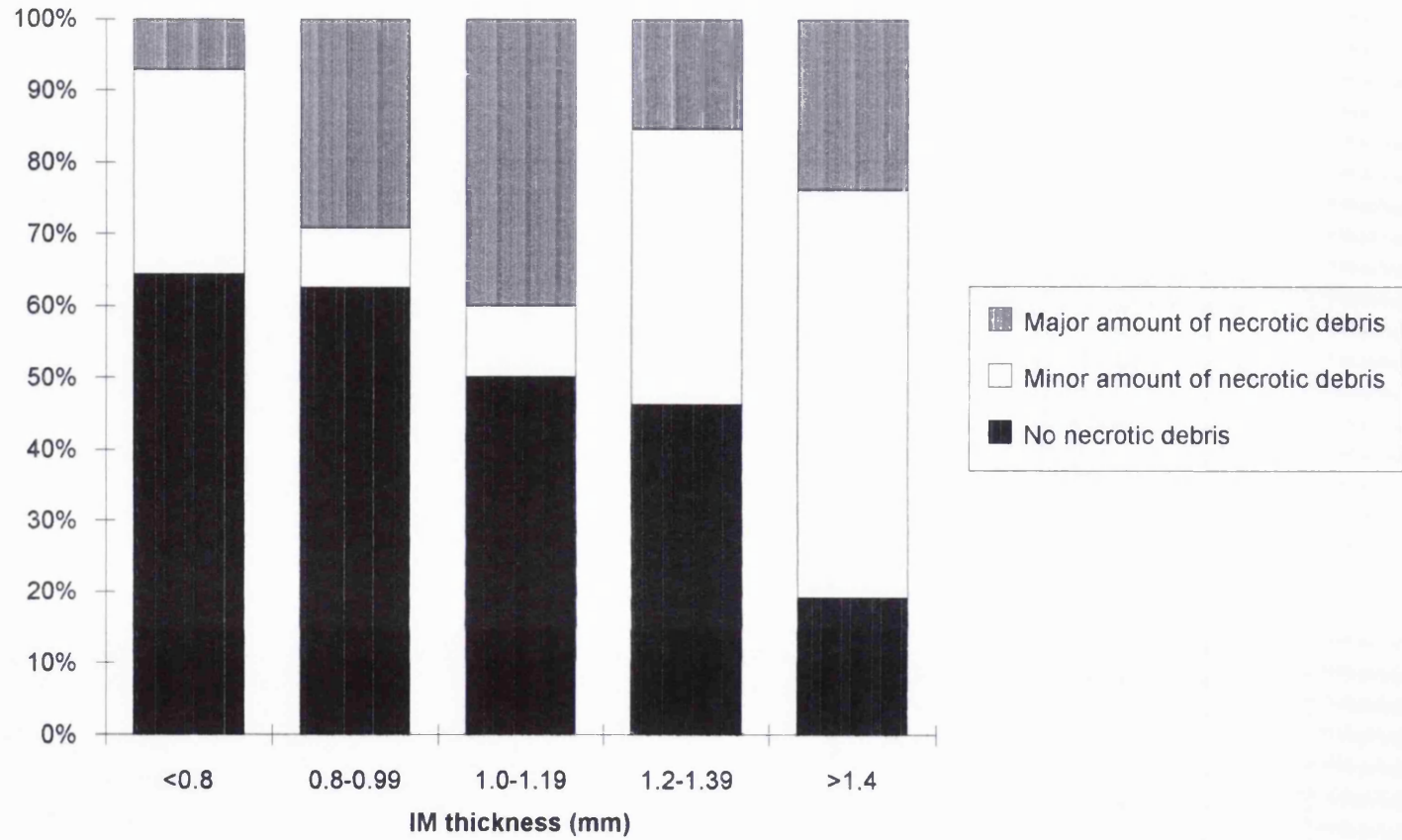
Percentage of histological sections showing fibrous change



**Fig 8.19**

**The relationship between the presence of fibrous change and the IM thickness**

Percentage of histological sections containing necrotic debris



**Fig 8.20**

**The relationship between the presence of necrotic debris and IM thickness**



with increasing IM thickness the proportion of vessels containing necrotic debris increases.

#### **8.4 Discussion**

*i) Which histological layer or layers correlate best with the ultrasound IM layer?*

In this study the correlation between the ultrasonic IM thickness and the combined intima and medial layers on histology was excellent with a correlation coefficient of 0.86. This confirms the findings of Pignoli et al. (Pignoli P(ii))<sup>1986</sup>.

*ii) Which layer is responsible for ultrasonic IM thickening?*

Changes in IM thickness occur due to changes in the intimal layer, while the medial layer remains relatively unchanged

*iii) Is there any correlation between the thickness of the first echogenic line and the thickness of the intima on histology?*

No

*iv) Is measurement of the ultrasonic IM thickness valid for both the near and far walls of the vessel?*

There is a disparity between the IM thickness measured on the near and far walls. This may be due to several factors. Firstly, this may be due a problem with the contrast resolution of our ultrasound probe. Ultrasound passing from fluid to solid gives a distinct interface, and hence the distinct first echogenic line. However, if neighbouring tissue areas are similar in consistency and closely spaced, tissue differentiation is more difficult as the reflected and scattered echoes from each tissue will overlap and interfere. The interference produces fluctuations in the resultant signal and hence a speckle pattern appearance in the neighbouring areas of the image. The speckle is in effect noise and can cause errors in the estimation of the IM thickness.

A second cause of error may be a design fault in our experiment. In order to assess the IM thickness of the near and far walls, the arteries were rotated in the water bath through 180°, but despite marking the vessel, it was difficult to do this accurately as the vessels were not rigid, and tended to flex on rotation, despite being pressurized. This may account for some of the discrepancies in the measurement of near and far wall thickness,



in particular, the measurement of the thickest regions, where the angle of the beam in relation to the diameter the vessel can result in major discrepancies in plaque thickness. A way to overcome this would be to develop a rigid frame to hold the vessel in relation to the probe.

*iv) What histological changes are observed with increasing ultrasonic IM thickness?*

The histological features of plaque formation: cholesterol crystals, medial atrophy, fatty change, necrotic debris, and fibrous change were all found in arteries with an IM thickness of less than 0.8mm. An IM thickness of less than 1mm is not considered to be a plaque, yet in this study, half of the specimens with an IM thickness of less than 1mm showed features thought to represent plaque formation (cholesterol crystals, medial atrophy, fatty change and fibrosis) and a few vessels even contained necrotic debris. With increasing thickness, the proportion of vessels showing these features increased and once the IM thickness exceeded 1.4mm all vessels contained cholesterol crystals, and the majority contained marked degrees of fibrosis. This would suggest that IM thickening is a manifestation of early plaque formation.

In conclusion, ultrasonic IM thickness is an accurate measure of the arterial wall IM thickness and changes in the intima-media thickness of a vessel are the manifestations of early plaque formation.

## **PART III**

METHODS USED IN CLINICAL STUDIES

PRESENTED IN PART IV

CHAPTER 8

ULTRASOUND IMAGING AND MEASUREMENTS  
AS DEVELOPED BY THE AUTHOR

## **9.1 Introduction**

The methods used in Part IV are described in this chapter.

High Resolution ultrasound was used to examine the carotid arterial tree according to the technique described below to determine intima-media thickness and plaque characteristics. These were compared with previously described methods of assessing arterial wall stenosis: noninvasive ultrasonic biopsy and duplex scanning with doppler waveform analysis. In the patient groups, the overall atherosclerotic status was assessed by the use of chest wall mapping, the 1 min treadmill and ankle brachial pressure indices. The risk factors were assessed using standard questionnaires and blood tests described below.

## **9.2 Imaging**

All imaging (duplex Doppler and high resolution ultrasound imaging) was performed using an ATL Ultramark 4. Duplex doppler examination was performed using a multifrequency annular probe set at an imaging frequency of 7.5MHz and a Doppler frequency of 5MHz. High resolution ultrasound examination was performed using a dedicated 7.5 MHz linear ultrasound probe. The machine was preset on the following settings: Dynamic range 47db, power 50%, FPS 28, Reject 1, Edge 2, grey scale 4, and smooth F3.

### **9.21 Position of the patient:**

All imaging was performed with the patient supine on the couch. For the examination of the carotid arteries, the head was rotated 45° away from the side to be studied, with the ipsilateral sternomastoid muscle relaxed and the arms by the patients side. The femoral arteries were examined with the hips extended and the legs straight, with a slight external rotation of the foot

### **9.22 High Resolution ultrasound examination**

#### **9.221 B-mode pictures of the carotid arteries**

##### **a) Longitudinal views**

With the head rotated away from the side to be examined, the common carotid artery was identified by placing the linear probe over the sternomastoid muscle, in line with the belly of the muscle. On identifying the artery, the probe was moved distally up the neck to identify the origin of the carotid bulb. By adjusting the position of the probe so that the ultrasound beam was perpendicular to the artery wall, the near and far walls of the carotid artery could be clearly defined. The distal 4 cm of common carotid artery was imaged and screen captures taken in the anterolateral plane immediately proximal to the origin of the bulb. The probe was then moved up the neck and placed anterior to sternomastoid, as high as possible behind the angle of the mandible. The position of the probe was adjusted until the carotid bifurcation was clearly imaged and screen captures taken (anterior view). The probe was then rotated through 90° to lie over the posterior border of sternomastoid, in line with the artery. The position of the probe was again adjusted until the artery walls were clearly defined, and screen captures were taken of the lateral view of the artery.

#### b) Transverse views.

The probe was then rotated to lie at right angles to the line of the artery. The position of the origin of the bifurcation and the flow divider were identified and, after adjustment of the probe so that the anterior and posterior walls could be clearly seen, a view of the bifurcation immediately proximal to the flow divider was taken.

#### **9.222 M-mode pictures of the carotid arteries**

The common carotid artery was visualised in the longitudinal plane, immediately proximal to the bifurcation and the M-mode switched on. This splits the screen into the B-mode picture and an adjacent M-mode picture. The M-mode picture displays a 3 second trace adjacent to the real-time B-mode image. Using the tracker ball, the cursor was placed across the longitudinal view of the artery (positioned parallel to the probe) and the arterial wall at this site was tracked through several cardiac cycles. A screen capture was taken of the trace with the clearest view of the echogenic lines. This process was repeated for the bulb, immediately distal to the flow divider. Care was taken to position the probe so that it lay parallel to the lie of the artery. The examination was repeated on the opposite side.

### **9.223 B-mode pictures of the common femoral artery.**

The common femoral artery was examined immediately proximal to its bifurcation. Longitudinal and transverse views were selected at this site and screen captures taken.

### **9.224 M-mode pictures of the common femoral artery.**

An M-mode trace was taken 1cm proximal to the bifurcation.

The examination was repeated on the opposite side.

## **9.23 Data capture**

All views were captured as screen captures. These were stored on to 3 1/2" floppy discs prior to downloading on to a WORM drive for permanent storage. Screen captures were also printed on thermal paper for storage with the patients records.

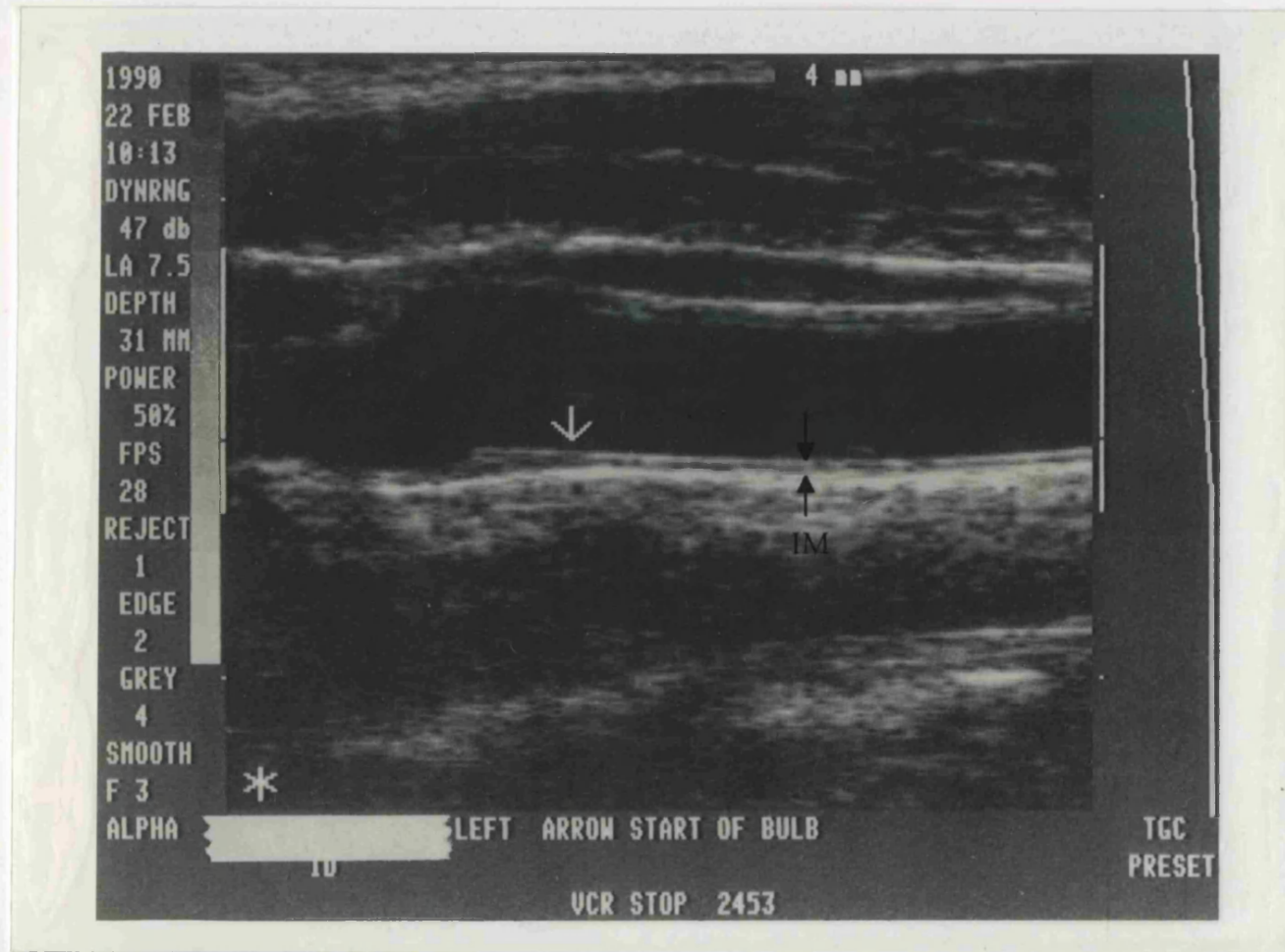
## **9.24 Measurements:**

All measurements were taken on a calc-comp @ measuring board using GWBasic software. The calibration for each measurement was taken from the calibration bar at the side of the screen captured picture. All measurements were taken from the screen capture prints, after magnification of the image to fill the screen.

### **9.241 Intima-media thickness:**

This is taken as the distance from the luminal surface of the first echogenic line to the luminal aspect of the second echogenic line (Fig.9.1) All measurements were taken on the far wall of the artery, in relation to the ultrasound probe. The average of three measurements taken at the site of maximal thickness was recorded. The sites at which the intima-media thickness was measured were:

- a) 1-3cm from the carotid artery bifurcation,
- b) at the bulb origin,



**Fig 9.1**

**Ultrasound picture of the common carotid artery showing the IM layer and the bulb origin**

- c) mid bulb,
- d) 1-3cm proximal to the femoral artery bifurcation (fig 9.2)

**9.242 Arterial wall excursions:**

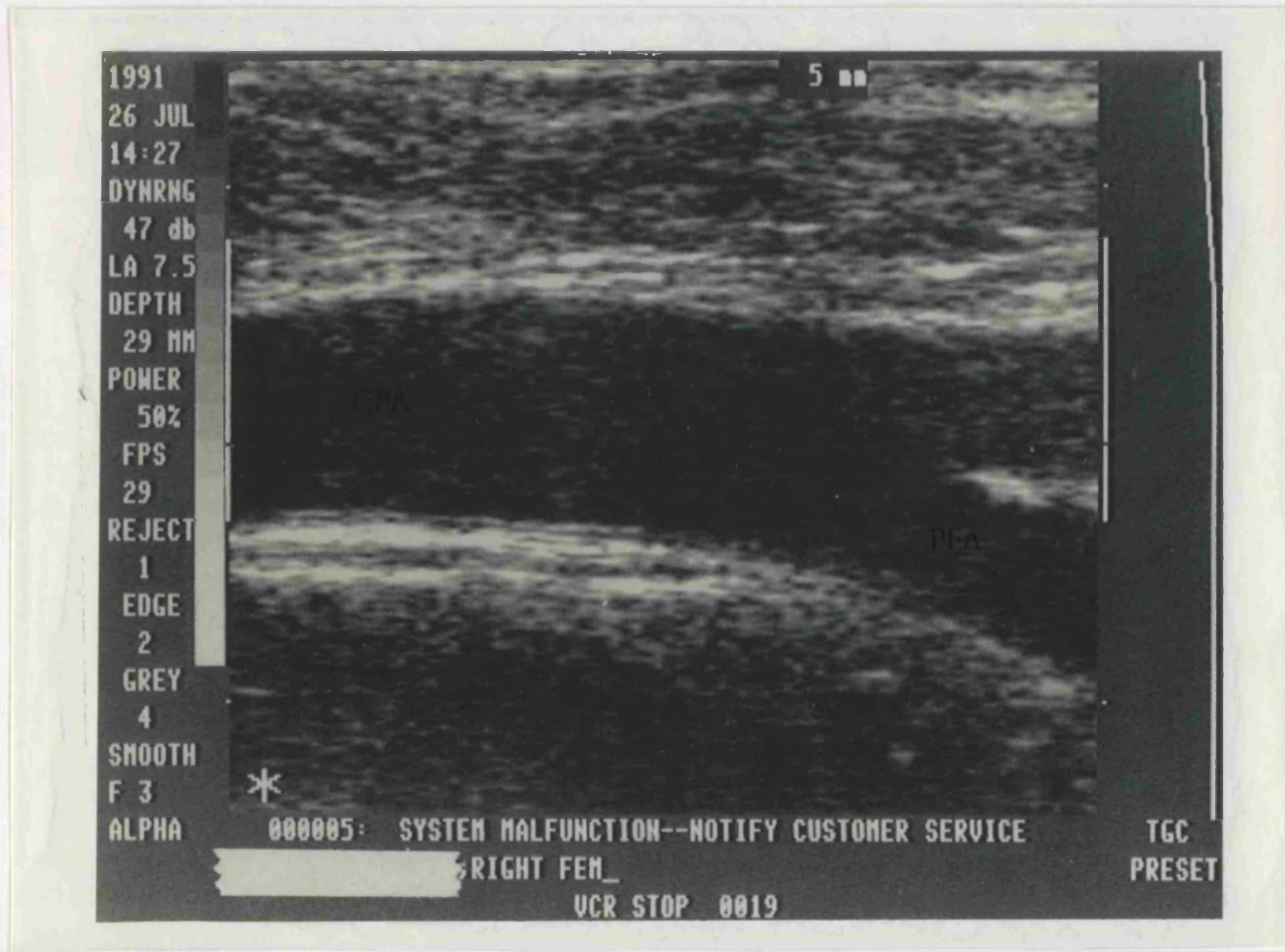
The end-diastolic and peak systolic diameters of the artery were measured from the M-mode picture (fig 9.3). Each measurement was repeated three times and the mean taken. Where possible, three cardiac cycles were measured.

**9.243 Reproducibility of IM thickness measurements:**

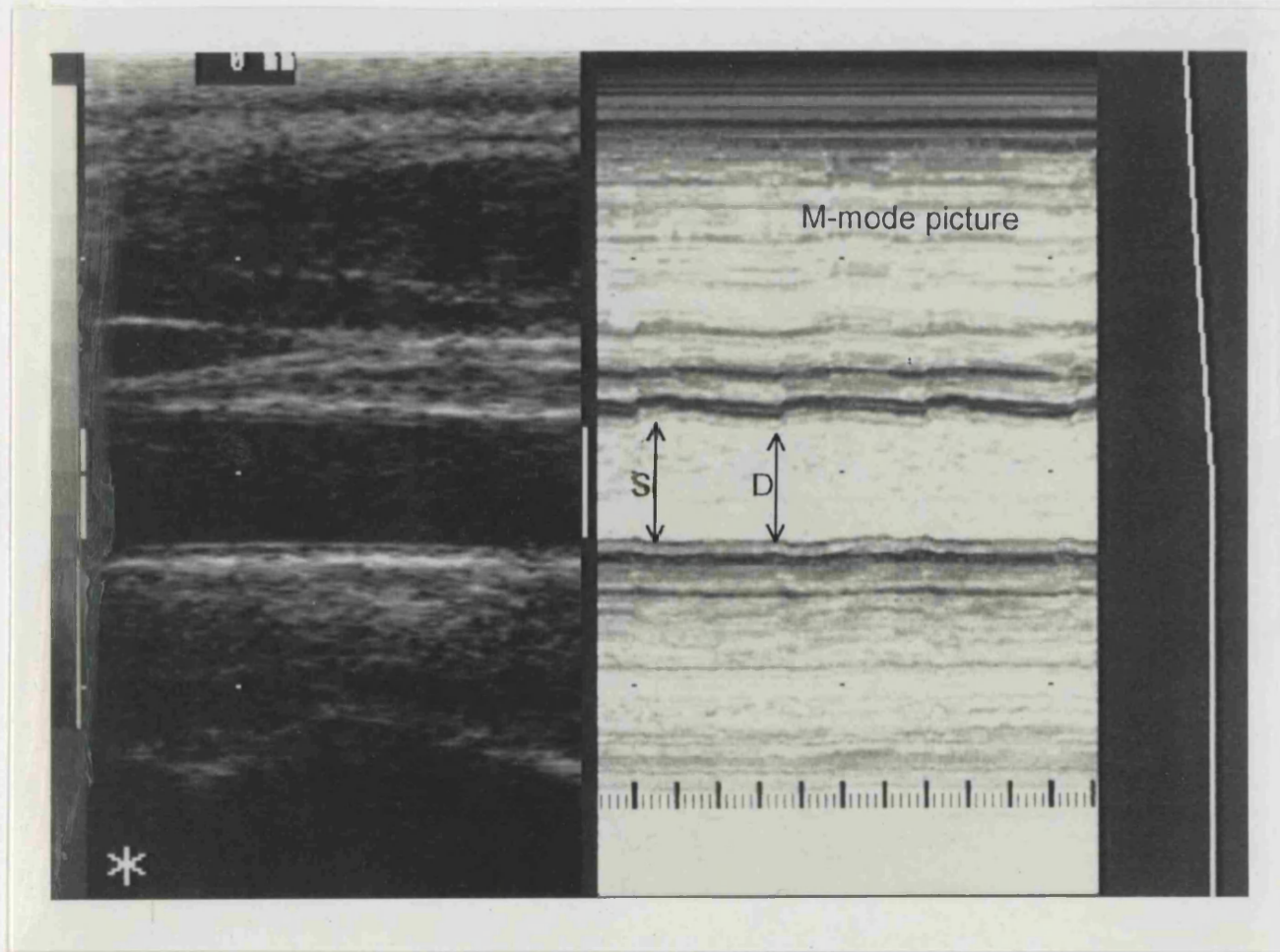
The reproducibility of measurements of IM thickness, systolic diameter and diastolic diameter were assessed by comparing measurements taken at a defined point on a single image, by three observers on three successive occasions (Table 9.1) and by comparing the measurements taken by two sonographers on six healthy vessels on three successive occasions (Table 9.2).

The 95% confidence limits in our laboratory for the measurement of the IM thickness are from -0.15mm to +0.15mm of the observed value (coefficient of variation of 15.9% for the measurement of 0.51mm).





**Fig 9.2** Ultrasound picture of the common femoral artery at its bifurcation



**Fig 9.3** M-mode picture of the common carotid artery

**Table 9.1**     **The reproducibility of measurements of IM thickness, systolic diameter and diastolic diameter, taken at a defined point on a single image, by three observers on three successive occasions.**

	IM thickness	M-M systole	M-M diastole
Mean (mm)	0.58	7.15	6.41
95% Confidence limits	0.08	0.19	0.18
Coefficient of variation (%)	6.89	7.31	6.39

**Table 9.2 The reproducibility of measurements taken by two sonographers on six healthy vessels on three successive occasions:**

	Sonographer	Occasion	All variables	
	CV (%)	CV (%)	CV (%)	$\pm 2sd$
IM thickness	12.41	13.31	15.93	0.15
M-M systole	5.45	6.45	7	0.95
M-M diastole	5.95	6.9	7.54	0.93

## 9.24 Derived values:

### Distensibility and Compliance

Distensibility (DC) = Relative change in volume per unit pressure change

$$\begin{aligned} &= \frac{\Delta V/V}{\Delta P} \\ &= \frac{\Delta A/A}{\Delta P} \end{aligned}$$

$$\text{as } \Delta A/A = \frac{\pi [(Ds/2)^2 - (Dd/2)^2]}{\pi (Dd/2)^2}$$

$$\text{Distensibility} = \frac{2d/D}{\Delta P} \quad (\text{ignoring } d^2 \text{ which is negligible})$$

$\text{mm}^3/\text{KPa}$

Compliance (CC) = Absolute change in volume / change in pressure

$$\begin{aligned} &= \frac{\Delta V}{\Delta P} \\ &= \frac{\Delta A}{\Delta P} \end{aligned}$$

$$\text{as } \Delta A = \pi [(D+d/2)^2 - (D/2)^2]$$

$$= \frac{\pi dD}{2\Delta P} \quad \text{mm}^3/\text{KPa}$$

$$\text{Elastic modulus} = E_p = K \times (P_s - P_d)/(d/D)$$

$$\text{Stiffness Index} = \beta = (\ln(P_s/P_d))/(d/D)$$

CHAPTER 10

PREVIOUSLY REPORTED METHODS

## **10.1 Duplex scanning and Doppler waveform analysis**

### **10.1 Method**

All duplex scans were performed on an ATL Ultramark IV model, using the 7.5 MHz setting of a multiple frequency annular probe. The time gain control, power, edge, grey scale and smooth settings were all preset. All Doppler spectral waveforms were taken using a 2x3mm sample volume placed in the centre of the lumen of the vessel, with the incident angle of the ultrasound beam at 60° to the axis of the vessel

The patients were positioned supine with the head straight, without a pillow, on an examination couch. The head was slightly rotated to the opposite side without producing tension in the ipsilateral sternomastoid muscle. The transducer was placed in contact with the skin using coupling gel and the cervical vessels were scanned in a longitudinal and transverse plane from the supraclavicular fossa to the mastoid process. Having defined the anatomy, Doppler velocity recordings were made at six sites: the proximal and distal portions of the common carotid artery, the external carotid artery and the proximal, middle and distal portions of the extracranial internal carotid artery. All scans were performed by experienced ultrasonographers and the lesions graded according to Strandness criteria (Blackshear et al, 1979; Roederer et al, 1984; Williams et al, 1986) (Table 10.1).

### **10.12 Validation studies in our department**

Using the modified Strandness criteria, 358 internal carotid artery duplex scans in which adequate two-plane angiography was available were compared (Williams et al, 1986). The sensitivity, specificity, positive predictive value and negative predictive value and accuracy achieved is shown in table 10.2

**Table 10.1      Criteria for grading internal carotid artery stenosis from duplex scanning spectral waveform analysis.**

<u>Grade</u>	<u>Percentage stenosis</u>	<u>Criteria</u>
A	No stenosis	Peak velocity in internal carotid artery <120cm/s No spectral broadening during the acceleration phase of systole
B	1-15%	Peak velocity in internal carotid artery <120cm/s Spectral broadening during the acceleration phase of systole Window present above the level of the end diastolic frequency
C	16-49%	Peak velocity in internal carotid artery <120cm/s Spectral broadening throughout systole No window present above the level of the end-diastolic frequency
D	50-79%	Peak velocity in internal carotid artery >120cm/s Spectral broadening throughout systole No window.
D+	80-99%	Peak velocity in internal carotid artery >120cm/s Spectral broadening throughout systole No window. End-diastolic velocity >200 cm/s
E	Occlusion	No flow signal in an adequately visualized internal carotid artery with characteristic low or reversed flow in the common carotid artery.



**Table 10.2 The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of duplex scanning in the detection of carotid artery disease when compared to two plane angiography.**

	Sensitivity	Specificity	PPV	NPV	Accuracy
To discriminate between normal and abnormal vessels	96%	83%	96%	82%	94%
To discriminate between a stenosis of less than or greater than 15%	97%	96%	97%	96%	97%
To discriminate between a stenosis of less than or greater than 50%	95%	94%	91%	97%	95%
To discriminate between a patent or an occluded vessel	100%	100%	100%	100%	100%

## **10.2 Noninvasive arterial ultrasound biopsy.**

### **10.21 Method**

Using the 7.5 MHZ high resolution ultrasound probe on the ATL Ultramark IV, both common carotid and common femoral arteries were examined over 3cm immediately proximal to the bifurcation and the carotid bulb was also examined for plaque. This examination was carried as part of the high resolution ultrasound examination described in 9.1.

### **10.22 Reporting of results**

Each artery was graded from I to VI according to the appearance of the far wall on ultrasound (Table 10.3). The artery was also allocated a score (0-10) and the total score per patient was calculated.

### **10.24 Interrelationship between the ultrasound and duplex tests in carotid artery disease.**

The tests described above grade the carotid artery at different stages of the atherosclerotic disease process. Ultrasonic biopsy grades I - IV would be graded as normal vessels on duplex scanning, whereas an ultrasonic biopsy grades V and VI could have a stenosis of 1 - 99% on duplex scanning.

**Table 10.3** Ultrasound biopsy grades and arterial ultrasound score

<u>Grade</u>	<u>Criteria</u>	<u>AUS</u>
I	Normal - Three ultrasonic layers clearly separated (Two echogenic layers separated by an echolucent layer). No disruption of the lumen-intimal interface for at least 5mm.	0
II	Interface disruption - lumen-intimal interface disruption at intervals of less than 5mm	2
III	Intima-media granulation - granular echogenicity of the deeper, normally echolucent intima-media layer.	4
IV	Plaque without haemodynamic disturbance - Wall thickening and increased density involving all ultrasonic layers. No haemodynamic disturbance on duplex (sample volume in the centre of the lumen)	6
V	Stenotic plaque - Wall thickening and increased density involving all ultrasonic layers. Haemodynamic disturbance detected on duplex (sample volume in the centre of the lumen)	8
VI	Stenotic plaque and the presence of symptoms (cardiac, cerebral or peripheral vascular symptoms)	10

### **10.3 Electrocardiographic chest wall mapping (ECG-CWM) stress test**

#### **10.31 Method**

##### **10.311 Preliminary cardiac assessment**

A conventional 12-lead supine electrocardiogram was initially performed in order to exclude recent silent myocardial infarction or cardiac arrhythmias which would constitute a contra-indication to undertaking an ECG stress test; or conduction abnormality such as bundle branch block that would render the exercise-induced changes in ST morphology difficult to interpret.

##### **10.312 Preparation for chest wall mapping**

After skin preparation with methyl alcohol, shaving hairy areas as necessary, sixteen disposable press-stud electrodes were placed in four vertical rows around the chest wall, the first row (A) in line with the right sternoclavicular joint, the second row (B) in line with the left sternoclavicular joint, the third row (C) along the anterior axillary line and the fourth row (D) down the back, along a line 6 cm medial to the posterior axillary line. Electrode C3 was placed over the fifth intercostal space and corresponded to the conventional V5 position: this determined the horizontal levels of A3, B3 and D3. The distance between B3 and C3 was used to determine the distance between the horizontal rows.

##### **10.313 Exercise protocol**

The exercise stress test was performed on a bicycle ergometer (Mijnhardt KEM2, Holland) at a constant speed of 60 rpm. The patient was asked to pedal with a starting workload of 50 watts for 2 minutes, this was increased to 75 watts for a further two minutes, and thereafter by 25 watt increments every minute until the end point, determined by the maximum predicted heart rate for the patient's age or the development of symptoms.

### **10.314 Electrocardiographic recording and blood pressure monitoring**

The test was monitored by a cardiologist with standard resuscitation equipment on standby. ECG recordings were made from the 16 electrodes, at rest and every minute during and after exercise, until the ECG changes and the heart rate returned to the baseline pre-exercise state. A standard three channel ECG recorder (Picker International, Cambridge Instrument; CD 6000) with the addition of an electronic switch facilitated recording of three channels in each vertical row in rotation during the test.

Blood pressure was monitored with a conventional mercury sphygmomanometer cuff at rest and every minute during and after exercise. The patients were asked to hold the bicycle bars without applying a firm handgrip in order to minimise the isometric element.

### **10.315 Criteria for stopping the test:**

The test was stopped if the patient developed symptoms (chest pain, claudication, dizziness or exhaustion), ECG changes (ST segment depression equal to or greater than 3mm or threatening arrhythmias), or a fall in systolic blood pressure.

### **10.32 Reporting of results**

The resting ECG was reported as positive in the presence of Q waves on the ECG tracings at rest using chest wall mapping, indicating previous infarction in the territory of that coronary artery. A Q wave was considered significant if its duration was 40 msec or more, or its amplitude equal to or greater than 25% of the amplitude of R wave.

The exercise test was reported as positive if horizontal or downsloping ST segment depression of 1mm or more below the isoelectric line, at least 80 msec after the J point, developed during exercise or in the postexercise period.

The exercise test was reported as negative if 85% of the target heart was achieved but no ST segment depression or other ECG changes occurred.

The exercise test was reported as inconclusive if the target heart rate was not achieved.

### **10.33 Validation studies**

Chest wall mapping of ST segment changes and of U waves during and after exercise, and of Q waves at rest was performed using 16 electrocardiographic electrodes and bicycle ergometry in 150 patients presenting to our department with chest pain suggestive of angina. All patients underwent coronary angiography (Salmasi et al, 1983). The presence or absence of significant (50% stenosis) coronary artery disease was detected by chest wall mapping with exercise stress testing with a sensitivity of 90% and a specificity of 88% compared with coronary angiography. The absence of significant coronary artery disease and the presence of single, double or triple vessel disease was correctly predicted in 70% of patients. Errors occurred in 25% of patients because the disease was missed or falsely diagnosed in one coronary artery. Errors in more than one vessel occurred in only 5% of patients.

## **10.4 One minute treadmill**

### **10.41 method**

After resting for 10 minutes on a couch, the arm and ankle systolic pressures were measured using a doppler probe and a pressure cuff. The measurements were taken from both arms and both legs. The subject then walked on a treadmill (4km/h, 10% slope) for 1 minute and returned to the couch. The ankle pressures were measured 30s and 90s following exercise and compared to the brachial pressure.

### **10.42 Reporting of results**

The ankle brachial pressure index was calculated as the ankle systolic blood pressure divided by the maximum brachial systolic pressure:

$$I = \frac{\text{Ankle systolic pressure}}{\text{Brachial systolic pressure}}$$

The treadmill was considered positive if the post exercise index fell below 0.8

### **10.5 Risk factor questionnaire**

All volunteers and all the patients studied completed a questionnaire based on the Rose cardiovascular questionnaire (Rose and Blackburn, 1968) to identify risk factors, and to exclude symptomatic cerebrovascular disease, cardiac disease and peripheral vascular disease (except the group of known claudicants) (Appendix)

### **10.6 Blood Pressure measurement**

The blood pressure was measured once the patient had rested for at least ten minutes. The brachial blood pressure was measured manually using a sphygmometer. Three measurements were taken from each arm, and the mean for each arm calculated. The highest mean was used in subsequent calculations.

### **10.7 Blood tests**

Fasting bloods were taken from the patient groups and tested routinely for cholesterol, HDL, LDL, and triglycerides.

CHAPTER 11

THE ULTRASONIC CHARACTERISTICS OF  
CAROTID AND FEMORAL ARTERIES IN NORMAL  
SUBJECTS



## **11.1 Introduction**

As discussed in chapter 3.2, the intima-media thickness of the common carotid artery has been shown to be increased in the presence of risk factors associated with atherosclerosis, namely cigarette smoking, LDL cholesterol concentration, age, systolic blood pressure and a history of ischaemic heart disease (Salonen and Salonen, 1990). Intima-media thickening is therefore assumed to be a forerunner of plaque formation. These studies include measurements of intima-media thickness which vary from 0.5mm to 4mm or more, and do not distinguish between the presence of discrete plaques and diffuse intima-medial thickening.

The aim of this chapter was to examine the relationship between intima-media thickness and the presence of discrete plaques in the carotid and femoral bifurcations, to determine the inter- and intra-vessel variation in intima-media thickening, the role of local factors such as vessel diameter and general factors such as age and risk factors for atherosclerosis on intima-media thickening.

Thus the questions posed were:

- i) Is the intima-media thickness related to plaque?*
- ii) Is the intima-media thickness related to age?*
- iii) Is the intima-media thickness related to the site at which it is measured?*
- iv) Is the intima-media thickness related to the diameter of the vessel?*
- v) Is the intima-media thickness related to the arterial ultrasound score?*
- vi) Can the intima-media thickness be predicted from known risk factors?*
- v) Can intima-media thickness be used as a risk factor to predict the presence of plaque?*

## **11.2 Material and methods**

### **11.21 Material**

One hundred and forty three volunteers were examined. The volunteers were recruited as part of a health screening service offered by the department of occupational health at St. Mary's Hospital.

### **11.22 Method**

As described in part III, all volunteers were screened with a health questionnaire to exclude a past history of cardiovascular, cerebrovascular and peripheral vascular symptoms. Both carotid and femoral arteries were imaged with high resolution ultrasound in B-mode and M-mode (as described in Part III) and the blood pressure was measured immediately after the ultrasound examination while the subjects were resting

### **11.23 Measurements**

The intima-media thickness of the far wall of the common carotid artery, the origin of the bulb, the far wall of the carotid bulb (beyond the flow divider) and the far wall of the common femoral artery were measured from the B-mode pictures. The peak systolic and end-diastolic diameter of the common carotid artery, the mid-bulb region and the common femoral artery were measured from the M-mode pictures. The presence of plaque was noted. Both carotid and femoral arteries were scored according to the Belcaro classification (see Part III). All measurements were taken in the first 106 adult volunteers, subsequently only the common carotid artery intima-media thickness was measured and the bifurcations examined for plaque.

## **11.3 Results**

The mean (and standard deviation) intima-media thickness of all arteries is shown in table 11.1

### **11.31 Intima-media thickness and its relationship to plaque**

Fifty-one subjects had at least one plaque in the four bifurcations examined (carotid and femoral). Of the 35 subjects with plaques at the carotid bifurcations, 9 had plaques in both bifurcations, and 26 had plaques in a single carotid bifurcation; in all but one of these, the plaques were confined to the region of the bulb. 16 subjects had plaques in the femoral bifurcations only (Table 11.2).

**Table 11.1** Variation in IM thickness at the sites studied (all arteries)

	n	mean IM thickness $\pm$ 1SD	Range
Common carotid artery	143	0.64 $\pm$ 0.16	0.4 - 1.15
Origin of bulb	96	0.84 $\pm$ 0.23	0.44 - 2.12
Mid bulb	106	0.64 $\pm$ 0.19	0.3 - 1.22
Common femoral artery	105	0.7 $\pm$ 0.25	0.38 - 1.5

The IM thickness at the bulb origin is significantly thicker than the IM thickness of the common carotid artery, the mid bulb and the common femoral artery ( $t = 7.27$ ,  $p < 0.001$ ,  $t = 6.88$ ,  $p < 0.001$  and  $t = 4.0$ ,  $p < 0.001$  respectively). The IM thickness of the common femoral artery is also thicker than both the common carotid artery and the mid bulb region ( $t = 2.22$ ,  $p < 0.02$ , and  $t = 2.34$ ,  $p < 0.02$ )

**Table 11.2**    **Distribution of plaque**

	Number
Carotid arteries only	11
Femoral arteries only	16
Both carotid and femoral arteries	24

The mean intima-media thickness in those subjects with plaques present in any of the four bifurcations was significantly greater than those without plaque (table 11.3, Fig 11.1), the difference was greater still for individual arteries with plaque in the ipsilateral bifurcation (table 11.4, fig 11.2).

Only 6% of subjects with a mean CCA intima-media thickness of 0.58mm or less had plaques, compared to 50% of subjects with an IM thickness of between 0.59-0.82mm, and all subjects with an IM thickness of 0.82mm and over (table 11.5, fig 11.3)

### **11.32 The relationship between intima-media thickness and age.**

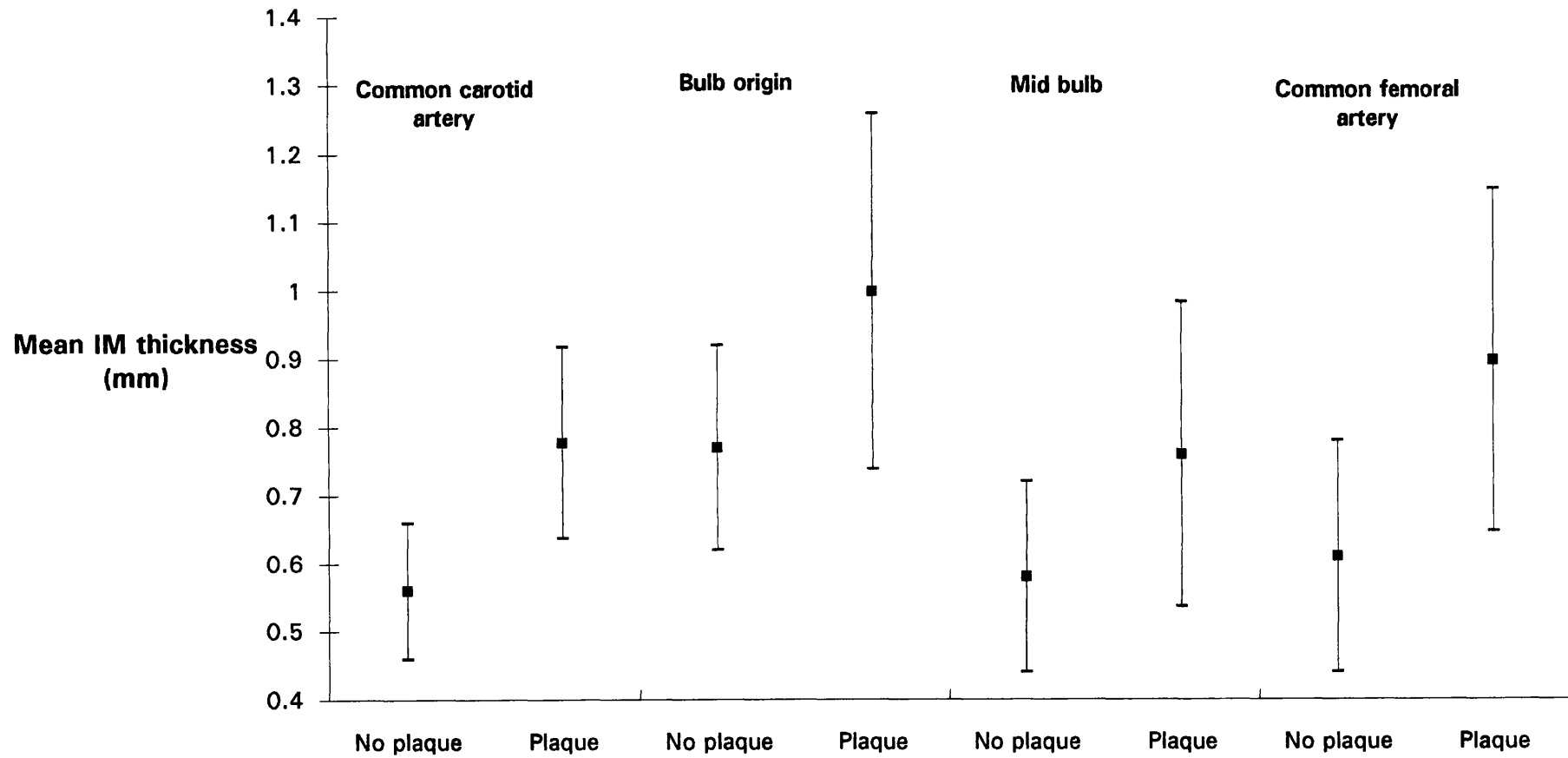
The correlation between the mean intima-media thickness of the common carotid artery and age is shown in figure 11.4. The mean and standard deviation of the intima-media thickness of the common carotid artery in the presence and absence of plaque for each decade of age is shown in table 11.6 and figures 11.5 and 11.6. In all age groups the presence of plaque is associated with a thickened CCA intima-media, this only reaches a level of significance for two decades (40-49 and 50-59) as too few individuals have plaques under 40 and only a few are plaque free over 60.

The correlation between the mean intima-media thickness at the bulb origin and age is shown in figure 11.7 and the intima-media thickness of the bulb origin in the presence and absence of plaque for each decade of age is shown in figure 11.8. Though the bulb origin is thicker in the presence of plaque, the difference is not statistically significant.

The correlation between the mean intima-media thickness of the common femoral artery and age is shown in figure 11.9. and the intima-media thickness of the CFA in the presence and absence of plaque for each decade of age is shown in figure 11.10. Again, though the CFA is thicker in the presence of plaque, this only reaches a level of statistical difference for the 40-49 age group ( $t=5.86$ ,  $p<0.001$ ).

**Table 11.3 Variation in mean IM thickness at the sites studied with and without plaque at any of the four (carotid and femoral) bifurcations examined**

	Plaque		No plaque		Mann Whitney U test	
	n	mean IM thickness $\pm$ 1sd	n	mean IM thickness $\pm$ 1sd	t	p value
Common carotid artery	51	0.78 $\pm$ 0.14	92	0.56 $\pm$ 0.11	9.69	<0.001
Origin of bulb	31	1.01 $\pm$ 0.26	63	0.76 $\pm$ 0.15	4.96	<0.001
Mid bulb	35	0.77 $\pm$ 0.22	69	0.58 $\pm$ 0.14	4.65	<0.001
Common femoral artery	35	0.91 $\pm$ 0.24	69	0.61 $\pm$ 0.17	6.6	<0.001

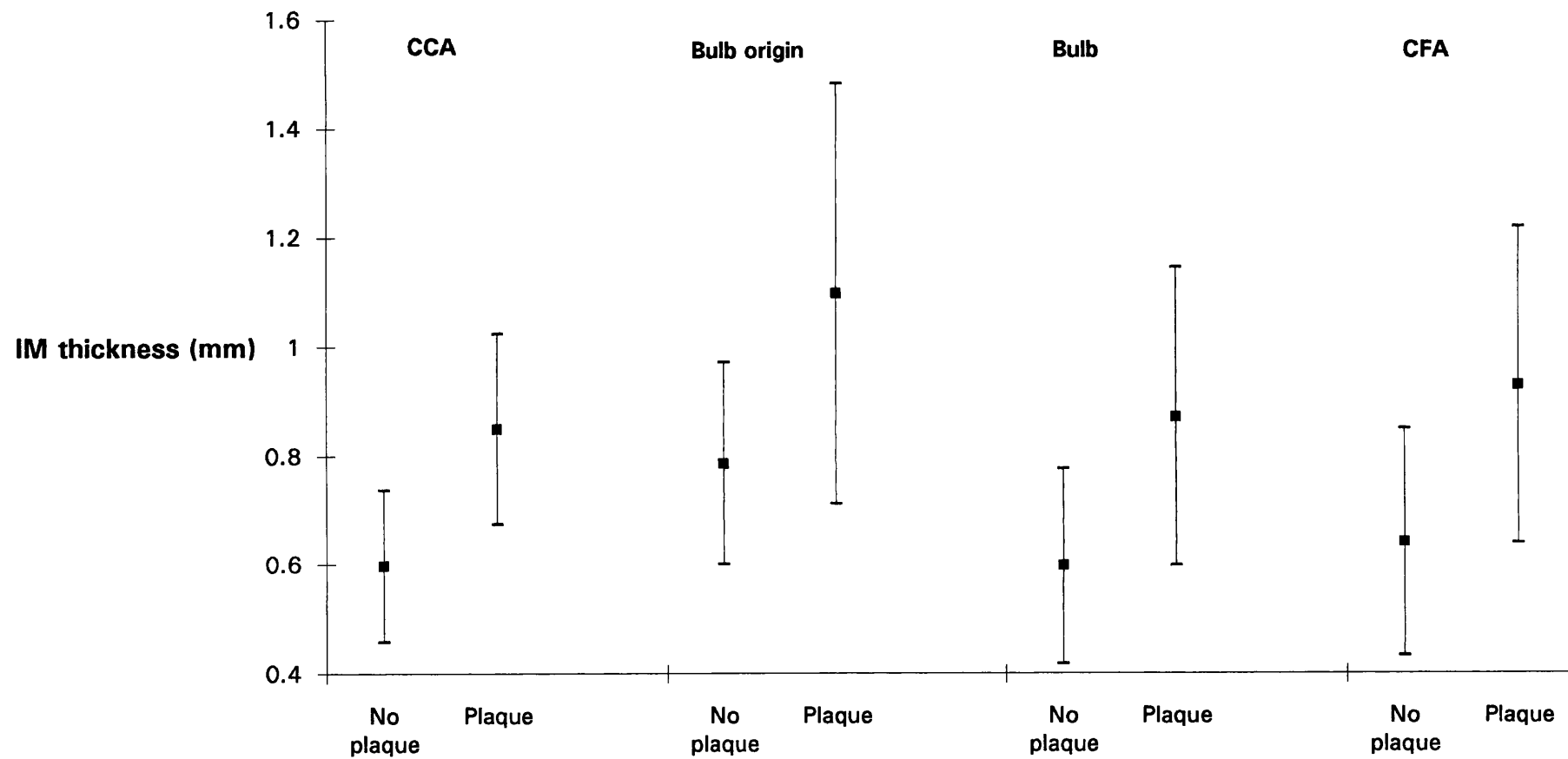
**Fig 11.1**

**Mean (+/- 1sd) IM thickness with and without plaque at any one of the four bifurcations**

**Table 11.4 Variation in IM thickness in individual arteries with and without plaque at the ipsilateral bifurcation**

	Plaque in the ipsilateral bifurcation		No plaque		Mann-Whitney U test	
	n	mean IM thickness $\pm$ 1 sd	n	mean IM thickness $\pm$ 1 sd	t	p value
Common carotid artery	45	0.85 $\pm$ 0.17	241	0.59 $\pm$ 0.14	9.37	<0.001
Origin of bulb	29	1.1 $\pm$ 0.39	154	0.78 $\pm$ 0.19	4.36	<0.001
Mid bulb	30	0.87 $\pm$ 0.27	174	0.6 $\pm$ 0.18	5.48	<0.001
Common femoral artery	38	0.94 $\pm$ 0.24	164	0.63 $\pm$ 0.21	7.41	<0.001





**Fig 11.2**

**IM thickness (+/- 1sd) with and without plaque in the ipsilateral bifurcation**

**Table 11.5 Relationship between the presence of plaque and the mean IM thickness of the common carotid artery.**

Mean CCA IM thickness	No plaque	Plaque	Total
Up to 0.58mm	64	4 (6%)	68
0.59mm-0.82mm	29	29 (50%)	58
0.83mm and over	0	18 (100%)	18

$\chi^2 = 64.14$ , to 2 degrees of freedom  $p < 0.001$

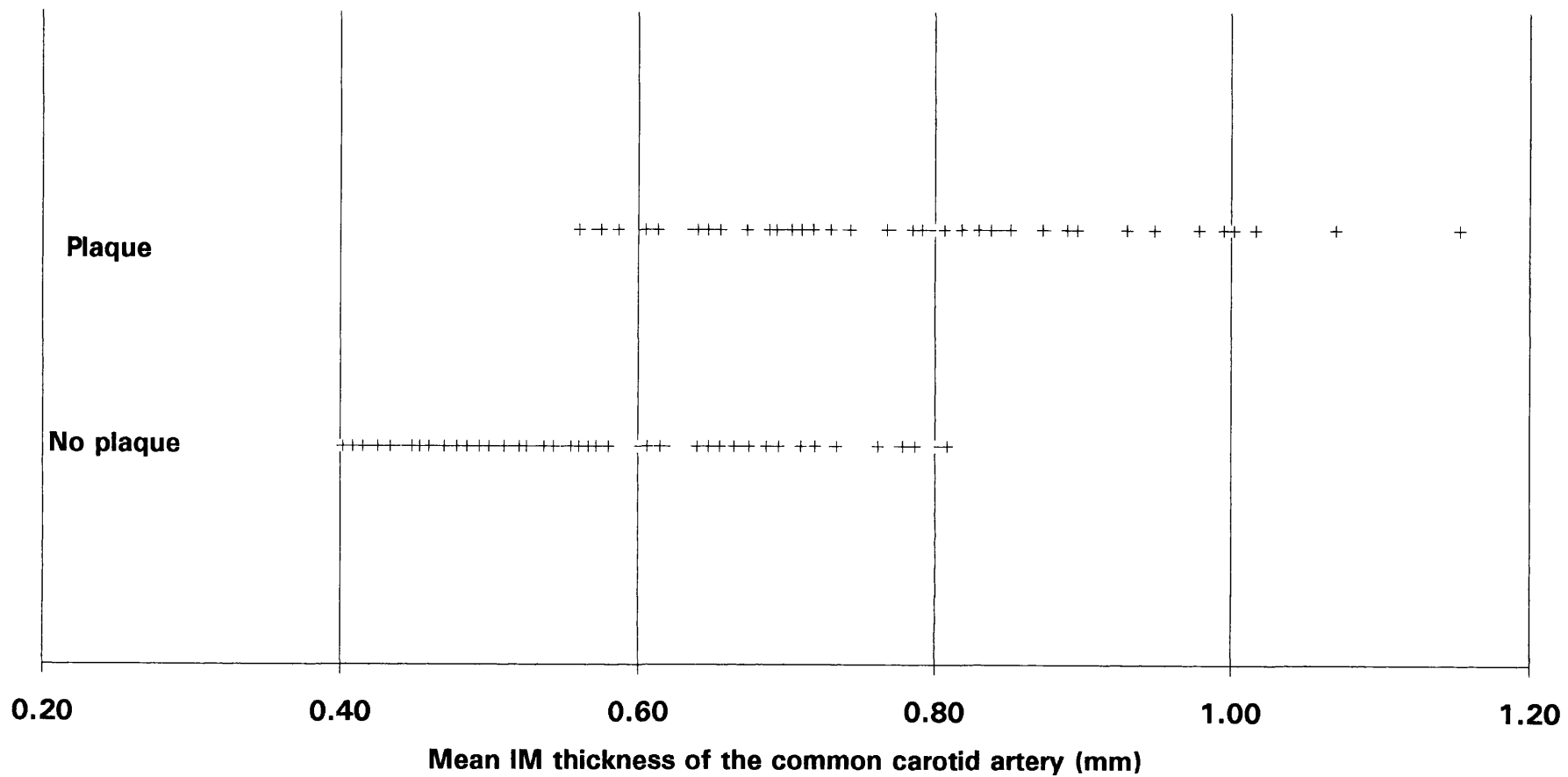
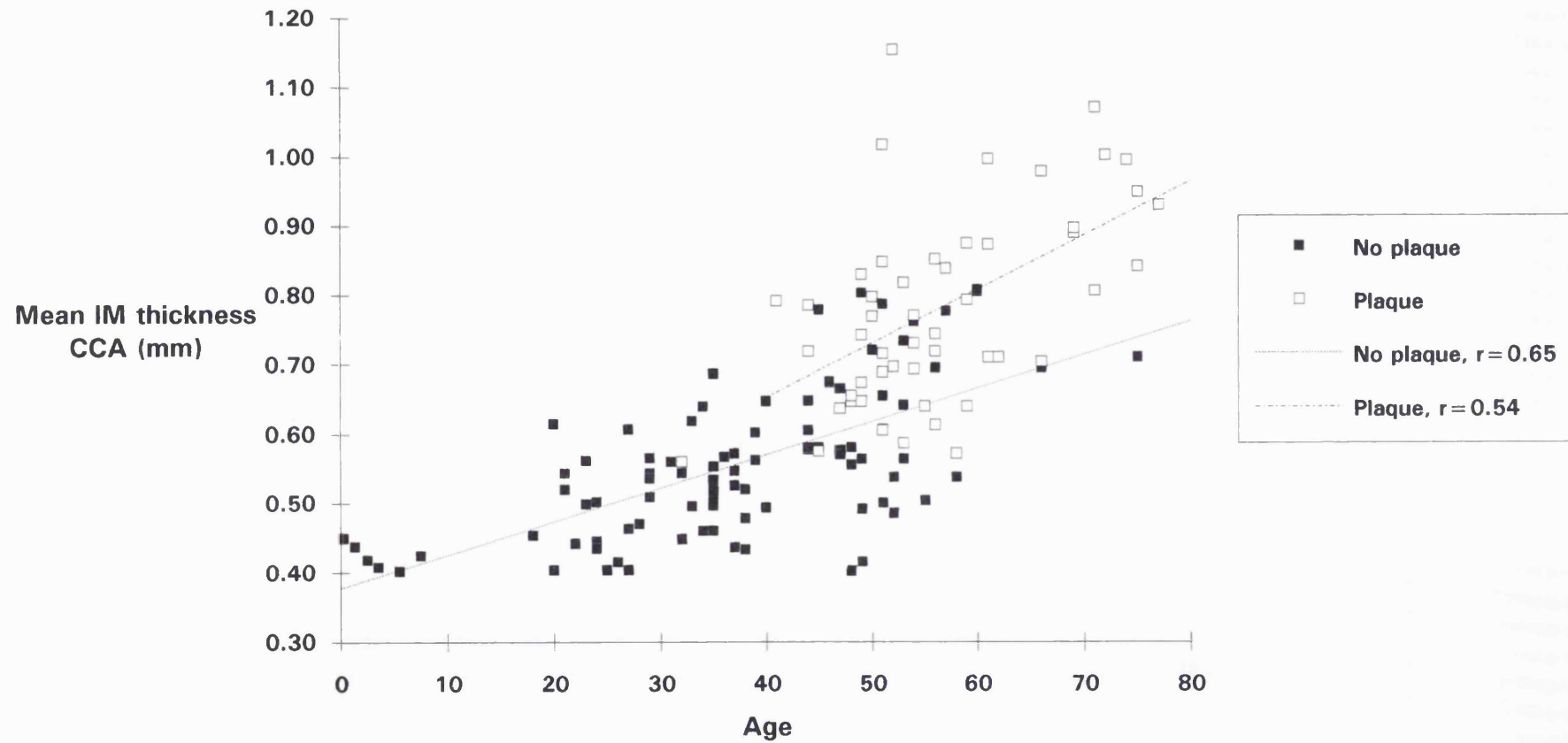


Fig 11.3

Distribution of IM thickness of the CCA with and without plaque

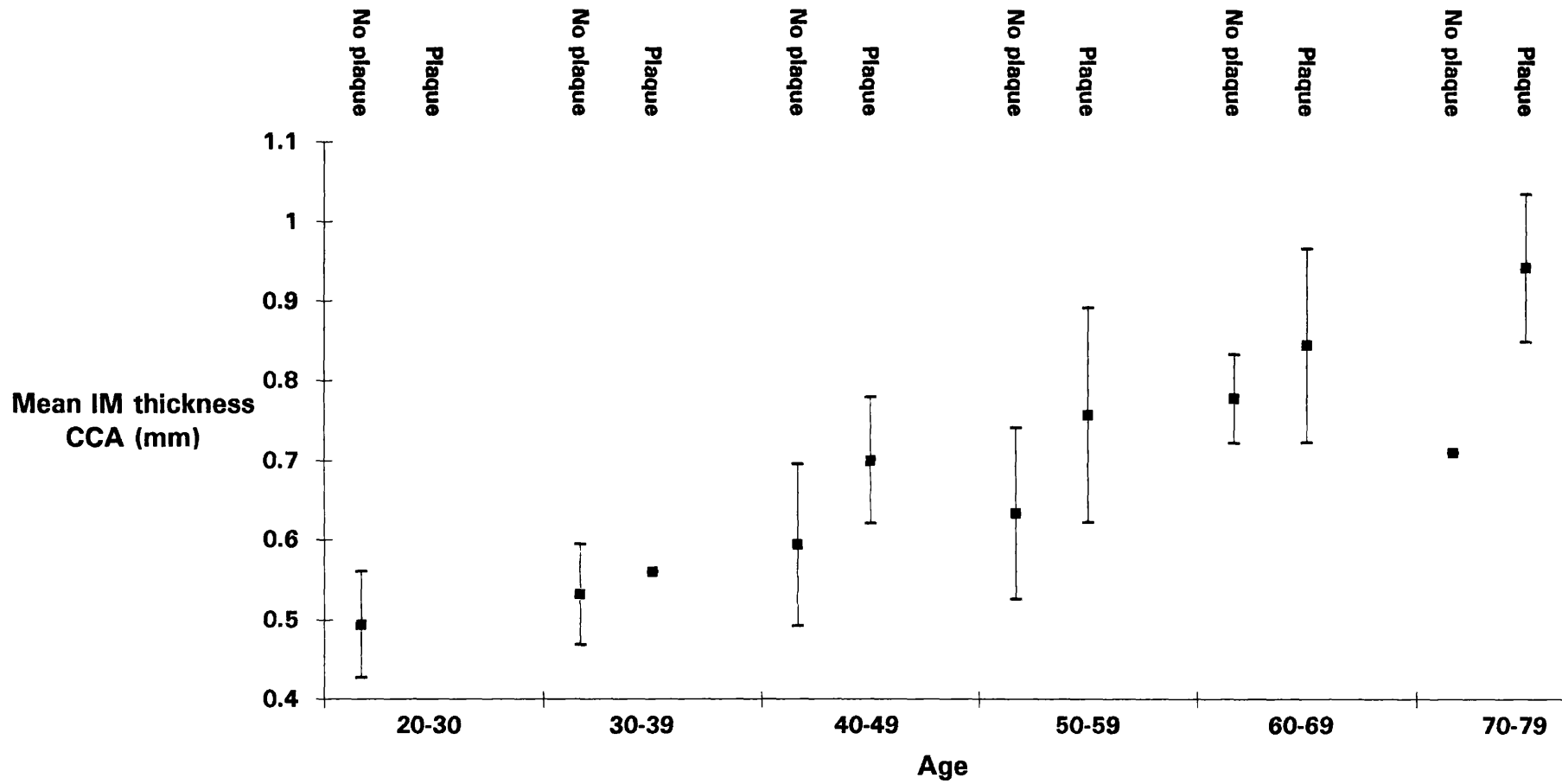


**Fig 11.4**

**Correlation between IM thickness of the CCA, age and the presence of plaque**

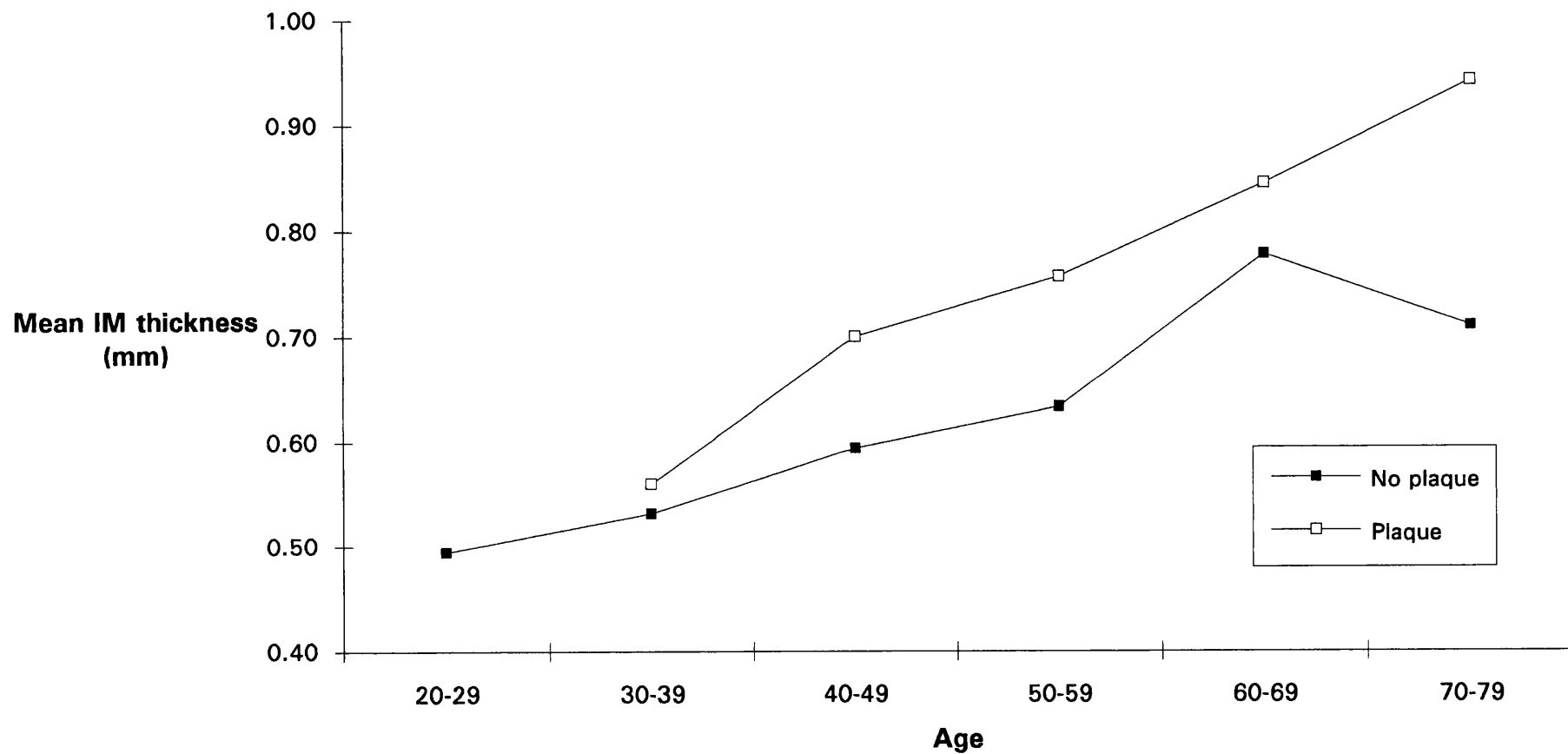
**Table 11.6 Mean IM thickness of the CCA by decade and the presence of plaque**

Age by decade	Mean IM without plaque (mm)	sd	n	Mean IM with plaque (mm)	sd	n	t	p value
20-29	0.49	0.07	20			0		
30-39	0.53	0.06	25	0.56		1	2.26	
40-49	0.59	0.1	20	0.7	0.08	11	3.22	<0.001
50-59	0.63	0.11	15	0.76	0.14	24	3.15	<0.001
60-69	0.78	0.06	4	0.84	0.12	8	1.31	NS
70-79	0.71		1	0.94	0.09	7	6.63	<0.001



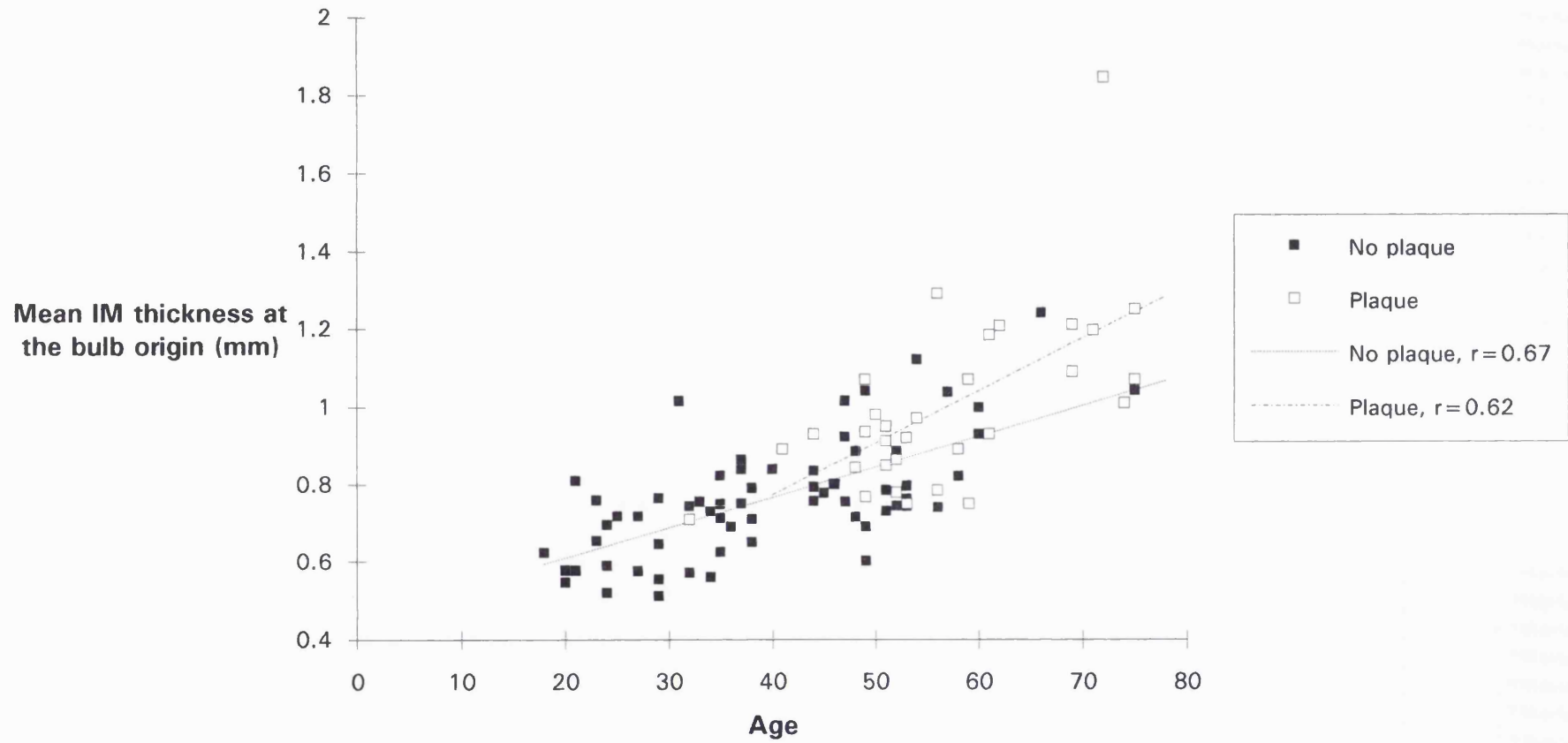
**Fig 11.5**

**Mean (+/- 1sd) IM thickness of the CCA by decade and the presence of plaque**



**Fig 11.6**

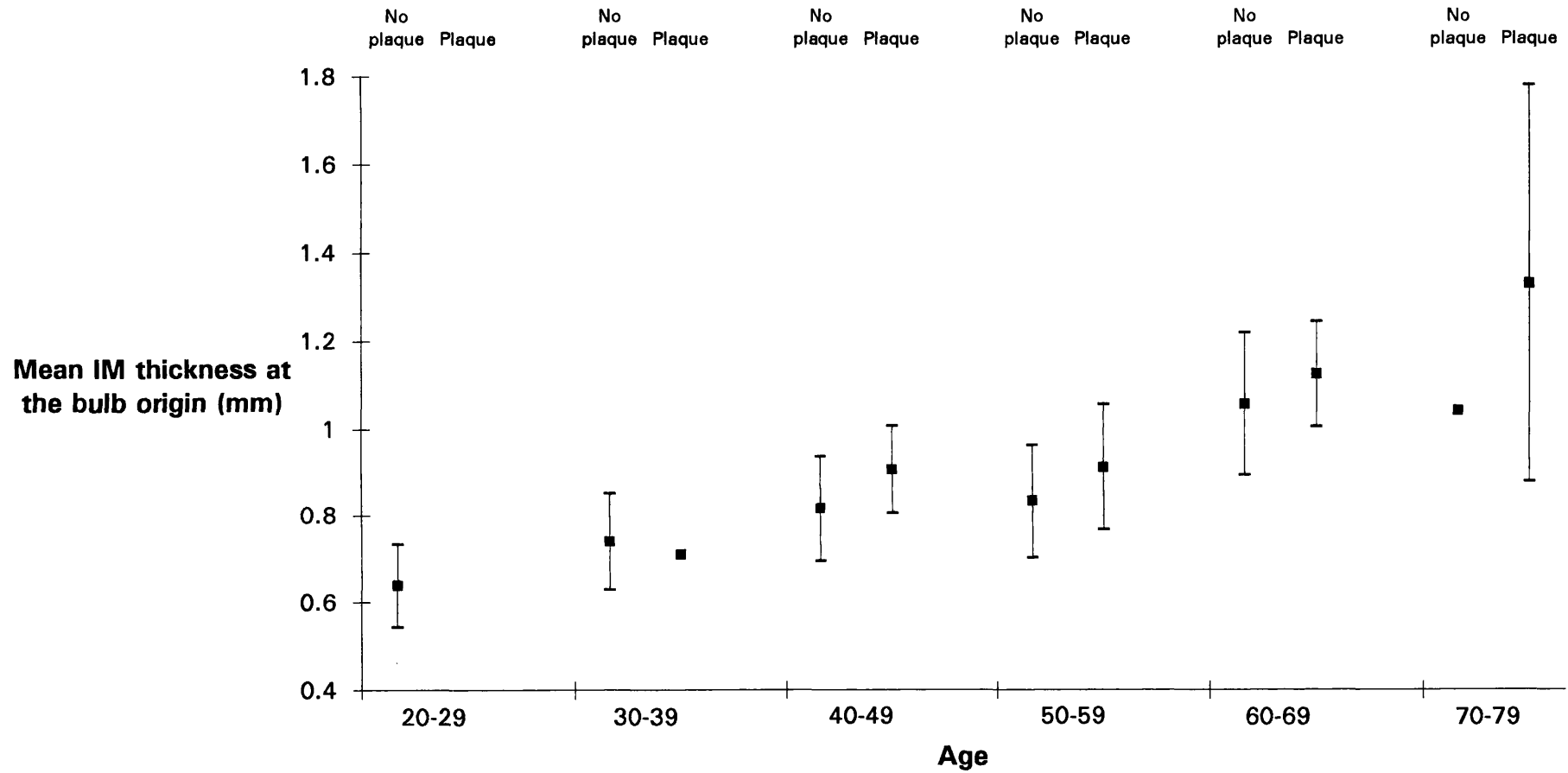
**Mean IM thickness of the CCA by decade and the presence of plaque**

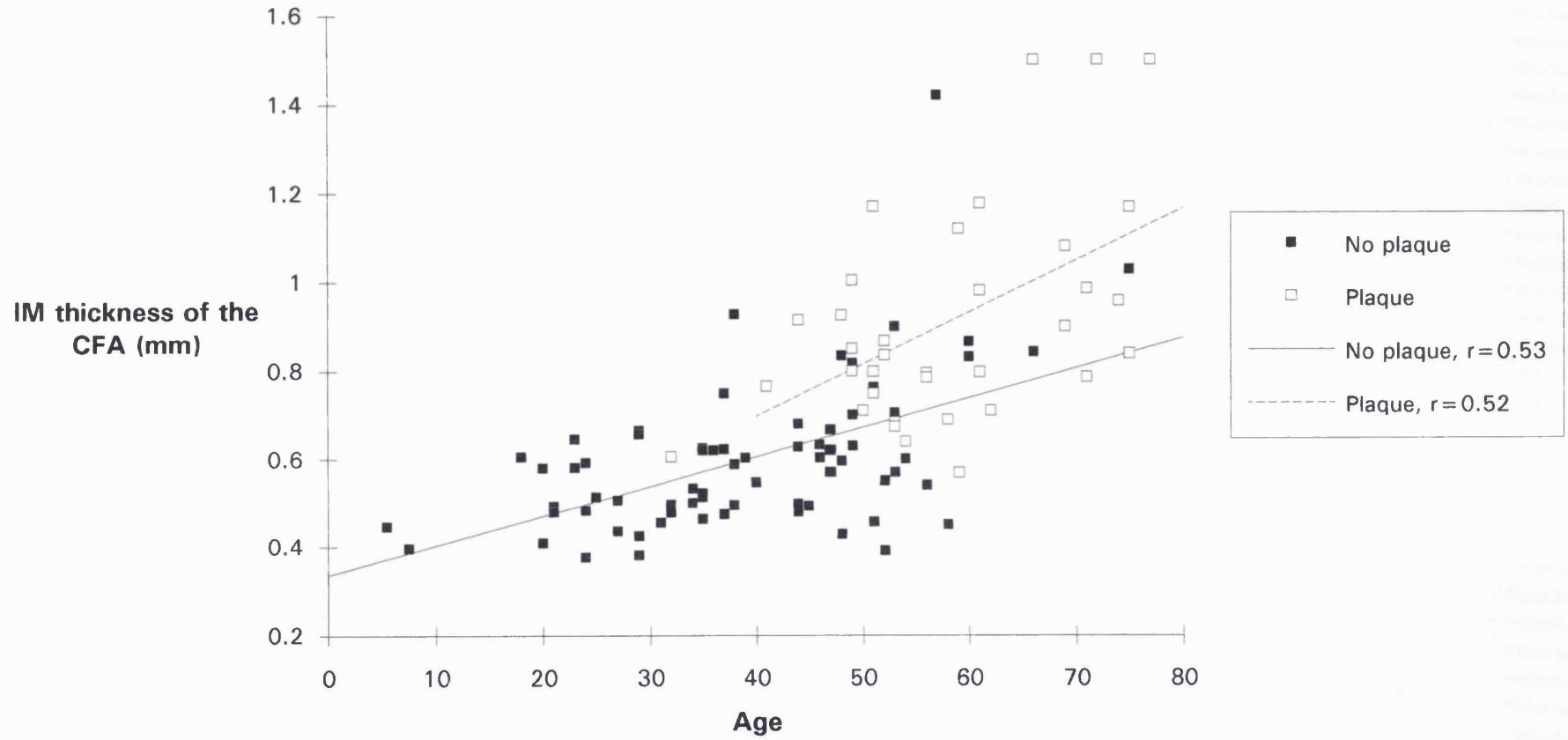


**Fig 11.7**

**Correlation between the mean IM thickness at the bulb origin and age**

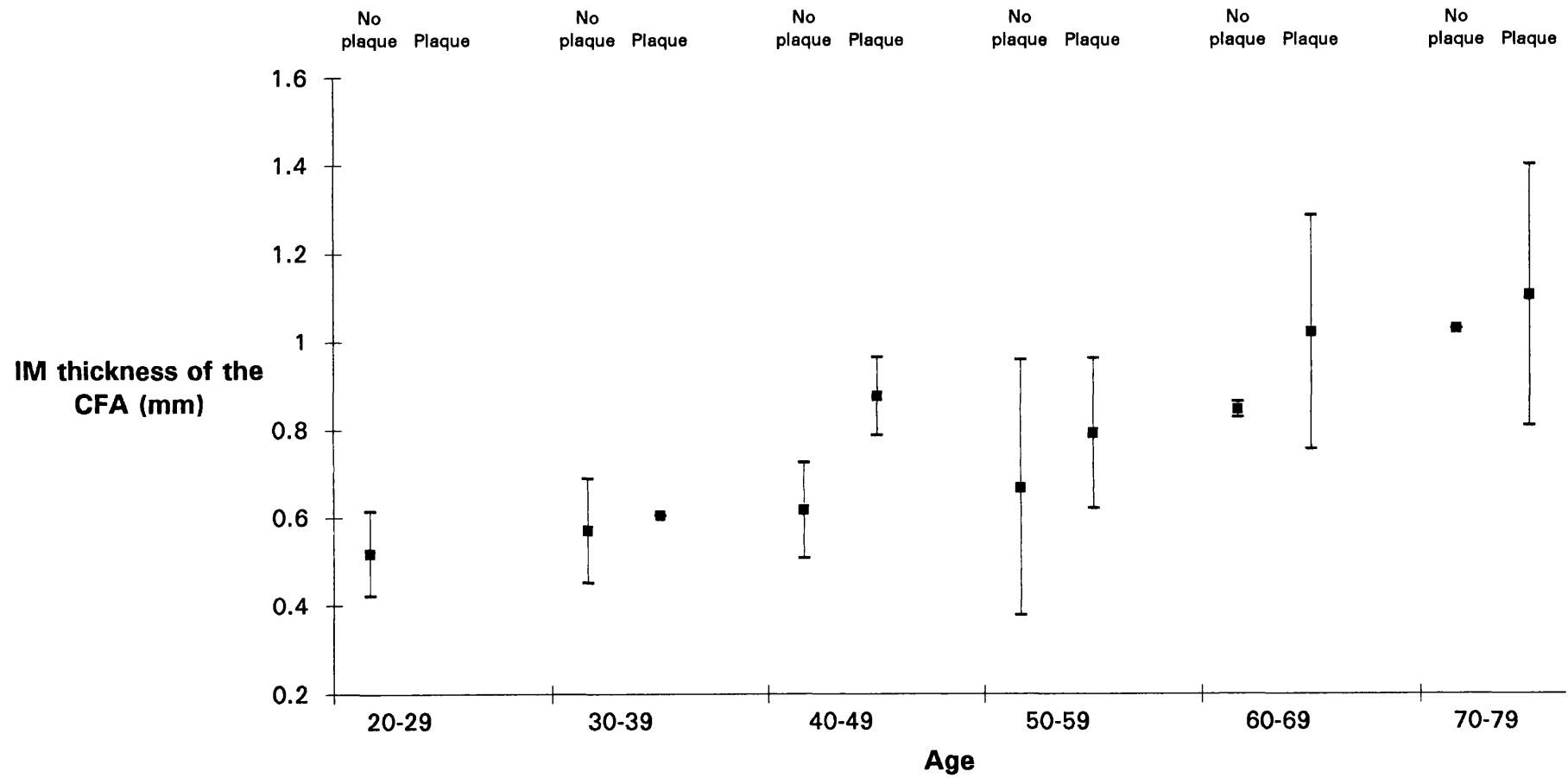


**Fig 11.8****Mean (+/- 1sd) IM thickness at the bulb origin by decade and the presence of plaque**



**Fig 11.9**

**Correlation between IM thickness of the CFA and age**



**Fig 11.10**

**Mean (+/- 1sd) IM thickness of the CFA by decade and the presence of plaque**

### **11.32 Intervessel variation**

#### **a) Left to right common carotid artery**

The correlation between intima-media thickness of the left and right common carotid arteries is shown in figure 11.11. The correlation coefficient is 0.75 ( $t=13.37$  to 141DF,  $p<0.001$ ). The mean difference between the left and right common carotid arteries is  $0.002 \pm 0.12$ mm. The scatter is shown in figure 11.12 as the difference against the mean.

#### **b) Left to right common femoral artery**

The correlation between the IM thickness of the left and right common femoral arteries is shown in figure 11.13. The mean difference between the left and right common femoral arteries is  $0.05 \pm 0.21$ . and the scatter is shown in figure 11.14 as the difference against the mean.

#### **d) Common carotid artery to bulb origin**

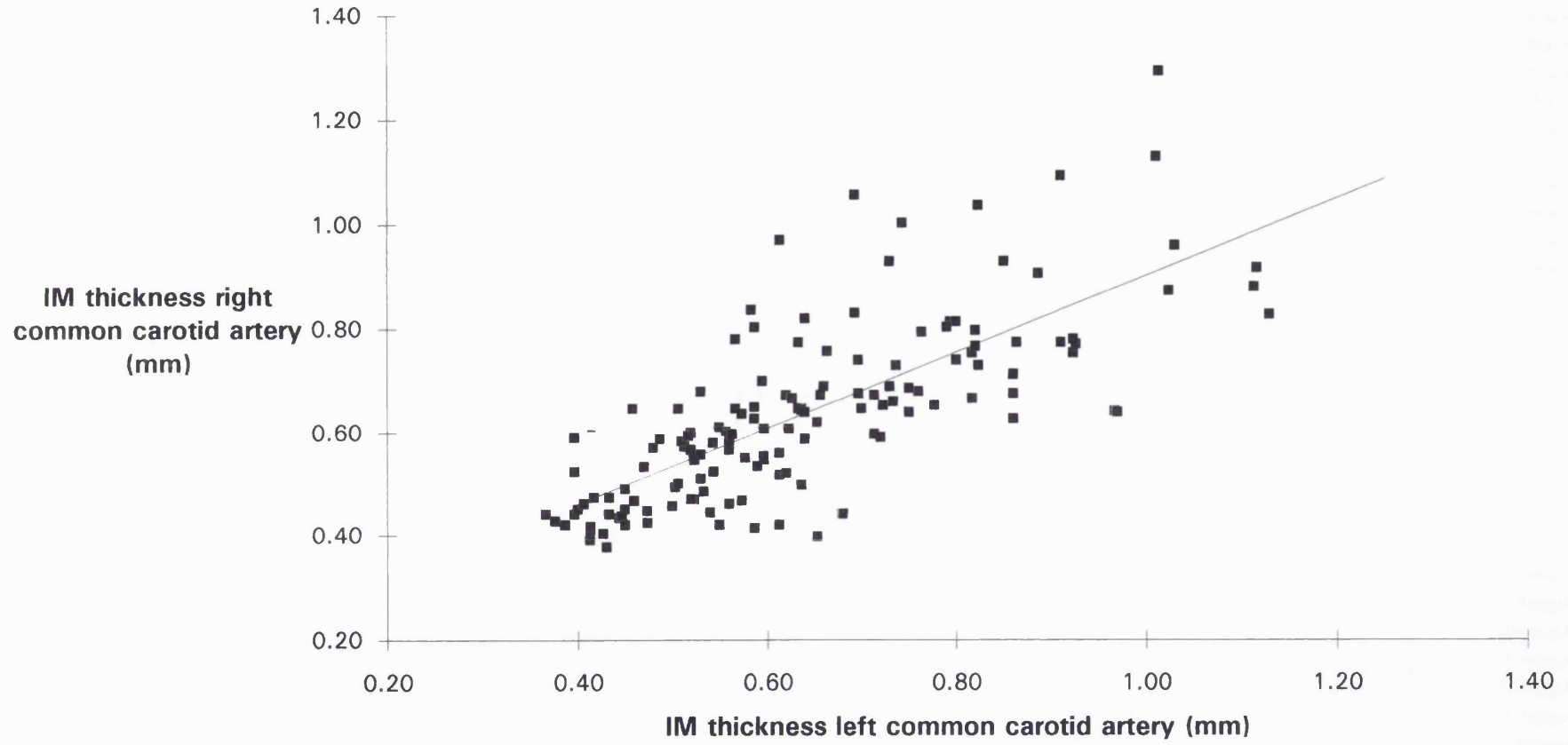
The IM thickness of the common carotid artery is compared to the thickness at the bulb origin of the ipsilateral vessel in figure 11. 15. In the absence of plaque, the mean difference between the IM thickness of the CCA and the IM thickness at the bulb origin is  $0.19 \pm 0.15$  mm. This relationship holds true even if bifurcations containing plaque are included (Mean difference =  $0.19 \pm 0.19$  mm) (Fig 11.16).

#### **c) Common carotid artery compared to mid bulb and femoral artery IM thickness**

In the absence of plaques, the mean IM thickness of the common carotid artery is not significantly different from the mean IM thickness in the mid bulb region (Mean difference =  $0.02 \pm 0.14$  mm) or the mean IM thickness in the common femoral artery (Mean difference =  $0.03 \pm 0.17$ mm) (Fig 11.17)

### **11.33 The relationship between vessel diameter and plaque**

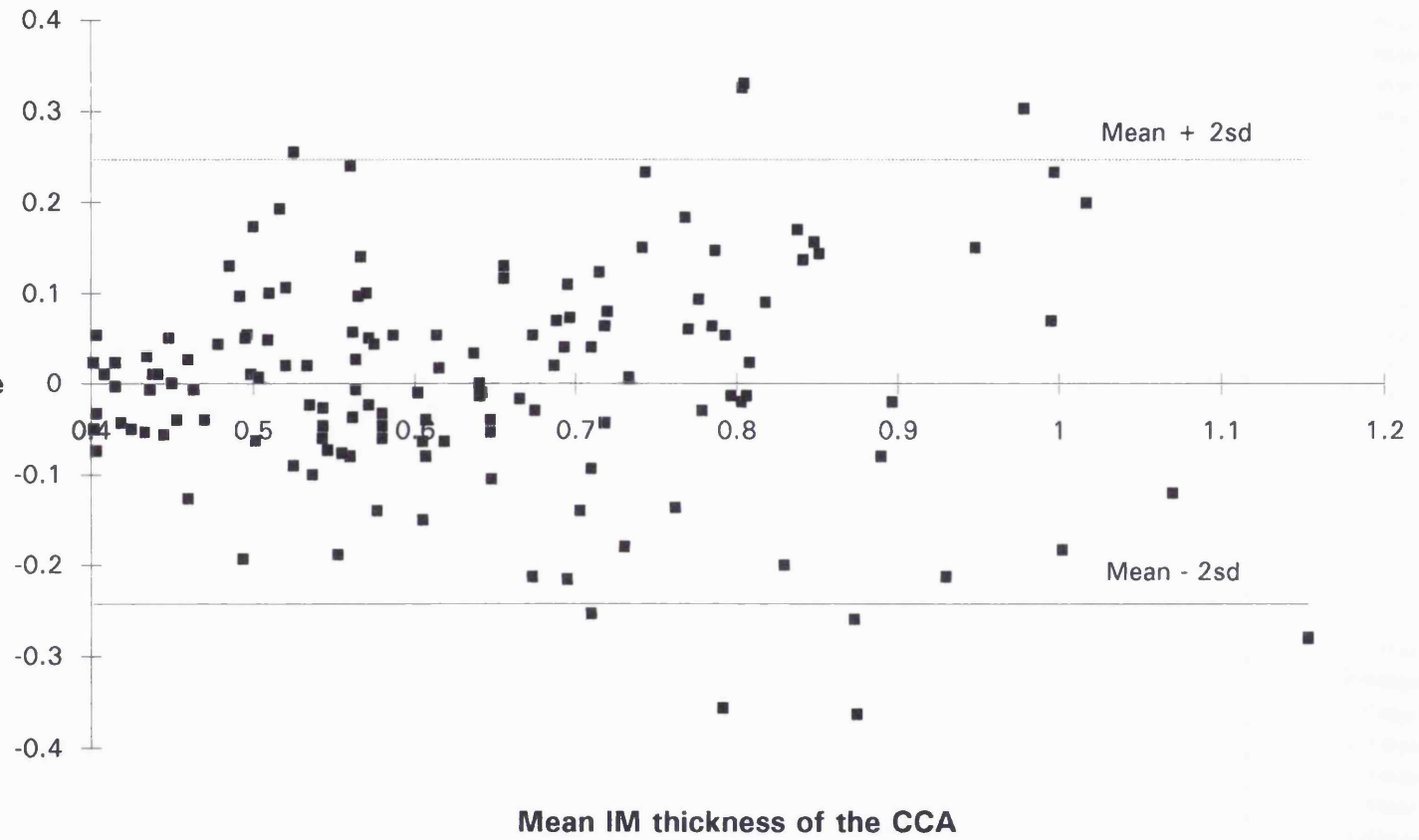
The mean vessel diameter of the CCA, bulb and CFA in the presence of plaque is compared to the mean vessel diameter in the absence of plaque in table 11.5. Though vessel diameter increases with age, the CCA diameter in individuals over 40 without plaque is significantly less than the CCA diameter of individuals with plaque (fig 11.18).



**Fig 11.11**

**Comparison between the IM thickness of the left and right carotid arteries**

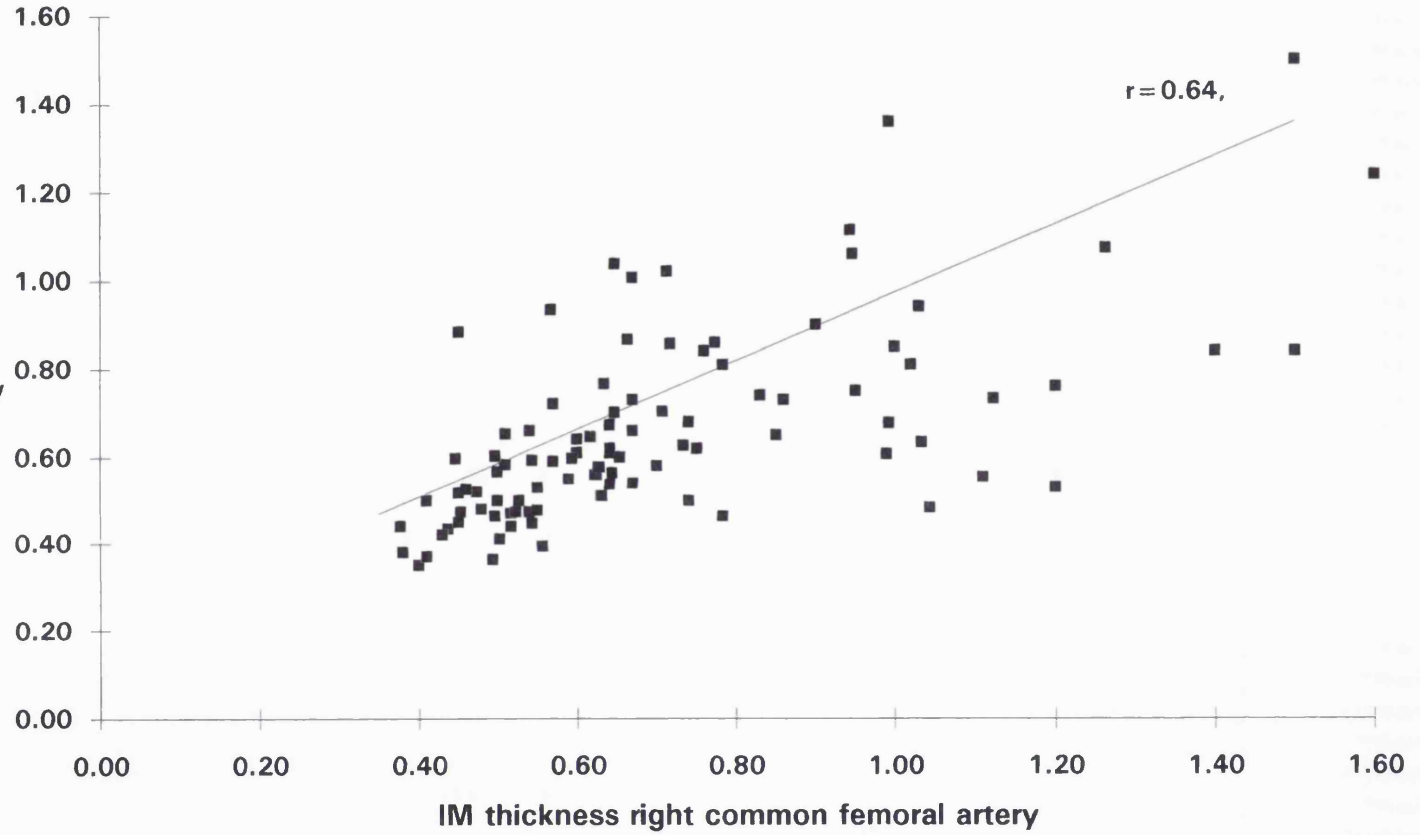
Difference in IM thickness between the right and left CCA



**Fig 11.12**

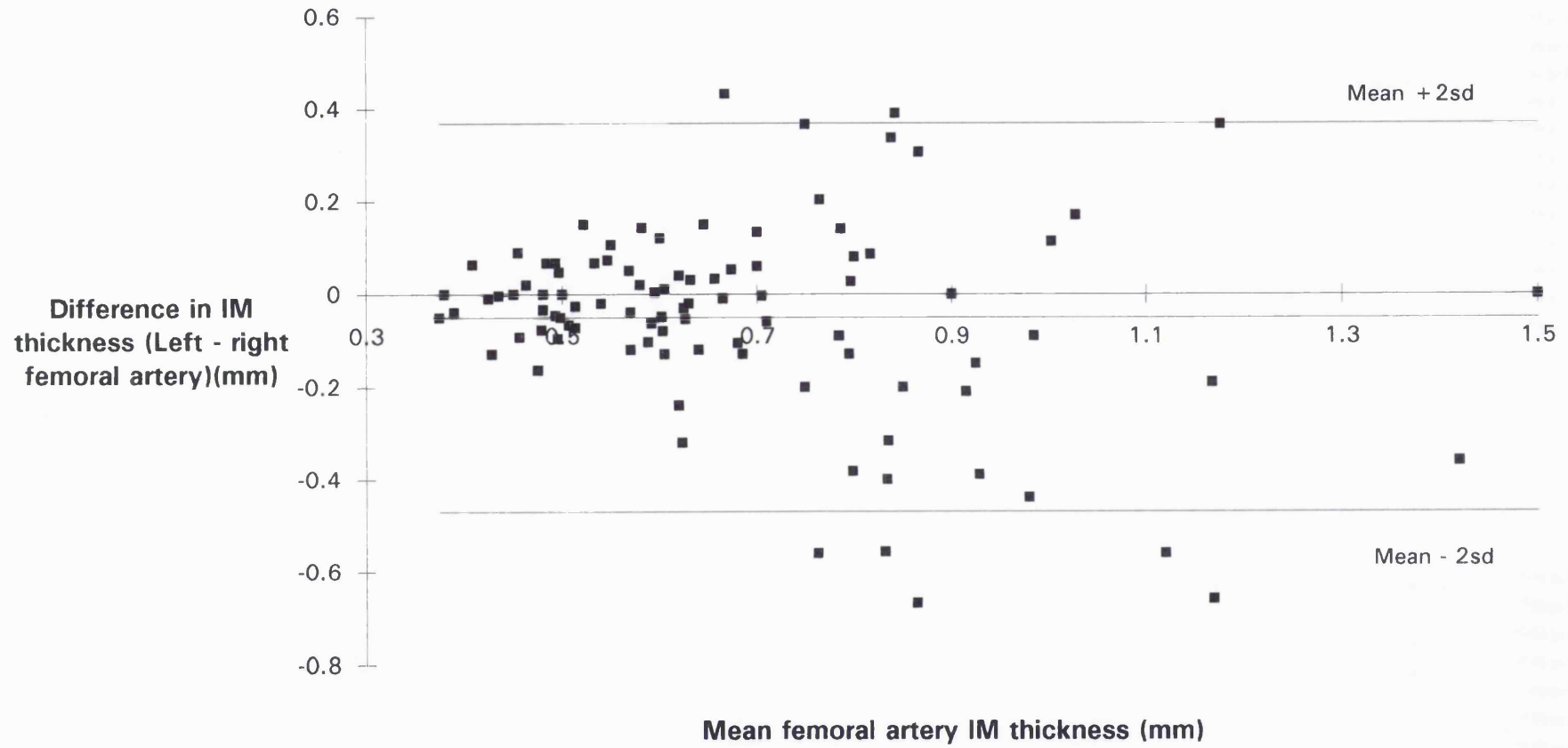
**The difference against the mean of the left and right CCA IM thickness**

IM thickness left  
common femoral artery



**Fig 11.13**

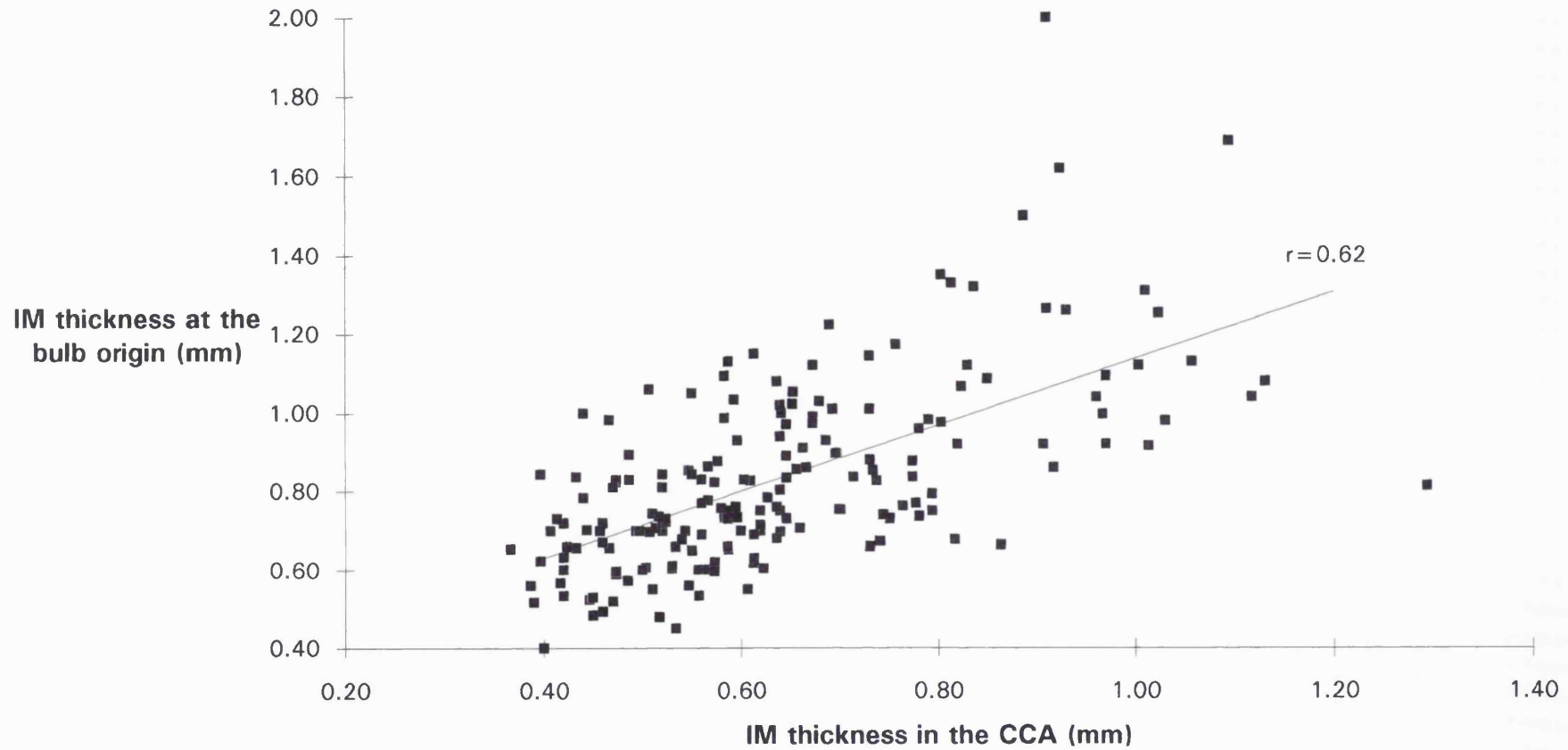
**Correlation between the intima-media thickness of the right and left common femoral arteries**



**Fig 11.14**

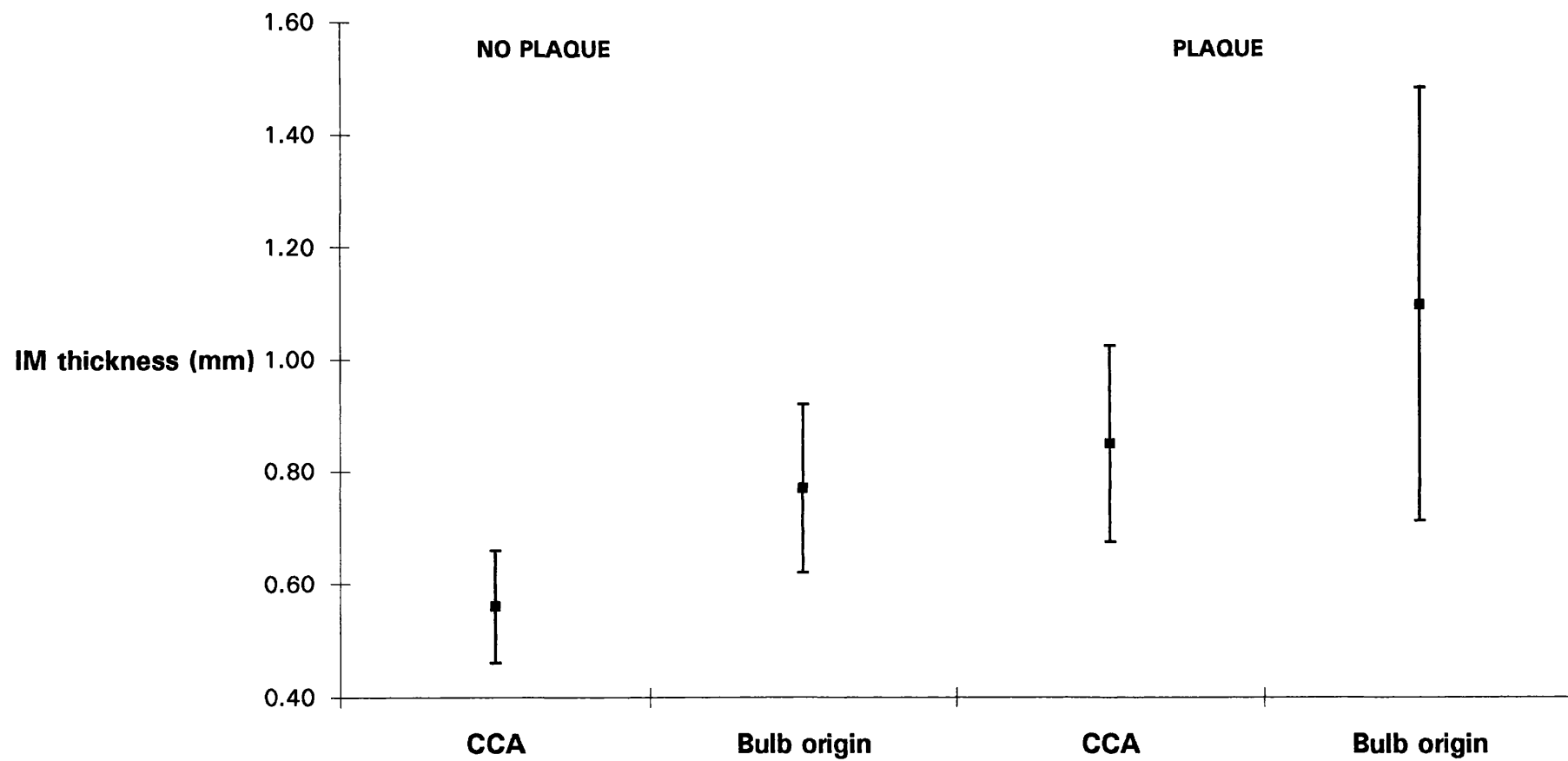
**The difference against the mean of the left and right common femoral arteries**





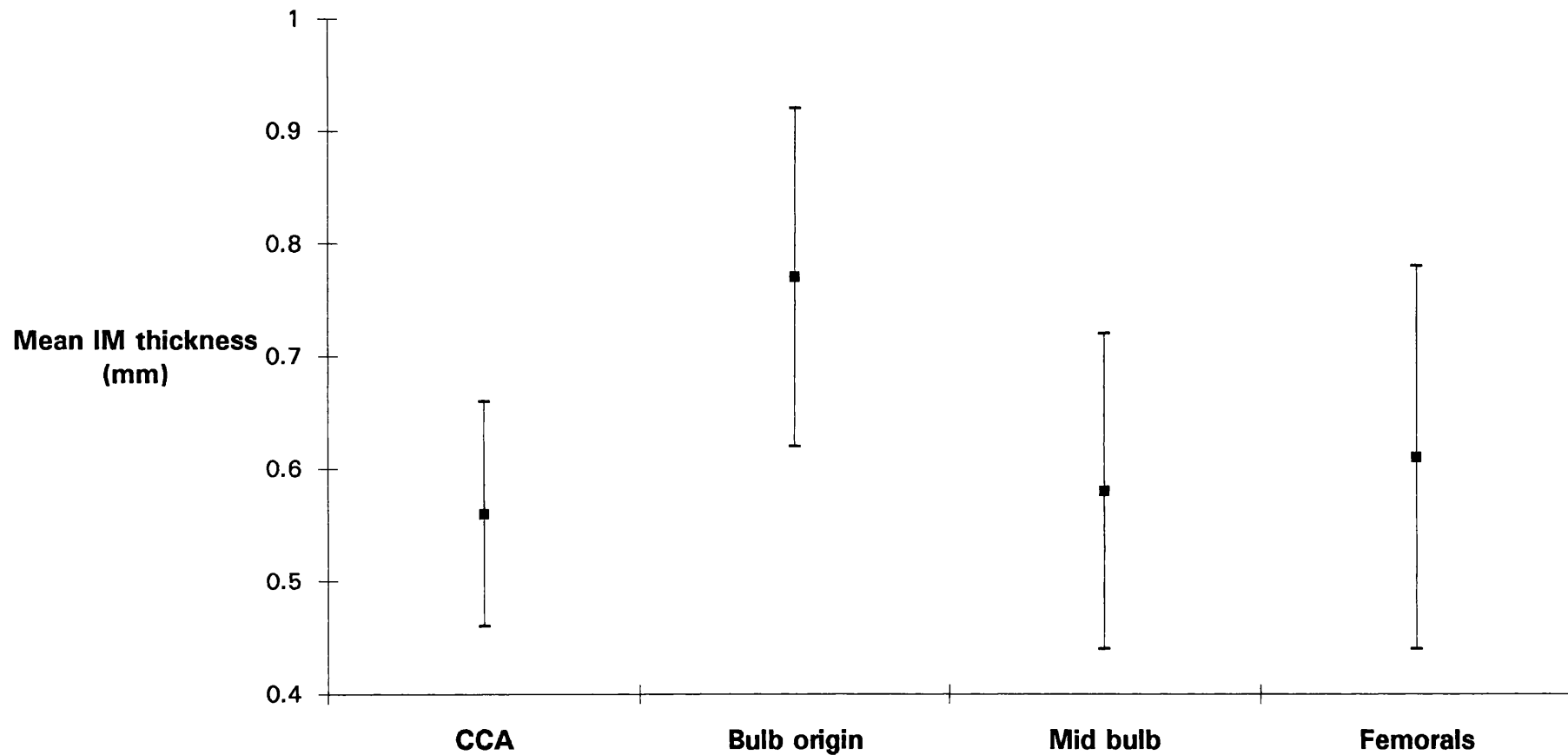
**Fig 11.15**

**Correlation between the IM thickness in the CCA and at the bulb origin**



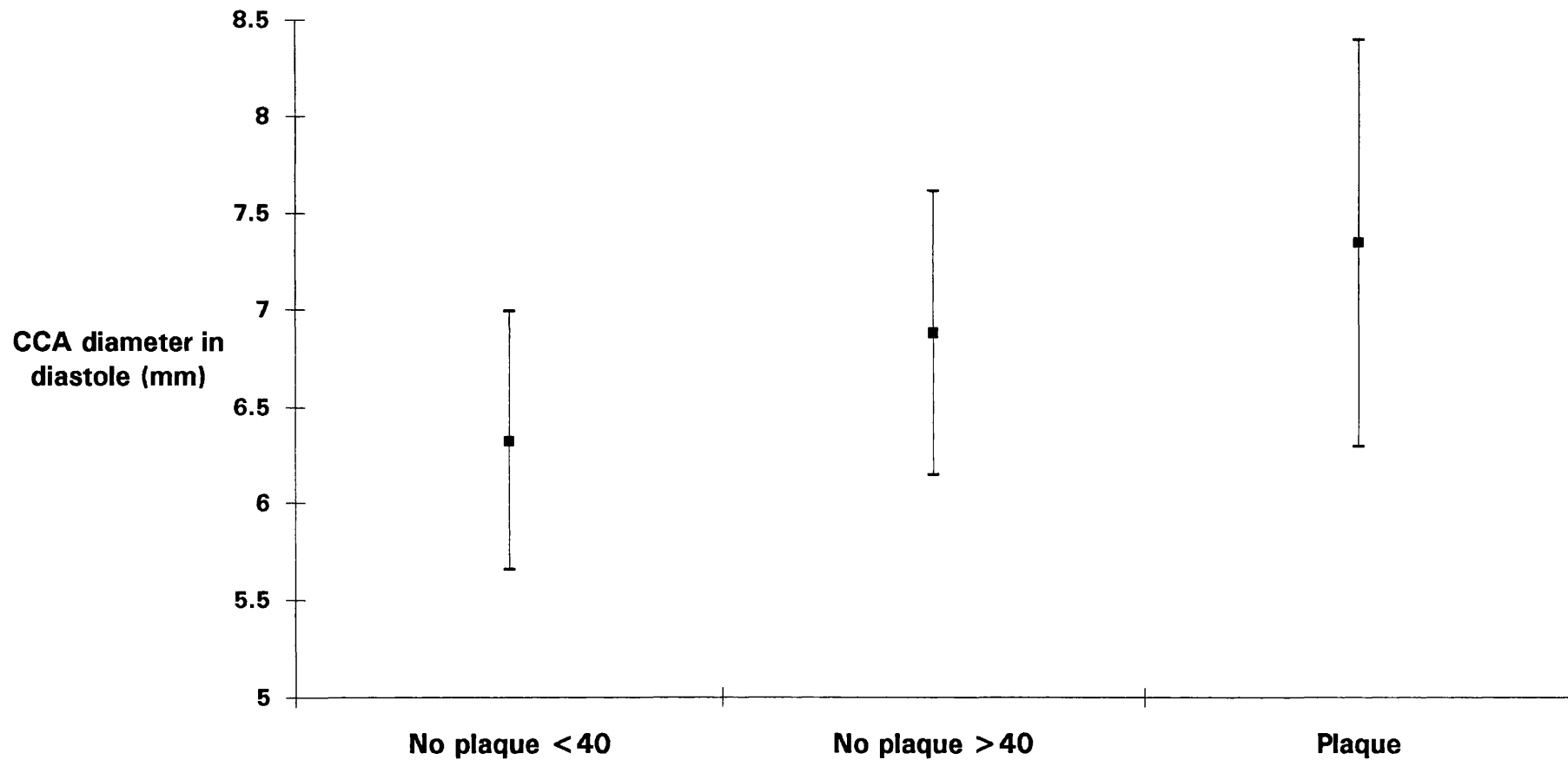
**Fig 11.16**

**Relationship between plaque and the mean IM thickness in the CCA and at the bulb origin**



**Fig 11.17**

**Mean IM (+/- 1sd) thickness at the sites in the carotid and femoral arteries in the absence of plaque**



**Fig 11.18**

**Mean (+/- 1sd) CCA diameter, and its relationship to age and the presence of plaque**

**Table 11.7 The mean vessel diameter of the CCA, bulb and CFA in the presence of plaque is compared to the mean vessel diameter in the absence of plaque**

	With plaque		Without plaque		t	p
	mean vessel diameter $\pm 1$ sd	n	mean vessel diameter $\pm 1$ sd	n		
CCA	6.76 $\pm$ 0.7	128	7.19 $\pm$ 0.92	35	2.62	<0.005
Bulb	8.18 $\pm$ 1.16	74	8.44 $\pm$ 1.14	22	0.94	NS
CFA	8.89 $\pm$ 2.18	62	8.78 $\pm$ 2.5	26	0.19	NS

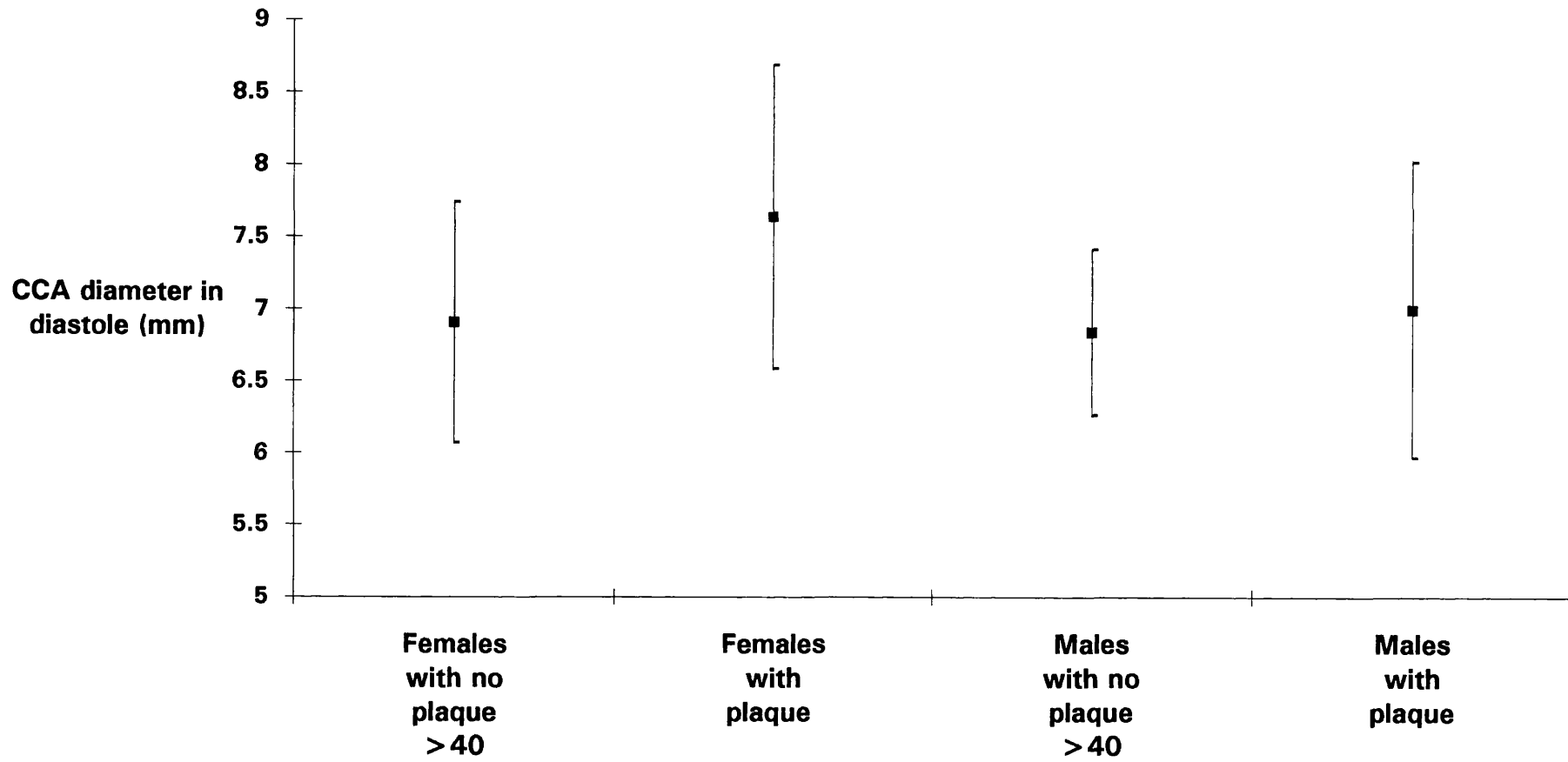
.This difference is seen in both men and women (fig 11.19). The diastolic diameter of the bulb and the CFA increases with age but not with plaque (figs 11.20 and 11.21).

#### **11.34 The relationship between intima-media thickness and arterial ultrasound score.**

The intima media thickness of the CCA increases with each grade of the arterial ultrasound score (AUS). The mean ( $\pm 1$ sd) IM thickness of the CCA for each grade of the AUS is shown in figure 11.22. IM thickness of the CCA correlates with AUS grade ( $r=0.73$ ,  $t=17.8$ ,  $p<0.001$ ). Like the IM thickness, the AUS grading is age related (Fig 11.23).

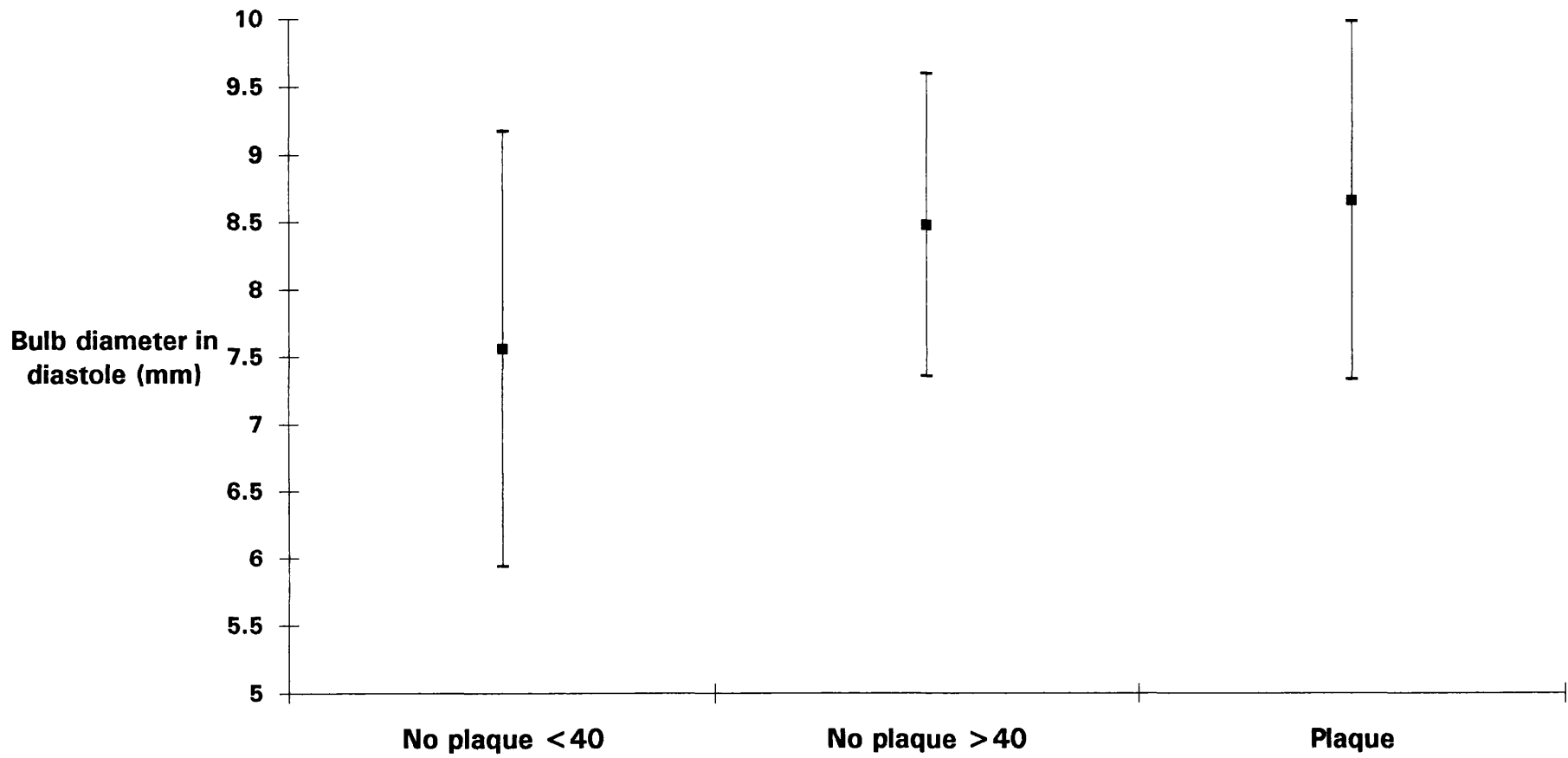
In the femoral arteries, the IM thickness also increases with AUS grade. The mean ( $\pm 1$ sd) IM thickness of the CFA for each grade of the AUS is shown in figure 11.24. IM thickness of the CFA correlates with AUS grade ( $r=0.68$ ,  $t= 13.2$ ,  $p<0.001$ ) and is age related (Fig 11.25).

The arterial ultrasound score is the sum of the scores for each carotid and each femoral artery. This correlates very well with the mean IM thickness of all 4 vessels ( $r=0.84$ )(Fig 11.26) and age (Fig 11.27).



**Fig 11.19**

**Mean +/- 1 sd CCA IM thickness in men and women with and without plaque**



**Fig 11.20**

**Mean (+/- 1sd) bulb diameter in diastole according to age and the presence of plaque**



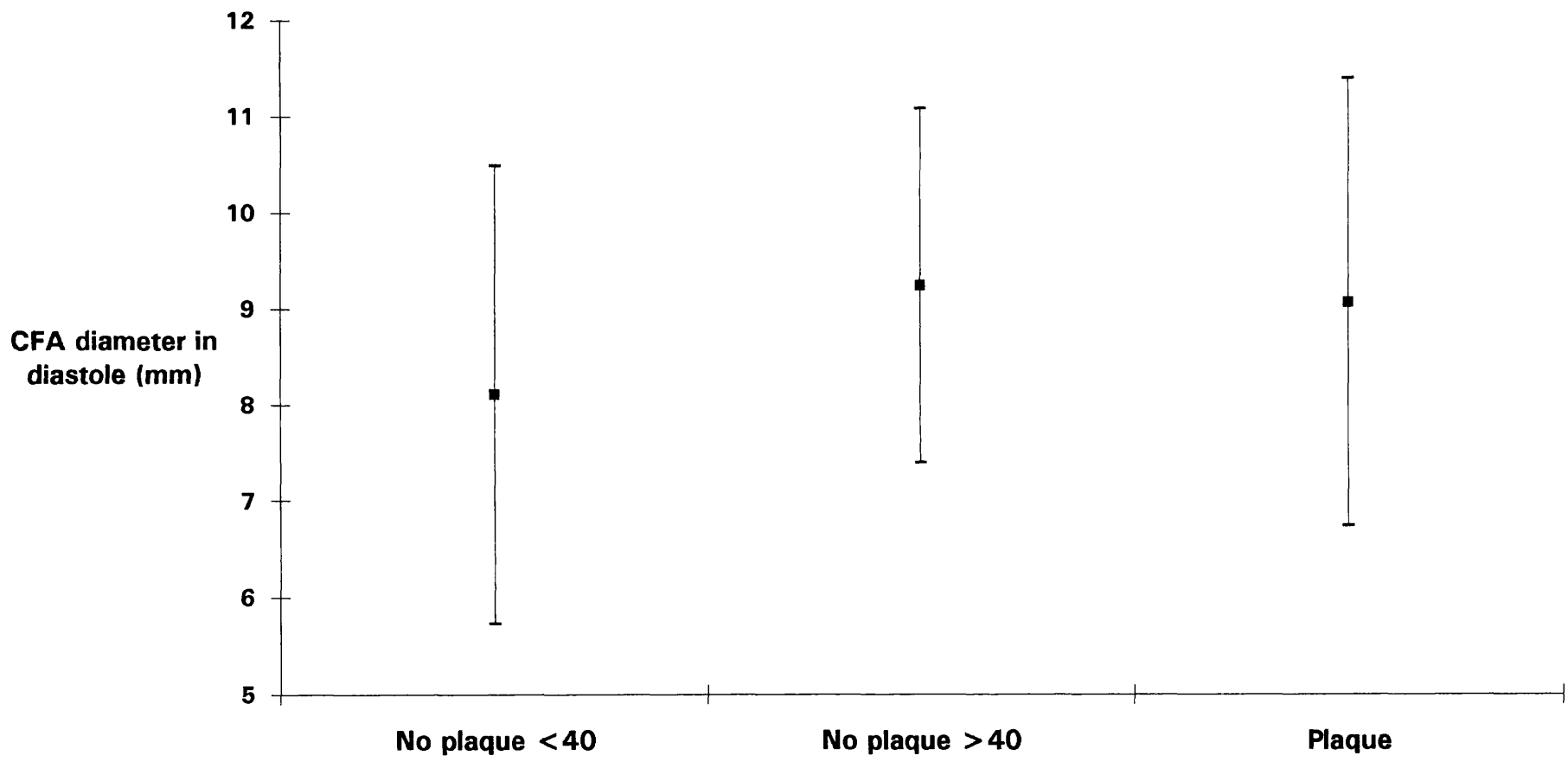
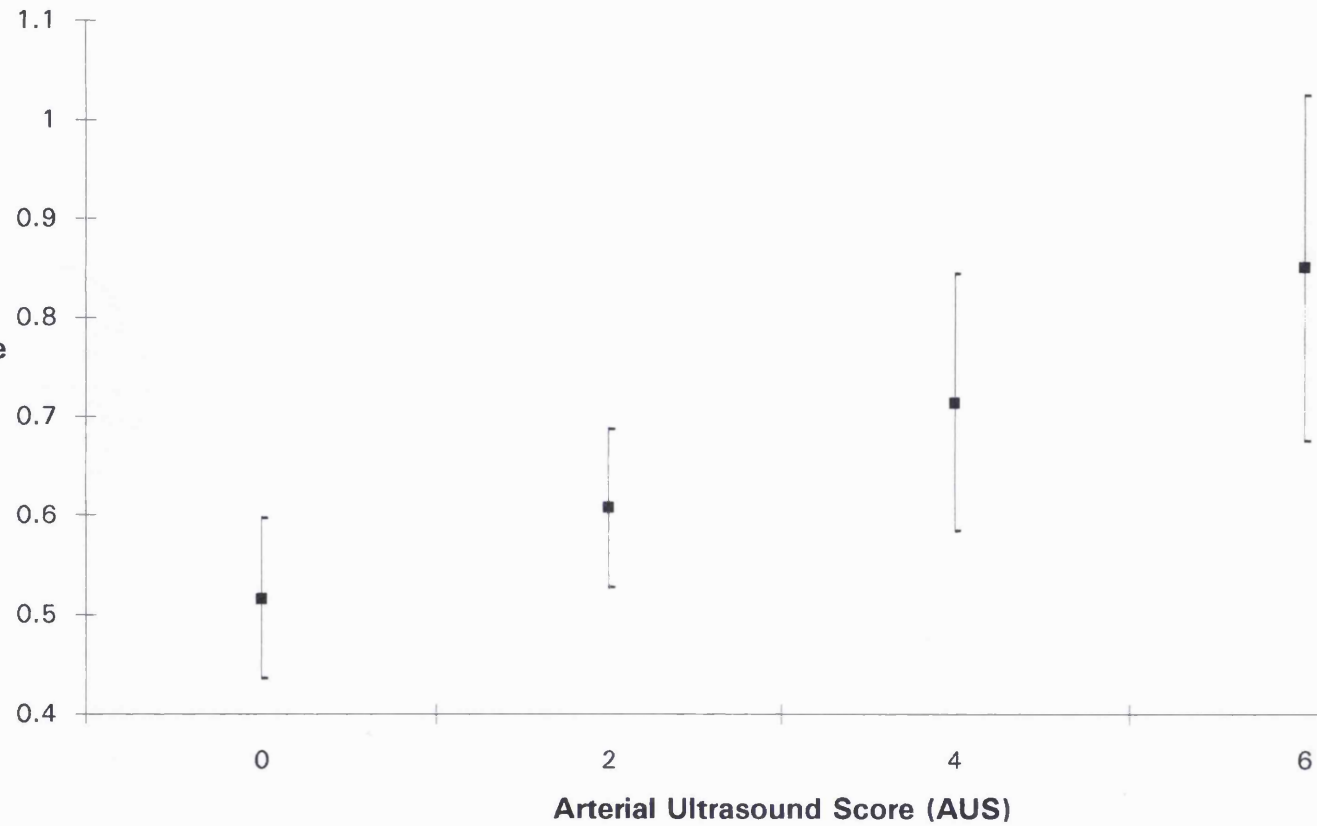


Fig 11.21

Mean (+/- 1sd) CFA diameter and the presence of plaque

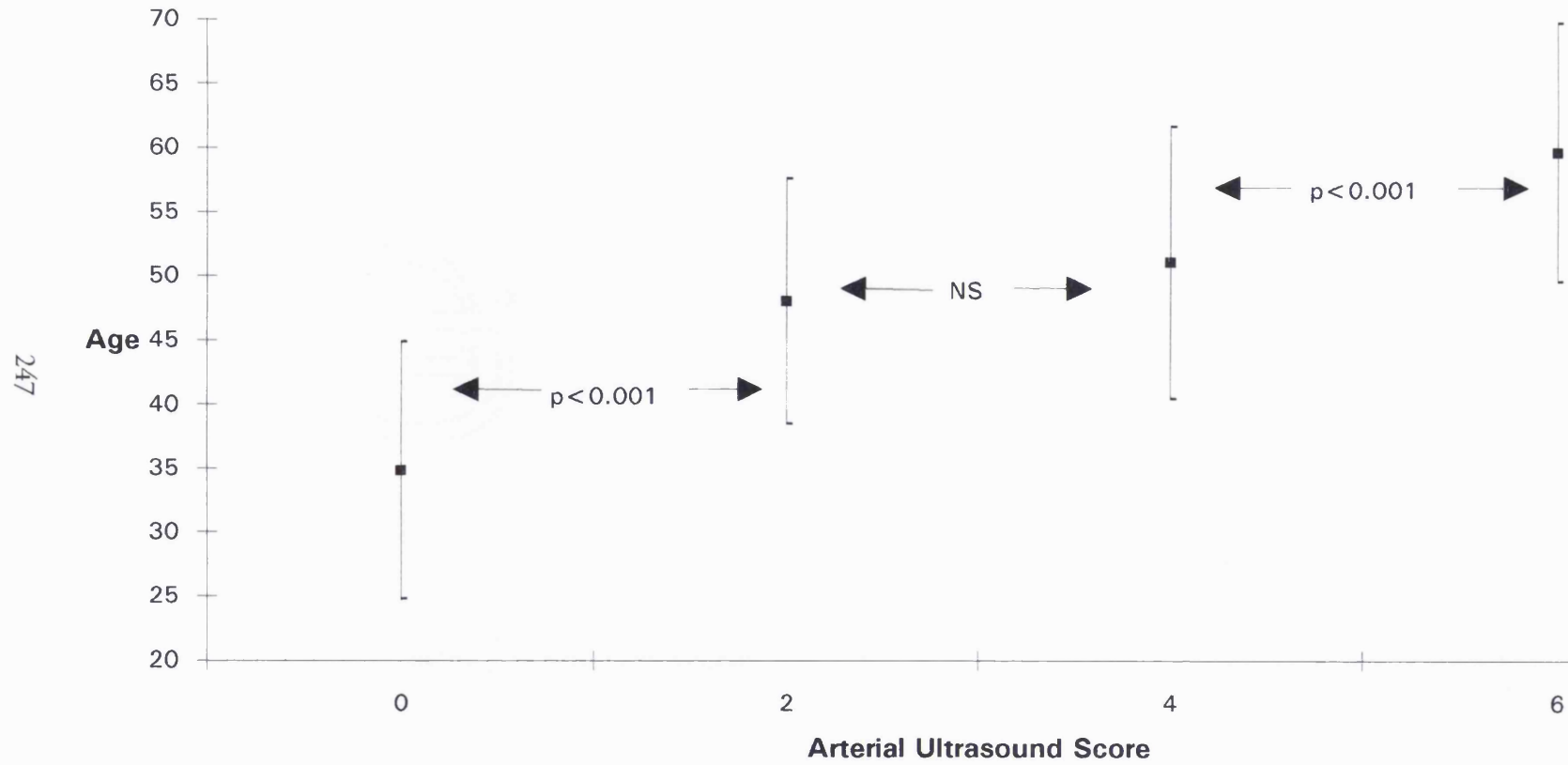
246

IM thickness of the CCA (mm)



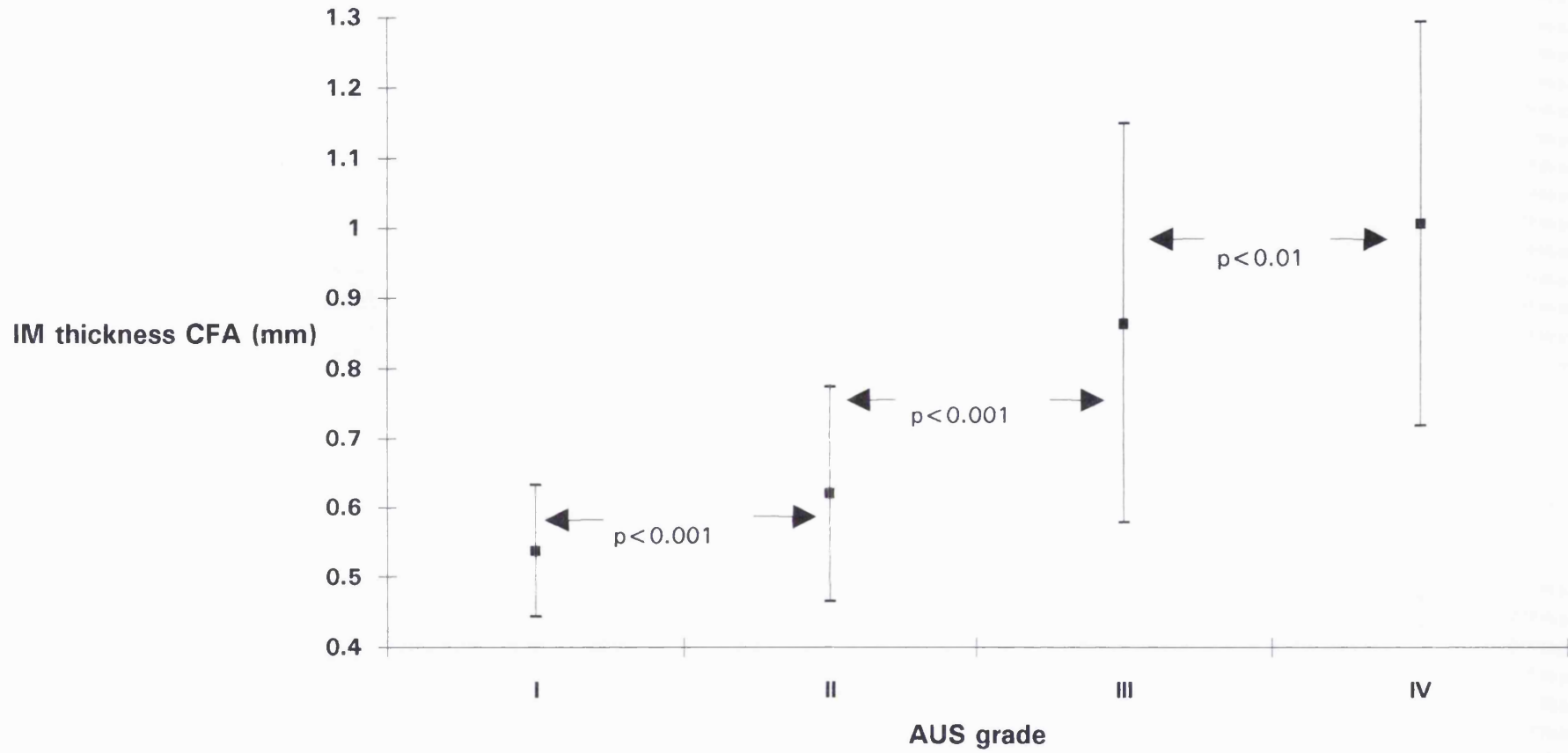
**Fig 11.22**

**Mean (+/- 1sd) IM thickness for each grade of the Arterial Ultrasound Score**



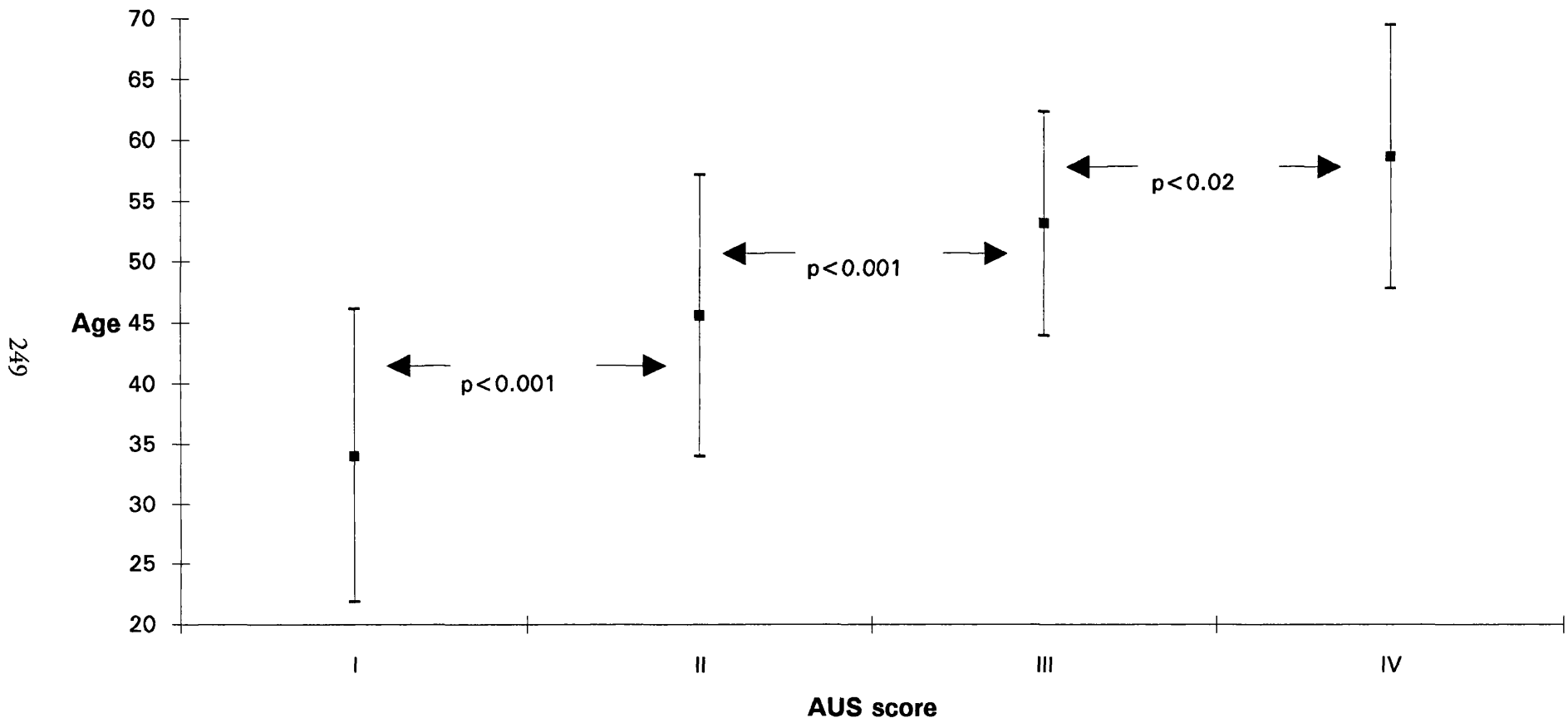
**Fig 11.23**

**Mean age (+/- 1sd) for each grade of the Arterial Ultrasound Score**



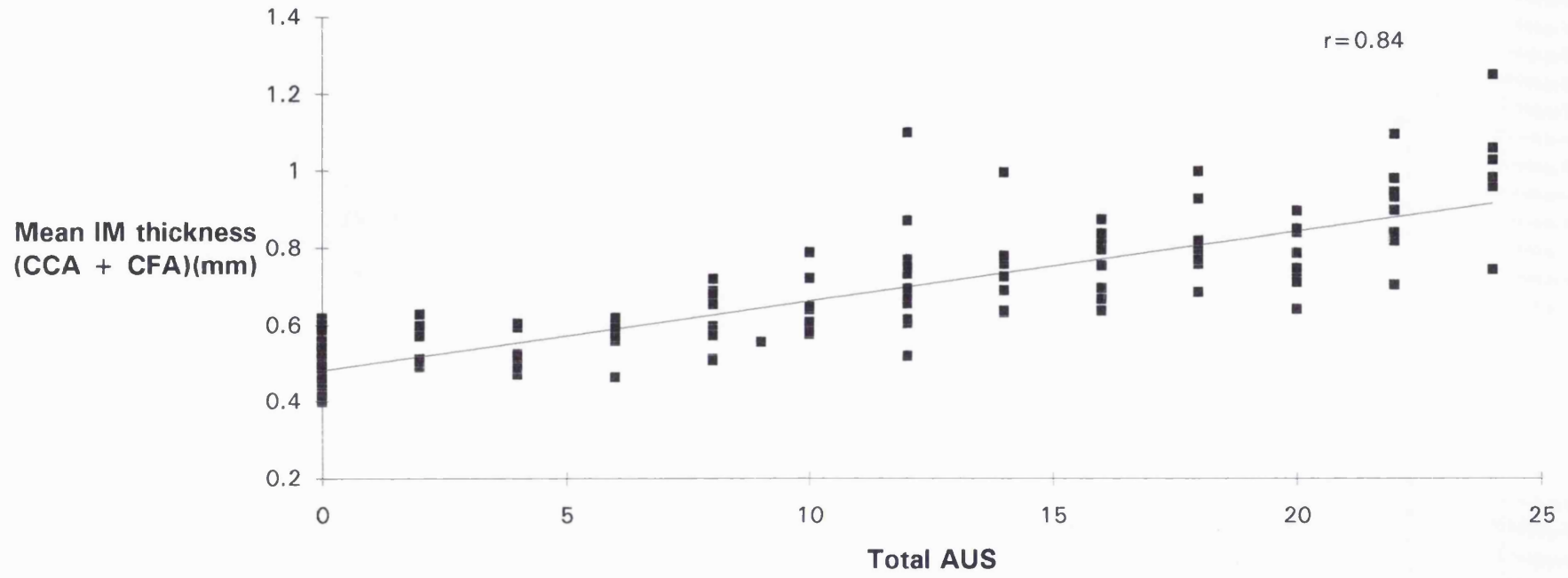
**Fig 11.24**

**Mean (+/- 1sd) IM thickness of the CFA for each grade of the AUS**



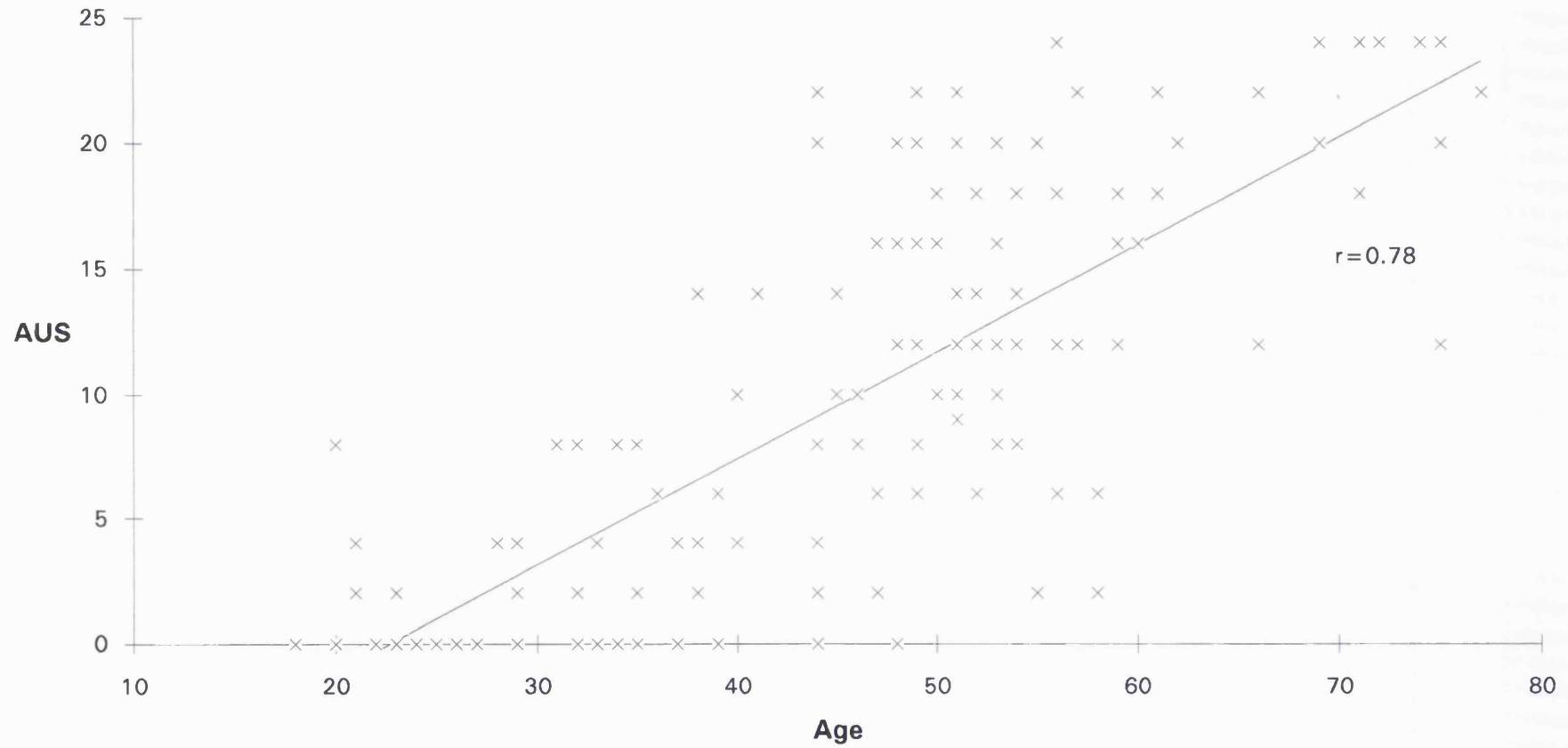
**Fig 11.25**

**Mean (+/- 1sd) age for each grade of the AUS in the CFA**



**Fig 11.26**

**Correlation between overall mean IM thickness and the AUS**



**Fig 11.27**

**Correlation between age and the AUS**

### **11.35 The relationship between Intima-media thickness and risk factors**

Multiple regression analysis was performed using sex, age, systolic blood pressure, diastolic blood pressure and pack years as independent variables. Table 11.8 shows the predictive strength of each of these variables. The mean IM thickness can be predicted from :

$$\text{Mean CCA IM} = 0.2806 + 0.017 \text{ sex} + 0.00648 \text{ age (years)} + 0.000991 \text{ systolic BP (mmHg)} + -0.001037 \text{ diastolic BP (mmHg)} + 0.00166 \text{ pack years}$$

The multiple correlation coefficient (R) for the above formula is 0.77

However, the strongest factors are age and sex and alone these can be used to predict IM thickness in the following formula:

$$\text{Mean CCA IM} = 0.318 + 0.00684 \text{ age (years)} + 0.00167 \text{ packyears.}$$

The multiple correlation coefficient (R) for this formula is 0.76 and in an analysis of variance  $F=64.36$ ,  $p<0.0001$ .

Multiple linear regression analysis was also performed after the CCA IM thickness was corrected for age. Using an age correction of 50, the formula became:

$$\text{IM thickness} = 0.668 + 0.02 \text{ sex} + 0.0005 \text{ systolic BP} + -0.001 \text{ diastolic BP} + 0.0015 \text{ pack years}$$

but the correlation for this formula was poor ( $R=0.3$ ,  $F=3.31$ ,  $p=0.0125$ )

### **11.36 The relationship between plaque, intima-media thickness and risk factors.**

To assess the predictive power of known risk factors on the presence of plaque at any of the four bifurcations, multiple regression analysis was performed using sex, age, systolic blood pressure, diastolic blood pressure and pack years as independent variables. Table 11.9 shows the predictive strength of each of these variables. As with CCA IM thickness,



**Table 11.8 Multiple linear regression of independent variables contributing to IM thickness and the significance of each component.**

		F	p value
	c=0.280652		
Sex	b1 = 0.017148	0.92	0.34
Age (years)	b2 = 0.00648	73.22	<0.0001
Systolic BP	b3 = 0.000991	1.41	0.24
Diastolic BP	b4 = -0.001037	0.55	0.46
Pack years	b5 = 0.001659	12.33	<0.0005

**Table 11.9 Multiple linear regression of independent variables contributing to the prediction of the presence of plaque and the significance of each component.**

		F	p value
	$c = -0.66049$		
Sex	$b1 = 0.080182$	1.49	0.22
Age (years)	$b2 = 0.012875$	21.48	<0.0001
Systolic BP	$b3 = 0.004446$	2.11	0.15
Diastolic BP	$b4 = -0.0026$	0.26	0.61
PY	$b5 = 0.004836$	7.79	0.01

Plaque =  $-0.660469 + 0.080182 \text{ sex} + 0.012875 \text{ age (years)} + 0.004446 \text{ systolic BP} +$   
 $-0.0026 \text{ diastolic BP} + 0.004836 \text{ packyears.}$

$r = 0.612, F = 16.37, p < 0.0001$

**Table 11.10 Multiple linear regression of independent variables (including mean CCA IM thickness) contributing to the prediction of the presence of plaque and the significance of each component.**

		F	p value
	c=-1.059512		
Sex	b1 = 0.0558	0.84	0.36
Age (years)	b2 = 0.003661	1.32	0.25
Systolic BP	b3 = 0.003037	1.14	0.29
Diastolic BP	b4 = -0.001125	0.06	0.81
PY	b5 = 0.002478	2.19	0.14
Mean IM thickness	b6 = 1.421845	24.04303	<0.0001

age and pack years were the strongest predictive variables and the likelihood that plaque was present could be predicted by the following formula:

$$\text{Plaque} = -0.364 + 0.015 \text{ age (years)} + 0.0049 \text{ pack years}$$

The multiple correlation coefficient for this formula is (R) 0.60

If IM thickness is added as an independent variable the predictive strength of the variables changes and only CCA IM thickness and age remain significant (Table 11.11).

With CCA IM thickness, the formula predicting the presence of plaque becomes:

$$\text{Plaque} = -0.834 + 0.005 \text{ age (years)} + 0.0024 \text{ pack years} + 1.48 \text{ CCA IM thickness.}$$

The multiple correlation coefficient (R) for this formula is 0.68. (F=29.55, p<0.0001)

Multiple regression analysis was repeated after correcting the IM thickness for age (to 50). Plaque could best be predicted from the following formula:

$$\text{plaque} = -1.712 + 0.00843 \text{ sex} + 0.01 \text{ systolic BP} + -0.0012 \text{ diastolic BP} + 0.0048 \text{ pack years} + 1.25 \text{ corrected IM thickness}$$

The strength of the variables is shown in table 11.12

**Table 11.11 Multiple linear regression of independent variables (including CCA IM thickness) contributing to the prediction of the presence of plaque and the significance of each component - best subset**

		F	p value
	c=-0.834897		
Age	b1 = 0.005078	3.346	0.0695
Pack years	b2 = 0.002432	2.121	0.1474
Mean CCA IM thickness	b3 = 1.47945	26.55	<0.0001

**Table 11.12 Multiple linear regression of independent variables contributing to the prediction of the presence of plaque and the significance of each component -(after correction of CCA IM thickness to a standard age of 50).**

		F	p value
	c=-1.711898		
Sex	b1 = 0.00843	0.01607	0.9
Systolic BP	b2 = 0.009961	12.524	0.0005
Diastolic BP	b3 = -0.001163	0.04955	0.8242
Packyears	b4 = -0.004776	7.15089	0.0084
Corrected IM thickness	b5 = 1.251217	15.5158	0.0001

R= 0.59, F = 14.73, p<0.0001

## **11.4 Discussion**

### ***i) Is the intima-media thickness related to plaque?***

Yes. The mean intima-media thickness in those subjects with plaques present in any of the four bifurcations was significantly greater than those without plaque. Furthermore, mean CCA intima-media thickness can be used to predict the presence of plaque as plaques are found in only 6% of subjects with a mean CCA intima-media thickness of 0.58mm or less had plaques, compared to 50% of subjects with an IM thickness of between 0.59-0.82mm, and all subjects with an IM thickness of 0.82mm and over.

### ***i) Is the intima-media thickness related to age?***

Yes. The intima media thickness in the common carotid artery, at the bulb origin and in the common femoral artery increases with age, even in the absence of plaque. In the presence of plaque the increase is greater still in both the CCA and the CFA, but not at the bulb origin.

### ***iii) Is the intima-media thickness related to the site at which it is measured?***

In the absence of plaques, the mean IM thickness of the common carotid artery is not significantly different from the mean IM thickness in the mid bulb region or the mean IM thickness in the common femoral artery. However, the mean IM thickness at the bulb origin is significantly thicker than all three sites. This may be due to the fact that it is the site of the earliest plaque formation, prior to the development of plaques at the other sites examined.

The advantage of the using the mean CCA IM thickness is that this site is involved in plaque late, if at all, whereas in the bulb and at the bifurcation of the common femoral arteries, plaques may obscure the intima-media.

### ***iv) Is the intima-media thickness related to the diameter of the vessel?***

Vessel diameter increases with age, and in the common carotid artery, the diameter of the vessel is significantly less in individuals aged 40 and over who do not have plaques in any of the four bifurcations, compared to individuals who do. This difference is seen in both men and women. Similar findings have been reported in the coronary artery

(Glagov et al, 1987) and they propose that this is an adaptive response of the vessel to the presence of potentially lumen narrowing plaque.

Though the diameter of the bulb and the CFA increase with age, they do not appear to increase with plaque.

***v) Is the intima-media thickness related to the arterial ultrasound score?***

Yes. The mean intima media thickness of both the CCAs and the CFAs correlate well with the AUS grade

***vi) Can the intima-media thickness be predicted from known risk factors?***

Yes, the strongest factors are age and pack years, and these can be used to predict IM thickness.

Our results are similar to the results reported by Salonen and Salonen in their population study of eastern Finnish men, (Salonen and Salonen, 1991) though they also showed that IM thickness correlated with ambulatory pulse pressure, S-LDL cholesterol and a history of ischaemic heart disease. Furthermore, in a two year follow-up, the group reported that maximum progression was observed in men who were current smokers and that pack years of smoking was one of the strongest predictors (after age) of the progression of common carotid intima-medial thickening (Salonen and Salonen, 1990). In a study of matched pairs, the ARIC study investigators compared individuals with a maximum IM thickness of 2.5mm or more or bilateral thickening over the 90th percentile of maximum IM thickness, and controls with an IM thickness below the 75th percentile, the odds ratio based on matched pairs was 4.4 for smokers and 1.5 for hypertension. Hypertension was not found to be relevant in this study, possibly because the sample is small and the majority were normotensive.

Both the Finnish study and the ARIC investigators have also shown a correlation with serum LDL cholesterol and IM thickness but this was not examined in the study reported above.



***v) Can intima-media thickness be used as a risk factor to predict the presence of plaque?***

Like IM thickness, the strongest factors predicting the presence of plaque are age and pack years of smoking, however, if IM thickness is used in the regression formula, this is found to be the strongest predictor of the presence of plaque.

In conclusion, the IM thickness of the arteries of healthy individuals increases with age, smoking habit and the presence of plaque. In the absence of plaque, there is no significant difference between thickness measurements in the common carotid artery, the mid bulb and the femoral artery, but once plaque is present, the common carotid artery is the most reliable site of measurement as it is least often affected by plaque and can be used to predict the presence of plaque at any of the four bifurcations.

CHAPTER 12

COMPLIANCE , DISTENSIBILITY, THE ELASTIC  
MODULUS AND THE STIFFNESS INDEX OF  
NORMAL ARTERIES

## **12.1 Introduction**

Intima-media thickness and plaque formation are structural changes of the artery which may alter or be altered by arterial wall movement. Using the M-mode of the ultrasound probe, it is possible to track the arterial wall in order to assess arterial wall stiffness.

The aim of this study was to assess ability of this method to determine arterial wall movement and changes associated with risk factors for atherosclerosis.

Thus the questions posed were

- i) Do compliance, distensibility, elastic modulus and the stiffness index in the carotid and femoral arteries alter with age?*
- ii) Do compliance, distensibility, elastic modulus and the stiffness index in the carotid and femoral arteries change in the presence of plaque?*
- iii) How do changes in arterial wall movement relate to known risk factors?*
- iv) How do our results compare to other methods of measuring arterial wall movement and the derived parameters?*

## **12.2 Method**

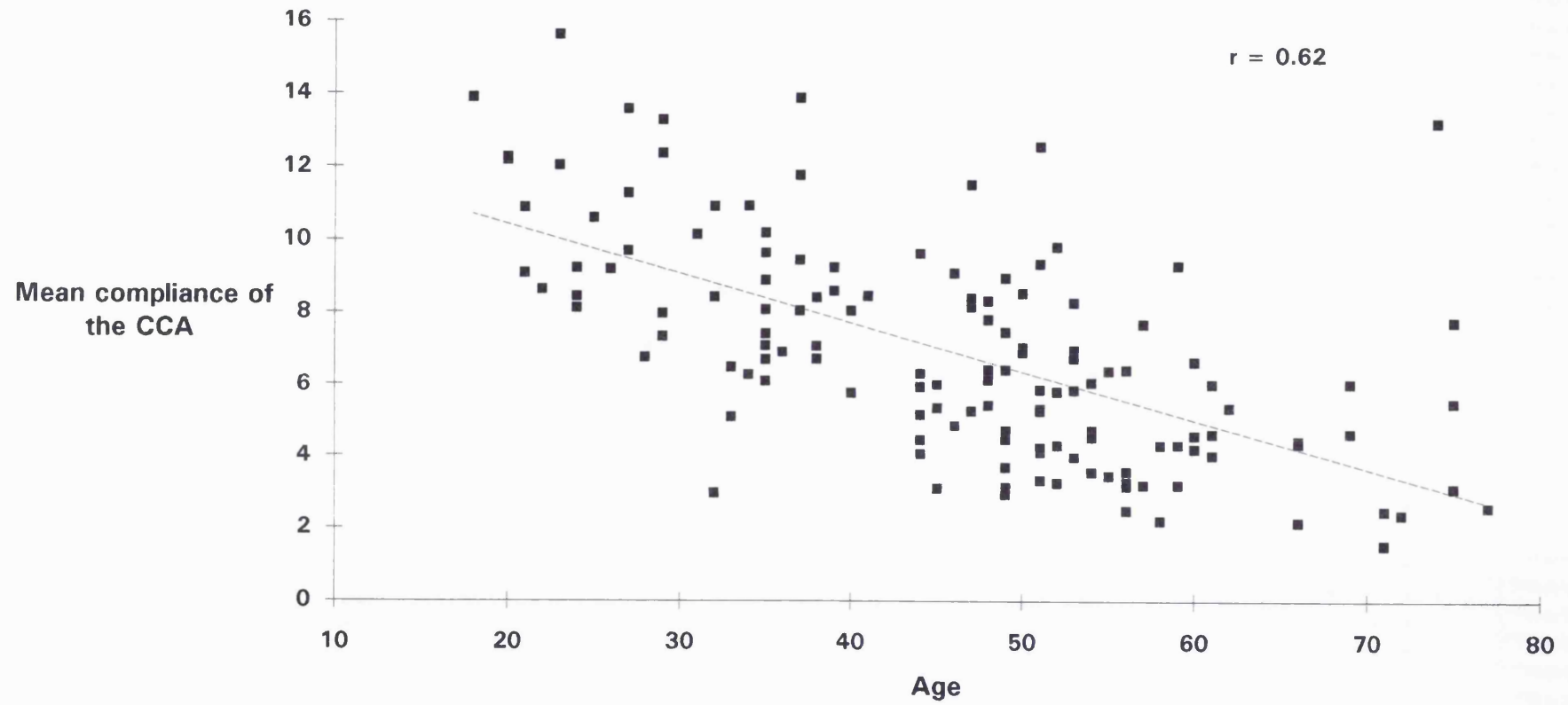
The method is described in Part III, Chapter 9

## **12.3 Results**

### **12.31 Compliance, distensibility, elastic modulus and the stiffness index of the common carotid artery**

#### **a) with age**

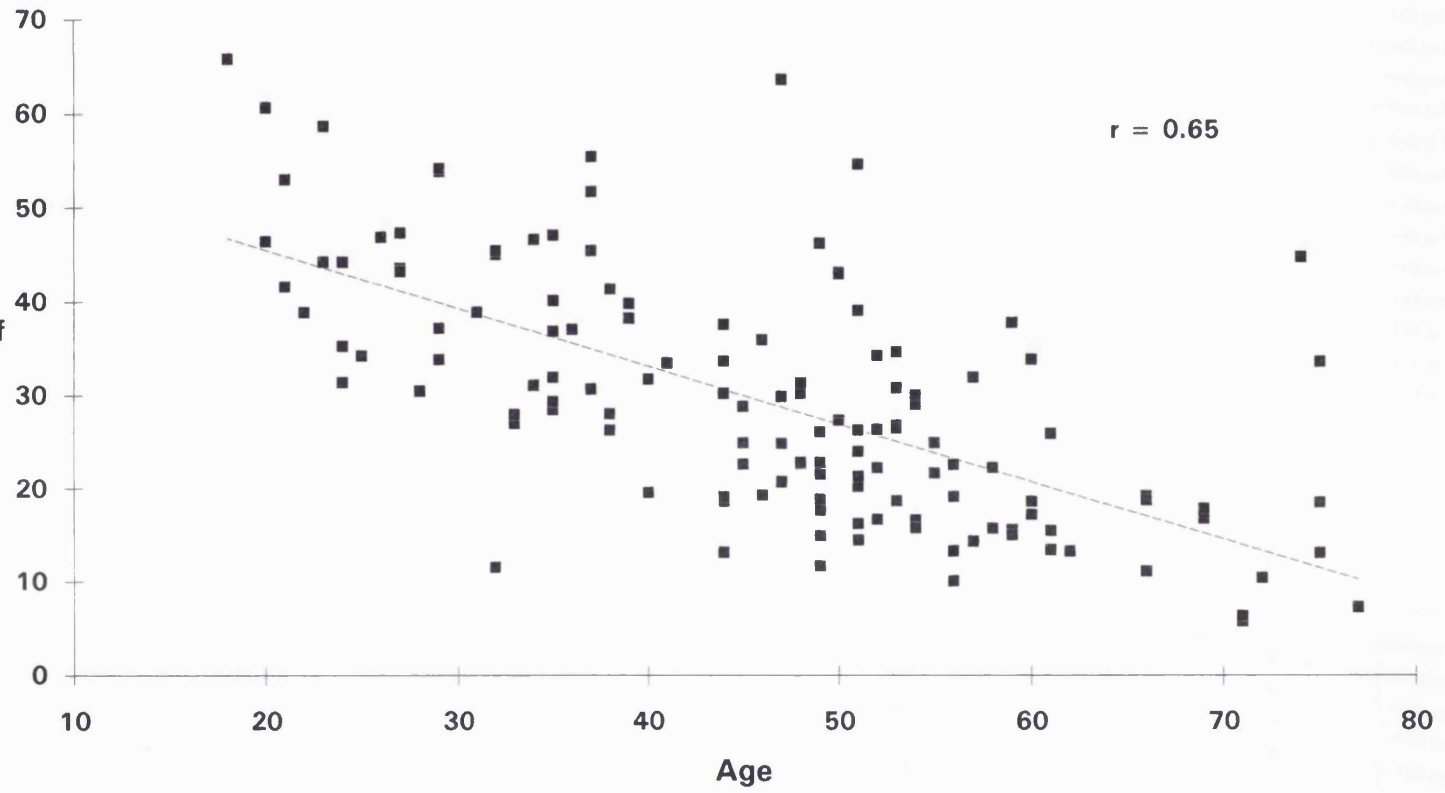
The correlation between the mean compliance and the mean distensibility of the CCA and age is shown in figures 12.1 and 12.2. The elastic modulus and the stiffness index of the CCA have an exponential relationship with age, and in the absence of plaque, the correlation coefficient for the regression line is 0.78 and 0.73 respectively (Figs 12.3 and 12.4)



**Fig 12.1**

**Correlation between mean compliance of the CCA and age**

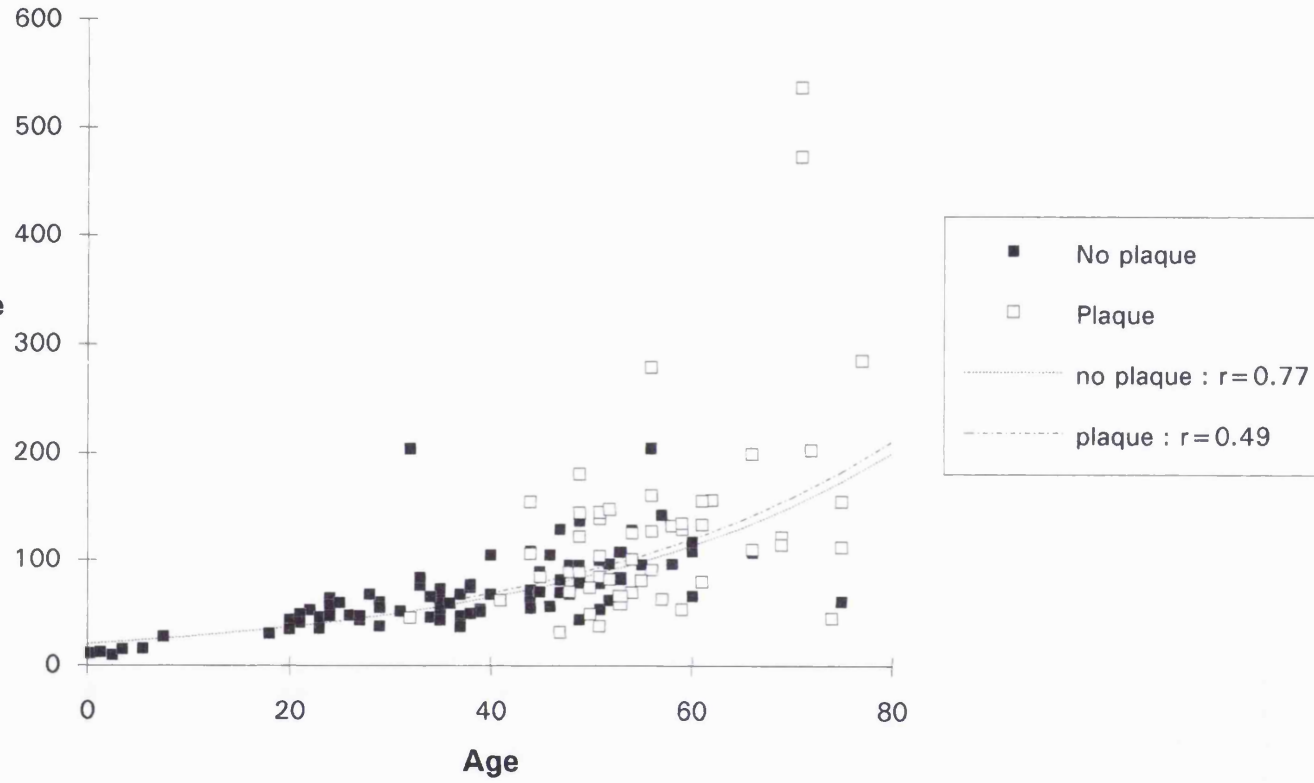
Mean distensibility of the CCA



**Fig 12.2**

**Correlation between mean distensibility of the CCA and age**

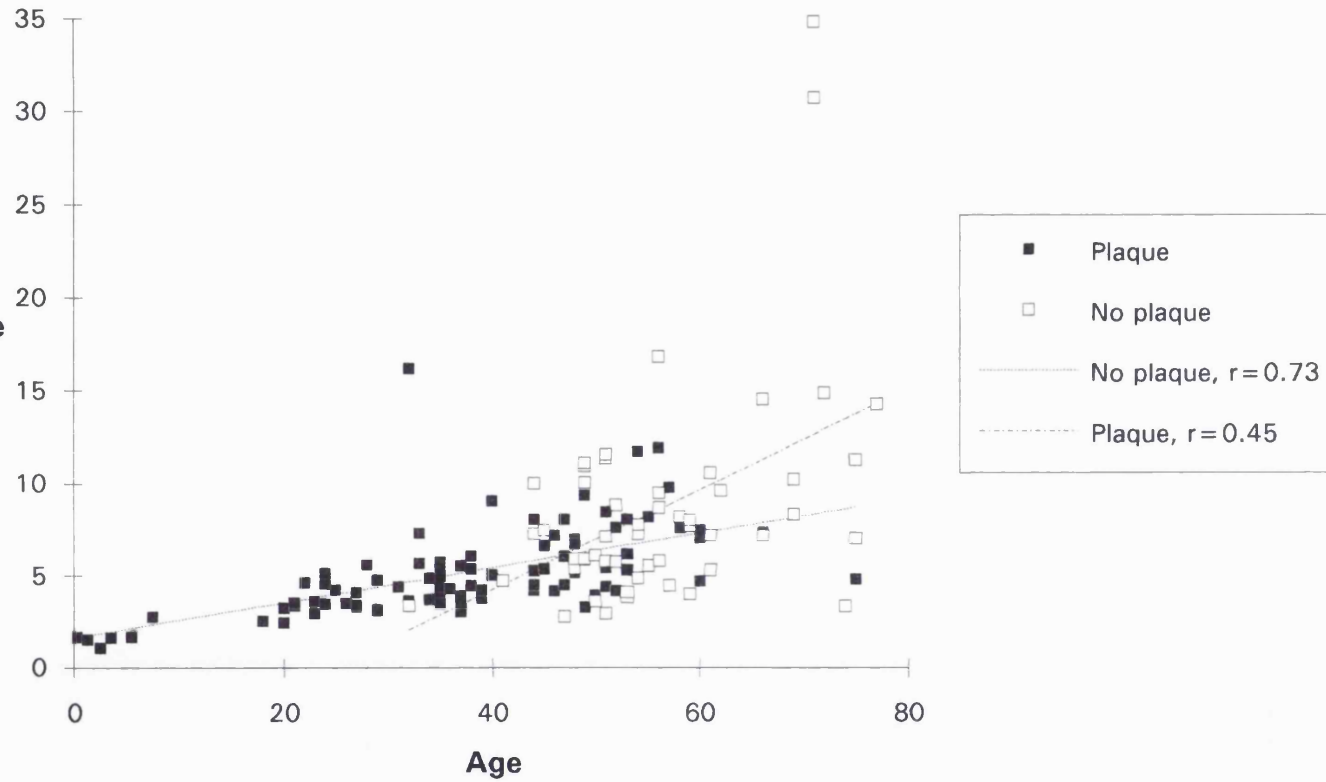
Elastic modulus of the common carotid artery (Ep)



**Fig 12.3**

**Correlation between the elastic modulus of the CCA and age**

Stiffness Index of the CCA



**Fig 12.4**

**Correlation between the stiffness index of the CCA and age**

b) with plaque

Compliance and distensibility of the CCA falls with age and there is little difference in the mean compliance and distensibility of the CCA in individuals with plaque than those without plaque but over the age of 45 (Figs 12.5 and 12.6).

**12.32 Compliance, distensibility, elastic modulus and the stiffness index of the carotid bulb**

a) with age

The mean compliance and the mean distensibility of the carotid bulb fall exponentially with age (Figs 12.7 and 12.8), The elastic modulus and the stiffness index also increase exponentially with age ( $r= 0.78$  and  $0.75$  respectively) (Figs 12.9 and 12.10)

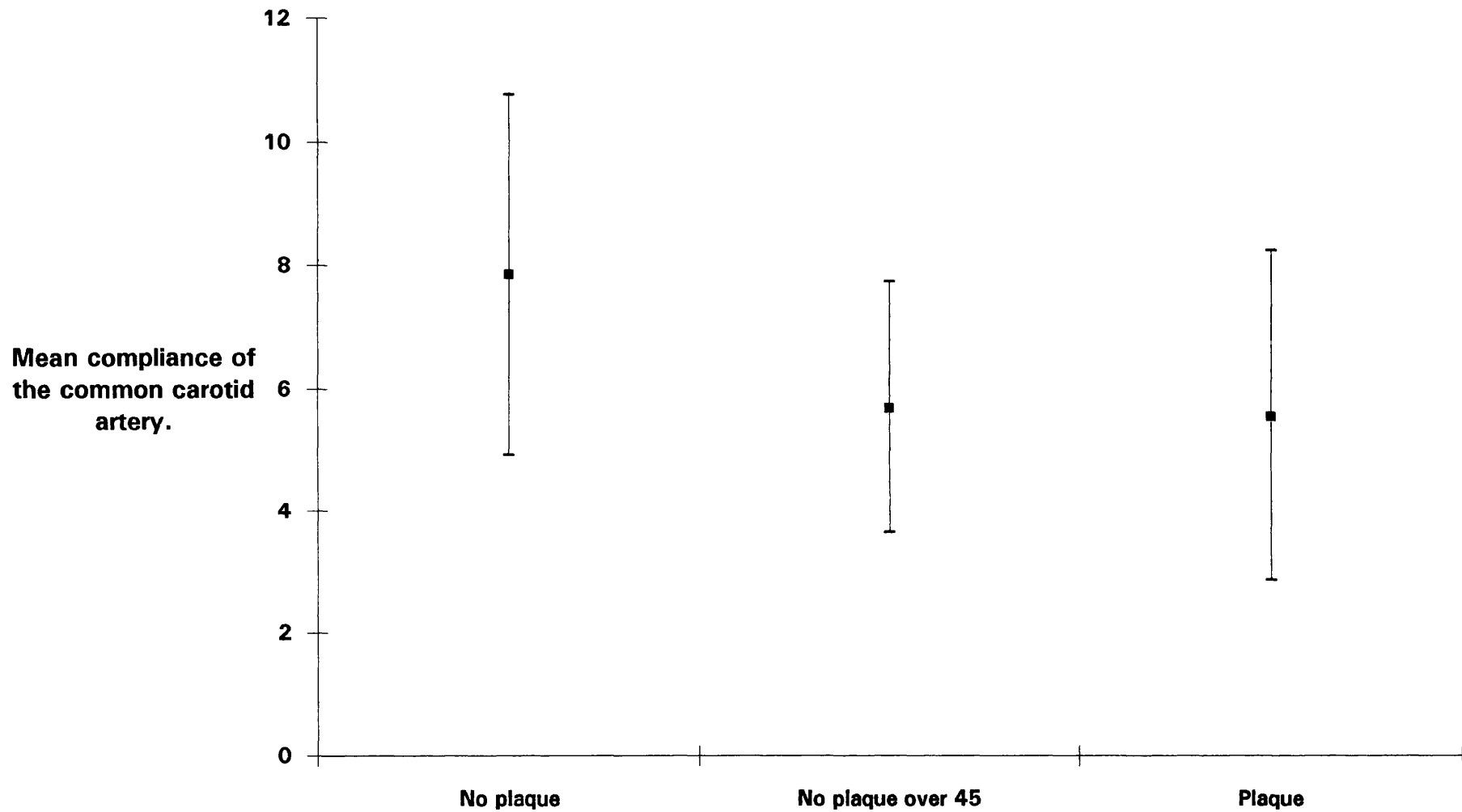
b) with plaque

By the age of 45, there is no difference between the mean compliance and the mean distensibility of individuals with plaque compared to those without (Figs 12.11 and 12.12). Once plaque is present, no correlation is seen between the elastic modulus and the stiffness index and age.

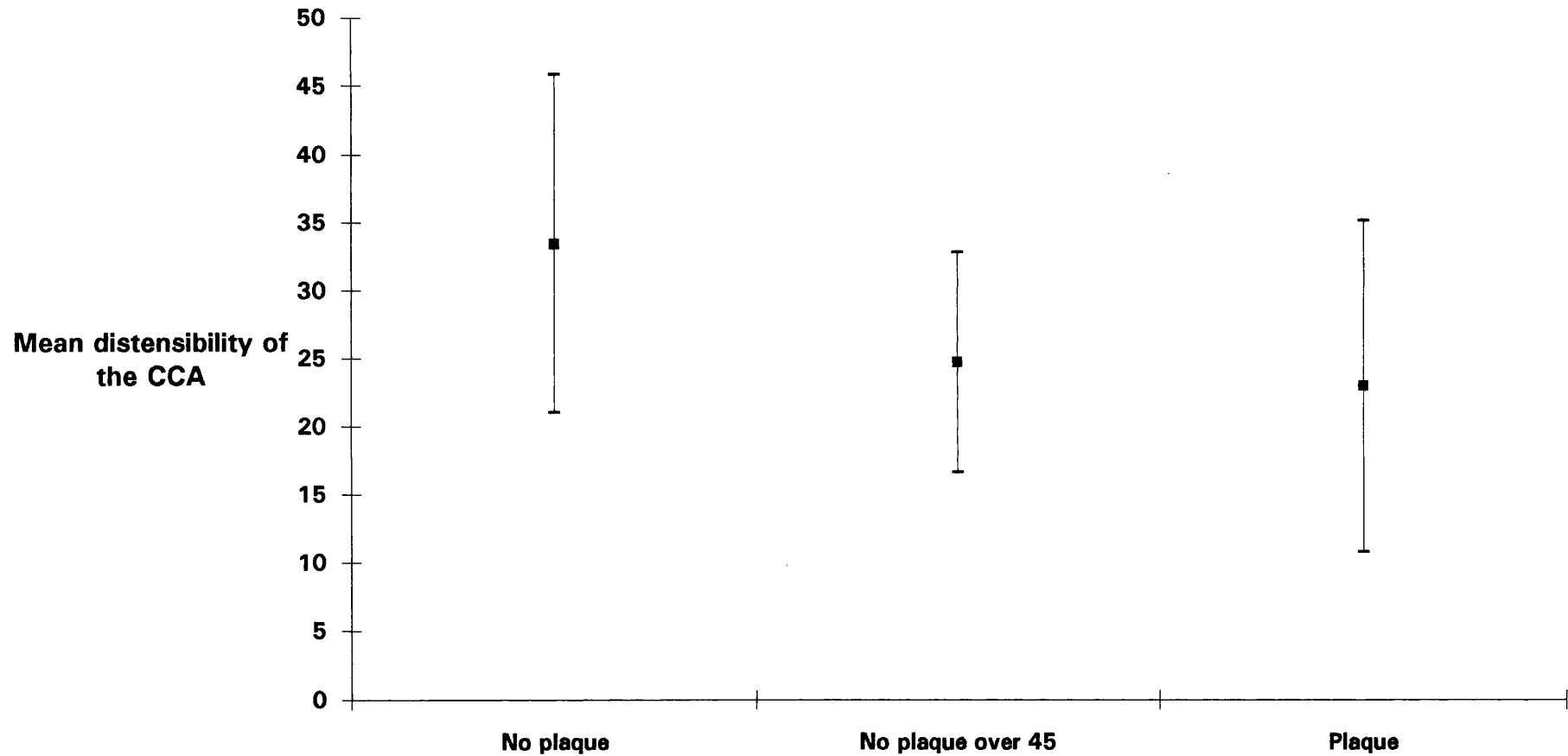
**12.33 Compliance, distensibility, elastic modulus and the stiffness index of the common femoral artery**

There is no correlation between the mean compliance, the mean distensibility, the elastic modulus and the stiffness index of the CFA and age (Figs 12.13, 12.14, 12.15, and 12.16), irrespective of the presence of plaque (Figs 12.17 and 12.18).

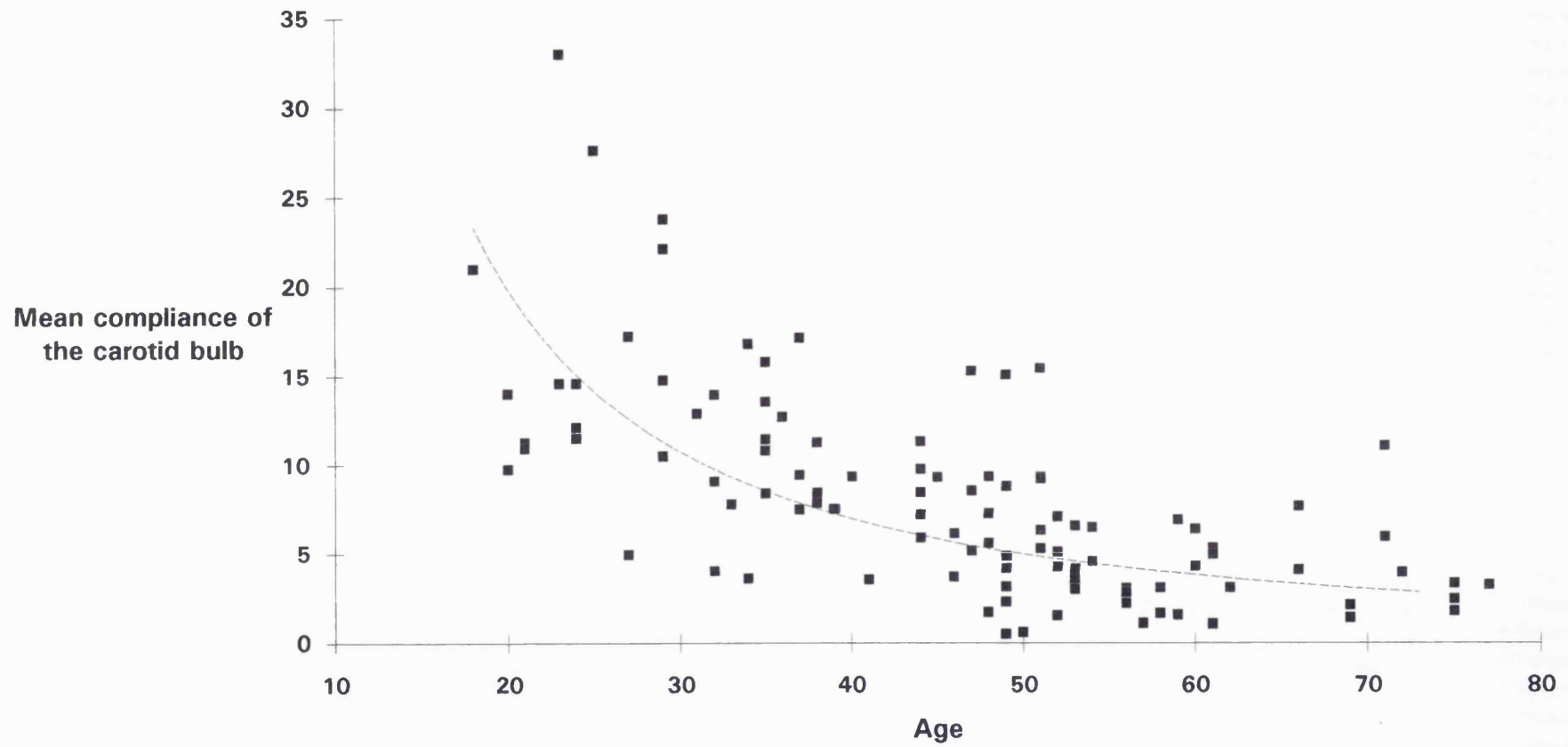




**Fig 12.5 Comparison between the mean ( $\pm$  1sd) compliance of the CCA in young individuals, older individuals without plaque, and individuals with plaque.**

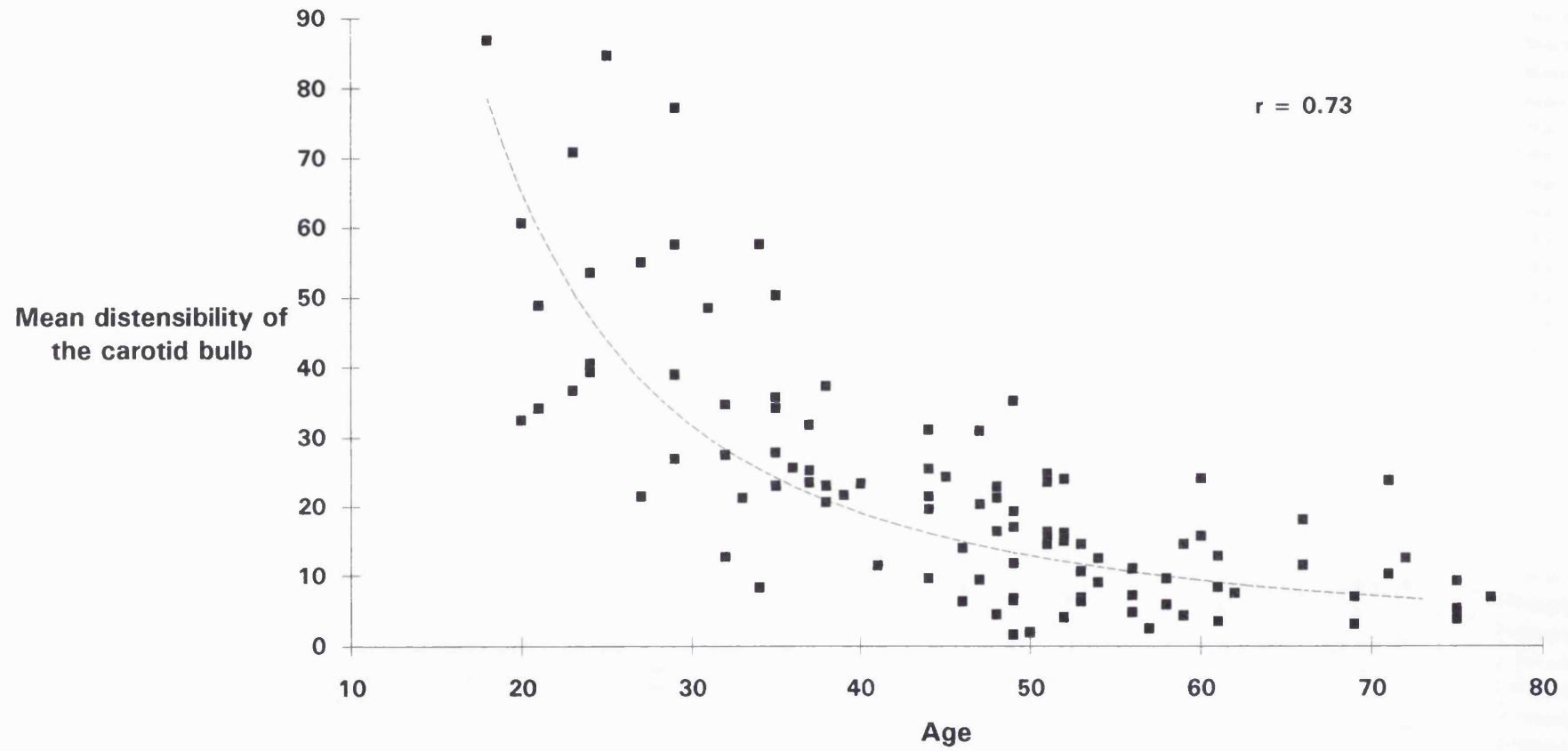


**Fig 12.6 Comparison between the mean ( $\pm 1$ sd) distensibility of the CCA in young individuals, older individuals without plaque and individuals with plaque**



**Fig 12.7**

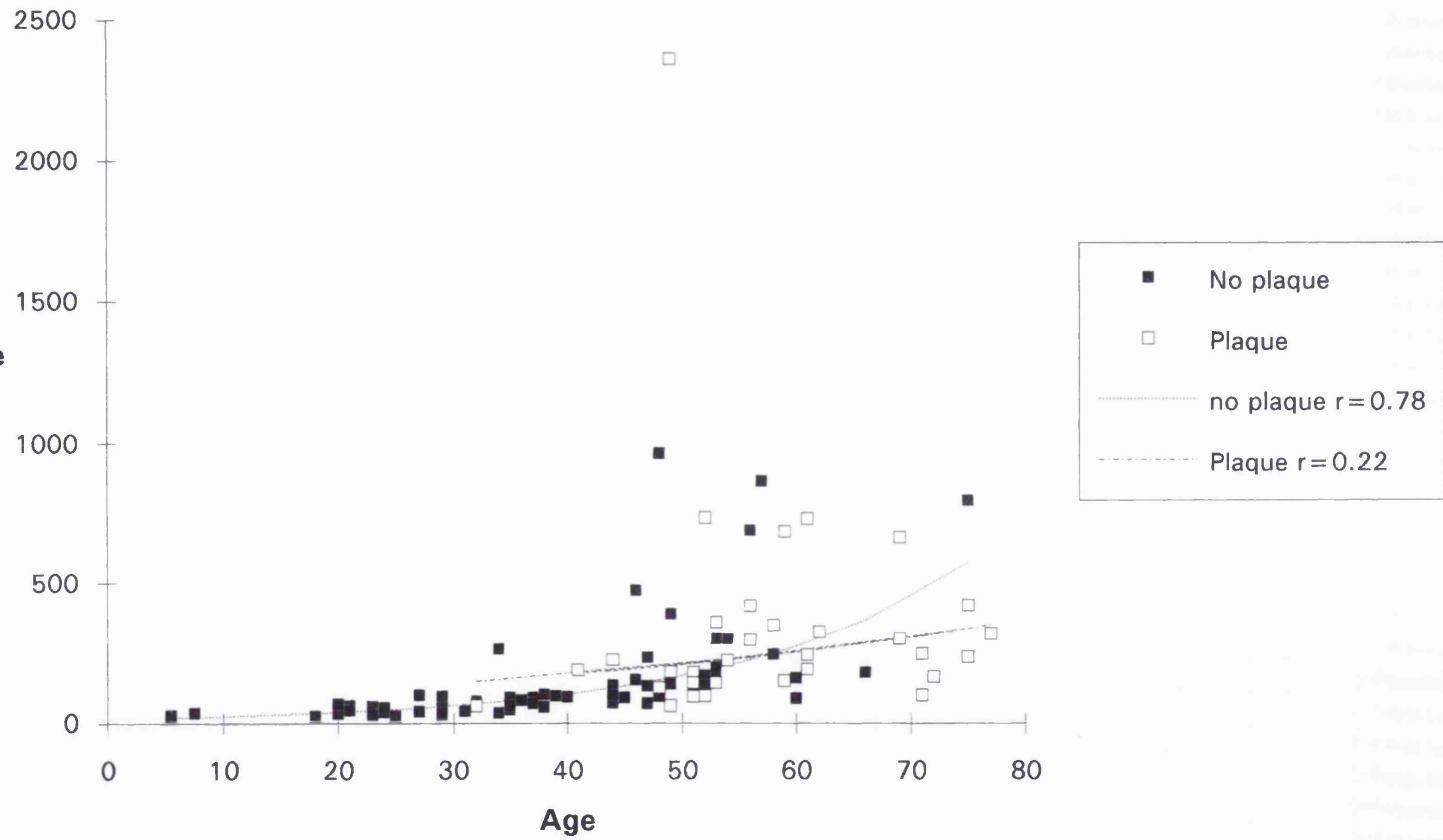
**Correlation between mean compliance of the carotid bulb and age.**



**Fig 12.8**

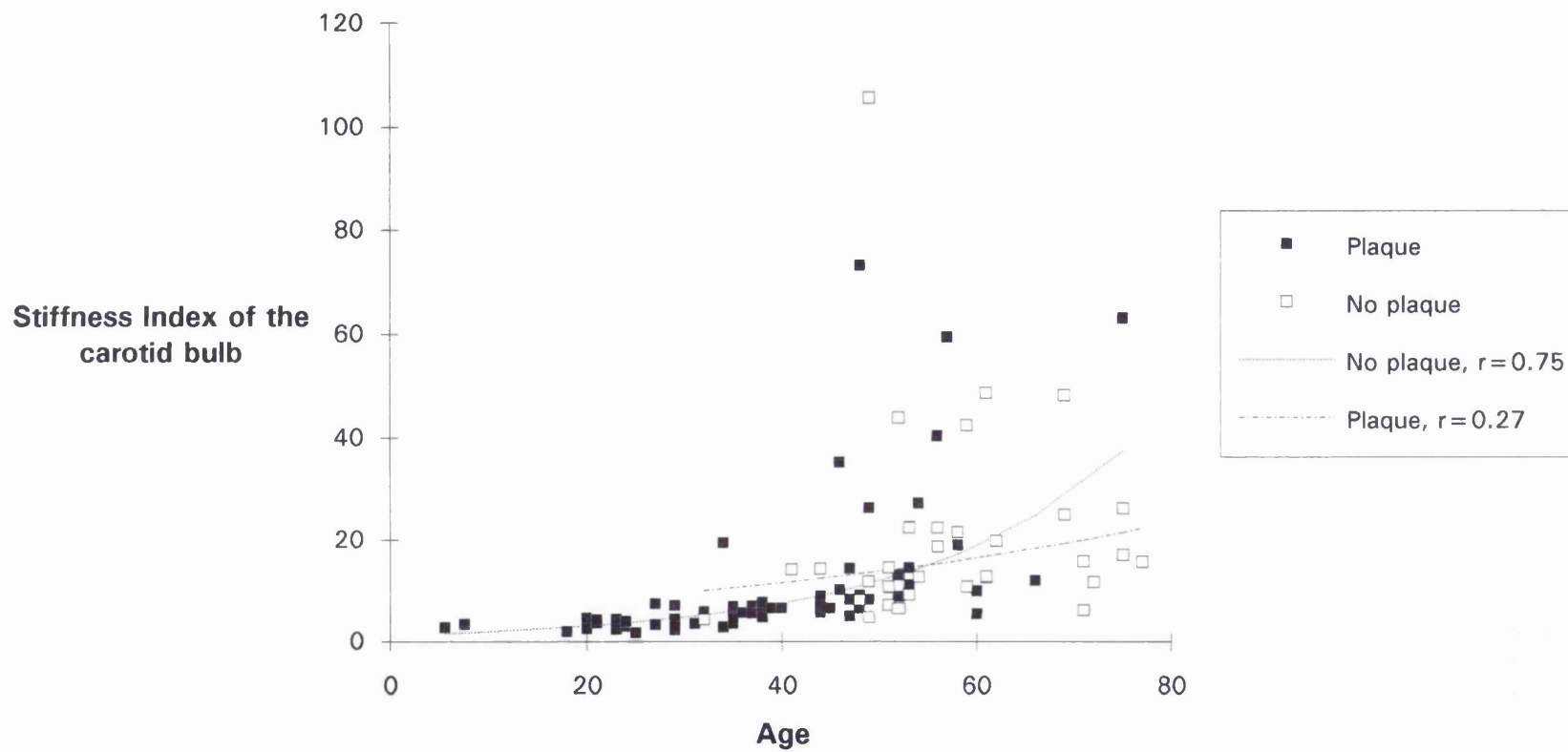
**Correlation between the mean distensibility of the carotid bulb and age.**

Elastic modulus of the  
carotid bulb ( $E_p$ )

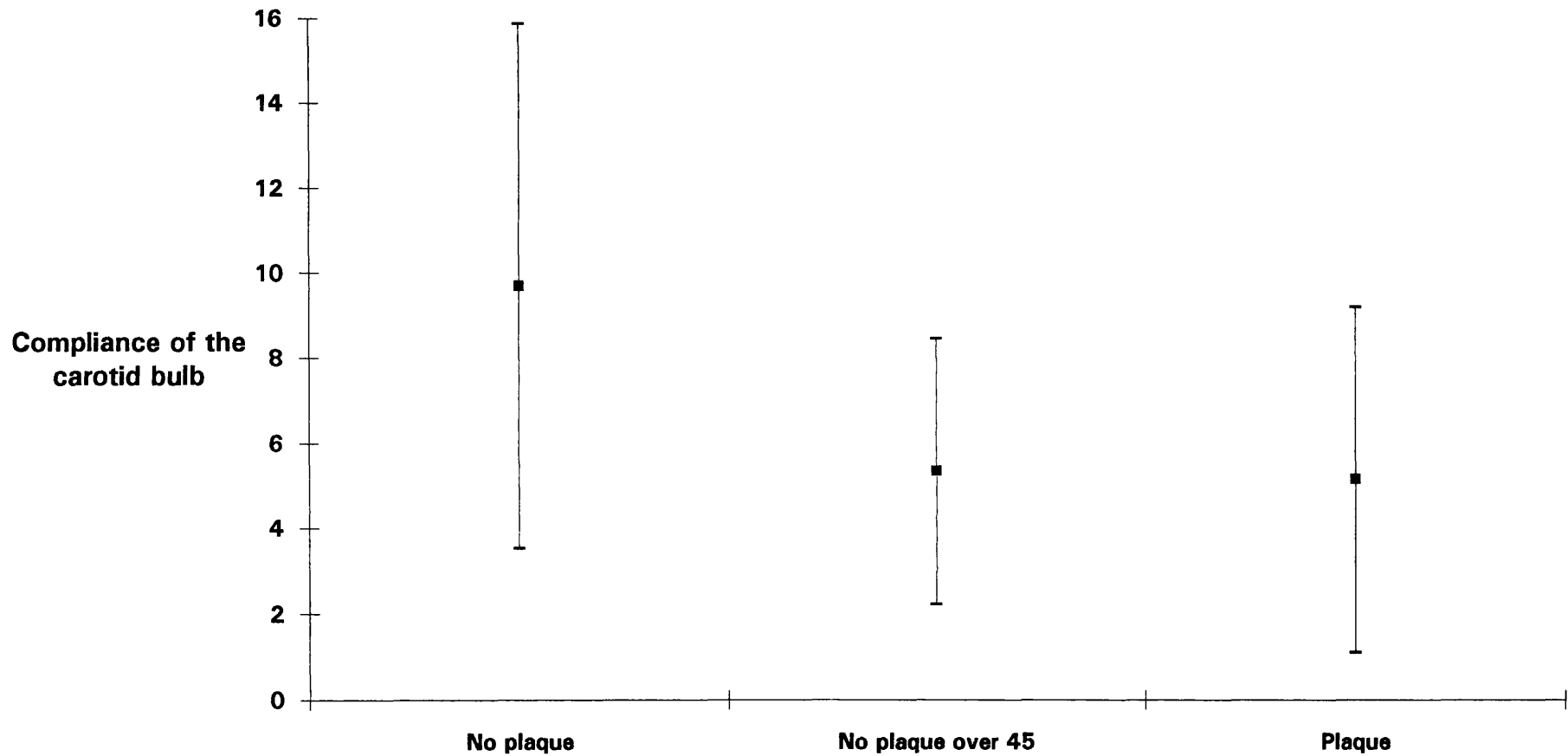


**Fig 12.9**

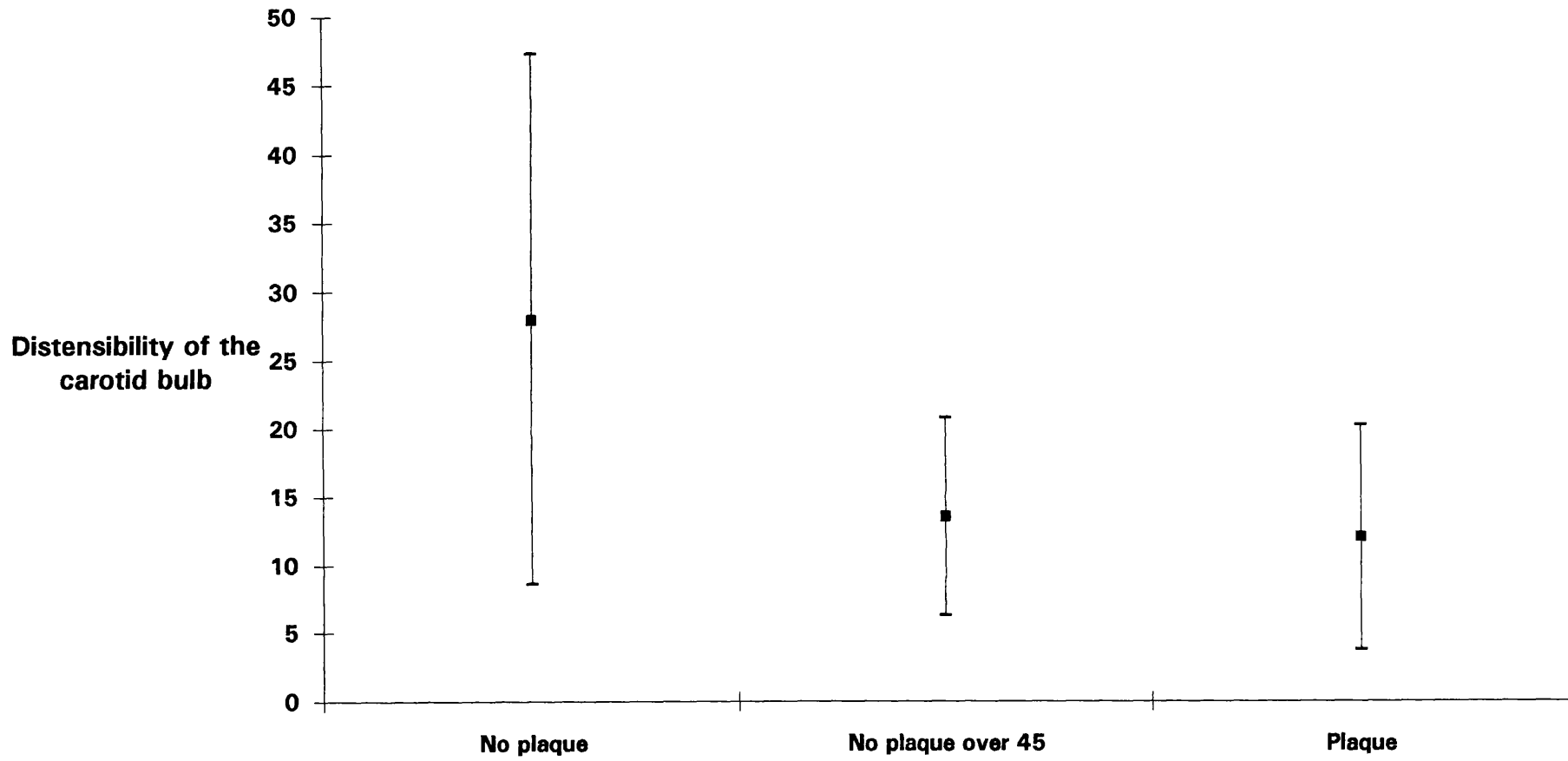
**Correlation between the elastic modulus of the carotid bulb, the presence of plaque and age.**



**Fig 12.10** Correlation between the stiffness index of the carotid bulb, the presence of plaque and age

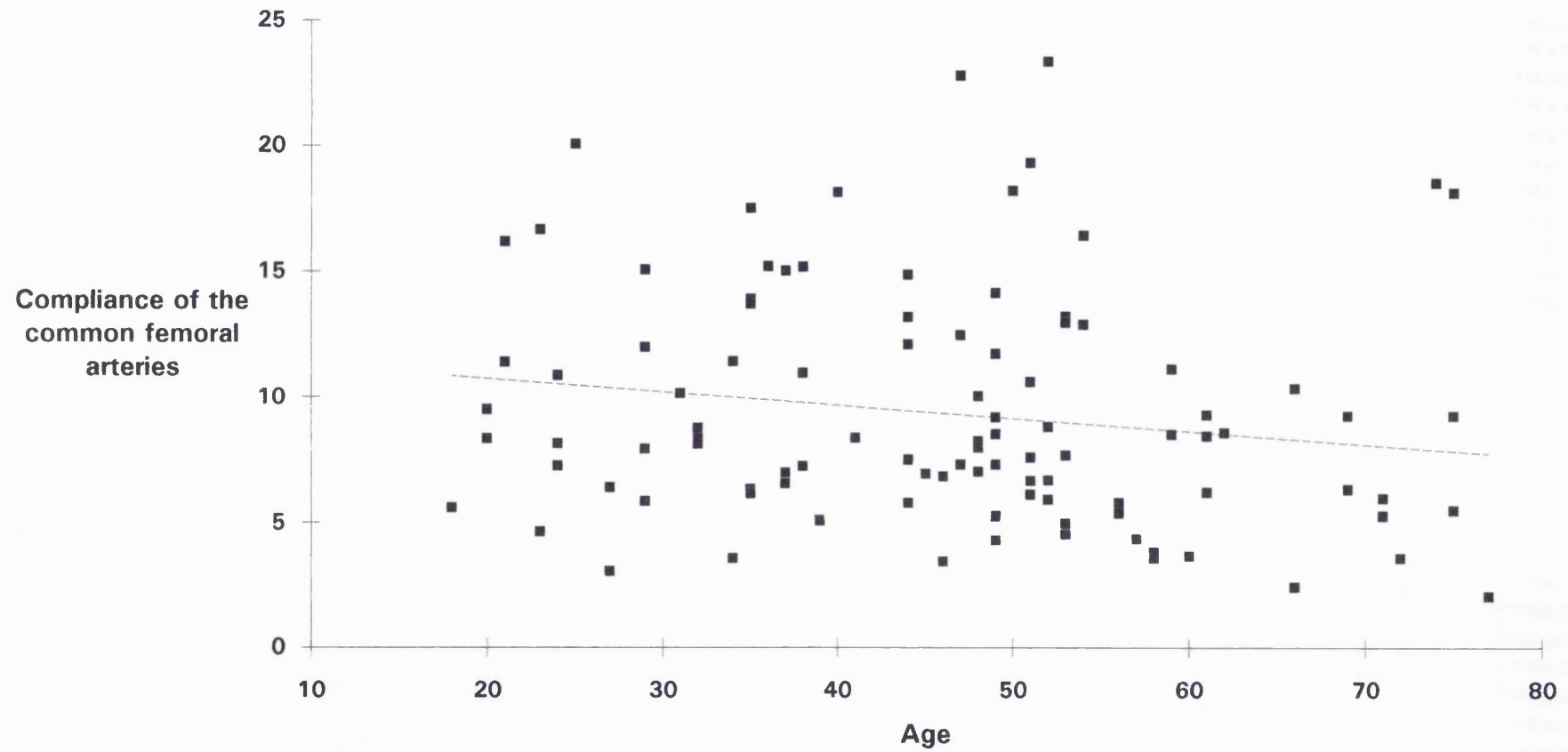


**Fig 12.11 Comparison between the mean ( $\pm$  1sd) compliance of the carotid bulb in young individuals, older individuals without plaque and individuals with plaque.**



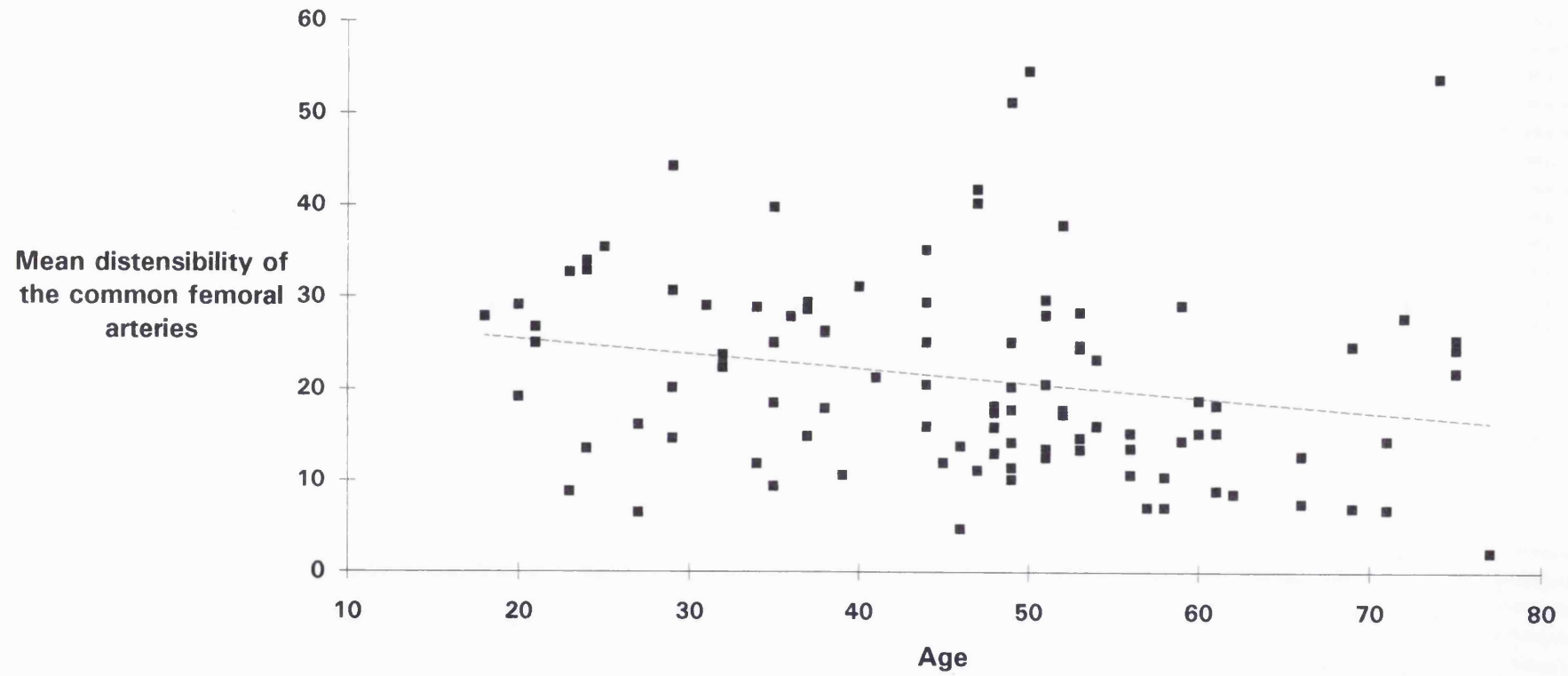
**Fig 12.12 Comparison between the mean ( $\pm$  1sd) distensibility of the carotid bulb in young individuals, older individuals without plaque and individuals with plaque**





**Fig 12.13**

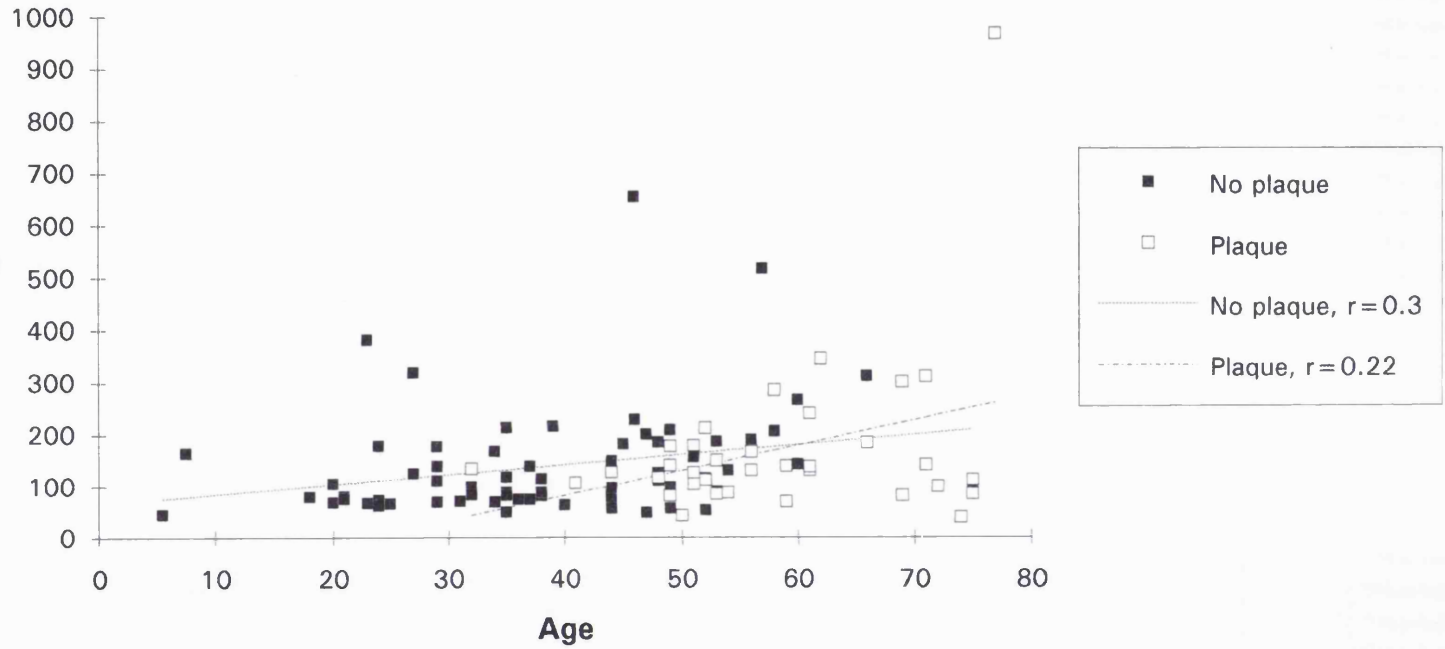
**Correlation between the mean compliance of the CFA and age**



**Fig 12.14**

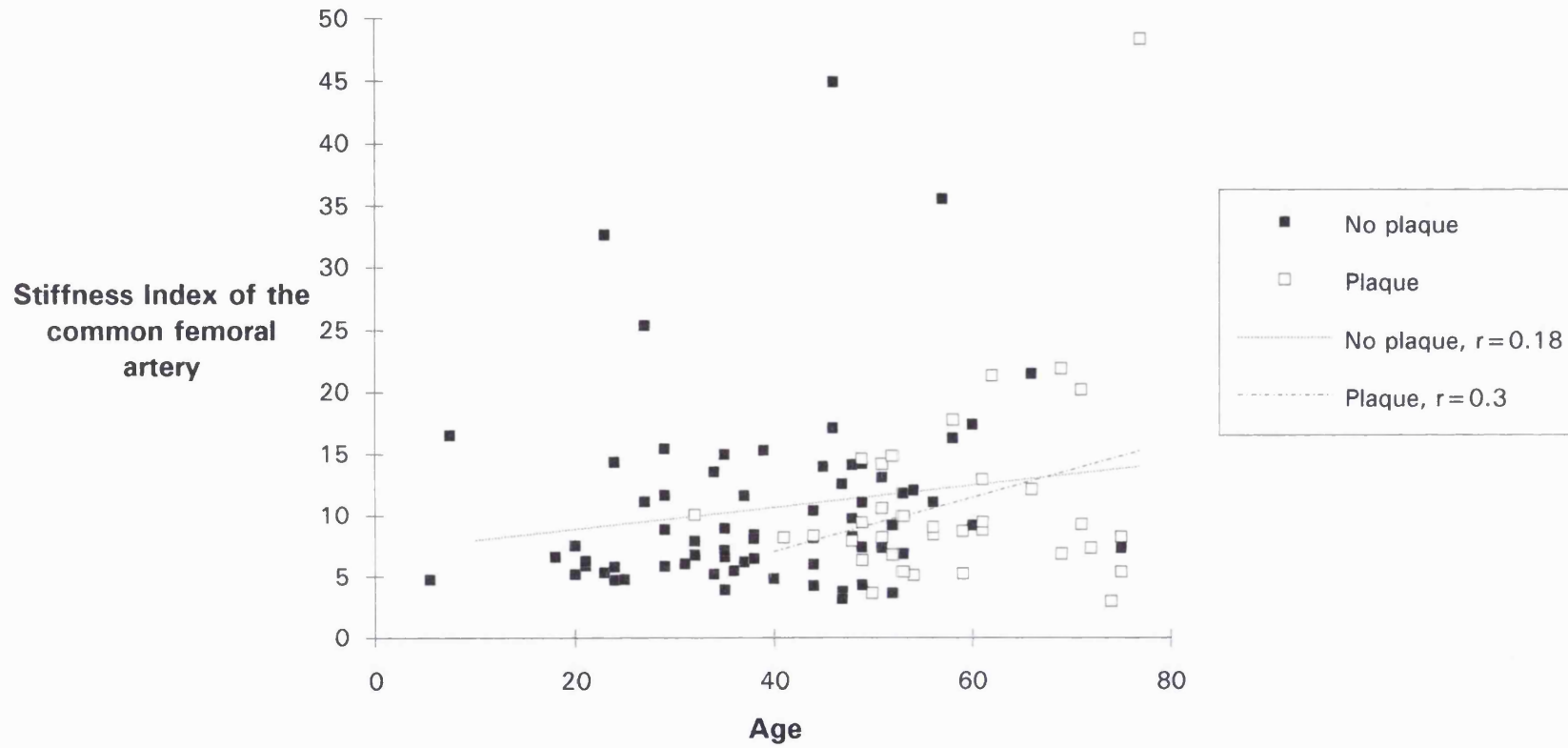
**Correlation between the mean distensibility of the CFA and age**

Elastic modulus of the femoral artery



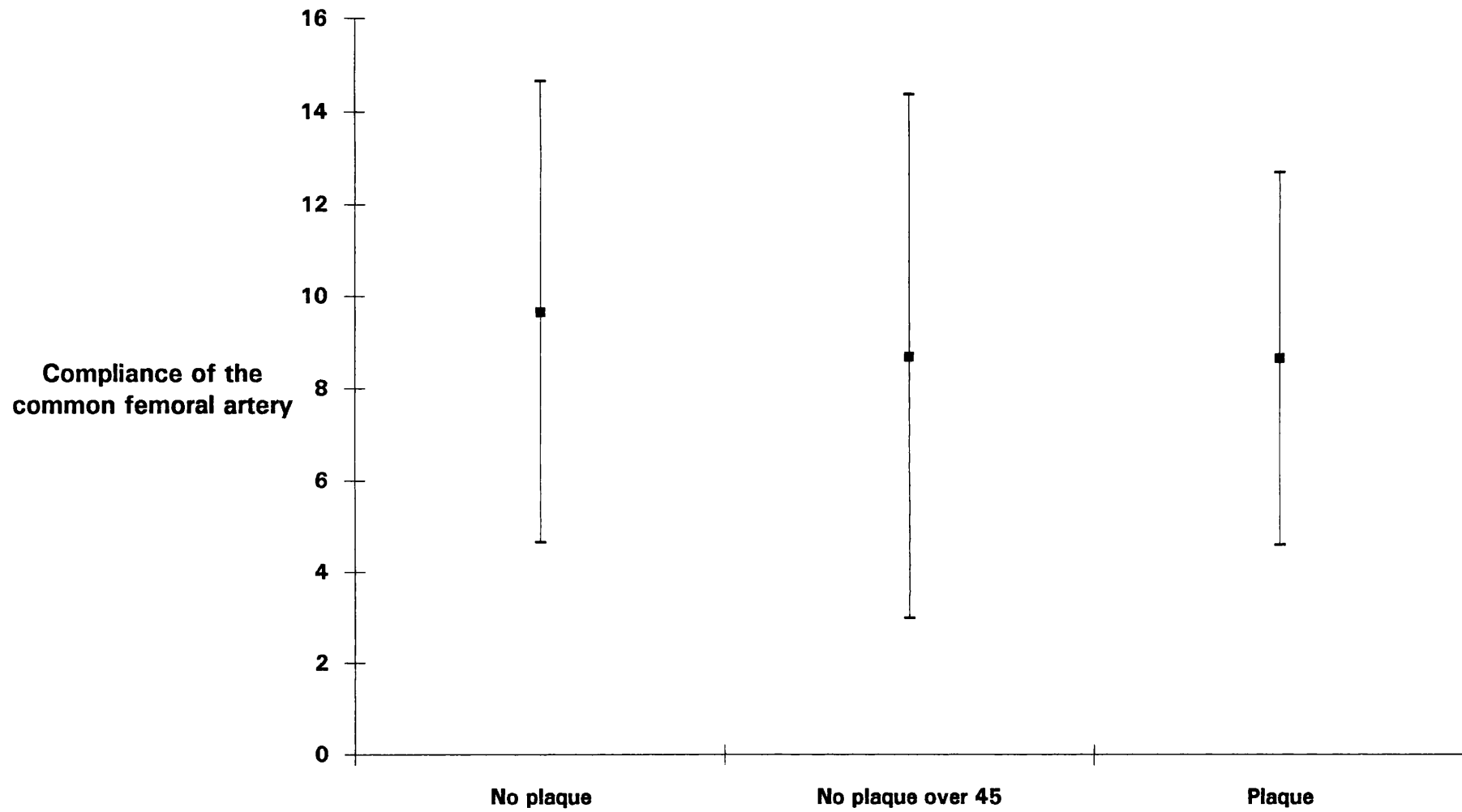
**Fig12.15**

**Correlation between the elastic modulus of the CFA and age**

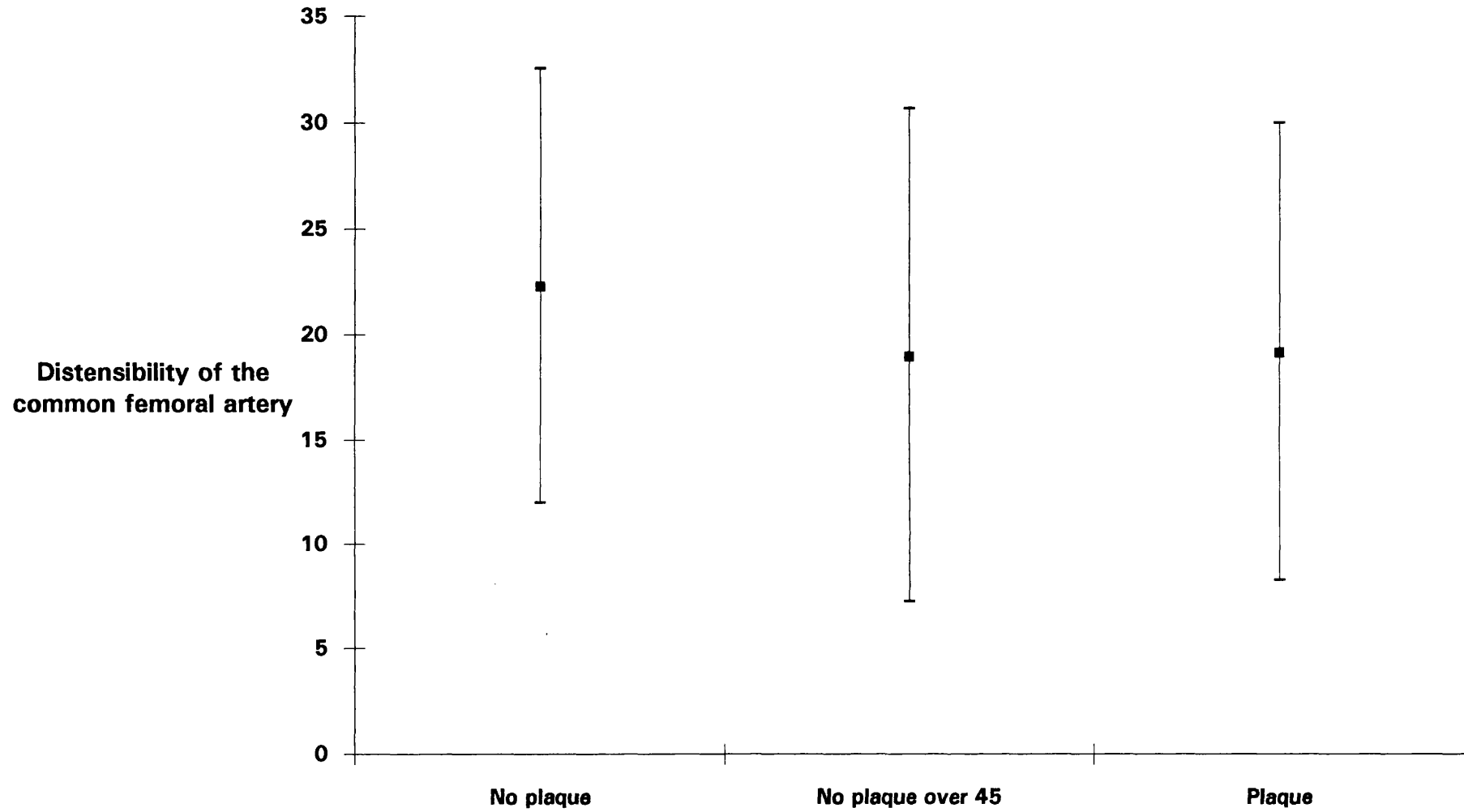


**Fig 12.16**

**Correlation between the stiffness index of the CFA, the presence of plaque and age.**



**Fig 12.17 Comparison between the mean (+/- 1sd) compliance of the femoral artery in young individuals, older individuals without plaque and individuals with plaque**



**Fig 12.18 Comparison between the mean distensibility of young individuals, older individuals without plaque and individuals with plaque.**

### **12.34 The relationship between arterial movement in the common carotid artery and known risk factors.**

As compliance, distensibility, and the elastic modulus are formulae relating to the individual's blood pressure or a derivative thereof, and blood pressure is a known risk factor in atherosclerosis, multiple regression analysis was performed using the relative change in diameter ( $d/D$ ) as the dependent variable and pulse pressure, diastolic pressure, age, sex and packyears of cigarette smoking as independent variables. Table 12.1 shows the contribution of each variable in predicting  $d/D$  and the significance of each component. The derived formula predicting the relative change in diameter of the CCA is:

$$d/D \text{ (mm)} = 0.194 + -0.002 \text{ sex} + -0.0017 \text{ age} + 0.0003 \text{ PP} + -0.00068 \text{ diastolic BP} \\ + 0.0002 \text{ packyears}$$

The main contributing factors are age, diastolic BP and packyears and the formula can be reduced to involve these factors only, with only a minimal reduction in the multiple correlation coefficient

$$d/D \text{ (mm)} = 0.198 + -0.0016 \text{ age} + -0.00061 \text{ diastolic BP} + 0.000205 \text{ packyears.}$$

The multiple correlation coefficient for this formula is 0.78

From the above formula, the relative change in diameter of the vessel decreases with age and diastolic pressure, and increases with the cigarette smoking habit.

**Table 12.1 Multiple linear regression of independent variables contributing to the prediction of common carotid artery d/D and the significance of each component.**

Variable		F	p value
	$c = 0.194152$		
Sex	$b_1 = -0.002147$	0.29	0.59
Age (years)	$b_2 = -0.001735$	105	<0.0001
Pulse pressure (mmHg)	$b_3 = 0.000313$	2.82	0.1
Diastolic BP (mmHg)	$b_4 = -0.00068$	9.64	0.002
Pack years	$b_5 = 0.000212$	4.05	0.05

Analysis of variance:  $F=42.06$ ,  $p<0.0001$

Multiple correlation coefficient (R) = 0.78



**12.35 The relationship between arterial movement in the carotid bulb and known risk factors.**

Multiple regression analysis was performed using the relative change in the carotid bulb diameter as the dependent variable against age, sex, pulse pressure, diastolic pressure and pack years as the independent variables. Table 12.2 shows the contribution of each variable in predicting d/D and the significance of each component. The main contributing factors are age and pulse pressure and the derived formula predicting the relative change in diameter of the carotid bulb can be reduced to :

$$\text{Carotid bulb } d/D = 0.126 + -0.0024 \text{ Age} + 0.00085 \text{ PP}$$

**12.36 The relationship between arterial movement in the femoral artery and known risk factors.**

Multiple regression analysis was performed using the relative change in diameter of the femoral artery as the dependent variable, and age, sex, pulse pressure, diastolic pressure and packyears, but the correlation was poor ( $r=0.17$ ).

**Table 12.2 Multiple linear regression of independent variables contributing to the prediction of carotid bulb d/D and the significance of each component.**

Variable		F	p value
	$c = 0.143267$		
Sex	$b1 = -0.004473$	0.35	0.56
Age (years)	$b2 = -0.002284$	54.75	<0.0001
Pulse pressure (mmHg)	$b3 = 0.00087$	5.56	0.02
Diastolic BP (mmHg)	$b4 = -0.000312$	0.51	0.48
Pack years	$b5 = 0.000044$	0.05	0.82

Analysis of variance : F 16.38,  $p < 0.0001$

Multiple correlation coefficient (R) = 0.73

## **12.4 Discussion**

### ***i) Do compliance, distensibility, elastic modulus and the stiffness index in the carotid and femoral arteries alter age?***

Yes but the three areas studied behave in different ways.

#### **a) The common carotid artery**

The compliance and distensibility of the common carotid artery decreases with age in a linear fashion, whereas the stiffness index and the elastic modulus increase in an exponential fashion with age. This is similar to the findings of Baskett and colleagues, though they report an exponential relationship, this discrepancy can be accounted for by the fact that their study consisted of a large number of children, and the fall in compliance during childhood and adolescence is more marked than in later life (Baskett et al, 1992).

#### **b) The carotid bulb**

In the carotid bulb, the greatest changes occur in the second and third decade with an exponential reduction in compliance and distensibility, matched by an exponential rise in the stiffness index and the elastic modulus.

Reneman and colleagues have previously reported similar differences between common carotid artery and carotid bulb distensibility; they also found that, in younger healthy subjects, the distensibility of the carotid bulb was greater than that of the common carotid artery, but the relationship was reversed with age (Reneman et al, 1985). Subsequently, they have reported that a ratio of distensibility of the carotid bulb to common carotid artery of less than 0.4 is indicative of atherosclerotic disease (van Merode et al, 1989)

#### **c) the femoral arteries**

In the femoral arteries, there is a trend towards a decrease in compliance and distensibility with age, but the scatter is very broad and the correlation poor. The same is true of changes in the elastic modulus and the stiffness index.

***ii) Do compliance, distensibility, elastic modulus and the stiffness index in the carotid and femoral arteries change in the presence of plaque?***

The presence of plaque does not significantly alter the compliance and distensibility at the three sites studied, as the main changes in arterial wall movement occur in the age range prior to the development of plaque.

***iii) How do changes in arterial wall movement relate to known risk factors?***

***a) The common carotid artery***

Using multiple regression analysis, age is the most powerful factor affecting the relative change in the diameter of the carotid artery. The carotid artery is also affected by components of the blood pressure, the most important being diastolic BP. Both age and diastolic pressure have a negative effect on the relative change in diameter of the artery. Smoking, however, has the reverse effect, a factor previously noticed by Riley and co-workers in the ARIC study (Riley et al, 1986).

***b) The carotid bulb***

Again age is the most important single factor which determines change in the arterial wall movement. The carotid bulb is also affected by pulse pressure. Both these factors have a negative effect on the relative change in diameter at the bulb. However, unlike the CCA, smoking habit has little effect on the carotid bulb, presumably because compliance falls rapidly in the first decades, before a significant number of packyears can be built up.

***c) The femoral artery.***

Multiple regression analysis did not contribute any further information.

***iv) How do our results compare to other methods of measuring arterial wall movement and the derived parameters?***

A number of methods to detect arterial wall compliance have been reported. Laogun and Gosling used Doppler-shifted ultrasound to measure pulse wave velocities and calculated the average compliance of various arterial segments. Their initial studies concentrated on the aorta and iliacs and confirmed previous autopsy reports that compliance of arteries decreased with age (Laogun and Gosling, 1982) and they have recently similar results in the common carotid artery (Baskett et al, 1990). Changes in the arterial wall movement

of the carotid artery with age have also been examined using wall tracking devices (Riley, 1986). Using our method of measuring carotid artery movement, we have shown similar trends in arterial wall compliance and distensibility that have been demonstrated in studies using wall tracking equipment but the individual variability is great. Riley has looked at this in the Bogalusa Heart Study and found that the variability within the population is three times greater than that which can be attributed to measurement variability alone (Riley, 1990). Our coefficient of variation for measurement of the carotid artery diameter is 7% (see Table 9.2).

In conclusion, the M-mode image of a conventional ultrasound machine can be used to examine the arterial wall compliance, distensibility and elastic modulus of the carotid and femoral arteries, though correlations with known risk factors can only be demonstrated in the carotid arteries.

CHAPTER 13

THE INTIMA-MEDIA THICKNESS AND ARTERIAL  
WALL MOVEMENT OF NON-INSULIN  
DEPENDENT DIABETICS

### **13.1 Introduction**

Diabetes is associated with a decreased life expectancy due to accelerated atherosclerotic cardiovascular and cerebrovascular disease in diabetics. The risk factors for cardiovascular disease in the general population are well recognized (Kannel) and include cigarette smoking, hypertension, elevated LDL (low density lipoprotein) cholesterol, lowered HDL (high density lipoprotein) cholesterol and possibly raised serum triglycerides. These risk factors are also thought to operate in the genesis of diabetic macrovascular disease but the degree to which each is operative is open to debate.

The aim of this study was to assess the value of the intima-media thickness in predicting the presence of macrovascular disease and discrete plaques in patients with NIDDM and to correlate IM thickness and risk factors with macrovascular disease.

Thus the questions asked were:

- i) Can IM thickness be used to predict the presence of macrovascular disease in NIDDM?*
- ii) Can IM thickness be used to predict the presence of plaques in NIDDM ?*
- iii) What is the relationship between IM thickness, macrovascular disease and known risk factors?*
- iv) How do compliance, distensibility, and the stiffness index alter in NIDDM patients?*

### **13.2 Method**

One hundred and one non-insulin dependent diabetics (NIDDM) who were asymptomatic of cardiovascular or cerebrovascular disease were examined as part of the recruitment procedure to a long term, double blind, controlled study of bezafibrate retard versus placebo for the prevention of vascular disease in NIDDM. Patients were asked to complete a Rose cardiovascular questionnaire and had both a duplex and a high resolution ultrasound examination of their carotids and femorals, a treadmill study and chest wall mapping (see Part III, chap 8 and 9).

Exclusion criteria are listed in table 13.1

**Table 13.1**    **Exclusion criteria**

Classification of D,D+, or E on carotid duplex scanning (see Part III, chap 9)

Symptomatic peripheral vascular disease

History of myocardial infarct

Stable or unstable angina pectoris

Congestive heart failure (NYHA III or IV)

Clinically relevant arrhythmia or conduction disturbance

History of PTCA or coronary artery bypass surgery

Severe diabetic microangiopathy

Severe diabetic neuropathic conditions



### **13.3 Results**

Forty female and sixty-one male NIDDM were studied. In Table 12.2, the risk factor profiles of the men and women included in this study are compared. There was no significant difference between men and women in the IM thickness at two sites (CCA and bulb origin), however the IM thickness of the CFA was significantly thicker in men. Men differed from women in the lipid profiles, with a lower total cholesterol, HDL cholesterol and LDL cholesterol and a higher triglyceride concentration than women.

The intima-media (IM) thickness and arterial ultrasound scores (AUS) for men and women are compared in table 13.3. There was no significant difference between men and women in the mean IM thickness of the CCA or the AUS.

#### **13.31 Comparison between patients with evidence of macrovascular disease and those without.**

Nineteen patients had macrovascular disease, defined as a positive pre-exercise ECG, a positive chest wall mapping stress test or a post exercise Doppler index of 0.8 or less. Table 13.4 compares the characteristics of these patients with those who had no evidence of macrovascular disease. Patients with macrovascular disease were significantly older and had smoked more than those without but due to broad range the latter does not reach a level of statistical significance. There was no significant difference in the mean body mass index, or the systolic and diastolic blood pressure. The NIDDMs with macrovascular disease had a significantly higher total cholesterol level and a significantly lower LDL cholesterol than those without macrovascular disease.

There is a trend for the intima-media thickness of the NIDDMs with macrovascular disease to be thicker than those without, however this only reaches a level of significance at the bulb origin. The predictive power of the IM thickness is poor in this group of NIDDM patients, as only 14% of patients with an IM thickness 0.59mm-0.82mm have macrovascular disease, and only 27% of patients with an IM thickness 0.83mm and over have macrovascular disease (Table 13.5).

**Table 13.2 Comparison between male and female NIDDM**

	Female NIDDM n=40	Male NIDDM n=61		
	Mean $\pm$ 1 sd	Mean $\pm$ 1 sd	t	p value
Age	51.7 $\pm$ 6.8	51.3 $\pm$ 7.56	0.34	NS
Body mass Index	30 $\pm$ 4.5	28 $\pm$ 5	2.05	<0.05
Systolic BP (mmHg)	129 $\pm$ 19	131 $\pm$ 17.2	0.68	NS
Diastolic BP (mmHg)	82 $\pm$ 10	84 $\pm$ 9.22	0.99	NS
Pack years	15.6 $\pm$ 22	15.69 $\pm$ 25	0.79	NS
LIPID PROFILE				
Cholesterol (mmol/l)	6.02 $\pm$ 1.05	5.58 $\pm$ 0.97	2.15	<0.02
Triglycerides (mmol/l)	2.04 $\pm$ 1.05	2.64 $\pm$ 1.59	2.16	<0.02
HDL (mmol/l)	1.19 $\pm$ 0.32	0.94 $\pm$ 0.22	4.09	<0.005
LDL (mmol/l)	3.92 $\pm$ 0.98	3.46 $\pm$ 0.92	2.32	<0.02
Fibrinogen (mmol/l)	5.14 $\pm$ 1.52	4.51 $\pm$ 1.01	2.08	<0.02

**Table 13.3** IM thickness and AUS results for male and female NIDDM

	MEN			WOMEN				
	Mean	Range	sd	Mean	Range	sd	t	p value
IM CCA (mm)	0.84	0.49 - 1.65	0.25	0.83	0.5 - 1.46	0.22	0.21	NS
IM CFA (mm)	0.97	0.43 - 1.56	0.33	0.83	0.4 - 1.5	0.31	2.17	<0.02
AUS	14.6	4 - 26	5.5	13.78	2 - 24	5.2	0.76	NS

**Table 13.4 Comparison between patients with MVD and those without**

	With MVD n=19	Without MVD n=82	t	p value
	Mean $\pm$ 1 sd	Mean $\pm$ 1 sd		
Age	65 $\pm$ 4	50 $\pm$ 8	4.72	<0.005
Body mass index	27.6 $\pm$ 3.9	29.7 $\pm$ 8.5	1.6	NS
Systolic BP (mmHg)	131 $\pm$ 18	130 $\pm$ 18	0.33	NS
Diastolic BP (mmHg)	83 $\pm$ 9	83 $\pm$ 10	0.01	NS
Smoking (packyears)	23.5 $\pm$ 33	12 $\pm$ 21	1.35	NS
<b>Intima-media thickness measurements</b>				
CCA IMT (mm)	0.86 $\pm$ 0.16	0.79 $\pm$ 0.23	1.42	NS
Bulb origin IMT (mm)	1.09 $\pm$ 0.32	0.92 $\pm$ 0.27	2.04	<0.05
CFA IMT (mm)	0.99 $\pm$ 0.35	0.88 $\pm$ 0.32	1.12	NS
<b>LIPIDS</b>				
Total Cholesterol (mmol/l)	6.4 $\pm$ 1.08	5.61 $\pm$ 0.94	2.93	<0.005
Triglyceride (mmol/l)	2.07 $\pm$ 1.35	2.46 $\pm$ 1.41	1.13	NS
HDL (mmol/l)	1.07 $\pm$ 0.29	1.03 $\pm$ 0.28	0.5	NS
LDL (mmol/l)	4.38 $\pm$ 0.98	3.49 $\pm$ 0.9	3.61	<0.005
Fibrinogen (mmol/l)	5.38 $\pm$ 0.74	4.83 $\pm$ 1.32	2.2	<0.02

**Table 13.5 Relationship between IM thickness and macrovascular disease in the NIDDM**

Mean CCA IM thickness	No Macrovascular disease	Macrovascular disease	Total
Up to 0.58mm	6	0 (0%)	6
0.59mm-0.82mm	46	8 (14%)	54
0.83mm and over	30	11 (27%)	41

### **13.32 Comparison between patients with plaque in at least one bifurcation and those without.**

Table 13.6 compares the characteristics of individuals with plaques in at least one bifurcation and those without. There was no significant difference in age sex, body mass index, blood pressure and smoking habit, or lipid profile. However, the intima-media thickness was significantly thicker at all sites in the presence of plaque (Fig 13.1)

Only 6 patients had a CCA intima-media thickness <0.58mm and none of these had plaques at the bifurcations. Of the rest, two-thirds of individuals had plaques at the bifurcations (table 13.7).

### **13.33 The relationship between intima-media thickness and age**

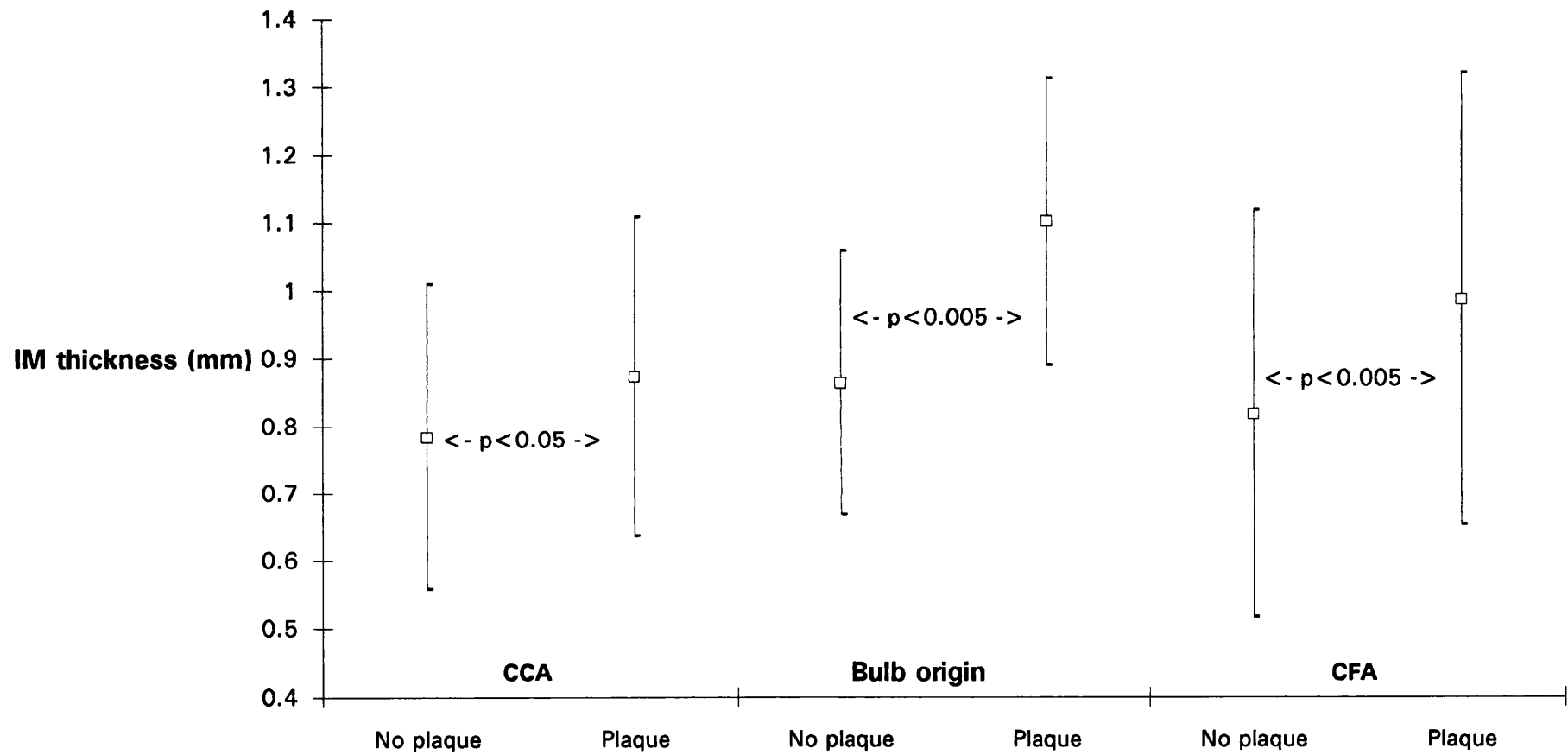
The correlation between age and intima-media thickness in NIDDM is poor. In the absence of plaque, the correlation coefficient for the mean IM thickness of the CCA is only 0.22 (Fig. 1. .2). The correlation is no better for the origin of the bulb, mid bulb or common femoral arteries ( $r=0.24$ ,  $r=0.16$ , and  $r=0.26$ , respectively)

### **13.34 The relationship between intima-media thickness and risk factors**

Multiple regression analysis was performed using sex, age, smoking habit (pack years), systolic blood pressure, diastolic blood pressure, body mass index, the presence of macrovascular disease, and lipids (Total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) as independent variables. Poor correlation was found between the mean intima media thickness of the CCA and the above risk factors.

**Table 13.6 Comparison between NIDDM patients with plaques at the bifurcations compared to those without.**

	With plaque n=60	Without plaque n=41	t	p value
	Mean $\pm$ 1 sd	Mean $\pm$ 1 sd		
Age	52 $\pm$ 7.7	50.7 $\pm$ 6.5	0.94	NS
Body mass index	31.1 $\pm$ 16.7	29.6 $\pm$ 10.7	0.56	NS
Systolic BP (mmHg)	130 $\pm$ 17	131 $\pm$ 19	0.21	NS
Diastolic BP (mmHg)	82.5 $\pm$ 10	84 $\pm$ 10	0.76	NS
Smoking (packyears)	16 $\pm$ 26	12 $\pm$ 21	0.01	NS
<b>Intima-media thickness measurements</b>				
CCA IMT (mm)	0.87 $\pm$ 0.23	0.78 $\pm$ 0.23	1.86	<0.05
Bulb origin IMT (mm)	1.09 $\pm$ 0.21	0.88 $\pm$ 0.19	4.97	<0.0005
CFA IMT (mm)	0.98 $\pm$ 0.33	0.81 $\pm$ 0.3	2.72	<0.0005
<b>LIPIDS</b>				
Total Cholesterol (mmol/l)	5.8 $\pm$ 1.03	5.72 $\pm$ 1.02	0.37	NS
Triglyceride (mmol/l)	2.57 $\pm$ 1.5	2.15 $\pm$ 1.24	1.51	NS
HDL (mmol/l)	0.99 $\pm$ 0.29	1.1 $\pm$ 0.82	1.65	NS
LDL (mmol/l)	3.63 $\pm$ 0.93	3.67 $\pm$ 1.03	0.2	NS
Fibrinogen (mmol/l)	4.89 $\pm$ 1.07	4.59 $\pm$ 1.47	1.05	NS

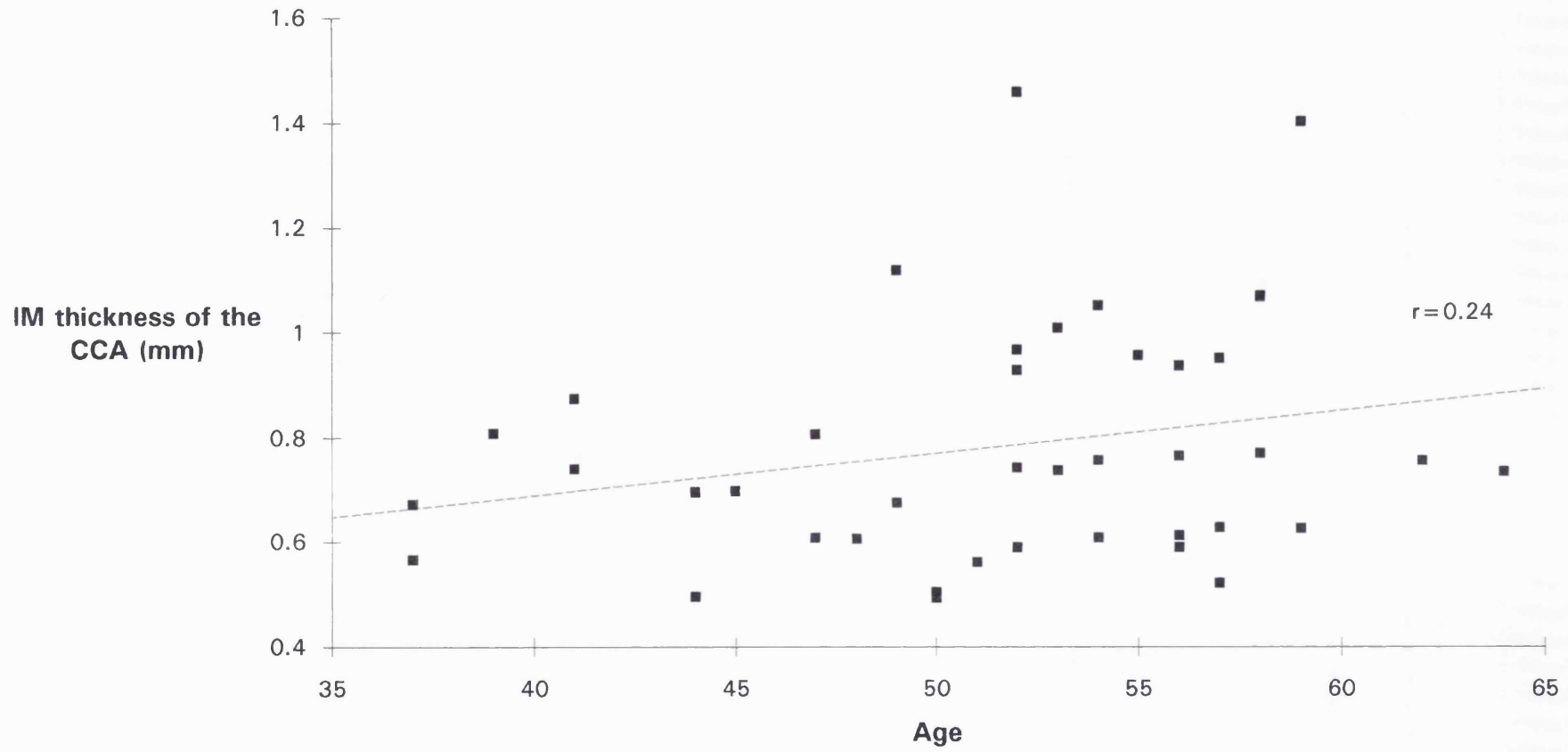


**Fig 13.1 Mean (+/- 1sd) IM thickness with and without plaque in the CCA, at the bulb origin and in the CFA**



**Table 13.7 Relationship between IM thickness and plaque in the NIDDM**

Mean CCA IM thickness	No plaque	Plaque	Total
Up to 0.58mm	6	0 (0%)	6
0.59mm-0.82mm	22	33 (60%)	55
0.83mm and over	13	27 (67.5%)	40



**Fig 13.2**

**The relationship between CCA intima-media thickness and age in NIDDM without plaques**

### **13.35 The relationship between plaque, macrovascular disease and risk factors**

Multiple regression analysis was performed using sex, age, smoking habit (pack years), systolic blood pressure, diastolic blood pressure, body mass index, and lipids (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) as independent variables. The presence of both plaque and macrovascular disease were best predicted by age and sex. The presence of macrovascular disease was best predicted by the following formula :

$$\text{MVD} = -1.014 + 0.0146 \text{ age} + 0.122 \text{ sex} \text{ (Table 13.8)}$$

and the presence of plaque was best predicted by the following formula:

$$\text{Plaque} = 0.1933 + 0.0136 \text{ age} + -0.3 \text{ sex} \text{ (Table 13.9)}$$

### **13.36 Compliance, distensibility, and the stiffness index in NIDDM**

#### **a) common carotid artery**

The correlation between the mean compliance and the mean distensibility of the CCA and age for individuals without plaques is shown in figures 13.3 and 13.4 . The correlation coefficients for the regression lines are 0.46 and 0.42 respectively The stiffness index of the CCA rises with age, and the correlation coefficient for the regression line is 0.33. (Fig 13.5)

In the presence of plaque, the correlation between compliance and age is poor ( $r=0.16$ ) and the compliance of these individuals was not significantly different from those without plaque. A similar relationship was observed for the distensibility and the stiffness index of the CCA.

#### **b) carotid bulb**

In the absence of plaque, the mean compliance and the mean distensibility of the carotid bulb fell exponentially with age ( $r=0.35$  and  $r=0.38$  respectively ) while the stiffness index increased exponentially with age ( $r= 0.37$  ). There was no significant difference between individuals with plaque and those without in the mean compliance, distensibility, or the stiffness index of the carotid bulb

**Table 13.8 Multiple linear regression of independent variables contributing to the prediction of macrovascular disease in NIDDM and the significance of each component.**

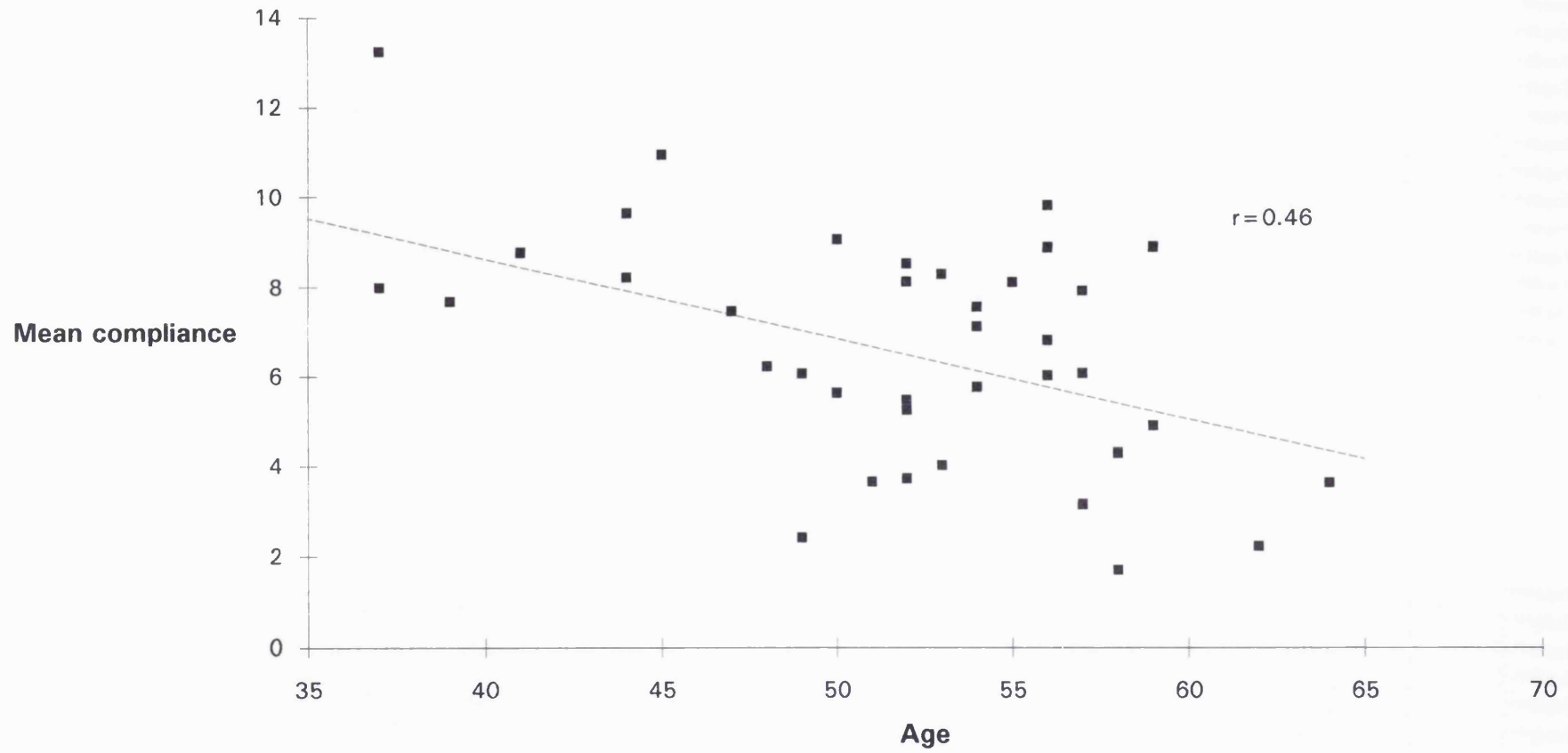
		F	p value
	c= -1.013923		
Age (years)	b1 = 0.014609	7.7	0.0066
Sex	b2 =0.122203	10.1	0.002

**(Multiple correlation coefficient R=0.43, and in an analysis of variance: F=10.8, p<0.01)**

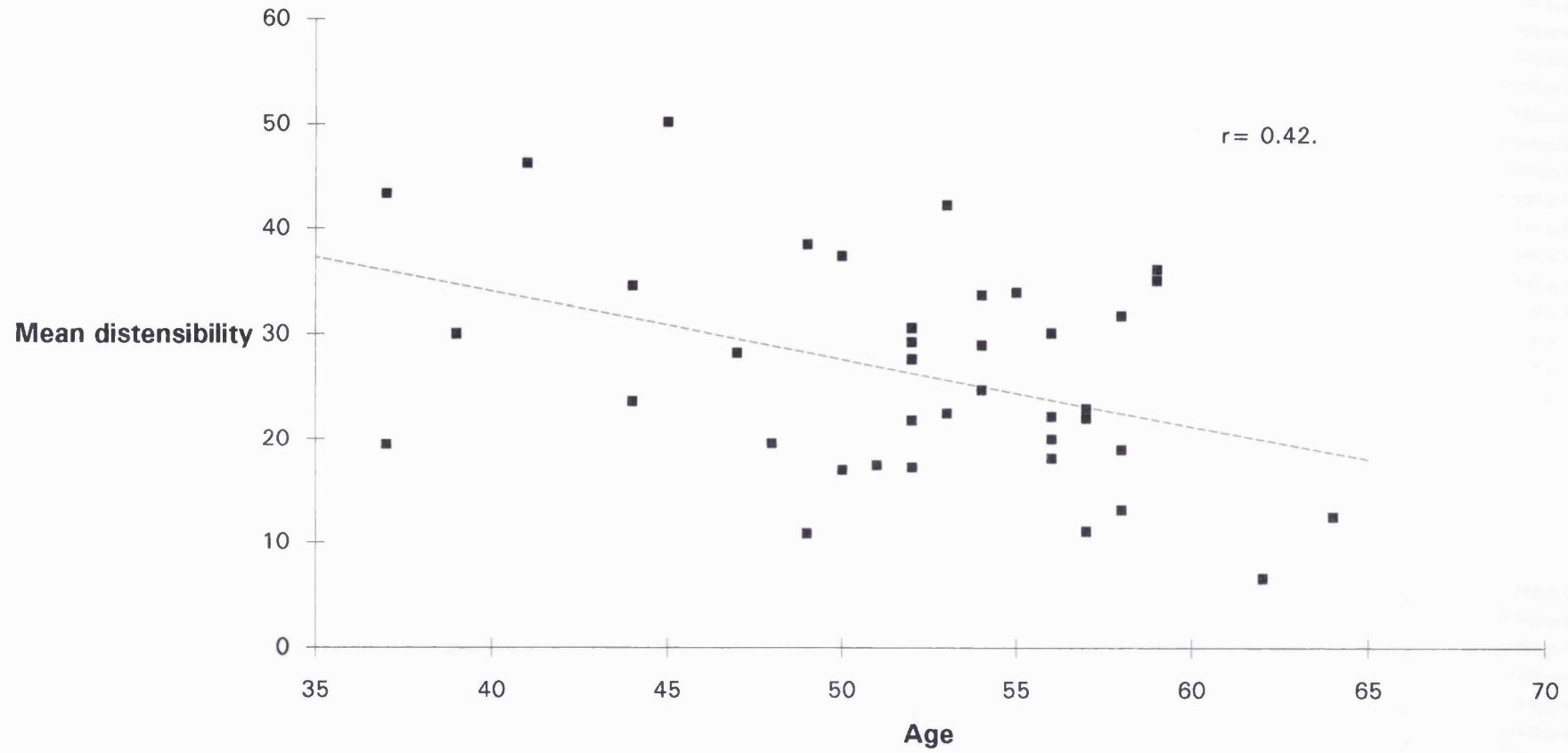
**Table 13.9 Multiple linear regression of independent variables contributing to the prediction of bifurcation plaques in NIDDM and the significance of each component**

		F	p value
	c=0.193341		
Age (years)	b1 = 0.013608	3.72	0.0564
Sex	b2 = - 0.300445	3.35	0.0702

(Multiple correlation coefficient R=0.26)

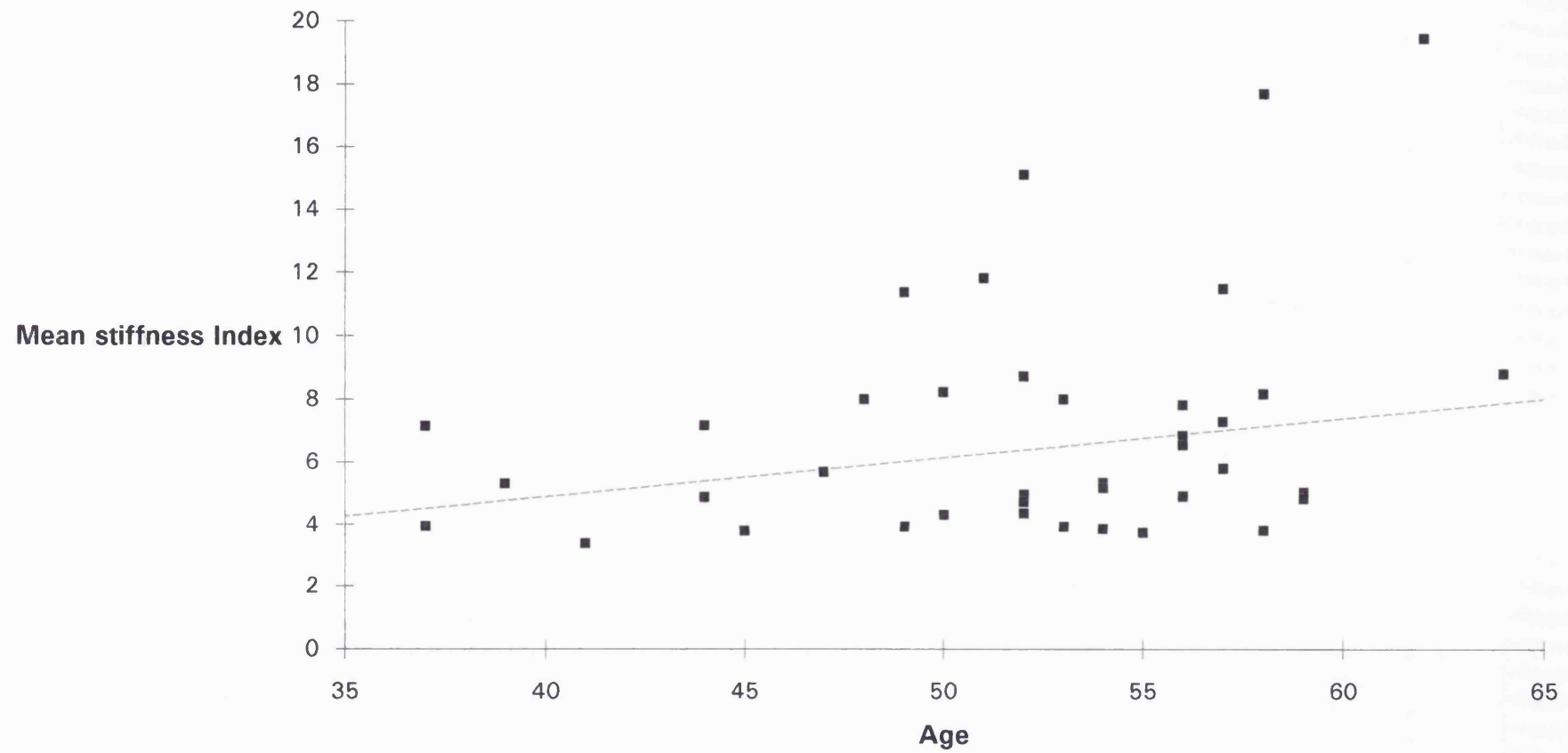


**Fig 13.3** The correlation between compliance of the CCA and age in NIDDM patients without plaques.



**Fig 13.4**

**The correlation between distensibility of the CCA and age, in NIDDM without plaques**



**Fig 13.5**

**Correlation between the stiffness index of the CCA and age in NIDDM patients without plaques**



c) common femoral artery

No correlation could be demonstrated between compliance, distensibility and the stiffness index of the common femoral artery and age in the absence of plaque. There was no significant difference between individuals with plaque and those without in the mean compliance, distensibility, or stiffness index of the common femoral artery.

## **13.4 Discussion**

### ***i) Can IM thickness be used to predict the presence of macrovascular disease in NIDDM?***

No. One fifth of our patients had evidence of macrovascular disease and the IM thickness was not a good predictor of its presence.

### ***ii) Can IM thickness be used to predict the presence of plaques in NIDDM?***

NIDDM patients have a thick intima-media layer on ultrasound and increasing IM thickness is not a good predictor of the presence of plaque. Though no patient with an intima media thickness less than 0.58mm had plaque, the majority of patients had an intima thickness greater than 0.58mm and there was no difference in the number of patients with plaque and an IM thickness of between 0.5mm and 0.82 mm and those with an IM thickness over 0.82mm. This is in contrast to the results reported in Chapter 11, where intima-media thickness was a good predictor of the presence of plaque in our volunteer group..

One explanation for the above observation is that the IM thickening represents a diffuse or global response to a disease process such as diabetes whereas plaques are a localised phenomena, in response to local flow disturbances. Alternatively, the extensive diffuse IM thickening observed in NIDDM may mask the presence of localised plaques.

### ***iii) What is the relationship between IM thickness, macrovascular disease and known risk factors?***

In NIDDM, the IM thickness correlates poorly with known risk factors including lipids.

The best predictors of both the presence of macrovascular disease and the presence of plaque were the age and the sex of the subject.

### ***iv) How does compliance, distensibility, and the stiffness index in the carotid and femoral arteries alter with age and the presence of plaque in NIDDM?***

Compliance and distensibility of the CCA fall with age in the absence of plaque, but there is little difference in the mean compliance and distensibility of the CCA in individuals with plaque compared to those without plaque. The stiffness index increases

in an exponential fashion, but again there is little difference between individuals with plaque and those without.

Compliance and distensibility of the carotid bulb falls exponentially in the absence of plaques whereas the stiffness index rise exponentially.

No correlation was observed between the arterial wall movement of the femoral artery and age, or the presence of plaque.

These findings are compatible with the results of arterial wall movement studies in the volunteer group, which also showed that, in the absence of plaque, compliance and distensibility of the carotid artery fell with age whereas the stiffness index increased with age but by the age of 45 there was little difference between individuals with plaques compared to those without.

CHAPTER 14

THE INTIMA-MEDIA THICKNESS AND ARTERIAL  
WALL MOVEMENT OF INSULIN DEPENDENT  
DIABETICS

## **14.1 Introduction**

Like NIDDM patients, insulin dependent diabetics (IDDM) have a very high incidence of macrovascular disease, however much of the mortality data for IDDM has to be derived from large studies in which the type of diabetes has to be inferred from use of insulin, or the age of onset of diabetes. Despite this, cross-sectional studies (Reckless) have shown the prevalence of all types of vascular disease is increased in insulin treated men and women and to be most marked in individuals with an elevated LDL-cholesterol.

In view of the high incidence of macrovascular disease in insulin dependent diabetes, we decided to study a group of insulin-dependent diabetic patients as a comparison to our NIDDM group. Therefore the aim of this study was to assess the value of the intima-media thickness in predicting the presence of macrovascular disease and discrete plaques in patients with NIDDM and to correlate IM thickness and risk factors with macrovascular disease.

Thus the questions asked were:

- i) Can the IM thickness be used to predict the presence of macrovascular disease in IDDM?*
- ii) Can the IM thickness be used to predict the presence of plaques in IDDM?*
- iii) What is the relationship between IM thickness and known risk factors?*
- iv) How do compliance, distensibility, elastic modulus and the stiffness index alter in IDDM patients?*

## **14.2 Method**

IDDM patients were recruited from the out-patients clinic. The presence of macrovascular disease was determined by clinical examination, Minnesota coded electrocardiography and Rose cardiovascular questionnaires (See Part III, method). These patients were also examined with high resolution ultrasound, the CCA IM thickness was measured and the carotid and femoral bifurcations were examined for plaque and the arterial ultrasound score (AUS). A fasting blood sample was taken for lipid profile estimation.

### **14.3 Results**

Fifty two insulin dependent diabetic patients were studied. The characteristics of this group of patients are shown in table 14.1. Male and female IDDM patients are compared in table 14.2 and there was no significant difference in age, body mass index, blood pressure, smoking habit, IM thickness, arterial ultrasound score, the incidence of macrovascular disease and the lipid profile.

#### **14.31 Comparison between patients with evidence of macrovascular disease and those without.**

In table 14.3, the IDDM patients with macrovascular disease are compared with those without. There was no significant difference in age and body mass index between patients with macrovascular disease and those without, however, patients with macrovascular disease had significantly higher blood pressures and a longer smoking habit. There was no significant difference in the lipid profiles between patients with and without macrovascular disease.

The IM thickness was significantly thicker in patients with macrovascular disease than those without. Furthermore, CCA IM thickness is a good predictor of the presence of macrovascular disease in patients with IDDM as shown in table 14.5.

#### **14.32 Comparison between patients with evidence of plaque and those without.**

The characteristics of individuals with plaques are compared to those without in table 14.5. Individuals with plaques were older and had significantly higher blood pressures, however there was no significant difference in their body mass index and their smoking habit. Individuals with plaques had significantly higher total cholesterol, but there was no significant difference in the rest of the lipid profile.

The IM thickness was thicker in the presence of plaque at all sites measured but this difference only reached a level of significance in the CCA and the CFA (Fig 14.1). CCA IM thickness is also a good predictor of the presence of plaque in IDDM (Table 14.6).

**Table 14.1 Risk factor profile for the IDDM patients**

	Mean $\pm$ 1sd	Range
Age	46.2 $\pm$ 13.57	24 - 75
Body mass Index	23.95 $\pm$ 2.76	20 - 35
Systolic BP (mmHg)	135 $\pm$ 21	95 - 180
Diastolic BP (mmHg)	77 $\pm$ 11	33 - 107
Pack years	13.72 $\pm$ 17.7	0 - 52
<b>LIPID PROFILE</b>		
Cholesterol	5.37 $\pm$ 0.9	3.49 - 7.96
Triglycerides	1.09 $\pm$ 0.68	0.26 - 3.94
HDL	1.58 $\pm$ 0.35	0.9 - 2.53
LDL	3.32 $\pm$ 0.84	1.74 - 5.63
Fibrinogen	4.34 $\pm$ 1	2.3 - 6.9

**Table 14.2 Comparison between male and female IDDM**

	Men n=29	Women n=23		
	Mean $\pm$ 1sd	Mean $\pm$ 1sd	t	p value
Age	47.4 $\pm$ 14.9	44.2 $\pm$ 11.6	0.87	NS
Body mass Index	23.9 $\pm$ 2.9	24 $\pm$ 2.6	0.48	NS
Systolic BP (mmHg)	133 $\pm$ 16.2	138 $\pm$ 25	0.9	NS
Diastolic BP (mmHg)	77 $\pm$ 10	77 $\pm$ 12	0.03	NS
Pack years	15.8 $\pm$ 18.5	11.7 $\pm$ 16.78	0.95	NS
<b>Intima-media thickness measurements</b>				
CCA IMT (mm)	0.76 $\pm$ 0.17	0.72 $\pm$ 0.21	0.71	NS
Bulb origin IMT (mm)	0.94 $\pm$ 0.27	0.88 $\pm$ 0.2	0.73	NS
CFA IMT (mm)	0.77 $\pm$ 0.3	0.87 $\pm$ 0.28	1.14	NS
Mean AUS	13.2 $\pm$ 6.9	11.8 $\pm$ 7.3	0.71	NS
Number with MVD	8/29	4/23		
<b>LIPID PROFILE</b>				
Cholesterol (mmol/l)	5.47 $\pm$ 0.85	5.23 $\pm$ 0.98	0.79	NS
Triglycerides (mmol/l)	1.16 $\pm$ 0.77	0.98 $\pm$ 0.5	0.89	NS
HDL (mmol/l)	1.58 $\pm$ 0.32	1.58 $\pm$ 0.4	0	NS
LDL (mmol/l)	3.37 $\pm$ 0.86	3.25 $\pm$ 0.83	0.49	NS
Fibrinogen (mmol/l)	4.23 $\pm$ 1.06	4.49 $\pm$ 0.93	0.84	NS



**Table 14.3 Risk factors and the presence of MVD in IDDM**

	MVD present n=12	MVD absent n=40		
	Mean $\pm$ 1sd	Mean $\pm$ 1sd	t	p value
Age	50.8 $\pm$ 13.6	44.5 $\pm$ 13.7	1.4	NS
Body mass Index	24.7 $\pm$ 3.7	24 $\pm$ 2.3	0.9	NS
Systolic BP (mmHg)	150 $\pm$ 18	130 $\pm$ 18	3.19	<0.001
Diastolic BP (mmHg)	84 $\pm$ 12	75 $\pm$ 8	2.31	<0.01
Pack years	23 $\pm$ 21	11 $\pm$ 16	1.8	<0.05
<b>Intima-media thickness measurements</b>				
CCA IMT (mm)	0.89 $\pm$ 0.19	0.7 $\pm$ 0.17	3.12	<0.001
Bulb origin IMT (mm)	1.14 $\pm$ 0.22	0.86 $\pm$ 0.22	3.06	<0.001
CFA IMT (mm)	0.95 $\pm$ 0.23	0.75 $\pm$ 0.3	1.96	<0.05
Mean AUS	15.8 $\pm$ 4.7	11.8 $\pm$ 7.5	2.17	<0.025
<b>LIPID PROFILE</b>				
Cholesterol (mmol/l)	5.64 $\pm$ 1.2	5.27 $\pm$ 0.77	0.98	NS
Triglycerides (mmol/l)	1.22 $\pm$ 0.66	1.03 $\pm$ 0.68	0.8	NS
HDL (mmol/l)	1.52 $\pm$ 0.52	1.6 $\pm$ 0.27	0.5	NS
LDL (mmol/l)	3.59 $\pm$ 1.07	3.22 $\pm$ 0.74	1.02	NS
Fibrinogen (mmol/l)	4.74 $\pm$ 0.79	4.15 $\pm$ 1.02	1.95	<0.05

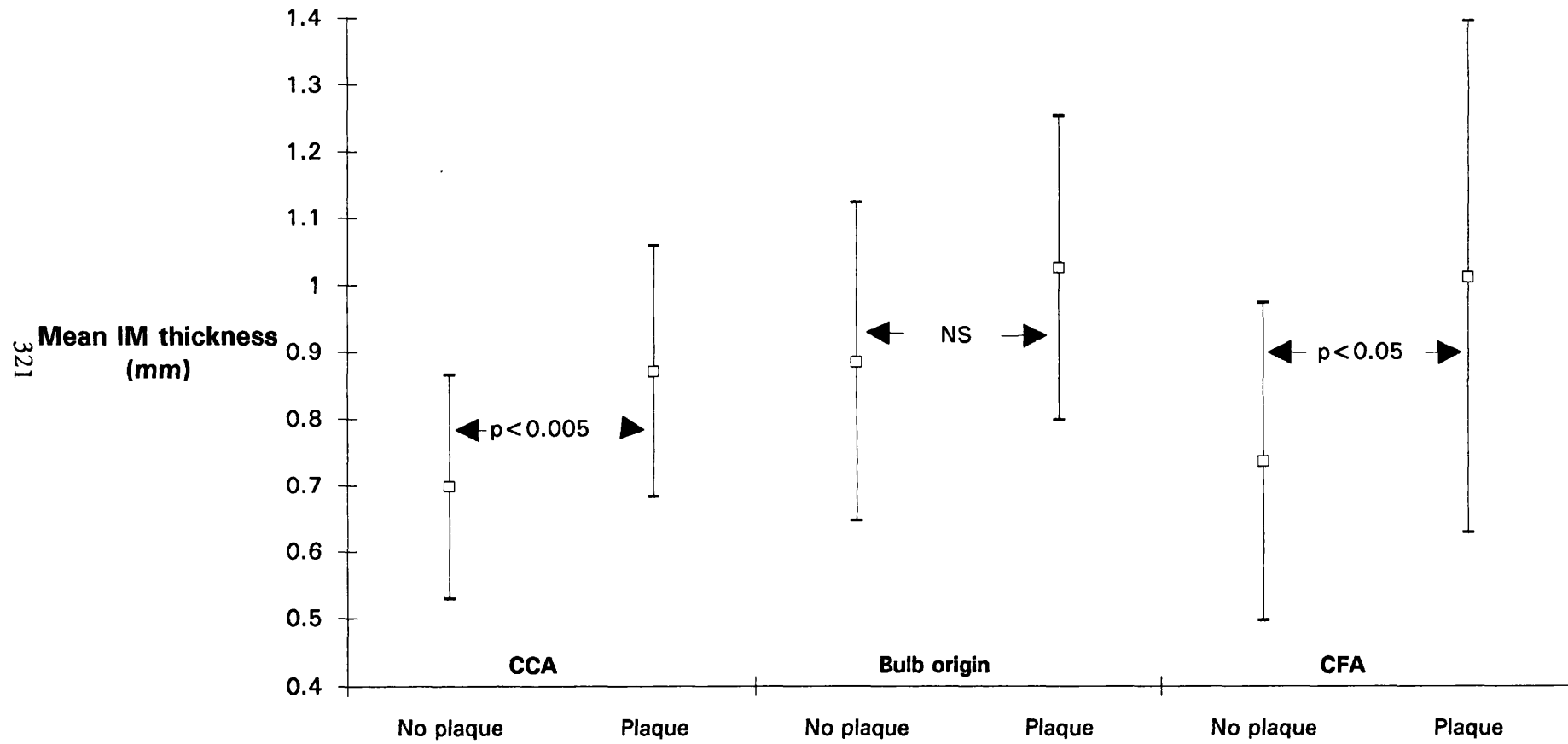
**Table 14.4 The relationship between IM thickness and the presence of macrovascular disease in IDDM**

Mean CCA IM thickness	MVD absent	MVD present	Total
Up to 0.58mm	10	0 (0%)	10
0.59mm-0.82mm	22	4 (15%)	26
0.83mm and over	8	8 (50%)	16

$\chi^2 = 10.4$  to 2 degrees of freedom  $p < 0.01$

**Table 14.5 Comparison between IDDM patients with and without plaque**

	Plaque present n=23	Plaque absent n=29		
	Mean $\pm$ 1sd	Mean $\pm$ 1sd	t	p value
Age	55.4 $\pm$ 12.4	42.6 $\pm$ 12.4	3.31	<0.001
Body mass Index	23.7 $\pm$ 2	24.1 $\pm$ 3	0.48	NS
Systolic BP (mmHg)	146 $\pm$ 23	131 $\pm$ 19	2.16	<0.025
Diastolic BP (mmHg)	83 $\pm$ 12	75 $\pm$ 9	2.03	<0.025
Pack years	14.6 $\pm$ 15.6	10.2 $\pm$ 14.7	0.87	NS
<b>Intima-media thickness measurements</b>				
CCA IMT (mm)	0.87 $\pm$ 0.19	0.7 $\pm$ 0.17	3.02	<0.005
Bulb origin IMT (mm)	1.03 $\pm$ 0.23	0.88 $\pm$ 0.23	1.52	NS
Bulb IMT (mm)	0.76 $\pm$ 0.2	0.69 $\pm$ 0.17	0.85	NS
CFA IMT (mm)	1.01 $\pm$ 0.38	0.73 $\pm$ 0.24	1.93	<0.05
Mean AUS	15 $\pm$ 7.8	11.8 $\pm$ 6.7	1.36	NS
<b>LIPID PROFILE</b>				
Cholesterol (mmol/l)	5.86 $\pm$ 0.71	5.17 $\pm$ 0.9	2.7	<0.005
Triglycerides (mmol/l)	0.95 $\pm$ 0.36	1.14 $\pm$ 0.76	1.13	NS
HDL (mmol/l)	1.64 $\pm$ 0.4	1.55 $\pm$ 0.33	0.73	NS
LDL (mmol/l)	3.79 $\pm$ 0.84	3.1 $\pm$ 0.77	2.5	NS
Fibrinogen (mmol/l)	4.69 $\pm$ 1.13	4.2 $\pm$ 0.93	1.32	NS



**Fig 14.1** Mean (+/- 1 sd) IM thickness with and without plaque in the CCA, at the bulb origin, and in the CFA

**Table 14.6** The relationship between CCA IM thickness and the presence of bifurcation plaques

Mean CCA IM thickness	No plaque	Plaque	Total
Up to 0.58mm	9	1 (10%)	10
0.59mm-0.82mm	17	9 (35%)	26
0.83mm and over	3	13 (81%)	16

$\chi^2 = 14.61$ , to 2 degrees of freedom  $p < 0.001$

### **14.33 The relationship between intima-media thickness and age**

The correlation between age and intima-media thickness in IDDM is moderately good. In the absence of plaque, the correlation coefficient for the mean IM thickness of the CCA is 0.58 (fig 14.2). The correlation between age and IM thickness at other sites is less good, the correlation coefficients for the origin of the bulb, mid bulb and CFA are 0.27, 0.41, and 0.52 respectively.

### **14.34 The relationship between intima-media thickness and risk factors**

Multiple regression analysis was performed using age, sex, packyears, diastolic BP, systolic BP and BMI as independent variables on the risk factors. The CCA IM thickness can be predicted from the following formula:

$$\text{Mean CCA IMT} = -0.326 + 0.0096 \text{ age} - 0.0011 \text{ py} + 0.02 \text{ sex} + 0.001 \text{ systolic BP} + 0.0033 \text{ diastolic BP} + 0.01 \text{ BMI}$$

The correlation coefficient for this formula is 0.80. The best subset is:

$$\text{Mean CCA IMT} = -0.069 + 0.0098 \text{ age} + 0.0047 \text{ packyears, and the correlation coefficient for this formula is 0.77}$$

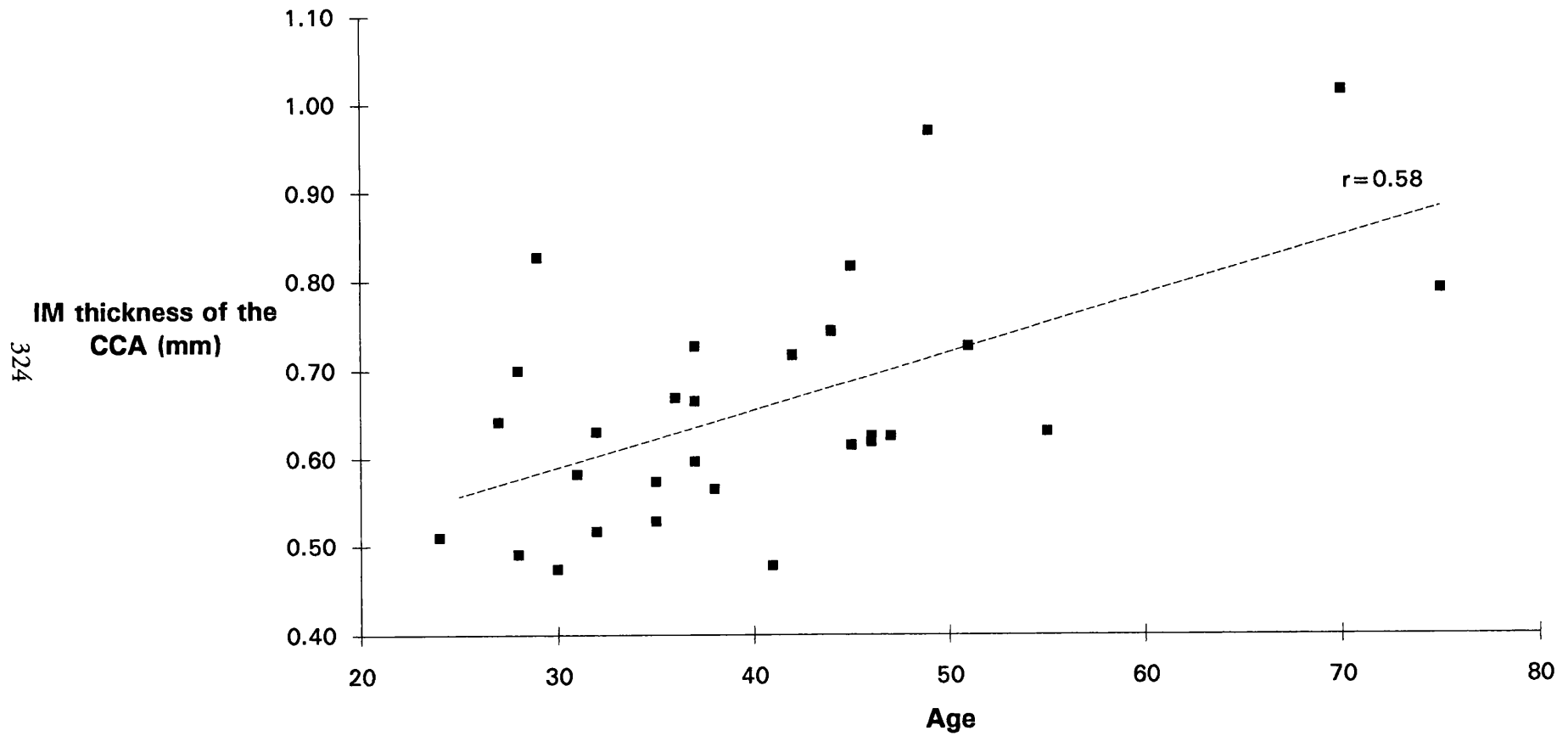
Lipid data were available for 43 IDDM. Table 14.8 shows the predictive strength of each variables if the lipid results are included in an the multiple regression analysis and CCA IM thickness can be predicted from the following formula:

$$\text{CCA IMT} = -0.256 + 0.01 \text{ age} + -0.0015 \text{ packyears} + 0.0186 \text{ sex} + 0.0016 \text{ systolic BP} + 0.0044 \text{ diastolic BP} + 0.0066 \text{ BMI} + -0.082 \text{ triglyceride} + 0.24 \text{ cholesterol} + -0.325 \text{ HDL3} + -0.247 \text{ HDL2} + -0.264 \text{ LDL}$$

The correlation coefficient for this formula is 0.85.

The most important factors are still age and packyears and the best subset is represented by the following formula:

$$\text{CCA IMT} = -0.189 + 0.01 \text{ age} + 0.006 \text{ packyears. The correlation coefficient for this formula is 0.81.}$$



**Fig 14.2**

**The relationship between intima-media thickness and age in IDDM without plaques**

**Table 14.7 Multiple linear regression of independent variables contributing to the prediction of the CCA IM thickness and the significance of each component in IDDM**

	$c = -0.256321$		
Age	$b1 = 0.010451$	$F = 32.4731$	$p < 0.0001$
Packyears	$b2 = -0.001527$	$F = 1.381947$	$p = 0.2462$
Sex	$b3 = -0.18646$	$F = 0.21417$	$p = 0.6459$
Systolic BP	$b4 = 0.001588$	$F = 1.333418$	$p = 0.2546$
Diastolic BP	$b5 = 0.004423$	$F = 4.002963$	$p = 0.0518$
Body mass index	$b6 = 0.006615$	$F = 0.674965$	$p = 0.4159$
Triglyceride	$b7 = -0.081209$	$F = 0.405799$	$p = 0.5275$
Total cholesterol	$b8 = 0.241833$	$F = 0.915104$	$p = 0.3441$
HDL3	$b9 = -0.325103$	$F = 1.482976$	$p = 0.2299$
HDL2	$b10 = -0.284716$	$F = 1.066964$	$p = 0.3074$
LDL	$b11 = -0.264518$	$F = 1.05971$	$p = 0.3090$

$F = 7.446, p < 0.0001. r = 0.85$



### **14.35 The relationship between plaque, macrovascular disease and risk factors**

Multiple regression analysis was performed using sex, age, smoking habit (pack years), systolic blood pressure, diastolic blood pressure, body mass index, and lipids (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) as independent variables.

#### **Macrovascular disease**

The presence of macrovascular disease is best predicted by:

$$\text{MVD} = -1.1 - 0.015 \text{ age} + 0.0078 \text{ py} + 0.1 \text{ sex} + 0.006 \text{ systolic BP} + 0.002 \text{ diastolic BP} + 0.002 \text{ BMI} + 1.19 \text{ CCA IM thickness (Table 14.8)}$$

and the correlation coefficient (r) for this formula is 0.61. The best subset is represented by :

$$\text{MVD} = -0.989 - 0.016 \text{ age} + 0.0079 \text{ py} + 0.1 \text{ sex} + 0.006 \text{ systolic BP} + 1.24 \text{ diastolic BP}$$

and the correlation coefficient (r) for this formula is still 0.61.

#### **Plaque**

The presence of plaque is best predicted by the following formula:

$$\text{Plaque} = -1.35 + 0.0088 \text{ age} + 0.0017 \text{ packyears} + 0.1 \text{ sex} - 0.00022 \text{ systolic BP} + 0.014 \text{ diastolic BP} - 0.008 \text{ BMI} + 0.63 \text{ CCA IM (table 14.9)}$$

the correlation coefficient for this formula is 0.61 and the best subset is represented by the following:

$$\text{Plaque} = -1.535 + 0.016 \text{ age} + 0.016 \text{ packyears} \quad (r=0.58)$$

Table 14.8 Multiple linear regression of independent variables contributing to the prediction of macrovascular disease in IDDM, and the significance of each component.

		F	p value
	c=-1.104256		
Age (years)	b1 = -0.01485	5.33	0.025
Pack years	b2 = - 0.007831	5.74	0.02
Sex	b3 = 0.098658	0.869	0.355
Systolic BP	b4 = 0.005625	2.63	0.111
Diastolic BP	b5 = 0.002224	0.144	0.705
BMI	b6 = 0.001629	0.0062	0.937
CCA IM thickness	b7 = 1.188662	7.085	0.01

Multiple correlation coefficient R= 0.61, F=3.76, p<0.003.

**Table 14.9 Multiple linear regression of independent variables contributing to the prediction of bifurcation plaques in IDDM, and the significance of each component**

		F	p value
	$c = -1.353791$		
Age (years)	$b_1 = 0.008837$	1.37	0.25
Pack years	$b_2 = -0.00168$	0.19	0.66
Sex	$b_3 = 0.107598$	0.75	0.39
Systolic BP	$b_4 = -0.00022$	0.003	0.96
Diastolic BP	$b_5 = 0.013706$	3.97	0.05
BMI	$b_6 = -0.008028$	0.11	0.74
CCA IM thickness	$b_7 = 0.634287$	1.46	0.23

Analysis of variance:  $F=3.81$ ,  $p=0.0026$

### **14.36 Compliance, distensibility and the stiffness index in IDDM**

The mean distensibility was significantly less in individuals with plaque than those without, and the mean stiffness index was significantly greater. However, these differences are abolished after adjustment for age (Table 14.10)

In IDDM, in the absence of plaque, the mean compliance and distensibility of the CCA decrease with age whereas the stiffness index increases with age (Figs. 14.3, 14.4, and 14.5). The correlation coefficient for each of these is 0.62, 0.62 and 0.71 respectively.

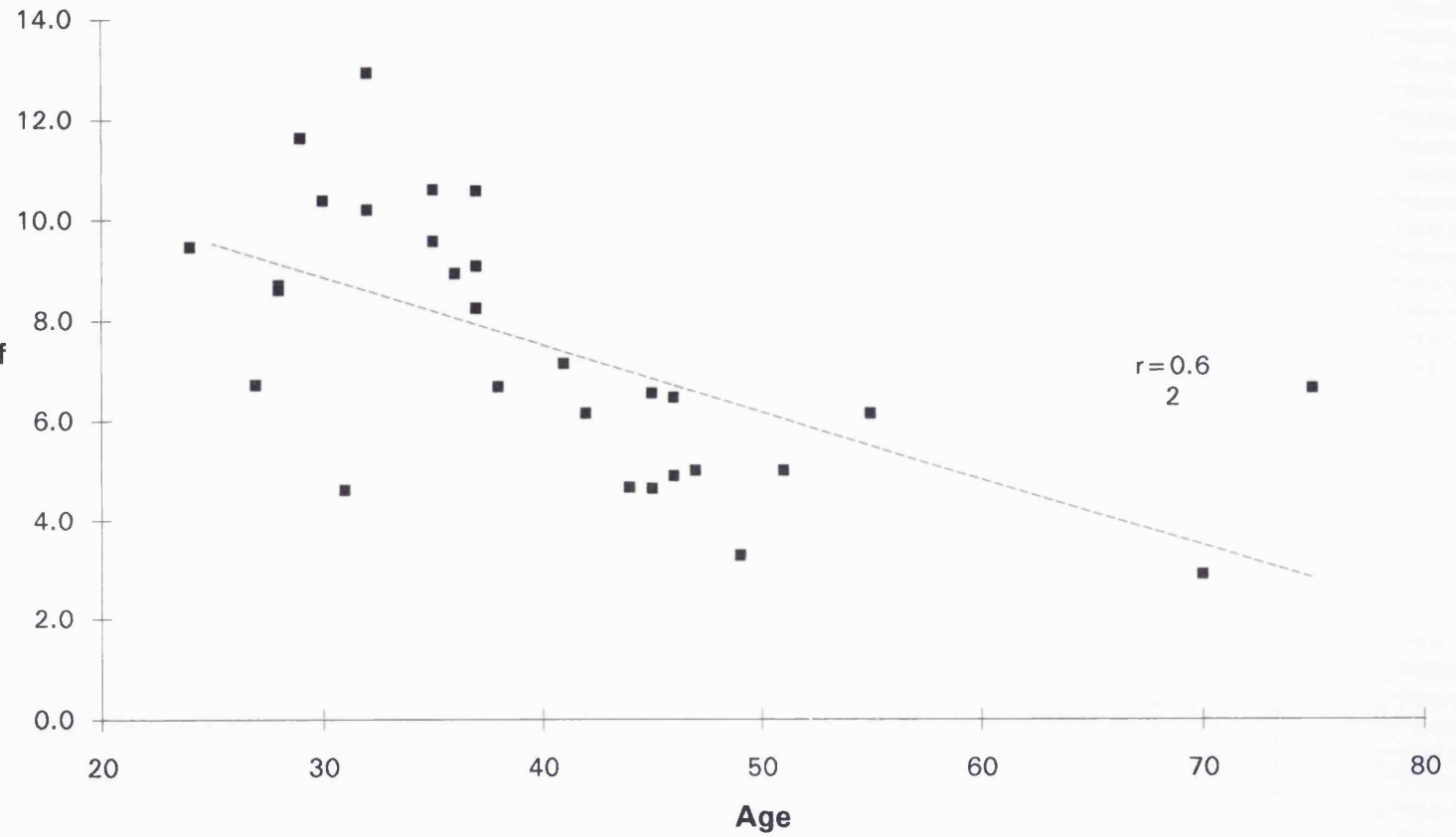
In the presence of plaque, the correlation between mean compliance, mean distensibility, the stiffness index and age was less good (the correlation coefficients for each of these are 0.43, 0.30 and 0.46 respectively.)

The arterial wall movement of the carotid bulb and the common femoral artery was not examined in this group of patients.

**Table 14.10 Comparison between IDDM with plaque and those without in arterial wall movement parameters.**

	Plaque	No plaque	t	p value
Mean compliance	6.2 ± 2.9	6.3 ± 2.6	1.54	NS
Age adjusted compliance	6.5 ± 2.8	6.2 ± 2	0.44	NS
Mean distensibility	23.3 ± 11.2	32.3 ± 11.8	2.77	<0.005
Age adjusted distensibility	24.6 ± 10.1	26.4 ± 9.2	0.64	NS
Mean stiffness index	7.3 ± 3.3	5.4 ± 2.1	2.4	<0.01
Age adjusted stiffness index	6.9 ± 2.9	6.5 ± 1.6	0.56	NS

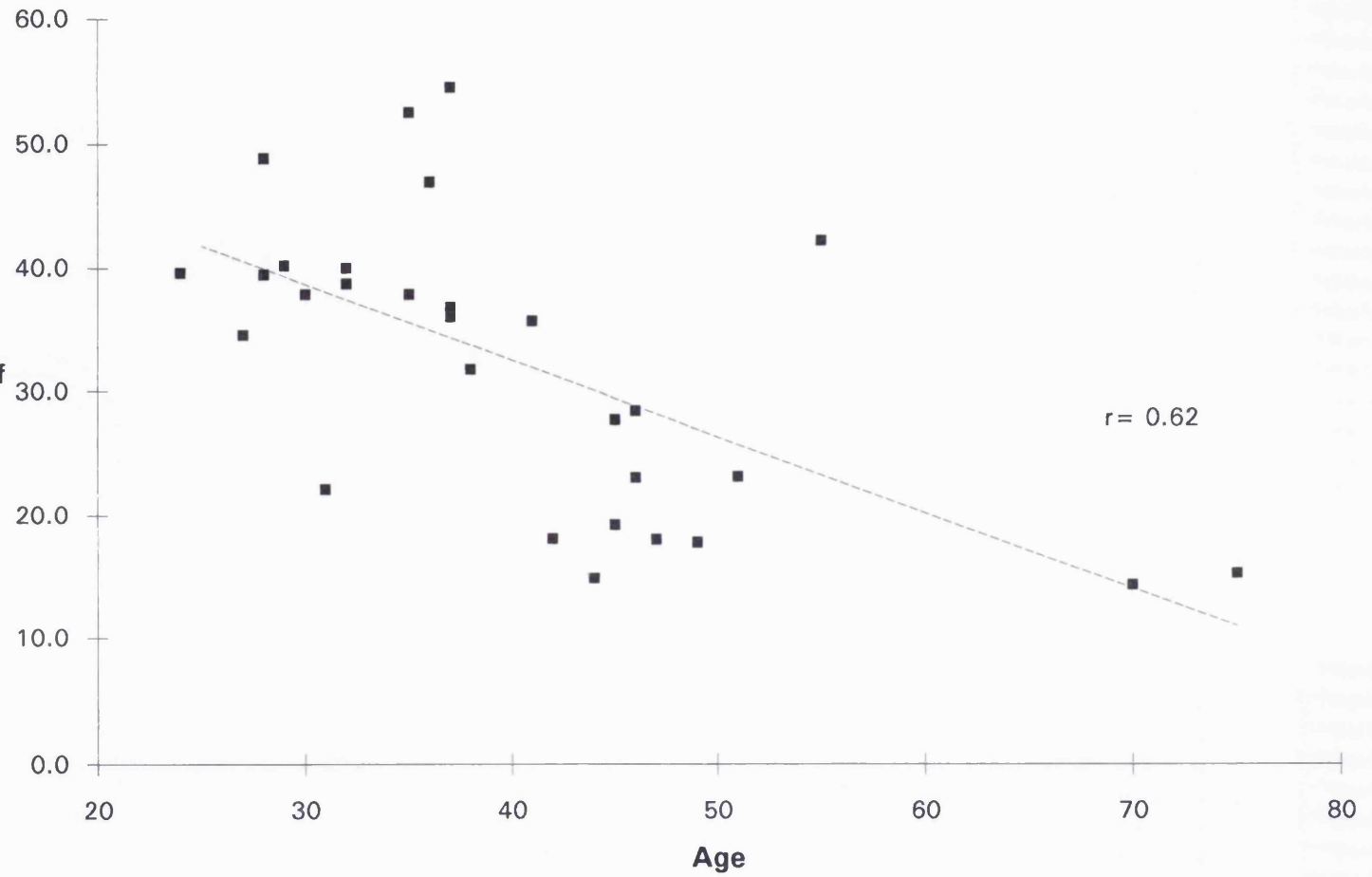
331  
Mean compliance of  
the CCA



**Fig 14.3**

**The correlation between compliance of the CCA and age, in IDDM without plaques.**

Mean distensibility of the CCA

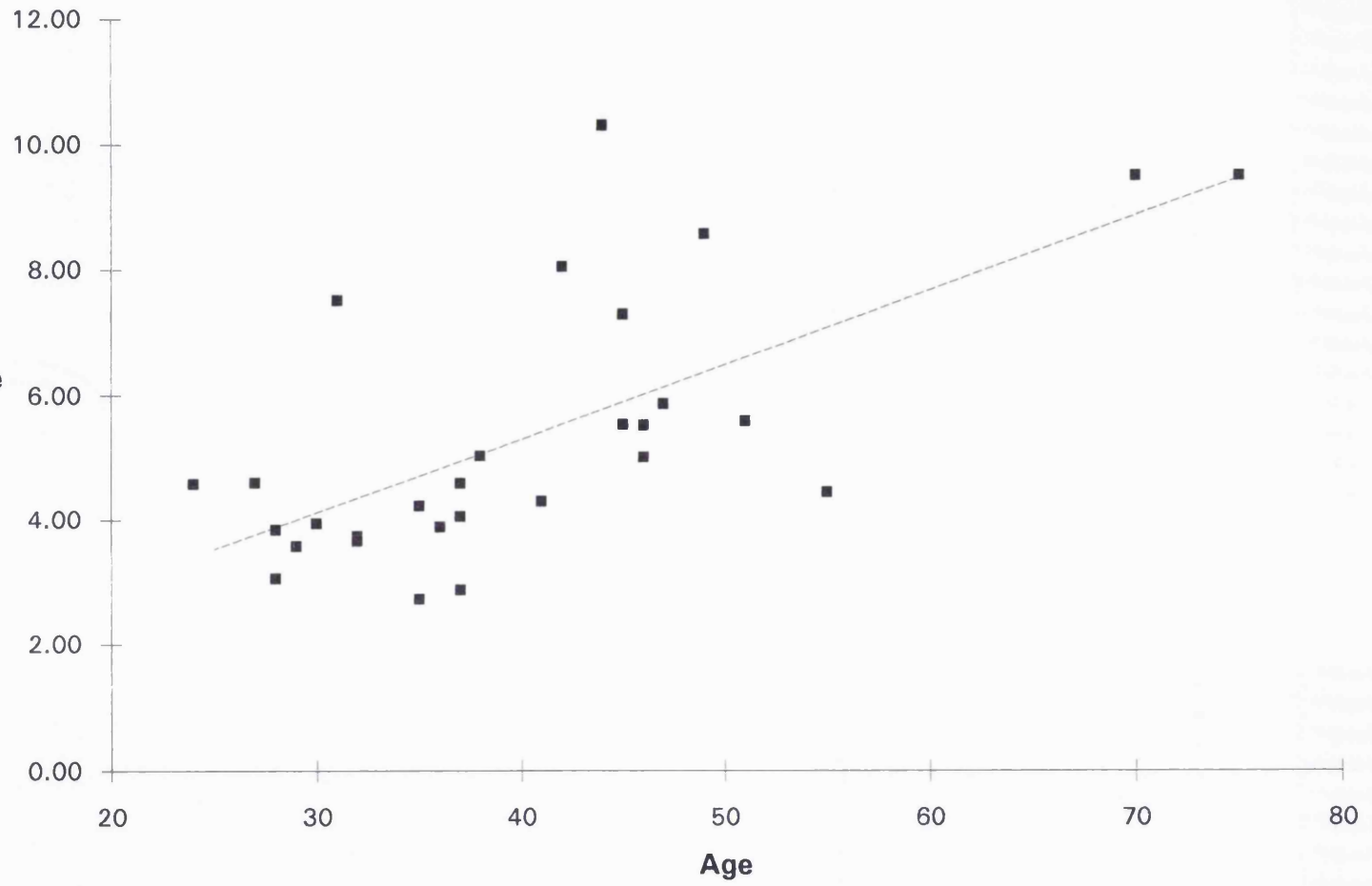


**Fig 14.4**

**The correlation between distensibility of the CCA and age, in IDDM without plaques.**

333

Stiffness Index of the CCA



**Fig 14.5**

**The correlation between the stiffness index of the CCA and age, in IDDM without plaques**



## **14.4 Discussion**

### ***i) Can the IM thickness be used to predict the presence of macrovascular disease in IDDM?***

Yes. IM thickness in IDDM correlates well with the presence of macrovascular disease. Only 13% of individuals with an IM thickness of less than 0.82mm had macrovascular disease compared to 50% of individuals with an IM thickness >0.82mm

### ***ii) Can the IM thickness be used to predict the presence of plaques in IDDM?***

Yes. The likelihood of plaque being present increase with CCA IM thickness, and only 10% of individuals with an IM thickness less than 0.52 mm had bifurcation plaques, as compared to 75% of individuals with an IM thickness greater than 0.82 mm

### ***iii) What is the relationship between IM thickness and known risk factors?***

In IDDM, the main risk factors determining intima-media thickness are age and smoking habit. The same holds true for the presence of plaque. No correlation was found between either the IM thickness or the presence of plaque and the lipid profile.

The main risk factors determining the presence of macrovascular disease were not only age and the number of packyears, but also sex and blood pressure.

CCA IM thickness did not contribute significantly to the presence of plaque or macrovascular disease in IDDM.

### ***iv) How do compliance, distensibility, elastic modulus and the stiffness index alter in IDDM patients?***

The compliance and distensibility of the CCA falls with age whereas the stiffness index increases.

Though there was a significant difference between the mean distensibility of the CCA in individuals with plaque and those without, compliance and distensibility of the CCA fall with age, patients with plaque were significantly older than those without. The stiffness index increased with age, and there was a significant difference between the mean stiffness of individuals with plaque and those without, again this difference could be accounted for by the age difference between the two groups

CHAPTER 15

THE INTIMA-MEDIA THICKNESS AND ARTERIAL  
WALL MOVEMENT OF HYPOPITUITARY  
PATIENTS

## **15.1 Introduction**

Like insulin and non-insulin dependent diabetic, hypopituitary patients on routine replacement therapy have a higher mortality rate (Rosen and Bengtsson, 1990) from vascular disorders, and a higher life-time incidence of vascular events. The reason why these patients should have an increased incidence of vascular disease is unknown, although growth hormone deficiency or endocrine replacement therapy may play a part.

The aim of this study was to assess the intima-media thickness of the carotid and femoral arteries of these patients and determine its relation to the presence of macrovascular disease and the presence of plaque and to assess its effect on the arterial wall movement.

thus the questions asked were :

- i) Can the IM thickness be used to predict the presence of macrovascular disease in hypopituitary patients?*
- ii) Can the IM thickness be used to predict the presence of plaques in hypopituitary patients?*
- iii) What is the relationship between IM thickness and known risk factors in hypopituitary patients?*
- iv) How do compliance, distensibility, elastic modulus and the stiffness index alter in hypopituitary patients?*

## **15.2 Method**

Hypopituitary patients were recruited from the endocrinology out patients clinic. The presence of macrovascular disease was determined by clinical examination, Minnesota coded electrocardiography and chest wall mapping in combination with the Rose cardiovascular questionnaire (See Part III, method). These patients were also examined with high resolution ultrasound and a fasting blood taken for lipid profile estimation.

### **15.3 Results**

Thirty-four patients with hypopituitarism were examined, 20 women and 14 men. The risk profile of this group of patients is shown in table 15.1 and men and women are compared in table 15.2. Men and women were similar in all risk factors except for body mass index and serum HDL cholesterol levels.

#### **15.31 The relationship between macrovascular disease and mean IM thickness of the CCA.**

The risk profiles are compared for patients with macrovascular disease and those without in table 15.3. The IM thickness is significantly thicker in patients with macrovascular disease than those without, these patients were also older, hypertensive and had smoked more. No significant difference was observed in the lipid profiles. In hypopituitary patients, CCA IM thickness is a good predictor of the presence of macrovascular disease (Table 15.4)

#### **15.32 The relationship between plaques and the mean IM thickness of the CCA.**

The characteristics of individuals with plaques are compared to those without in table 15.5. Individuals with plaques were also older, with higher systolic pressures and a longer smoking habit than those without. They also had a significantly thicker CCA IM thickness and mean AUS

The relationship between CCA IM thickness and the presence of plaque is examined in table 15.6. and CCA IM thickness is a good predictor of the presence of bifurcation plaques

#### **15.33 The relationship between the IM thickness, the presence of plaque and age**

The relationship between the IM thickness, the presence of plaque and age is shown in figure 15.1. In patients with hypopituitarism, the IM thickness increases with age. The slope is not significantly different in the presence of plaque (Fig 15.2).

**Table 15.1 Risk factor profile for hypopituitary patients**

	Mean $\pm$ 1sd	Range
Age	51.9 $\pm$ 15.8	26 - 75
Body mass Index	27.15 $\pm$ 3.72	20 - 35
Systolic BP (mmHg)	126 $\pm$ 16	100 - 160
Diastolic BP (mmHg)	75 $\pm$ 9	60 - 90
Pack years	13.74 $\pm$ 18.5	0 - 61
<b>LIPID PROFILE</b>		
Cholesterol	7.08 $\pm$ 1.96	3.74 - 10.74
Triglycerides	1.68 $\pm$ 0.82	0.76 - 4.34
HDL	1.3 $\pm$ 0.56	0.58 - 2.93

**Table 15.2 Comparison between male and female hypopituitary patients**

	Male n=14	Female n=20		
	Mean $\pm$ 1sd	Mean $\pm$ 1sd	t	p value
Age	50.7 $\pm$ 14.4	51 $\pm$ 15.4	0.06	NS
BMI	26 $\pm$ 4	29 $\pm$ 3	2.1	<0.02
Systolic BP (mmHg)	122 $\pm$ 13	127 $\pm$ 17	1.01	NS
Diastolic BP	77 $\pm$ 9	75 $\pm$ 9	0.68	NS
Pack years	13.9 $\pm$ 18.6	9.4 $\pm$ 16.4	0.73	NS
<b>Intima-media thickness measurements</b>				
CCA IMT (mm)	0.75 $\pm$ 0.16	0.73 $\pm$ 0.17	0.37	NS
AUS	14.3 $\pm$ 6.9	12.3 $\pm$ 7.8	0.78	NS
<b>LIPID PROFILE</b>				
Cholesterol (mmol/l)	7.3 $\pm$ 1.8	6.8 $\pm$ 2.2	0.6	NS
Triglycerides (mmol/l)	1.88 $\pm$ 1	1.42 $\pm$ 0.37	1.48	NS
HDL (mmol/l)	1.56 $\pm$ 0.63	1.01 $\pm$ 0.29	2.56	<0.01

**Table 15.3 Risk factors and the presence of macrovascular disease in hypopituitary patients**

	MVD present n=8	MVD absent n=26	t	p value
Age	65 ± 8	47 ± 13	4.66	<0.001
Body mass Index	28 ± 2.4	26 ± 7.2	1.49	NS
Systolic BP (mmHg)	134 ± 16	122 ± 14	1.8	<0.02
Diastolic BP (mmHg)	78 ± 7.9	76 ± 9.3	0.73	NS
Pack years	24 ± 23	7.5 ± 13	1.88	<0.05
<b>Intima-media measurements</b>				
Mean CCA IM thickness	0.9 ± 0.1	0.69 ± 0.16	4.9	<0.001
Mean AUS	21.8 ± 22	10.5 ± 6.3	7.23	<0.001
<b>LIPID PROFILE</b>				
Cholesterol (mmol/l)	7.5 ± 1.7	6.8 ± 2.08	0.71	NS
Triglycerides (mmol/l)	2.02 ± 1.3	1.5 ± 0.48	0.97	NS
HDL (mmol/l)	1.5 ± 0.7	1.22 ± 0.5	0.85	NS

**Table 15.4 The relationship between IM thickness and the macrovascular disease in hypopituitary patients**

Mean CCA IM thickness	MVD absent	MVD present	Total
Up to 0.58mm	6	0 (0%)	6
0.59mm-0.82mm	16	1 (6%)	17
0.83mm and over	4	7 (64%)	11



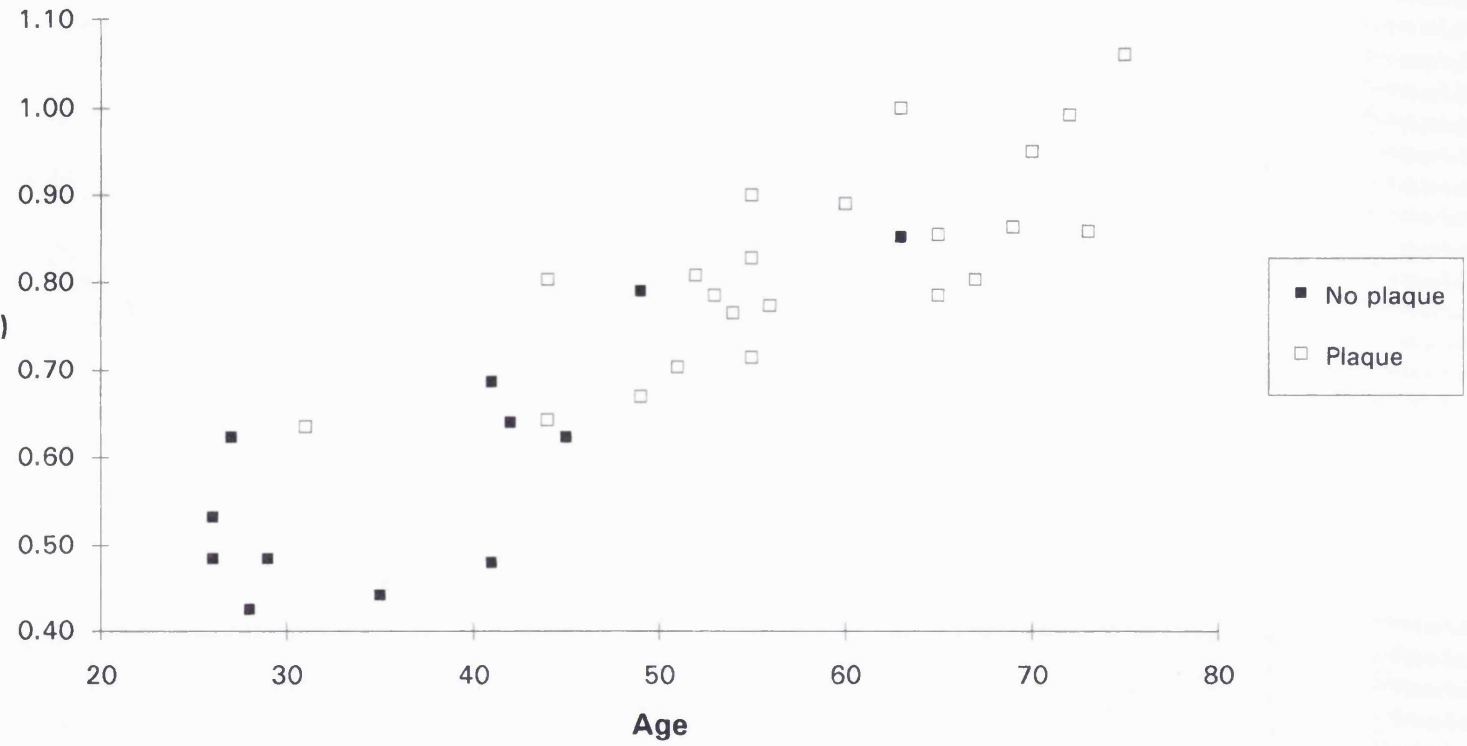
**Table 15.5 Comparison between hypopituitary patients with and without plaque**

	Plaque present n=22	Plaque absent n=12		
	Mean $\pm$ 1sd	Mean $\pm$ 1sd	t	p value
Age	58 $\pm$ 11	37.7 $\pm$ 8.1	5.06	<0.001
Body mass Index	27 $\pm$ 3.2	27 $\pm$ 6.5	0.1	NS
Systolic BP (mmHg)	132 $\pm$ 14	113 $\pm$ 10	4.56	<0.001
Diastolic BP (mmHg)	78 $\pm$ 9	73 $\pm$ 9	1.69	NS
Pack years	16 $\pm$ 19	1.8 $\pm$ 4.5	3.4	<0.001
<b>Intima-media measurements</b>				
Mean CCA IM thickness	0.82 $\pm$ 0.1	0.59 $\pm$ 0.15	5	<0.001
Mean AUS	17.6 $\pm$ 4.1	4.8 $\pm$ 4.1	8.64	<0.001
<b>LIPID PROFILE</b>				
Cholesterol (mmol/l)	7.7 $\pm$ 1.8	6.1 $\pm$ 1.9	2	<0.05
Triglycerides (mmol/l)	1.8 $\pm$ 0.9	1.5 $\pm$ 0.6	0.99	NS
HDL (mmol/l)	1.33 $\pm$ 0.6	1.25 $\pm$ 0.54	0.31	NS

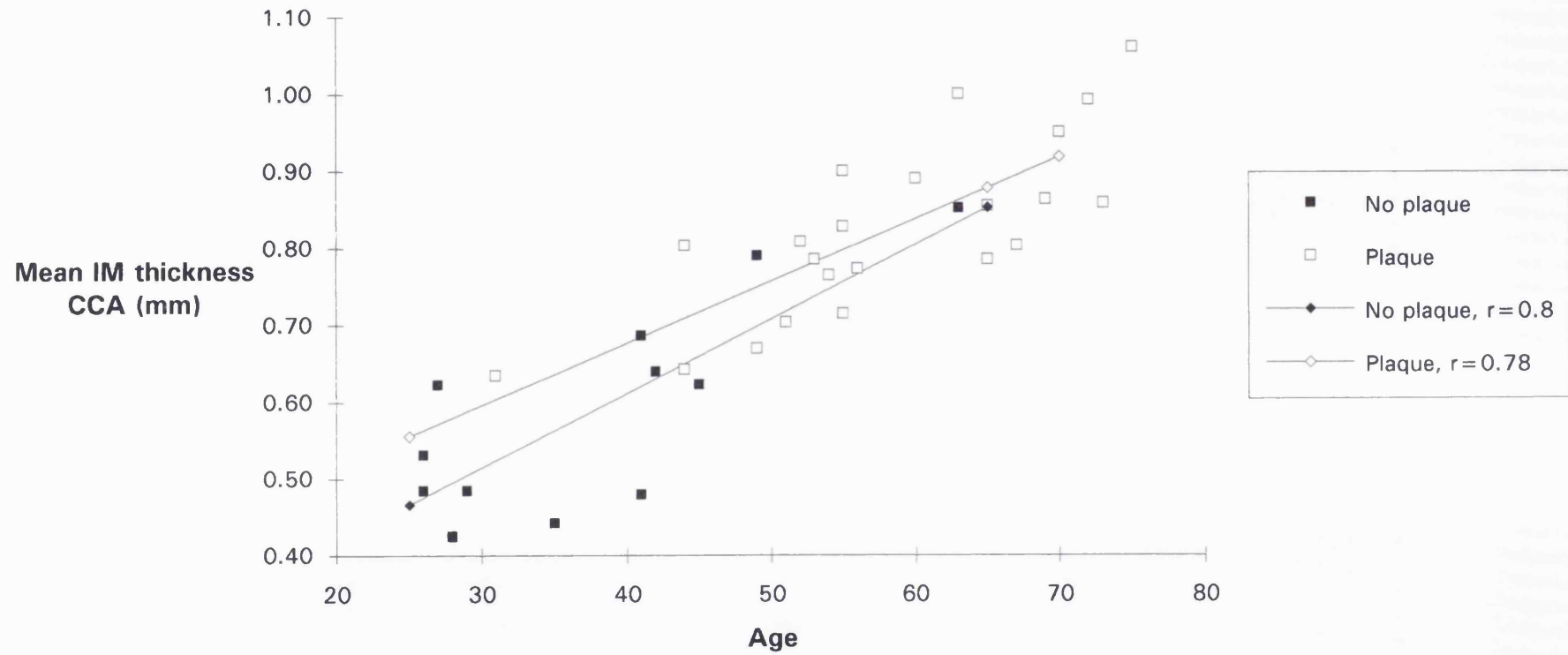
**Table 15.6** The relationship between CCA IM thickness and the presence of plaque

Mean CCA IM thickness	No plaque	Plaque	Total
Up to 0.58 mm	6	0 (0%)	6
0.59-0.82mm	5	12 (70%)	17
0.83mm and over	1	10 (90%)	11

Mean CCA IMT (mm)



**Fig 15.1** The relationship between IM thickness, the presence of plaque and age in patients with hypopituitarism



**Fig 15.2** The relationship between IM thickness, the presence of plaque and age in patients with hypopituitarism.

**15.34 The relationship between known risk factors and mean IM thickness of the CCA.**

Multiple regression analysis was performed using age, sex, BMI, systolic BP, diastolic BP and the lipid profile as independent variables. The CCA IM thickness can be predicted from the following formula:

$$\text{Mean CCA IMT} = -0.0002 + 0.04 \text{ sex} + 0.0095 \text{ age} + 0.0012 \text{ BMI} - 0.00003 \text{ PY} + 0.003 \text{ systolic BP} - 0.0007 \text{ diastolic BP} - 0.016 \text{ cholesterol} - 0.016 \text{ tryglycerides} - 0.0012 \text{ HDL. (table 15.7)}$$

The correlation coefficient for this formula is 0.94. The best subset is represented by the following formula:

$$\text{Mean CCA IMT} = 0.068 + 0.0019 \text{ age} + 0.009 \text{ BMI.}$$

The correlation coefficient for this formula is 0.91

**15.35 The relationship between the presence of plaque and risk factors**

Multiple regression analysis was performed using age, sex, BMI, systolic BP, diastolic BP and the lipid profile as independent variables. The the presence of plaque can be predicted from the following formula:

$$\text{Plaque} = -1.69 - 0.018 \text{ BMI} - 0.36 \text{ CCA IMT} + 0.02 \text{ age} + 0.005 \text{ packyears} + 0.28 \text{ sex} + 0.019 \text{ systolic BP} - 0.005 \text{ diastolic BP} + 0.01 \text{ cholesterol} - 0.167 \text{ tryglyceride} - 0.00003 \text{ HDL (table 15.8)}$$

The correlation coefficient for this formula is 0.9 and the best subset is represented by :

$$\text{Plaque} = -2.09 + 0.017 \text{ BMI} + 0.005 \text{ CCA IMT} + 0.19 \text{ age} + 0.015 \text{ packyears} - 0.17 \text{ sex}$$

and the correlation coefficient for this formula is 0.87.

**Table 15.7 Multiple linear regression of independent variables contributing to the prediction of the CCA IM thickness and the significance of each component in hypopituitary patients**

	$c = 0.001806$		
Age	$b1 = 0.009475$	$F = 27.354$	$p < 0.0001$
Packyears	$b2 = -0.000027$	$F = 0.0005$	$p = 0.9818$
Sex	$b3 = -0.040823$	$F = 0.7311$	$p = 0.4014$
Systolic BP	$b4 = 0.003324$	$F = 1.9289$	$p = 0.1771$
Diastolic BP	$b5 = -0.000726$	$F = 0.0348$	$p = 0.8537$
Body mass index	$b6 = 0.001227$	$F = 0.022522$	$p = 0.882$
Triglyceride	$b7 = -0.016463$	$F = 0.188292$	$p = 0.6684$
Total cholesterol	$b8 = -0.015617$	$F = 2.18427$	$p = 0.1530$
HDL3	$b9 = -0.001194$	$F = 0.0007$	$p = 0.9791$

In an analysis of variance  $F=10.7$ ,  $p=0.0001$

**Table 15.8 Multiple linear regression of independent variables contributing to the prediction of bifurcation plaques in hypopituitary patients, and the significance of each component**

	$c = -1.69$		
Age	$b1 = 0.019814$	$F = 2.566$	$p = 0.1228$
Packyears	$b2 = -0.005446$	$F = 1.409$	$p = 0.2474$
Sex	$b3 = .276083$	$F = 2.108$	$p = 0.16$
Systolic BP	$b4 = 0.019369$	$F = 3.816$	$p = 0.063$
Diastolic BP	$b5 = -0.005439$	$F = 0.13$	$p = 0.722$
Body mass index	$b6 = -0.01802$	$F = 0.323$	$p = 0.5754$
Triglyceride	$b7 = -0.167834$	$F = 1.285$	$p = 0.269$
Total cholesterol	$b8 = 0.01219$	$F = 0.076$	$p = 0.7854$
HDL	$b9 = -0.000035$	$F = 0.000$	$p = 1.0$

In an analysis of variance  $F = 4.84$ ,  $p = 0.0062$

**15.36 Compliance, distensibility, and the stiffness index of the common carotid artery in hypopituitary patients.**

The mean compliance was significantly greater in individuals without plaque compared to those with plaque, the same was true of the mean distensibility, and the mean stiffness index was significantly less. This difference persists even after age adjustment (table 15.8)

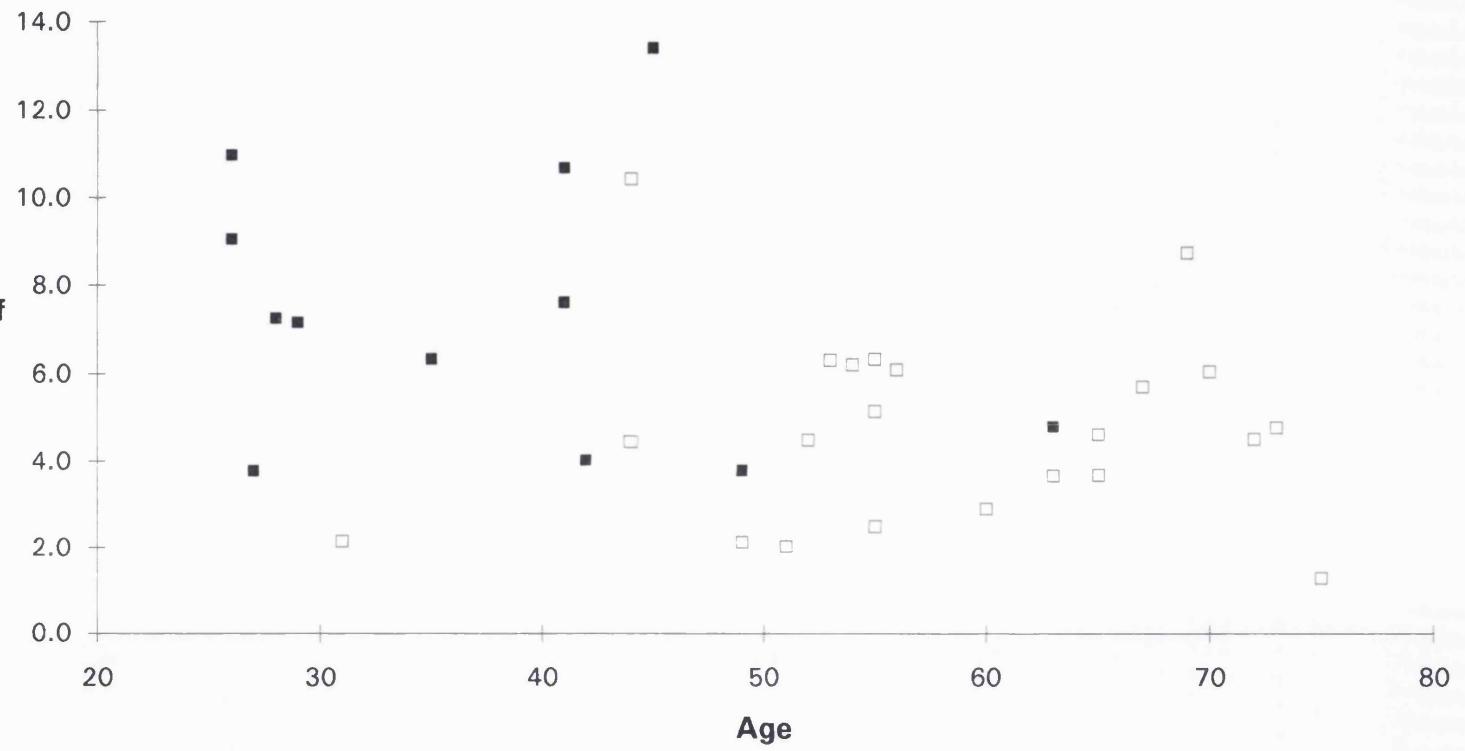
Both the distensibility and compliance of the CCA decrease with age (fig 15.3 and 15.4) but there were too few individuals without plaque in this patient group to permit a significant correlation. The stiffness index increases with age (fig 15.5), and again there were too few patients without plaque to permit a significant correlation.



**Table 15.9 Comparison between individuals with plaque and those without in arterial wall movement parameters.**

	Plaque	No plaque	t	p value
Mean compliance	4.7 ± 2.2	7.4 ± 3.2	2.6	<0.01
Age adjusted compliance	4.5 ± 2.4	6.7 ± 3.1	1.83	<0.05
Mean distensibility	18.4 ± 6.9	34.9 ± 15.8	3.44	<0.001
Age adjusted distensibility	20.3 ± 6.4	30.4 ± 15.2	2.2	<0.025
Mean stiffness index	8.7 ± 3.4	5.4 ± 1.9	3.77	<0.001
Age adjusted stiffness index	7.8 ± 3.1	6.3 ± 1.7	1.83	<0.05

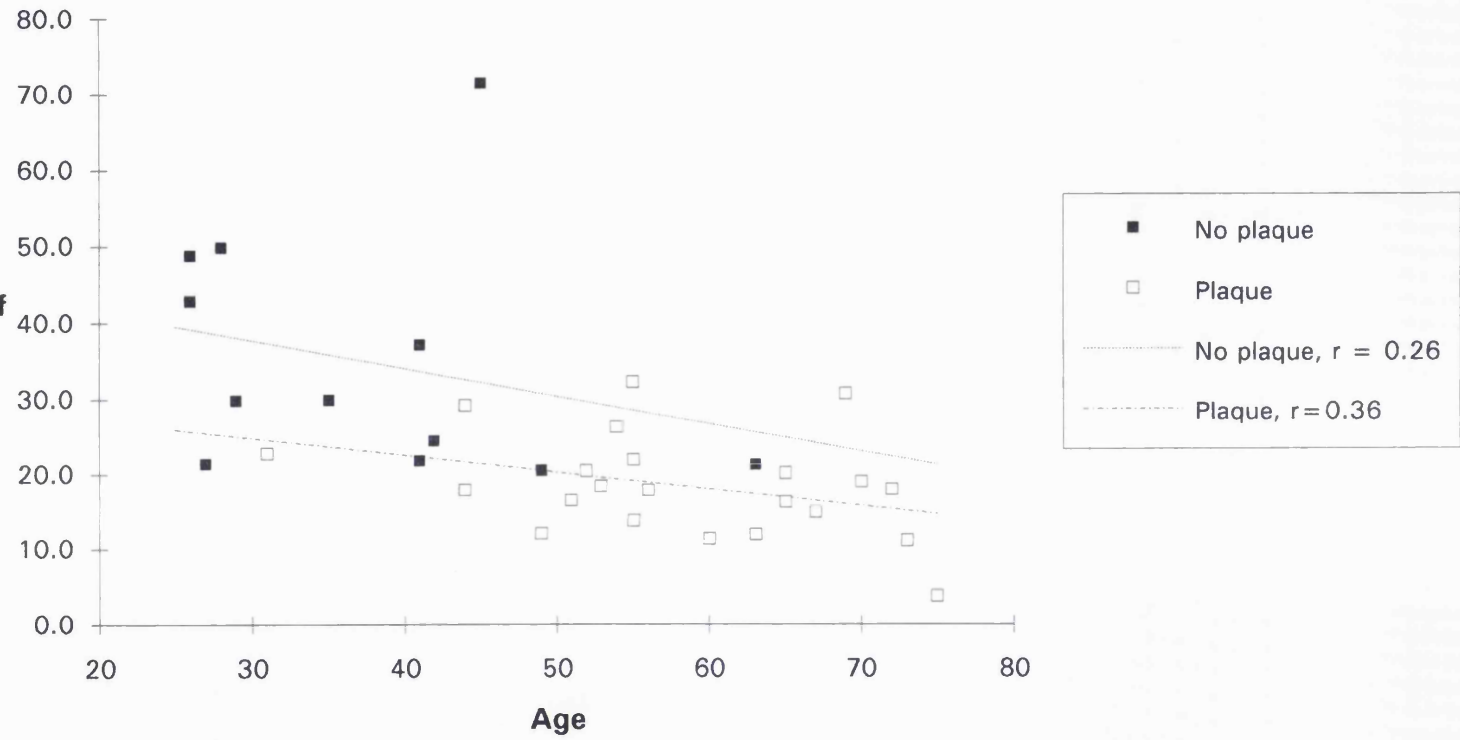
Mean compliance of  
the CCA



**Fig 15.3**

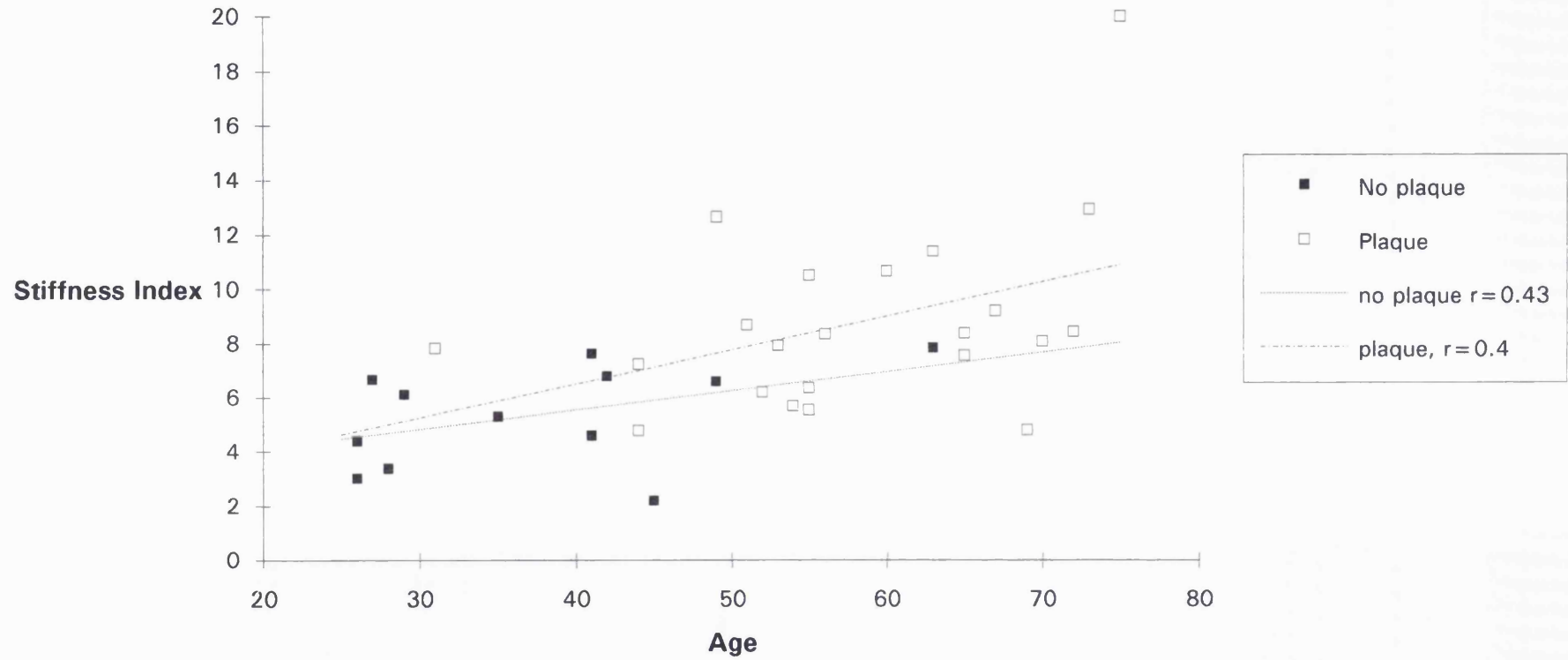
**The correlation between the mean compliance of the CCA in hypopituitary patients and age**

Mean distensibility of  
the CCA



**Fig 15.4**

**The correlation between mean distensibility of the CCA and age, in hypopituitary patients**



**Fig 15.5**

**The correlation between the stiffness index and age, in hypopituitary patients**

## **15.4 Discussion**

### ***i) Can the IM thickness be used to predict the presence of macrovascular disease in hypopituitary patients?***

Yes. None of the patients with a mean CCA IM thickness less than 0.58mm had macrovascular disease, and the majority of patients with macrovascular disease had a CCA IM thickness of greater than 0.82mm.

### ***ii) Can the IM thickness be used to predict the presence of plaques in hypopituitary patients?***

Yes. The likelihood of plaque being present increases with CCA IM thickness. None of the individuals with an IM thickness less than 0.58 mm had bifurcation plaques, whereas 70% of individuals with an IM thickness of between 0.58mm and 0.82mm had plaques, this rose further to 90% in individuals with an IM thickness greater than 0.82 mm.

### ***iii) What is the relationship between IM thickness, plaques and known risk factors in hypopituitary patients?***

The most significant factors determining IM thickness in hypopituitary patients were age and body mass index. Age, BMI and packyears of smoking were the most significant determinants for the presence of plaque. The lipid profile was not a significant factor in determining either IM thickness or the presence of plaque.

### ***iv) How do compliance, distensibility, elastic modulus and the stiffness index alter in hypopituitary patients?***

The mean distensibility and compliance of hypopituitary individuals was significantly greater and the mean stiffness index significantly less in individuals without plaque than those with plaque, though this difference was less after age adjustment.

Distensibility and compliance fall with age while the stiffness index increases, and there was no significant difference in the slope of the regression line for individuals with plaque compared to those without.

In conclusion, the most significant factors determining IM thickness in hypopituitary patients are age, smoking habit and obesity. and the CCA IM thickness is a good indicator for the presence of plaque, whereas CCA wall movement is not.

CHAPTER 16

INTIMA-MEDIA THICKNESS AND ARTERIAL  
WALL MOVEMENT

IN PATIENTS WITH SYMPTOMATIC  
PERIPHERAL VASCULAR DISEASE

## **16.1 Introduction:**

In the preceding chapters, I have discussed the ultrasonic characteristics of the carotid artery of a group of healthy volunteers and three patient groups with a known propensity to develop atherosclerosis. The aim of this chapter is to examine the same features in a group of patients with symptomatic peripheral vascular disease (defined as intermittent claudication with an ankle-brachial index at rest of less than 0.8) but without any symptoms of carotid artery disease.

The questions asked were:

- i) What is the relationship between intima-media thickness of the carotid artery and age in patients with symptomatic peripheral vascular disease?*
- ii) What is the relationship between intima-media thickness and carotid artery stenosis?*
- iii) What is the relationship between intima-media thickness and known risk factors?*
- iv) What is the relationship between arterial wall movement and age?*
- v) What is the relationship between arterial wall movement and CCA IM thickness in these patients?*

## **16.2 Material and Method:**

### **16.21 Material**

Patients with symptomatic peripheral vascular disease were recruited from the vascular out-patients at St Mary's Hospital

### **16.22 Method**

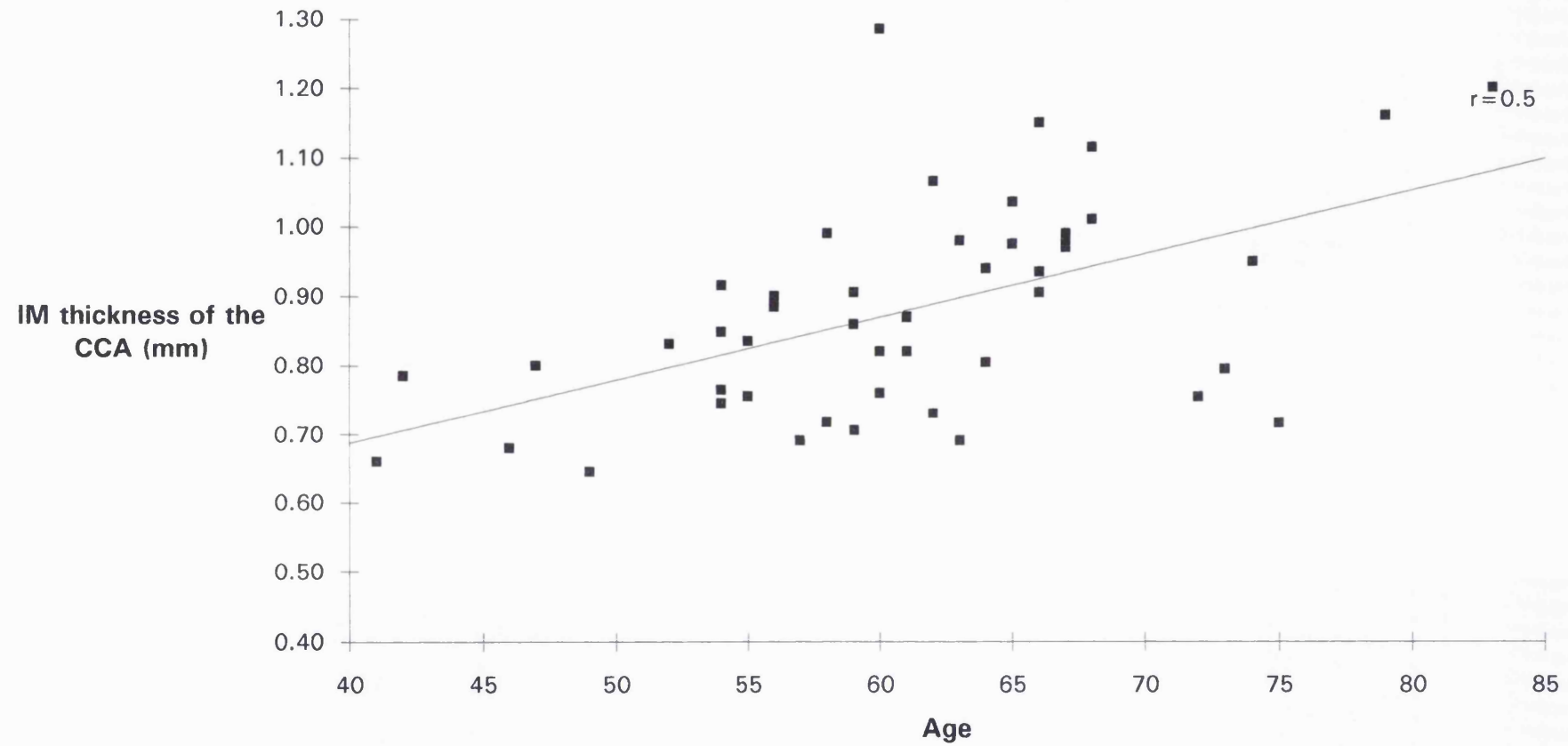
Patients were examined according to the methods described in Part III.

## **16.3 Results:**

### **16.31 The relationship between intima-media thickness and age**

- a) The common carotid artery:

The correlation between the CCA IM thickness and age is shown in figure 16.1. Despite the presence of peripheral vascular disease, IM thickness increased with age ( $r=0.5$ )



**Fig 16.1**      The correlation between age and IM thickness of the CCA in individuals with symptomatic PVD



b) The bulb origin:

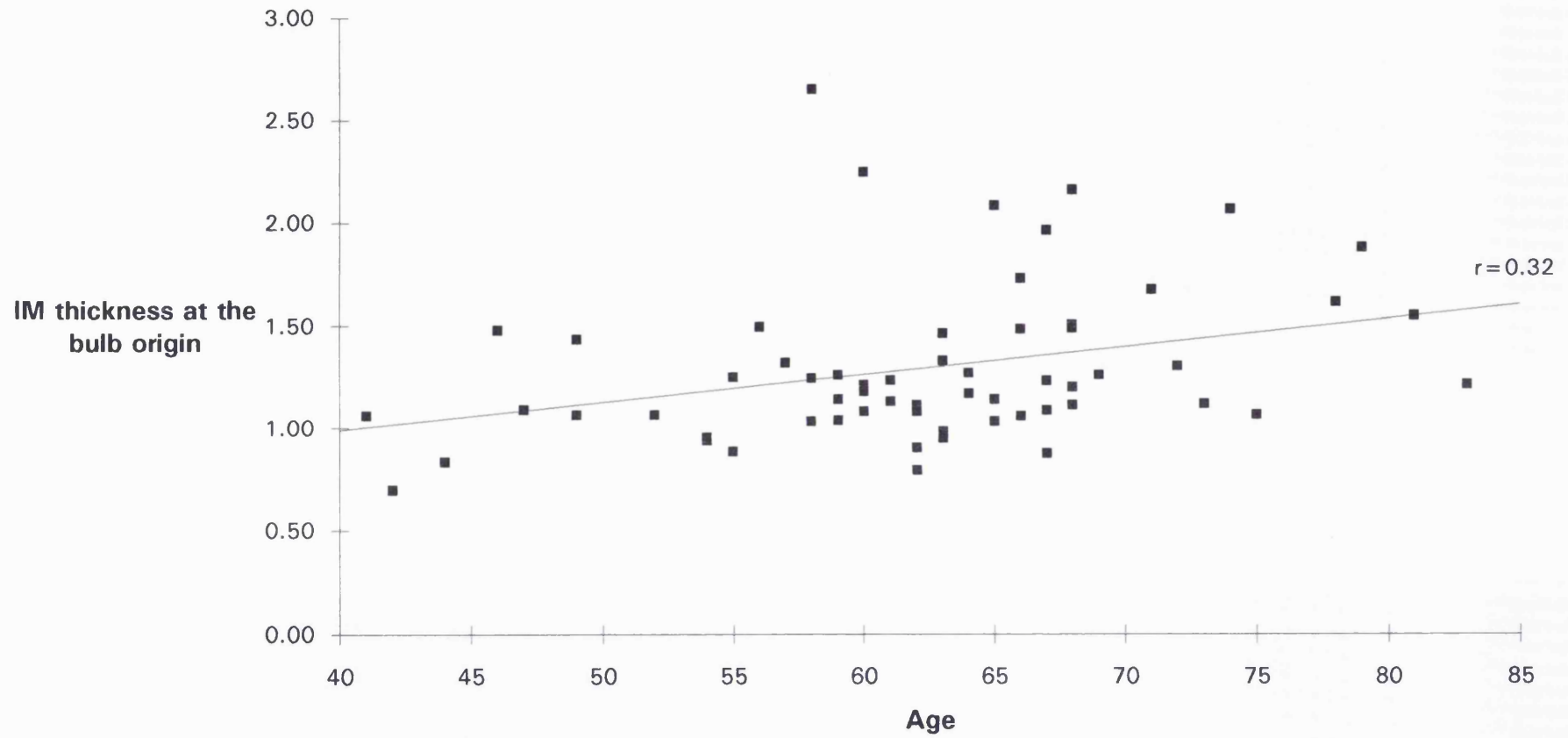
The correlation between the IM thickness at the bulb origin and age is poor (Fig 16.2,  $r=0.3$ ).

c) The bulb

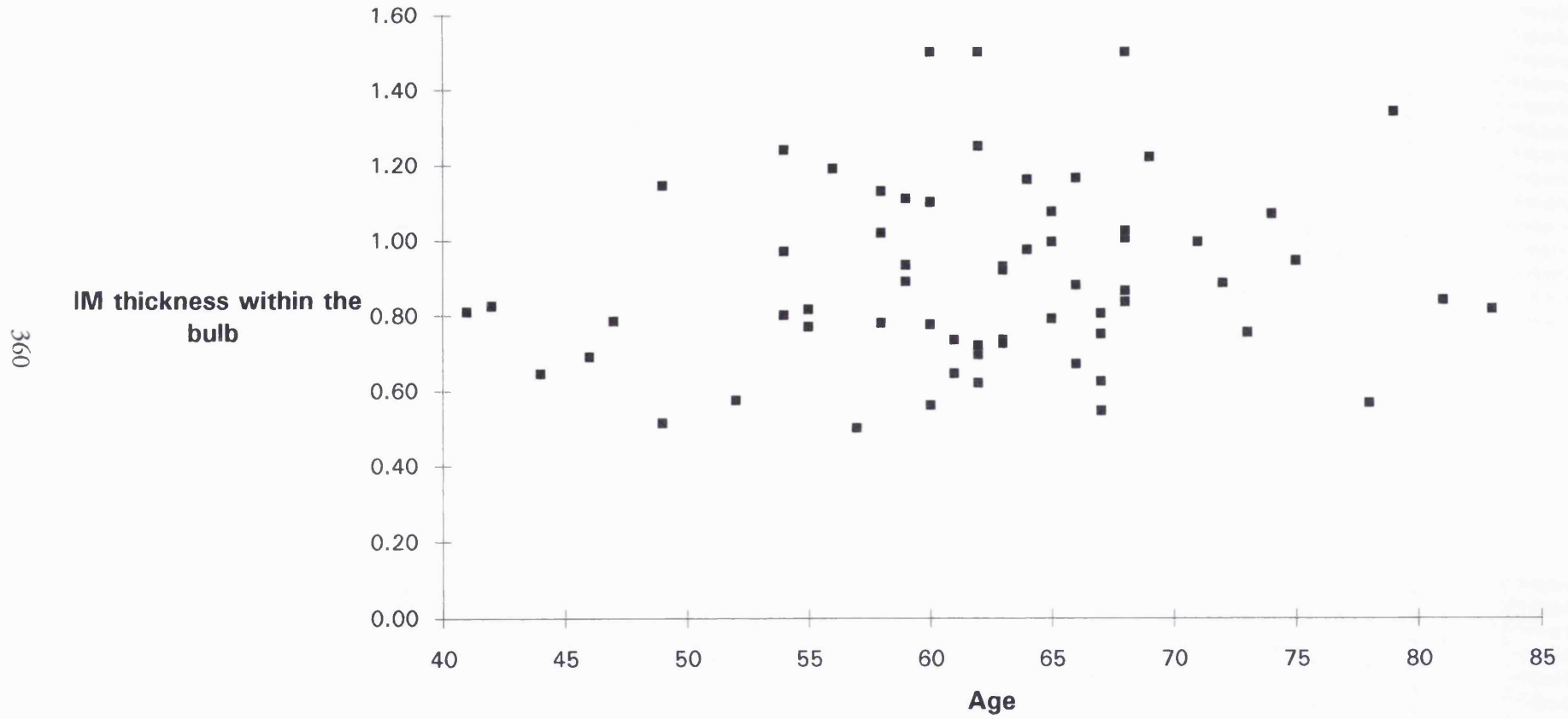
No correlation exists between the intima-media thickness in the bulb and age (Fig 16.3)

**16.32 The relationship between intima-media thickness of the common carotid artery and the degree of stenosis on duplex examination:**

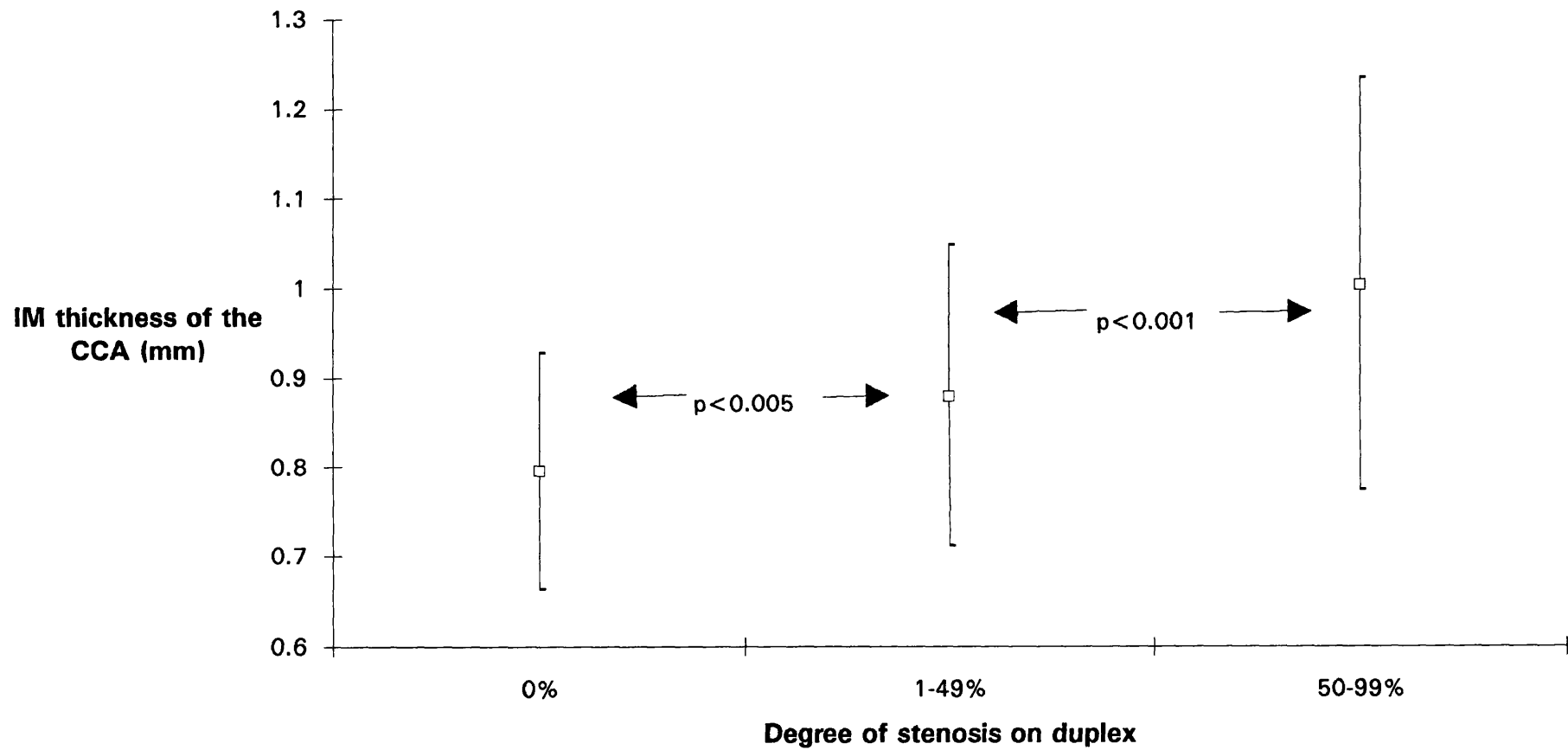
The intima-media thickness of the CCA is compared with the stenosis on duplex in figure 16.4. The IM thickness was significantly increased in patients with a stenosis on duplex of 1-15% (B according to the Strandness criteria - see Part III) in the ipsilateral carotid compared to patients with no stenosis. There was no significant difference in the intima-media thickness of individuals with a stenosis of 1-15% (B according to the Strandness criteria) and 16-49% (C according to the Strandness criteria) and, therefore, these were grouped together. The patients with a carotid artery stenosis of greater than 50% on duplex also had a significantly thicker IM thickness than patients with a stenosis of less than 50%.



**Fig 16.2** The correlation between IM thickness at the bulb origin and age in patients with symptomatic PVD



**Fig 16.3** The distribution of IM thickness in the bulb and its relation age in patients with symptomatic PVD.



**Fig 16.4** Comparison between the mean (+/- 1sd) IM thickness of the CCA and carotid artery stenosis on duplex.

### **16.33 The relationship between IM thickness of the CCA and known risk factors:**

Multiple regression analysis was performed using sex, age, systolic blood pressure, diastolic blood pressure and pack years as independent variables. Table 16.1 shows the predictive strength of each of these variables. The mean IM thickness of the CCA in patients with symptomatic peripheral vascular disease can be predicted from:

Mean CCA IM = 0.305 + 0.00749 age + 0.0017 pack years -0.0054 sex - 0.00114 systolic BP + 0.002487 diastolic BP.

The correlation coefficient for the above formula is 0.53

However, the strongest factors are age and pack years and alone these can predict IM thickness in the following formula:

Mean CCA IM = 0.387 + 0.007 age + 0.00177 pack years.

The correlation coefficient (r) for this formula is 0.5 and in an analysis of variance F=8.4, p<0.001.

### **16.34 The relationship between distensibility, compliance, the stiffness index and age:**

There was no correlation between age and CCA distensibility, compliance, or the stiffness index.

### **16.35 The relationship between distensibility, compliance, the stiffness index and IM thickness:**

There was no correlation between distensibility, compliance, the stiffness index and the IM thickness of the CCA.

**Table 16.1 Multiple linear regression of independent variables contributing to CCA IM thickness and the significance of each component**

		F	p value
	$c = 0.305368$		
Age	$b_1 = 0.007488$	10.39	$p = 0.0022$
Pack years	$b_2 = 0.001733$	5.18	$p = 0.027$
Sex (M=1, F=0)	$b_3 = -0.005393$	0.01	$p = 0.910$
Systolic BP	$b_4 = -0.001136$	0.98	$p = 0.326$
Diastolic BP	$b_5 = 0.002847$	2.24	$p = 0.14$

## **16.4 Discussion:**

***i) What is the relationship between intima-media thickness of the carotid artery and age in patients with symptomatic peripheral vascular disease?***

Despite the presence of peripheral vascular disease, the IM thickness of the carotid artery increases with age though the correlation is not as good as that of the volunteer group (chap 11.32). The correlation is best in the CCA, and poor at the bulb origin, this may be because changes occur early at the bulb origin, and the bulb origin becomes incorporated in bulb plaque with its associated changes in local blood flow patterns, and hence irregular growth. CCA IM thickening is a diffuse process, and is less subject to local flow disturbances.

***ii) What is the relationship between intima-media thickness and carotid artery stenosis?***

There is a strong relationship between the IM thickness of the CCA and the degree of stenosis in the carotid bulb. Though this could be explained by the presence of local plaques, a concerted effort was made to take this measurement away from the bulb origin and in a region without localised disease. If IM thickening is a diffuse process, then with progressive disease, greater IM thickening would be expected.

***iii) What is the relationship between intima-media thickness and known risk factors?***

The strongest relationship between IM thickness and known risk factors is with age and smoking habit, but this only accounts for half of the changes observed.

***iv) What is the relationship between arterial wall movement and age?***

There is no relationship between arterial wall movement and age in this group of patients.

***v) What is the relationship between arterial wall movement and CCA IM thickness in these patients?***

Again, no relationship could be observed in these patients.

In conclusion, the IM thickness of patients with symptomatic peripheral vascular disease is related to age and smoking habit as well as the degree of carotid artery stenosis. However, no such relationship is observed with the arterial wall movement, presumably because these arteries are extremely stiff.



CHAPTER 17

THE INTIMA-MEDIA THICKNESS

OF THE COMMON CAROTID ARTERY:

COMPARISON BETWEEN PATIENT GROUPS

## **17.1 Introduction:**

In chapter 11, the ultrasonic characteristics of carotid and femoral arteries in normal subjects was examined, and a close relationship was demonstrated between intima-media thickness and both plaque and age. Furthermore, it was shown that the IM thickness could be used to predict the likelihood of plaques being present at the bifurcations. In chapters 13,14 and 15, the value of the intima-media thickness in predicting the presence of discrete plaques or macrovascular disease was assessed in three patient groups at risk of developing atherosclerosis. Finally, chapter 16 examined the intima-media thickness of the carotid artery in a group of patients with symptomatic peripheral vascular disease but no symptoms of carotid disease.

The aim of this chapter is to compare our three patient groups with both our normal group and the patients with symptomatic peripheral vascular disease, and to assess the ability of IM thickness measurements to predict the presence of plaque and macrovascular disease.

Thus the questions asked were:

- i) How do the patient groups compare with the volunteer group in the relationship between CCA IM thickness and age?*
- ii) How do the patient groups compare with the volunteer group in the relationship between CCA IM thickness and the presence of plaque?*
- iii) Can CCA IM thickness be used to predict the presence of plaques?*
- iv) Can CCA IM thickness be used to predict the presence of macrovascular disease?*
- v) How do the patient groups compare with the volunteer group in the relationship between CCA IM thickness and risk factors?*

## **17.2 Method:**

The method is described in Part III.

Patient groups are compared using the student's t test, before and after correction for age (corrected to 50).

## **17.3 Results:**

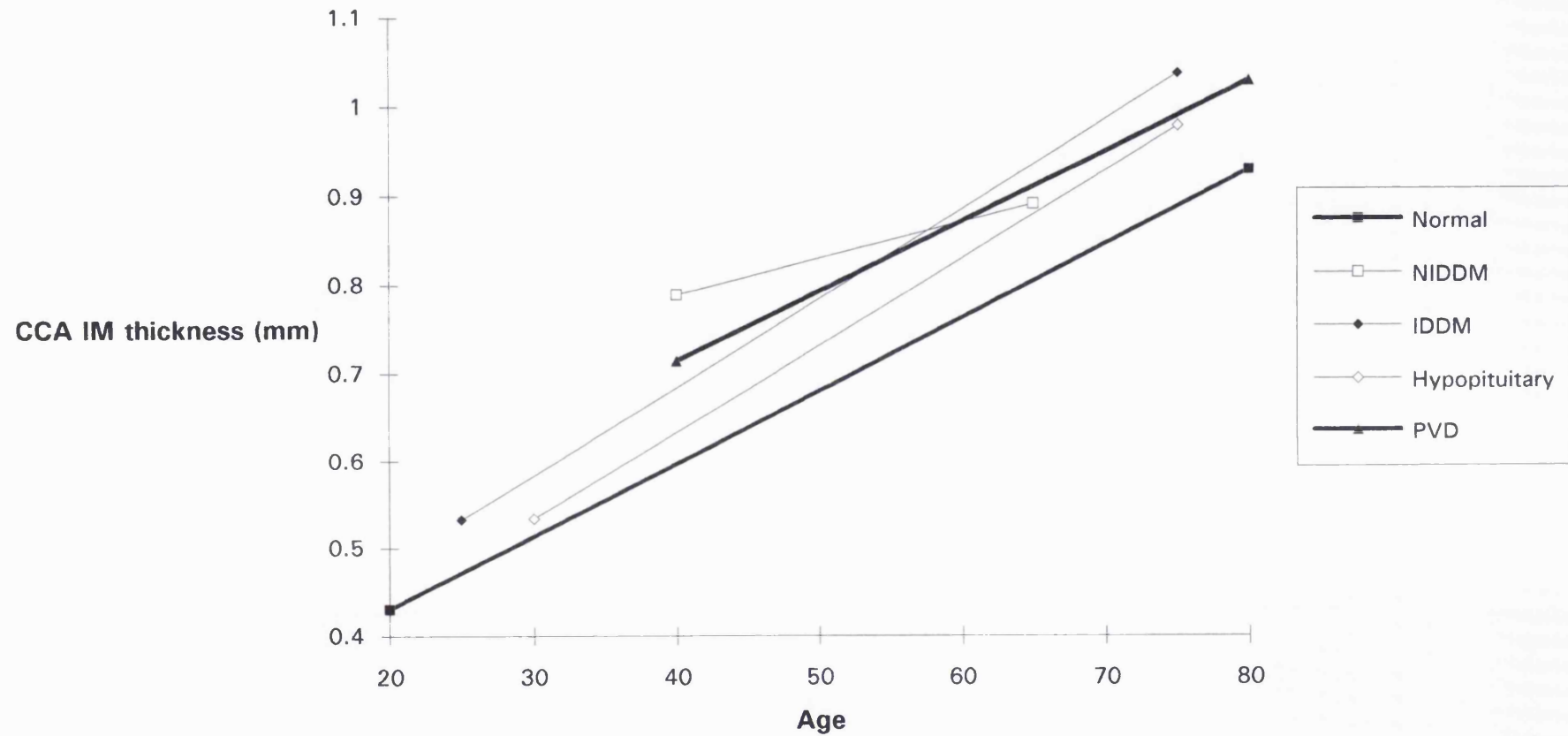
### **17.31 The relationship between CCA IM thickness and age:**

In figure 17.1, the regression lines for CCA IM thickness and age for the normal group is compared with patients with NIDDM, IDDM, hypopituitarism and symptomatic peripheral vascular disease, irrespective of the presence of plaque. In all groups, the IM thickness increases with age. The regression line for patients with peripheral vascular disease runs parallel to that of our group of healthy volunteers and slope of the regression lines for the hypopituitary patients and the patients with IDDM are steeper than the normal group, approaching that of patients with PVD with age.

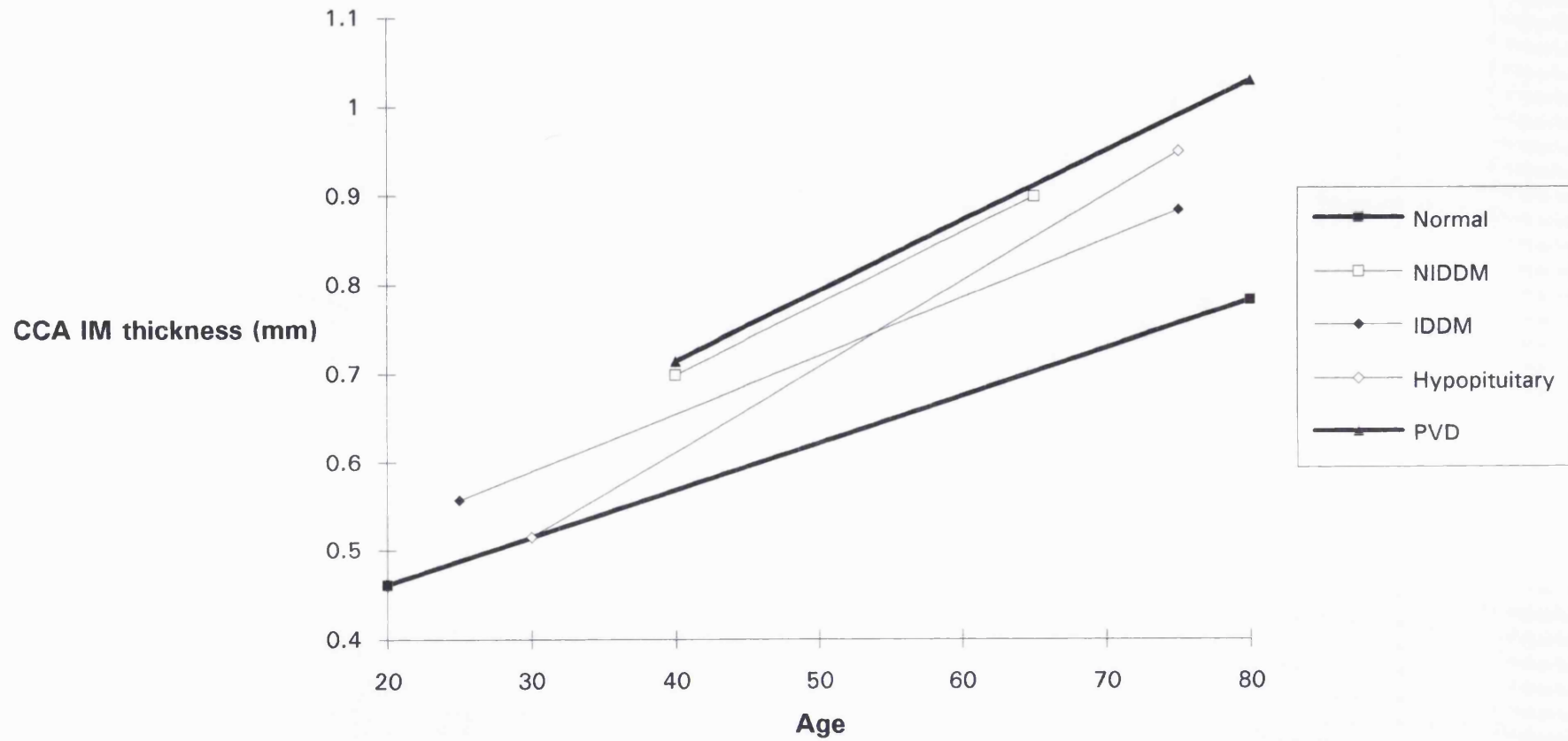
### **17.32 The relationship between CCA IM thickness and plaque:**

#### **a) No plaque**

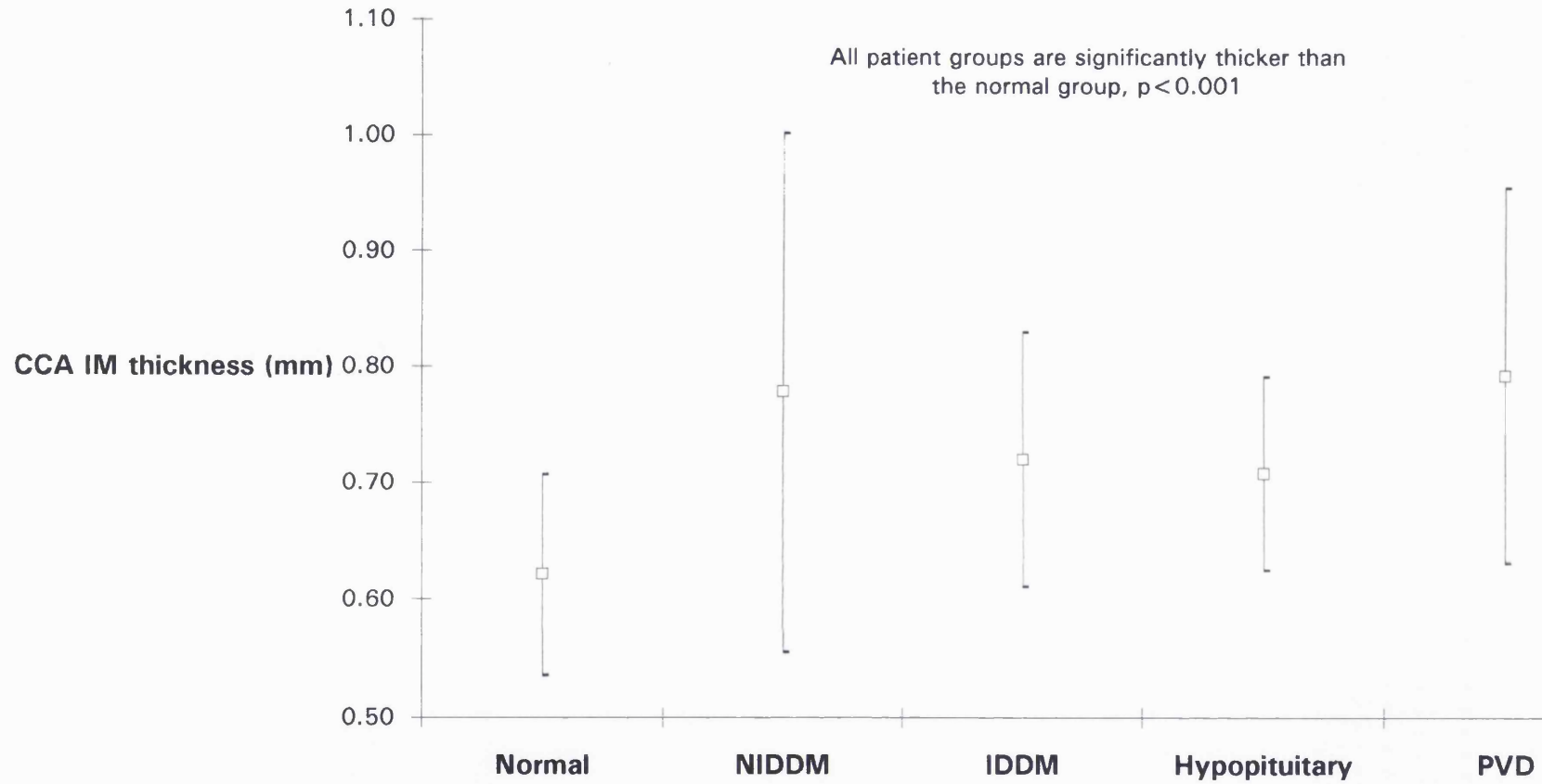
The regression lines for CCA IM thickness and age for the normal group, NIDDM, IDDM, and hypopituitary patients without plaque are compared with patients with symptomatic peripheral vascular disease in figure 17.2. The slopes are similar in all groups except the patients with hypopituitarism. All groups have relatively thicker CCA IM layer than the normal group, with the NIDDM patients having age related IM thickness similar to that of the patients with symptomatic peripheral vascular disease, while the IDDM patients lie half way between the normal group and patients with PVD. In figure 17.3, the mean CCA IM thickness is compared after correction for age (corrected to 50). The mean CCA IM thickness for each patient group was significantly thicker than that of the normal group (using students t test, comparing NIDDM to normal,  $t=4.35$ ,  $p<0.001$ , comparing IDDM to normal,  $t=4.41$   $p<0.001$ , and comparing hypopituitary patients to normal  $t=3.34$ ,  $p<0.001$ ). Though thicker than the normal group, the mean CCA IM thickness of the patients with IDDM and hypopituitarism were significantly thinner than those of the patients with PVD ( $t=2.55$ ,  $p<0.01$  and  $t=2.72$ ,  $p<0.005$  respectively), however, there was no significant difference between the mean CCA IM thickness in NIDDM patients in the absence of plaque and the mean CCA IM thickness of patients with symptomatic PVD ( $t=0.38$ , NS).



**Fig 17.1 Superimposition of regression lines of CCA IM thickness and age: comparison between normal and patient groups**



**Fig 17.2 Superimposition of regression lines of CCA IM thickness and age in the absence of plaque: comparison between normal and patient groups**



**Fig 17.3 Mean (+1sd) CCA IM thickness after correction for age: comparison between individuals and patients without plaque and patients with PVD**

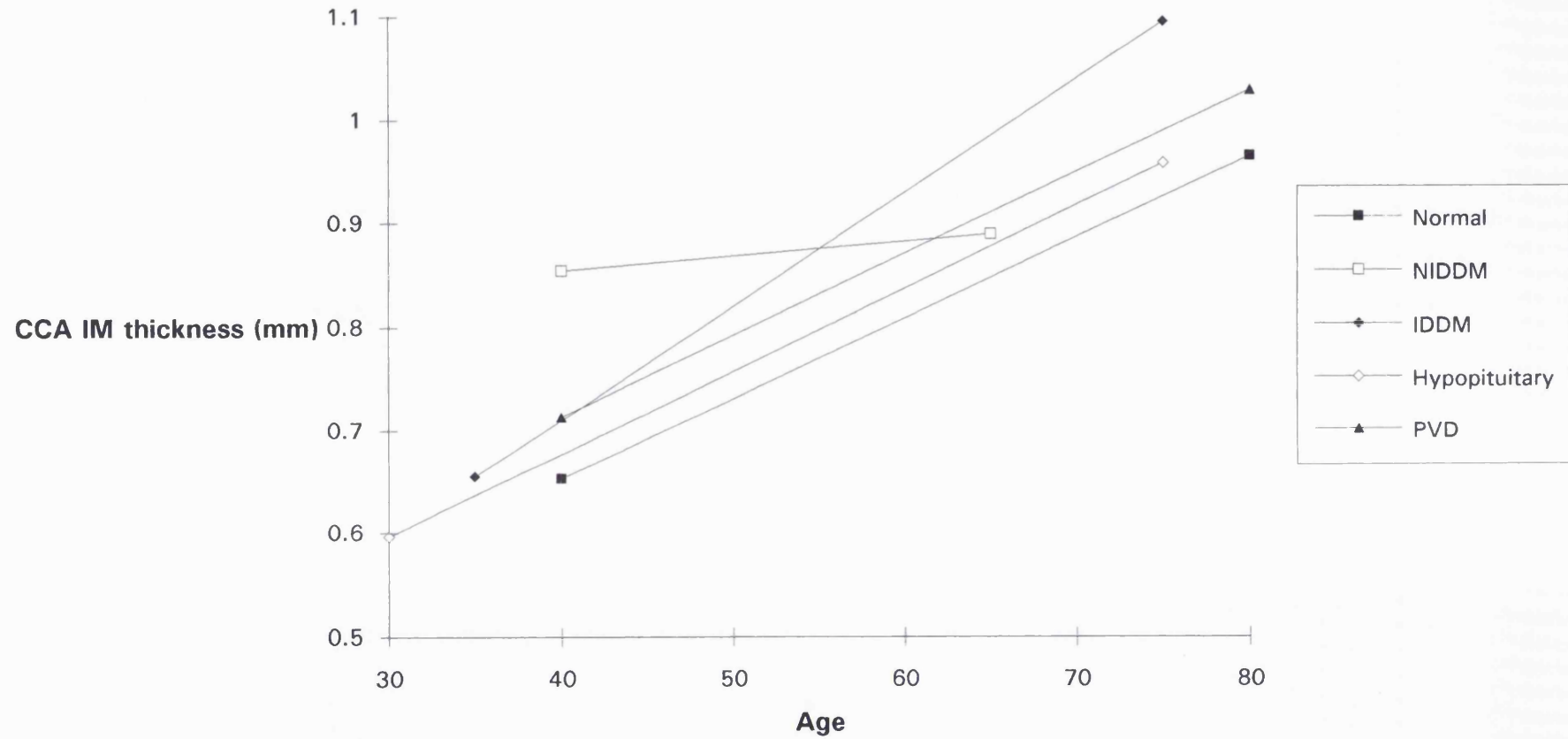
b) With plaque.

Patients with plaques are compared in figure 17.4. Despite the presence of plaque, the patient groups are all relatively thicker than the normal group and the age relationship still holds except for NIDDM patients who have a much thicker CCA IM in the younger patients but have a much flatter slope. After correction for age (all corrected to 50), the mean IM thickness of all groups was thicker in the presence of plaque (fig 17. 5), however the mean CCA IM thickness of the normal group was thinner than that of patients with PVD (fig 17. 6) ( $t=2.39$ ,  $p<0.01$ ) and there was no significant difference between patients with IDDM and hypopituitarism compared to patients with PVD, whereas the mean CCA IM thickness of patients with NIDDM was significantly thicker even than patients with symptomatic PVD ( $t=2.1$ ,  $p<0.02$ ).

The value of CCA IM thickness in predicting the presence of plaque is examined in table 17.1.

c) macrovascular disease

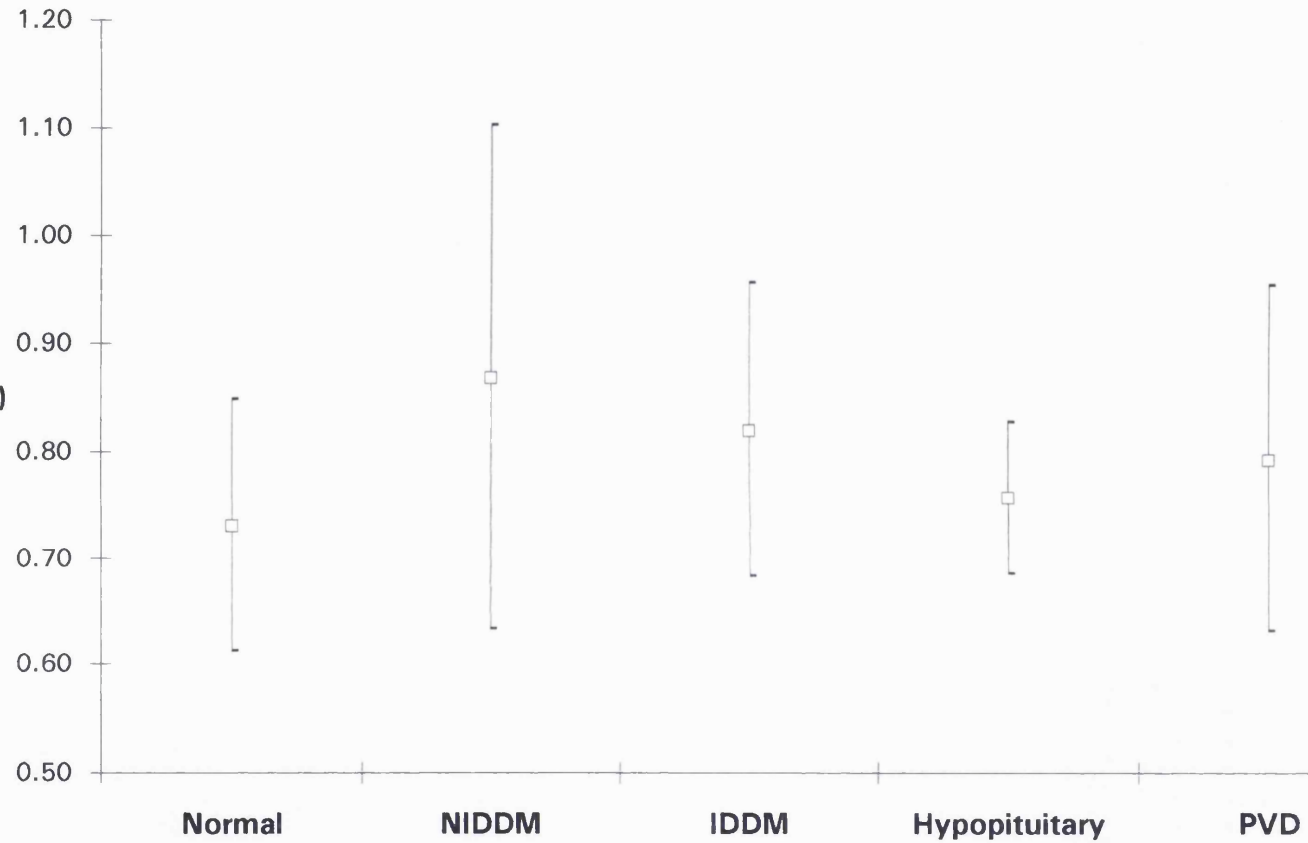
The value of CCA IM thickness in predicting the presence of macrovascular disease is examined in table 17.2.



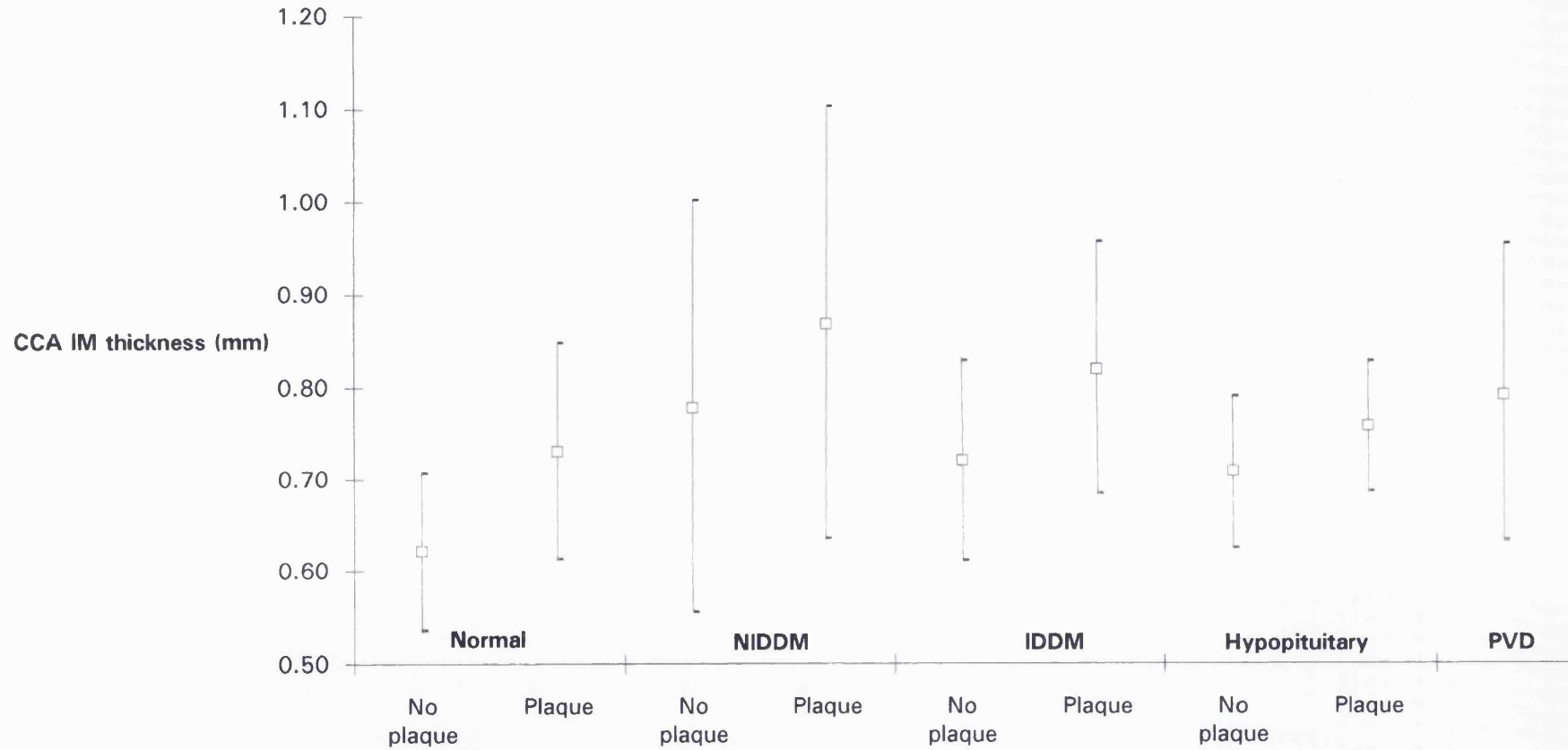
**Fig 17.4 Superimposition of regression lines of CCA IM thickness with age in the presence of plaque: Comparison between the normal and patient groups.**



CCA IM thickness (mm)



**Fig 17.5 Mean (+/- 1sd) IM thickness CCA after correction for age: comparison between individuals and patients with plaques and patients with PVD.**



**Fig 17.6 Mean (+/- 1sd) IM thickness after correction for age: comparison between individual and patients with and without plaques and patients with PVD.**

**Table 17.1**    **The relationship between CCA IM thickness and plaque**

Mean CCA IM thickness	No plaque	Plaque
< 0.58 mm	79	3 (3.6%)
0.58 - 0.82 mm	77	84 (52%)
> 0.82 mm	17	69 (80%)

$\chi^2 = 101.57$ , to 2 degrees of freedom,  $p < 0.001$

**Table 17.2** The relationship between CCA IM thickness and the presence of macrovascular disease

Mean CCA IM thickness	No MVD	MVD
< 0.58 mm	77	0 (0%)
0.58 - 0.82 mm	129	12 (8.5%)
> 0.82 mm	55	53 (49%)

$\chi^2 = 88.14$ , to 2 degrees of freedom,  $p < 0.001$

### **17.33 IM thickness and known risk factors.**

#### **a) all**

Multiple regression analysis using sex, age, smoking habit (pack years), systolic blood pressure, diastolic blood pressure, IDDM (disease present = 1), NIDDM (disease present = 1), and hypopituitarism (disease present = 1) as independent risk factors with IM thickness as the dependent variables. IM thickness could be predicted by the following formula:

CCA IM thickness = 0.17 + 0.0068 age + 0.00046 packyears + 0.014 sex + 0.0008 systolic BP + 0.0007 diastolic BP + 0.118 NIDDM + 0.088 IDDM + 0.059 hypopituitary.

The correlation coefficient (r) for this formula is 0.67 and in an analysis of variance F=32.9, p<0.0001. The best subset is represented by:

CCA IM thickness = 0.17 + 0.008 age + 0.0024 packyears and the correlation coefficient for this formula is 0.59.

#### **b) No plaque**

Multiple regression analysis was performed using the same independent variables after exclusion of all individuals with plaque. The following formula could be used to predict the CCA IM thickness:

CCA IM thickness = 0.217 + 0.0046 age + 0.0008 packyears - 0.007 sex + 0.0005 systolic BP + 0.0015 diastolic BP + 0.14 NIDDM + 0.08 IDDM + 0.062 hypopituitary.

The correlation coefficient (r) for this formula is 0.67 and in an analysis of variance F=15.6, p<0.0001 (table 17.3). The best subset is represented by:

CCA IM thickness = 0.36 + 0.0058 age + 0.13 packyears

and the correlation coefficient is virtually unchanged at 0.64.

**Table 17.3 Multiple linear regression of independent variables contributing to the prediction of the CCA IM thickness in the absence of plaque and the significance of each component**

	$c = 0.217112$		
Age	$b1 = 0.004575$	$F = 19.8$	$p < 0.0001$
Packyears	$b2 = 0.00083$	$F = 1.014$	$p = 0.3154$
Sex	$b3 = -0.006088$	$F = 0.075$	$p = 0.7833$
Systolic BP	$b4 = 0.000509$	$F = 0.274$	$p = 0.6015$
Diastolic BP	$b5 = 0.001451$	$F = 0.77$	$p = 0.3813$
Hypopituitary	$b6 = 0.062465$	$F = 1.68$	$p = 0.1964$
IDDM	$b7 = 0.080391$	$F = 5.41$	$p = 0.0212$
NIDDM	$b8 = 0.139337$	$F = 21.9$	$p < 0.0001$

## **17.4 Discussion**

### ***i) How do the patient groups compare with the volunteer group in the relationship between CCA IM thickness and age?***

In all the groups studied, the CCA IM thickness increased with age, though, in the absence of plaque, the patient groups had a significantly thicker CCA IM thickness than the volunteer group even after correction for age.

In all groups, the IM thickness increases with age, however, the regression line for patients with peripheral vascular disease runs parallel to that of our group of healthy volunteers and slope of the regression lines for the hypopituitary patients and the patients with IDDM are steeper than the normal group, hence these patients have an IM thickness similar to normal in their 20's to 40's but IM thickness increases faster than normal, becoming similar to that of patients with symptomatic with PVD with advancing years.

### ***ii) How do the patient groups compare with the volunteer group in the relationship between CCA IM thickness and the presence of plaque?***

Even in the absence of plaque, the patient groups have relatively thicker CCA IM layer than the normal group, with the NIDDM patients having age related IM thickness similar to that of the patients with symptomatic peripheral vascular disease, while the IDDM patients lie half way between the normal group and patients with PVD. This difference persists even after correction for age.

The mean IM thickness of all groups was thicker in the presence of plaque, however the mean CCA IM thickness of the normal group was thinner than that of patients with PVD and there was no significant difference between patients with IDDM and hypopituitarism compared to patients with PVD, whereas the mean CCA IM thickness of patients with NIDDM was significantly thicker even than patients with symptomatic PVD.

### ***iii) Can CCA IM thickness be used to predict the presence of plaques?***

Yes. The CCA IM thickness is a good predictor of the presence of plaque, as less than 4% of both patients and volunteers with a CCA IM thickness of less than 0.58 mm had plaques, whereas 80% with an IM thickness greater than 0.82mm had plaques.

***iv) Can CCA IM thickness be used to predict the presence of macrovascular disease?***

Yes. The CCA IM thickness is a good predictor of the presence of macrovascular disease, as no patients or volunteers with a CCA IM thickness of less than 0.58 mm had macrovascular disease, and only 8% with an IM thickness of between 0.58 and 0.82mm had macrovascular disease, whereas 49% of patients and volunteers with a CCA IM thickness over 0.82mm were affected.

***v) How do the patient groups compare with the volunteer group in the relationship between CCA IM thickness and risk factors?***

On multiple regression analysis, the main factors determining CCA IM thickness are age and packyears; the presence of IDDM, NIDDM or hypopituitarism does not significantly alter the correlation coefficient .

In conclusion, patients with NIDDM, IDDM and hypopituitarism have a significantly thicker CCA IM thickness than normal, whether or not plaques are present, and IM thickness can be used to predict the presence of plaques and macrovascular disease. NIDDM have a particularly thick IM, thicker even than patients with symptomatic PVD, suggesting additional factors may be involved in NIDDM which promote IM thickening.



CHAPTER 18

COMPLIANCE, DISTENSIBILITY, AND THE  
STIFFNESS INDEX:

COMPARISON BETWEEN PATIENT GROUPS

## **18.1 Introduction**

As discussed in chapter 11, compliance, distensibility, the elastic modulus and the stiffness index are all parameters which have been used to assess arterial wall movement in peripheral arteries. In our normal volunteers, we have shown that using the M-mode picture of the carotid artery mapped through three cardiac cycles, we can reproduce these results. We have subsequently used this to determine the arterial wall movement of our patient groups.

The aim of this chapter is to compare the arterial wall movement of each of the patient groups with our normal volunteer group, and to determine if using the M-mode picture to assess arterial wall movement offers any information of clinical significance. Thus the questions asked were:

- i) How do changes in arterial wall movement with age and with plaque compare among the patient groups and the volunteer group?*
- ii) Can arterial wall movement be used to differentiate patients at high risk of developing atherosclerosis from normal individuals?*

## **17.2 Method**

The method is described in Part III, and the derivations of the formulae used are discussed in 9.24

## **18.3 Results**

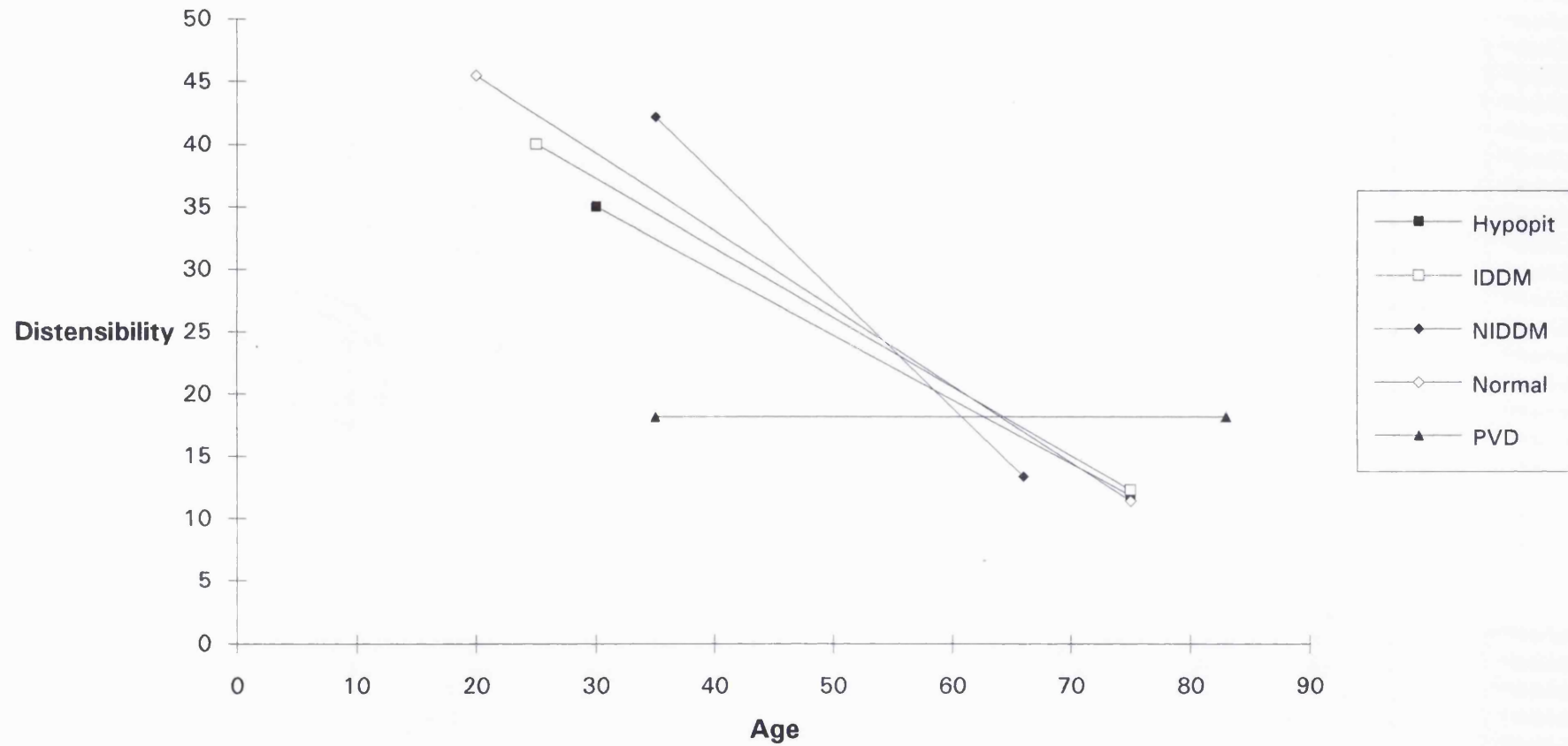
### **18.31 Compliance , distensibility, and the stiffness index of the common carotid artery**

#### **a) with age**

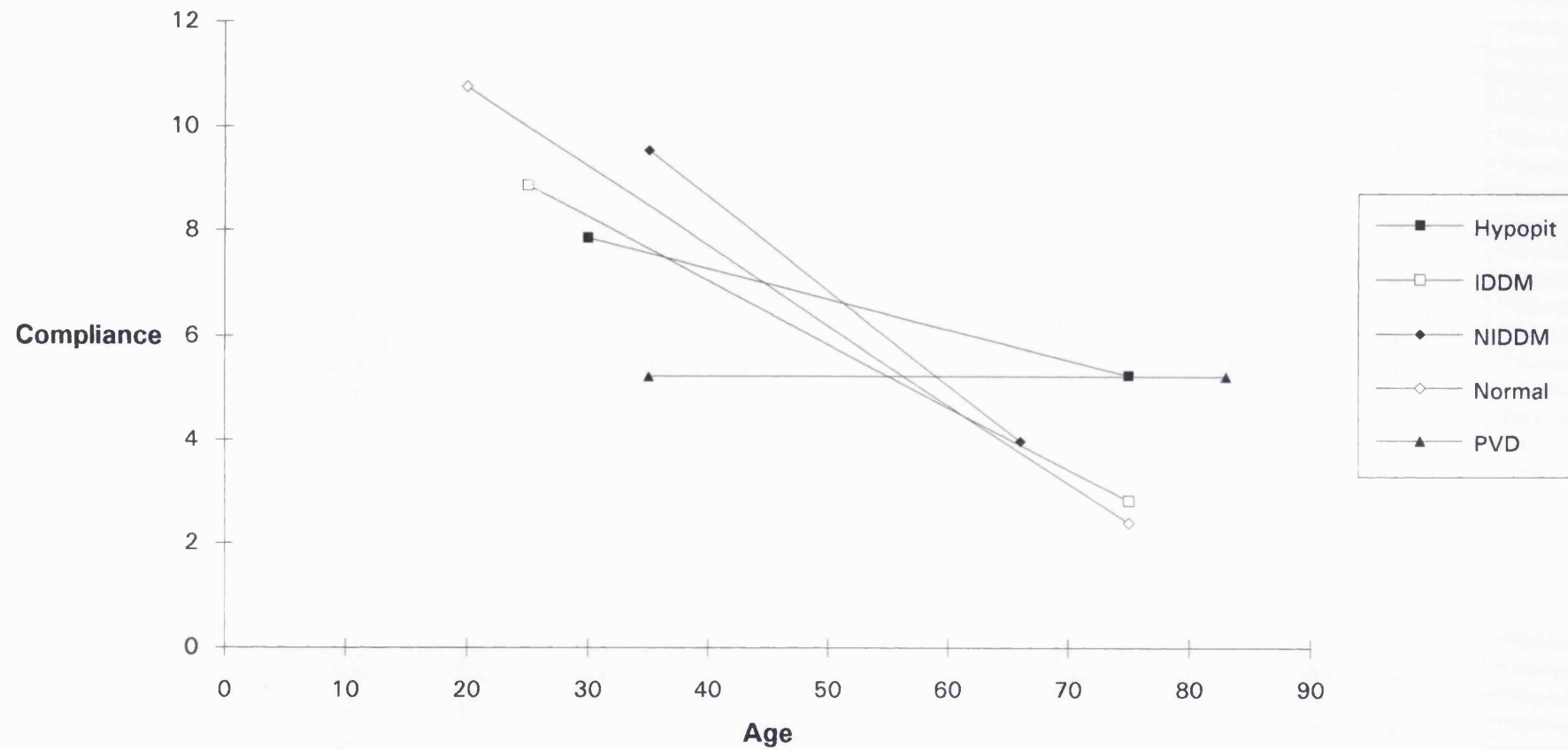
The regression lines for compliance, distensibility, and the stiffness index of the CCA in the absence of plaque are compared with the mean for patients with peripheral vascular disease in figures 18.1, 18.2, and 18.3. In all three patient groups (NIDDM, IDDM, and hypopituitary), the compliance and distensibility decreased with age, whereas the stiffness index increased. There was no significant difference between the patient groups (NIDDM, IDDM, and hypopituitary) and the normal volunteers.

#### **b) with plaque**

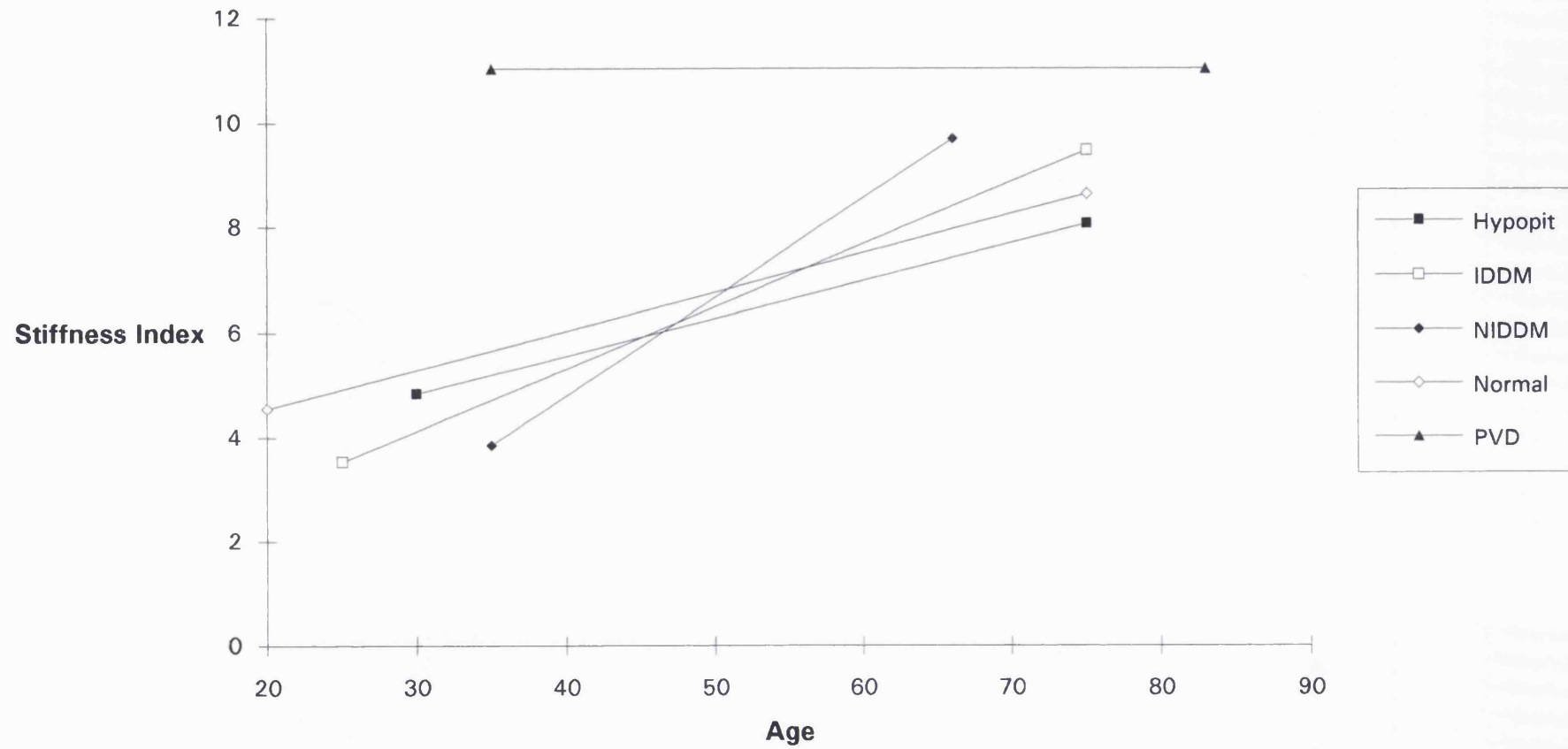
The mean distensibility, the mean compliance and the stiffness index of the CCA for the three patient groups after correction for age are compared for the presence of plaque in tables 18.1, 18.2 and 18.3 respectively, and there was no significant difference in arterial wall movement between individuals with plaque compared to those without. In contrast, all groups had a greater compliance and distensibility with a lower stiffness index than the group of patients with symptomatic peripheral vascular disease.



**Fig 18.1** The regression lines for mean distensibility in the absence of plaque are compared with the mean distensibility of patients with PVD



**Fig 18.2** The regression lines for mean compliance in the absence of plaque are compared with the mean compliance in the presence of PVD



**Fig 18.3** The regression lines for the mean stiffness index in the absence of plaque are compared to the mean stiffness index in the presence of PVD

**Table 18.1 Comparison between mean distensibility of the patient groups after correction for age.**

	No plaque			Plaque			t	p
	Mean	sd	n	Mean	sd	n		
Normal	*26.88	9.3	85	*26.56	10.99	51	0.17	NS
NIDDM	*27.62	9.32	40	*30.53	13.03	58	-1.29	NS
IDDM	*26.37	9.2	29	*25.1	10.32	23	0.46	NS
Hypopititary	*30.39	15.2	12	**22.59	7.19	22	1.68	NS
PVD				17.38	9.18	46		

All groups are significantly different from the symptomatic PVD group, \*p<0.005, and \*\*p<0.01

**Table 18.2 Comparison between mean compliance of the patient groups after correction for age.**

	No plaque			Plaque			t	p
	Mean	sd	n	Mean	sd	n		
Normal	*6.19	2.25	85	*6.33	2.55	51	-0.32	NS
NIDDM	*6.84	2.27	40	*7.28	2.62	58	-0.88	NS
IDDM	*6.17	2.04	29	*6.58	2.83	23	-0.58	NS
Hypopituitary	**6.7	3.07	12	5.29	2.37	22	1.38	NS
PVD				4.59	2.51	46		

All groups are significantly different from the symptomatic PVD group except the hypopituitary patients with plaque, \*p<0.005, and \*\*p<0.01.



**Table 18.3 Comparison between the mean stiffness index of the patient groups after correction for age.**

	No plaque			Plaque			t	p
	Mean	sd	n	Mean	sd	n		
Normal	*6.36	2.01	85	*7.67	5.4	51	-1.67	NS
NIDDM	*6.67	3.55	40	*6.8	5.32	58	-0.14	NS
IDDM	*6.5	1.58	29	*6.86	2.91	23	-0.54	NS
Hypopititary	*6.27	1.68	12	**8.01	5.91	22	-1.29	NS
PVD				12.27	9.35	46		

All groups are significantly different from the symptomatic PVD group, \*p<0.005, and \*\*p<0.02

## **18.4 Discussion**

*i) How do changes in arterial wall movement with age and with plaque compare among the patient groups and the volunteer group?*

In all three groups of patients studied, the compliance and distensibility decrease with age, whereas the stiffness index increases with age. The slope of the regression lines for all three patient groups was similar to that of the normal volunteers, even in the absence of plaque. With advancing age, the distensibility, compliance and stiffness index of the CCA approached that of patients with symptomatic peripheral disease.

*ii) Can arterial wall movement be used to differentiate patients at high risk of developing atherosclerosis from normal individuals?*

No. Not in this group of patients. This may be related to the age of our patient groups, as our study of normal volunteers showed that arterial wall movement tails off in an exponential fashion, with the maximum fall occurring in the first two decades. Most of our patients were in their forties and fifties.

Alternatively, if the changes are minor, this may not be detected, either because of the wide inter-individual variation in wall movement, or because our method was not sensitive enough to pick up these changes.

Though arterial wall movement did not distinguish between our patient groups and our normal volunteers, it is interesting that even those with asymptomatic plaques had significantly better carotid artery wall movement than our group of patients with symptomatic peripheral vascular disease.

CHAPTER 19

GENERAL DISCUSSION AND CONCLUSION

## **19.1 General discussion**

The aim of this thesis was to examine IM thickness and arterial wall distensibility, compliance and elastic modulus measured with high resolution ultrasound, and to assess the value of these measurements in the prediction of early atherosclerotic disease, either as bifurcation plaques or as occult macrovascular disease. Part I of the thesis reviewed the literature concerning the development of ultrasound and its use as a diagnostic tool in arterial disease (Chapter 1). As the main focus of this thesis concerned the use of high resolution ultrasound in the detection of early asymptomatic carotid artery disease, the literature relating to the pathology, natural history and risk factors associated with asymptomatic carotid artery disease was reviewed in chapter 2, and the literature relating to high resolution ultrasound and the measurement of intima-media thickness were examined in chapter 3. Finally other noninvasive methods of detecting occult atherosclerosis were discussed in chapter 4.

In Part II, a number of preliminary studies are reported. The aim of these studies was to identify the sites around the carotid bifurcation at which small plaques were most likely to occur and the variation in the site of early plaques. In the first study (chapter 5), four different bulb morphologies identifiable with angiography were described and 104 angiograms were examined by two observers. This study showed that the site of origin of the bulb varied, and the commonest type of bulb was only found in 40% of the angiograms studied. A second study on bulb morphology (chapter 6) confirmed that these bulb types could also be identified with high resolution ultrasound. A disparity was noted between the incidence of bulb types in the two studies, this could be explained by the ability of high resolution ultrasound to image the arterial wall, whereas, angiography can only image the lumen of the vessel. Though different bulb morphologies have not previously been recorded, geometric variations at the carotid bifurcation have (Fisher and Fieman, 1990). Bulb morphology may be dependent on the angle of take-off of the internal carotid artery in relation to the external carotid artery, this would alter the flow patterns in the region of the bulb and could account for the variability of the bulb origin in relation to the flow divider. Ultrasound, being restricted to the two-dimensional image, is not the right medium to pursue this hypothesis, and three dimensional flow models would be necessary to examine this further.

In a third study (chapter 7), the carotid bifurcation was examined with high resolution ultrasound. The bifurcation was divided into regions in relation to the flow divider and the regions were compared for the frequency of small and large plaques. This demonstrated that plaques occur predominantly in regions proximal to the flow divider in relation to the bulb origin. The site of early plaque formation varies with bulb morphology as the majority of plaques develop in relation the bulb origin. This is in contradiction to the previously reported studies that propose that plaques develop in areas of low shear stress associated with flow vortices that occur opposite to the flow divider (Ku and Giddens, 1983; Rindt et al, 1987, Reneman et al, 1988) but is supported by the work of Burrig and Hort (Burrig and Hort, 1988), in which they demonstrated an endothelial disarray with an associated ridge at the bulb origin. A similar area was observed opposite the flow divider at the site of early atherosclerotic lesions. If, as proposed above, the geometry of the carotid bifurcation and in particular the angle between internal and external carotid arteries is responsible for the position of the bulb origin, then as flow depends on a number of factors including the angle of divergence and the sharpness of flow divider, this area would be exposed to the areas of low shear stress and vortical flow discussed and be prone to the development of early plaque. The purpose of identifying sites of early plaque formation was to identify the sites which yield the largest number of small plaques, this information was used to identify small plaques in the volunteers and patients discussed in Part IV.

The last chapter in Part II (chapter 8) examined the correlation between histological intima-media thickness and the measurement of intima-media using high resolution ultrasound, and the associated histological changes. High resolution ultrasound studies of intima-media thickening have shown considerable variation in intima-media thickness, from 0.48mm to as thick as 4.09mm (Salonen and Salonen, 1991) and most do not differentiate between intima-media thickness and plaques in the common carotid artery, although an intima-media thickness of less than 1.2mm is not considered to represent a plaque (Salonen et al, 1986). Therefore, the purpose of this study was to examine the histological constituents of the intima-media of carotid arteries taken at post mortem and compare them with the ultrasound image. Arteries with an intima-media thickness of less than 0.8 mm were compared with arteries with progressively thicker intima-media layers. This study showed that the histological

features of plaque formation, namely cholesterol crystals, medial atrophy, fatty change, necrotic debris and fibrous change, were all found in the arterial wall of vessels with an intima-media thickness less than 0.8mm, yet this thickness would not be considered to represent a plaque. With increasing thickness, the proportion of vessels showing these histological features increased. Therefore, changes in intima-media thickness of a vessel are similar to those found in early plaque formation.

In part III, the methods used to measure intima-media thickness on the ultrasound image, and arterial wall excursion on the M-mode image were described (chapter 9), and the methods used to identify macrovascular disease were outlined (chapter 10).

Part IV consisted of a number of studies on normal healthy volunteers, three patient groups at high risk of developing premature atherosclerosis, and a group of patients with symptomatic peripheral vascular disease. The purpose of these studies was to assess the value of intima-media thickness measurement in predicting the presence of bifurcation plaques and macrovascular disease. M-mode ultrasound, available in modern duplex equipment, was used to measure arterial wall excursion, and hence to calculate compliance, distensibility and the elastic modulus; in order to assess the ability of these parameters to predict the presence of plaque and to compare the results from M-mode ultrasound examination with previously reported methods.

Chapters 11 and 12 examined the intima-media thickness and arterial wall compliance, distensibility and elastic modulus of the carotid and femoral arteries of a group of volunteers. As has previously been reported (Tell et al, 1989; Salonen and Salonen, 1991; ), age was found to be the major factor determining intima-media thickness. This was true of all the sites studied though the bulb origin was significantly thicker than the other sites measured. Cigarette smoking habit was also found to be a significant risk factor for intima-media thickening, independent of age. Though Salonen and Salonen have reported that systolic and diastolic BP are independent factors producing intima-media thickening in the CCA (with a 3.17 fold risk after adjusting for age) (Salonen and Salonen (ii), 1991), this was not found to be true in this study,

possibly because our cohort is a relatively small and the majority of the volunteers were normotensive.

The intima-media thickness of the common carotid artery in individuals with carotid bifurcation plaques was compared to those without. The intima-media thickness of the common carotid artery proved to be a good predictor of the presence of bifurcation plaque, irrespective of age, as only 6% of subjects with a mean CCA intima-media thickness of 0.58mm or less had bifurcation plaques, compared to 50% of subjects with an intima-media thickness of between 0.59-0.82mm, and all subjects with an intima-media thickness of 0.82mm and over.

Distensibility, compliance and the elastic modulus were also found to be age dependent with a decrease in arterial wall compliance and distensibility, and a concomitant rise in the elastic modulus and stiffness index with age. A linear relationship was observed between age and distensibility and compliance in the common carotid artery, as compared to the carotid bulb where the fall in distensibility and compliance with age is exponential. Using M-mode ultrasound, we have observed similar trends to those reported Reneman and colleagues using multi-gated pulsed Doppler, and Baskett and colleagues using Doppler shifted ultrasound to measure pulse wave velocities (Reneman et al, 1985; Baskett et al, 1990). These same researchers have also reported differences in carotid wall properties between men and women (Van Merode et al, 1988; Baskett et al, 1990), but this was not confirmed in this study. In the femoral arteries, the correlation between distensibility, compliance, the elastic modulus and age was poor. The presence of plaque did not significantly alter the distensibility, compliance or the elastic modulus of the arteries studied as arteries become progressively stiffer with age, and by the time plaques become evident, they are poorly compliant. Therefore, arterial distensibility, compliance, and the elastic modulus are not useful parameters for the prediction of early atherosclerosis elsewhere in the body. As intima-media thickness changes occur later in life and compliance and distensibility changes occur early, the value of M-mode measurement of arterial wall may be in screening individuals in their second and third decades to predict their risk of developing disease as Riley has shown that in teenagers, parental history of ischaemic heart disease is associated with a decrease in arterial elasticity (Riley et al, 1986).

The purpose of chapters 13-15 was to assess the ability of intima-media thickness measurement to predict the presence of bifurcation plaques or macrovascular disease in patients with known risk factors, therefore the intima-media thickness distensibility, compliance and elastic modulus were examined in three patient groups at risk of developing premature atherosclerosis, these were patients with non-insulin dependent diabetes mellitus (NIDDM), insulin dependent diabetes mellitus (IDDM), and patients with hypopituitarism. In all three groups of patients, the relationship between intima-media thickening and age was preserved. In both IDDM and hypopituitary patients, intima-media thickness was also a good predictor of the presence of plaque. Multiple regression analysis revealed age as the strongest risk factor followed by smoking habit in IDDM and BMI in hypopituitary patients. In contrast, the NIDDM patients were found to have a very thick CCA intima-media and the correlation between the presence of plaque and intima-media thickness was poor. Also, the correlation was poor between intima-media thickness and other risk factors (including lipids), on multiple regression analysis.

In chapter 16, the intima-media thickness and arterial wall movement of the CCA were examined in a group of patients with symptomatic peripheral vascular disease (PVD) and the healthy volunteer group was compared to all four patient groups in chapters 17 and 18. CCA intima-media thickness was significantly less in healthy volunteers than all patient groups after correction for age. In all five groups, intima-media thickness increased with age and in the absence of plaque, the regression line for patients with NIDDM was almost identical to that of patients symptomatic PVD, while the lines for patients with IDDM and hypopituitarism lay half way between the normal group and patients with symptomatic PVD.

## **19.2 Conclusion**

Measurement of CCA intima-media thickness using high resolution ultrasound is a quick, noninvasive yet reproducible examination and CCA intima-media thickness is a good predictor of both the presence of bifurcation plaque and macrovascular disease. The strongest factors determining intima-media thickness are age and smoking habit. The NIDDM patients have a particularly thick CCA intima-media which does not appear to be governed by the same factors as the intima-media in other patient groups. No



correlation was observed between lipid profiles and intima-media thickness in NIDDM, IDDM and hypopituitarism.

Arterial wall distensibility, compliance or elastic modulus did not distinguish between patients at risk of atherosclerosis and normal individuals as arterial wall movement decreased with age and there was no difference in the decay lines of the three patient groups at risk of developing atherosclerosis compared with the healthy volunteers. Patients with symptomatic PVD had a very poor compliance and distensibility, with no age related change, and the arterial wall movement of the volunteer group and the three at risk patient groups approached that of the symptomatic group in the 7th -8th decades.

### **19.3 Future Directions**

Intima-media thickness of the CCA has been shown to be a good predictor of the presence of plaque and macrovascular disease in selected populations, but to determine the value of this measurement in predicting overall cardiovascular mortality and morbidity, intima-media thickness measurements need to be performed in community based studies that assess known cardiovascular risk factors, and monitor cardiovascular morbidity. Such studies are already in progress in Finland (the Kuopio ischaemic heart disease risk factor study, (Salonen and Salonen, 1989) and the United States of America (the ARIC study, 1989), but need to be repeated in other countries including England as risk factors and prevalence of cardiovascular disease show marked geographical variations.

Intima-media thickness measurement also has the potential to be used in the monitoring of disease progression and regression, and is particularly suited to monitoring the effects of therapy. The NIDDM patients discussed above have been recruited in to a double blind controlled trial on the effect of Bezafibrate in treatment of diabetic hyperlipidaemia, this trial will last five years, and it is hoped that intima-media thickness changes will be observed with successful therapy. The multicentre isradipine/diuretic atherosclerosis study ( The MIDAS Research Group, 1989) is a similar interventional trial being carried out in the United States.

Intima-media thickness measurement has the potential to be a useful tool indicating an individuals atherosclerotic status. It is quick to do, noninvasive and

repeatable. Furthermore, it can be performed on a routine ultrasound machine equipped with a linear high resolution probe, and now that these machines are becoming increasingly portable, it need not be restricted to an X-ray department or vascular laboratory. In order to validate the efficacy of the CCA intima-media thickness in predicting an individuals atherosclerotic status, formal comparison with a gold standard needs to be performed, and as most of the mortality related to atherosclerosis is due to coronary atherosclerosis, CCA intima-media thickness needs to be compared with coronary angiography or a noninvasive indicator of coronary disease, such as ECG stress testing.

Though there is no role for compliance, distensibility and elastic modulus measurements in the age group at risk of having plaques or macrovascular disease, the work of Riley and colleagues suggest that this modality should not be dismissed as it may be important as a screening tool in a younger population, where changes occur that may predict future events. The M-mode is readily available on most ultrasound machines and further work needs to be done to assess the value of this in predicting the future development of atherosclerotic disease. Such a tool would be very useful in identifying those at risk that would benefit from preemptive treatment and risk factor reduction.

## LIST OF REFERENCES

- Abbott RD, Yin Y, Reed DM, Yano K.: Risk of stroke in male cigarette smokers. *N. Engl J. Med.* 1986;315:717-20
- AbuRhama AF and Robinson PA: Prospective Clinicopathophysiological Follow-up study of Asymptomatic neck bruit. *Am. Surg.* 1990; 56:108-113
- Ackerstaff RGA, Eikleboom BC, Hoeneveld H., Moll FL, Vries AR: Ultrasonic duplex scanning in atherosclerotic disease of the innominate, subclavian and vertebral arteries. A comparative study with angiography. *Ultrasound Med Biol.* 1984 10:409-18
- Ahn SS, Baker JD, Walden K, Moore WS :Which asymptomatic patients should undergo routine screening carotid duplex scan? *Am J Surg*1991,162:180-3
- Aldoori MI, Baird RN, Al-Sam SZ, Cole SEA, Mera S, Davies JD: Duplex scanning and plaque histology in cerebral ischaemia. *Eur. J. Vasc Surg.* 1987, 1:159-164
- Allen N: The significance of vascular murmurs in the head and neck. *Geriatrics* 1965; 20:525
- Ammar AD, Ernst RL, Lin JE, Travers H: The influence of repeated carotid plaque haemorrhages on the production of cerebrovascular symptoms. *J. Vasc Surg* 1986; 3:857-9
- Anitschkow N: Experimental atherosclerosis in animals. In *Atherosclerosis*. Ed Cowdry EV. MacMillan, New York. pp271-322, 1933
- Armitage P and Berry G.: *Statistical Methods in Medical Research*, Blackwell 1987
- Armstrong ML: Regression of atherosclerosis. *Atherosclerosis Review.* 1976; 1:137-82
- Aro A, Ventilainen E, Uusitupa M, Siitonen O, Korhonen, Pyorala K: Abnormalities in serum lipids and lipoproteins in newly diagnosed diabetics. *Diabetologia* 1981; 21: 244
- Asymptomatic Carotid Atherosclerosis Study Group (ACAS). Study design for randomised prospective trial of carotid endarterectomy for asymptomatic carotid stenosis. *Stroke* 1989; 20:844.
- Bagi P, Schroeder T, Sillesen H, and Lorentzen JE: Real time B-mode mapping of the great saphenous vein. *Eur J Vasc Surg.* 1989; 3:103-5
- Baker DW : Pulsed ultrasonic Doppler blood-flow sensing. *IEEE Journal of Sonics and Ultrasonics.* 1970; 17:170
- Baker JD. Post stress Doppler ankle pressures. *Arch Surg* 1978;113:1171-3
- Bandyk DF, Moldenhauer P, Lipchik E, Schreiber E, Pohl L, Cato R, Towne JB : Accuracy of duplex scanning in the detection of stenosis after carotid endarterectomy. *J. Vasc. Surg.* 1988; 8: 696-702
- Bandyk DF, Seabrook GR, Moldenhauer P, Lavin J, Edwards J, Cato R, Towne JB: Haemodynamics of graft stenosis. *J. Vasc. Surg.* 1988; 8: 688-695
- Baranowski A, Adams CWM, Bayliss OB, Bowyer DB: Connective tissue responses to oxysterols. *Atherosclerosis* 1982 41:255-66

- Barber F, Baker D, Nation A: Ultrasonic duplex echo-Doppler scanner. *IEEE Trans. Biomed. Eng.* 1974; 21: 109-113
- Barnes RW, Bone GE, Reinertson J, Slaymaker EE, Hokanson DE and Strandness DE: Noninvasive ultrasonic carotid angiography: prospective validation by contrast angiography. *Surgery* 1976; 80:328-35
- Barnett HJM. The contribution of multicentre trials to stroke prevention and treatment *Arch Neurol* 1990; 47(4):441-4
- Barrett-Connor E, Grundy SM, and Holdbrook MJ: Plasma lipids and diabetes mellitus in an adult community. *Am J Epidemiology* 1982; 115: 657-663
- Barrett-Connor E, Witztum JL, and Holdbrook MJ.: A community study of high density lipoproteins in adult non-insulin dependent diabetics. *Am J Epidemiology* 1983; 117: 186-192
- Baskett JJ, Lewis RR, Beasley MG and Goslin RG. Changes in carotid artery compliance with age. *Age and ageing* 1990;19:241-246
- Beach KW, and Strandness DE: Arteriosclerosis obliterans and associated risk factors in insulin dependent and non-insulin dependent diabetes. *Diabetes* 1980; 29: 882-888
- Beach KW, Brunzell JD, Conquest LL, and Strandness DE: The correlation of atherosclerosis obliterans with lipoproteins in insulin dependent and non-insulin dependent diabetes. *Diabetes* 1979; 28:836-840
- Belcaro G, Renton S, and Nicolaidis AN: Ultrasonic Biopsy. A new technique to follow up regression and progression of atherosclerosis. *Curr. Ther. Res.* 1990; 48 :116-127
- Belcaro G, Barsotti A, and Nicolaidis AN: Ultrasonic Biopsy - A noninvasive screening technique to evaluate the cardiovascular risk and to follow up the progression and regression of arteriosclerosis. *VASA* 1991; 20: 40-50
- Berens ES, Kouchoukos NT, Murphy SF, Wareing TH. Preoperative carotid artery screening in elderly patients undergoing cardiac surgery. *J Vasc Surg* 1992;15(2):313-21
- Bernstein EF and Fronek A. Current status of noninvasive tests in the diagnosis of peripheral arterial disease. *Surg Clin N.AM* 1982; 62:473-87
- Bharadvaj BK, Mabon RF, Giddens DP: Steady flow in a model human carotid bifurcation. *J Biomech* 1982; 15 (5):363-378
- Blackshear PL, Blackshear GL, Newell MK. The localisation of transient subendothelial water filled blisters in the in situ rabbit aorta as a result of reduction in pressure. In *Fluid dynamics as a localising factor for atherosclerosis*
- Blackshear WM, Phillips DJ, Thiele BC, Hirsch JH, Chikos PM, Marinelli MR, Ward KJ, Strandness DE. Detection of carotid occlusive disease by ultrasonic imaging and pulsed Doppler spectrum analysis. *Surgery* 1979; 86:698-706
- Bland JM and Altman DG :Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1 (8476): 307-310
- Blue SK, McKinney WM, Barnes RW, and Toole JF: Ultrasonic B-mode scanning for study of extracranial vascular disease. *Neurology* 1972; 22:1079-85.

- Bluth EI, Kay D, Merritt CRB, Sullivan M, Farr G, Mills NL, Foreman M, Sloan K, Schlater M, Stewart J: Sonographic characterisation of carotid plaque: detection of haemorrhage. *A.J.R.* 1986; 146:1061-5
- Bogousslavsky J, Despland PA and Regli F: Asymptomatic tight stenosis of the internal carotid artery: Long term prognosis. *Neurology* 1986; 36:861-3
- Bogousslavsky J, Van Melle G, Despland PA, and Regli F: Alcohol consumption and carotid atherosclerosis in the Lausanne Stroke Registry. *Stroke* 1990; 21:715-20
- Bolondi L, Gandolfi L, Arienti V, Caletti GC, Corcioni E, Gasbarrini G, and Labo G: Ultrasonography in the diagnosis of portal hypertension: Diminished response of the portal vessels to respiration. *Radiology* 1982 142 167-172
- Bom N, Lancee CT, Honkoop J, Hugenholtz PG. Ultrasonic viewer for cross sectional analysis of moving cardiac structures. *Biomed Eng* 1971; 6:500-3
- Bond MG, Wilmoth SK, Enevold GL, Strickland HL: 1989 *Am. J. Med* 86(supp 4a):33-36
- Bond MG, Strickland HL, Wilmoth SK, Safrit A, Phillips R, Szostak L. Intervention clinical trials using noninvasive ultrasound end points: the multicentre Isradipine/diuretic atherosclerosis study. *Journal of Cardiovascular Pharmacology* 1990;15 suppl1:s30
- Bonithon-Kopp C, Scarabin P-Y, Taquet A, Touboul PJ, Malmejac A, Guize L Risk factors for early carotid atherosclerosis in middle-aged french women. *Circulation* 1991; 11:966-72
- Borhani NO, Bond MG, Sowers JR, Canossa-Terris M, Buckalew V, Gibbons ME, Worthy AJ :The MIDAS: a study of the antiatherogenic properties of isradipine in hypertensive patients. *J. Cardiovasc. Pharmacol* 1991;18 suppl 3:s15-9
- Born GVR. Pathogenesis and properties of atherosclerotic lesions as exemplified by carotid plaques. In: *Diagnostic vascular ultrasound*. Eds Labs KH, Yager KA, Fitzgerald DE, Woodcock JP. Neverberg-Heusler D. Edward Arnold, London pp23-32, 1992.
- Brener BJ, Brief DK, Alpert J, Goldenkranz RJ, Parsonnet V. The risk of stroke in patients with asymptomatic carotid stenosis undergoing cardiac surgery: A follow-up study. *J. Vasc Surg* 1987;5:269-79
- Breslau PJ, Fell G, Phillips DJ, Thiele BL, and Strandness DE Jr.: Evaluation of carotid bifurcation disease: the role of common carotid artery velocity patterns. *Arch. Surg.* 1982; 117: 58-60
- Brown MS and Goldstein JL: Lipoprotein metabolism in macrophages, implications for cholesterol deposition in atherosclerosis. *Ann Rev Biochem* 1983; 52:223-261
- Brown MS and Goldstein JL: A receptor mediated pathway for cholesterol haemostasis. *Science* 1986; 232:34-47
- Brown TG: Direct contact ultrasonic scanning techniques for the visualisation of abdominal masses. In *Proceedings of the 2nd International Conference on Medical Electronics*. Iliffe and Sons Ltd. London 1960 p. 358
- Burns PN: Principles of deep Doppler ultrasonography. In: *Recent Advances in noninvasive diagnostic techniques in vascular disease*. Ed: EF Bernstein. The CV Mosby Co 1990

- Burrig K-F. and Hort W. Pathogenesis of carotid atherosclerosis. In Carotid artery plaques Eds Hennerici M., Sitzer G., Weger H-D. 1988 pp101-114
- Busuttill RW, Baker JD, Davidson RK and Machleder HI.: Carotid artery stenosis - Haemodynamic significance and clinical course. JAMA 1981 245:1438-41
- Camerota AJ. Correlation of B-Mode ultrasound of the carotid artery with arteriography and pathology. In Bond MG, Insull W, Glagov S, et al . (eds) Clinical diagnosis of atherosclerosis, Quantitative methods of evaluation 1982 pp 351-363 Springer-Verlag N
- Caplan LR, Gorelick PB, Hier DB. Race, sex and occlusive cerebrovascular disease: a review. Stroke 1986; 17 (4): 648-55
- Caracci BF, Zuckowski AJ, Hurley JJ, Naunheim KS, Auer AI.: Asymptomatic severe carotid stenosis J. Vasc. Surg. 1989;9:361-6
- Caro CG, Fitzgerald JM, Schroter RC: Atheroma and arterial wall stress. Observation, correlation and proposal of a shear dependent mass transfer mechanism of atherogenesis. Proc R Soc Lond B 1971; 177:109-159
- Caro C, Pedley T, Schroter R. The mechanics of the circulation. New York. Oxford University Press. 1978
- Carter SA. Response of ankle systolic pressure to leg exercise in mild or questionable arterial disease. N Eng J Med 1972; 287:578-82
- Cata R, Seabrook GR, Bandyk DF: Intraoperative duplex scanning following carotid artery reconstruction. J. Vasc. Surg. 1989; 13: 31-36
- Chambers BR and Norris JW: Clinical significance of asymptomatic neck bruits. Neurology 1985; 35:742-5
- Chambers BR and Norris JW : Outcome in patients with asymptomatic neck bruits. N. Eng J Med, 1986; 315:860-5
- Chan A, Beach KW, Martin DC and Strandness DE: Carotid artery disease in NIDDM diabetes. Diabetes care 1983;6:562-9
- Chilowsky C and Langevin MP: Procédes et appareils pour la production de signaux sous-marins dirigés et pour la localisation a distance d'obstacles sous-marins. Phil Mag 1927; 7(4): 417
- Colditz GA, Bonita R, Stampfer MJ, Willett WC, Rosner B, Speizer FE, Hennekens CH: Cigarette smoking and risk of stroke in middle aged women. N. Engl J. Med. 1988;318:937-41
- Colgan MP, Kingston W and Shanik DG.: Asymptomatic carotid stenosis: is prophylactic carotid endarterectomy justifiable? Br. J. Surg. 1985 ;72:313-4
- Colgan MP, Strode GR, Sommer JD, Gibbs JL, Sumner DS. : Prevalence of asymptomatic carotid artery disease: Results of duplex scanning in 384 unselected volunteers. J Vasc Surg 1988; 8: 674-8
- Collin J, Araujo L, Lindsell D. A community screening programme for abdominal aortic aneurysms. Eur J Vasc Surg. 1988; 2:83
- Cooperberg PL, Robertson WD, Fry P and Sweeney V.: High resolution real-time ultrasound of the carotid bifurcation. J. Clin. Ultrasound 1979 7:13-17

Cornhill JF and Roach MR :A quantitative study of the localisation of atherosclerotic lesions in the rabbit aorta. *Atherosclerosis* 1976; 23:489-501 1976)

Cossmann DV, Ellison JE, Wagner WH, Carroll RM, Treiman RL, Foran RF, Levin PM and Cohen JL: Comparison of contrast arteriography to arterial mapping with colour-flow duplex imaging in the lower extremities. *J Vasc Surg*; 1989; 10:522-9

Criqui MH, Fronck A, Barrett-Connor E, Klauber MR, Gabriel S, Goodman D. The prevalence of peripheral arterial disease in a defined population. *Circulation* 1985; 71:510-5

Criqui MH, Fronck A, Klauber MR, Barrett-Connor E, Gabriel S. The sensitivity, specificity, and predictive value of traditional clinical evaluation of peripheral arterial disease. *Circulation* 1985; 71:516-22

Crouse JR, Toole JF, McKinney WM, Digman MB, Howard G, Kahl FR, McMahan MR, Harpold GHI: Risk factors for extracranial carotid artery atherosclerosis. *Stroke* 1987;18:990-6

David TE, Humphries AW, Young JR and Beven EG: A correlation of neck bruits and arteriosclerotic carotid arteries. 1973 *Arch Surg* 107:729-31

Di Candio G, Campatelli A, Mosca F, Santi V, Casanova P, Bolondi L: Ultrasound detection of unusual spontaneous portosystemic shunts associated with uncomplicated portal hypertension. *J. Ultrasound Med*, 1985; 4: 297-305

Donald I and Brown TG: Demonstration of tissue interfaces within the body by ultrasonic echo sounding. *Br. J. Radiol* 1961; 34:539

Dorazio RA, Ezzet F, Nesbitt NJ: Long-term follow-up of asymptomatic carotid bruits. *Am.J. Surg.* 1980; 140:212-3

Duffield RG, Lewis B, Miller NE, Jamieson CW, Brunt JN, Colchester AC. Treatment of hyperlipidaemia retards progression of symptomatic femoral atherosclerosis. A randomised controlled trial. *Lancet* 1983; 2 : 639-42

Duguid JB. Thrombosis as a factor in the pathogenesis of coronary atherosclerosis. *J Pathol*, 1946; 58:207

Duncan GW, Lees RS, Ojemann RG and David SS: Concomitants of atherosclerotic carotid artery stenosis. *Stroke* 1977; 8:665-9

Dussik KT, Dussik F and Wyt L. : Auf dem Wege zur Hyperphonographie des Gehirnes. *Wien Med Wschr* 1947; 97:425

Ellis MR, Franks PJ, Cuming R, Powell JT, Greenhalgh RM. Prevalence, progression and natural history of asymptomatic carotid stenosis: is there a place for carotid endarterectomy? *Eur J Vasc Surg*1992;6(2):172-7

Erikson J and Thaulow E. Follow-up of patients with asymptomatic myocardial ischaemia. In *Silent myocardial ischaemia*. Eds: Rutishauser W., Roskamm H. Springer, Berlin 1984. p156

European Carotid Surgery Trialists Collaborative Group. MRC European carotid surgery trial: interim results for symptomatic patients with severe (70-99%) or with mild (0-29%) carotid stenosis. *Lancet* 1991; 337:1235-1243

Faggioli GL, Curl GR, Ricotta JJ. The role of carotid screening before coronary artery bypass. *J Vasc Surg*, 1990; 12:72J4-9

- Fagiotto A, Ross R, Harker L. Studies of hypercholesterolaemia in non-human primates. 1. Changes that lead to fatty streak formation. *Arteriosclerosis* 1984;4:323
- Farrar DJ, Green HD, Wagner WP, Bond MG: Aortic pulse wave velocity, elasticity and composition in a non-human primate model of atherosclerosis. *Circ Res.* 1978; 43:52-62
- Farrar DJ, Green HD, Bond MG, Wagner WP.:Reduction in pulse wave velocity and improvement of aortic distensibility accompanying regression of atherosclerosis in the Rhesus monkey. *Circ. Res.* 1980; 47:425-432
- Fell G, Phillips DJ, Chikos PM, Harley JD, Thiel-BL, Strandness DE Ultrasonic duplex scanning for disease of the carotid artery. *Circulation* 1979; 64:1191-1195
- Fields WS, Lemak NA, Frankowski RF, Bigelow RH: Controlled trial of aspirin in cerebral ischaemia. *Circulation* 1980; 62:90-96
- Fish PJ.: Multi-channel direction resolving Doppler angiography. Proceedings of the 2nd European Congress on Ultrasonics in Medicine *Excerpta Medica, Amsterdam* 1975. p. 153
- Fisher M, Fieman S. Geometric factors of the bifurcation in carotid atherogenesis. *Stroke.* 1990; 21 (2):267-71
- Flaherty JT, Pierce JE, Ferrans VJ, Patel DJ, Tucker WK, Fry DL. Endothelial nuclear patterns in the canine arterial tree with particular reference to haemodynamic events. *Circ Res* 1972; 30:23-33
- Ford CS, Crouse JR, Howard G, Toole JF, Ball MR, Frye J. The role of plasma lipids in carotid bifurcation atherosclerosis. *Ann Neurol.* 1985; 17:301-303
- Fowkes FGR, Housley E, Macintyre CC, Prescott RJ, Ruckley CV Variability of ankle and brachial systolic pressures in the measurement of atherosclerotic peripheral vascular disease. *J Epidem and Comm Health* 1988; 42:128-33
- Fowkes FG, Housley E, Macintyre CC, Prescott RJ, Ruckley CV Reproducibility of reactive hyperaemia test in the measurement of peripheral vascular disease. *Br J Surg.* 1988; 75:743-6
- Fowl RJ, Marsch JG, Love M, Patterson RB, Shukla R. Prevalence of haemodynamically significant stenosis of the carotid artery in an asymptomatic veteran population. *Surg. Gynecol & Obstet.* 1991;172:13-6
- Fox KM, Selwyn A, Oakley D. Shillingford JP.: The relation between the precordial pojection of ST segment changes after exercise and coronary angiographic findings. *Am J Cardiol.* 1979; 43:541-6
- Fox MJ, Tripolitis A, Kirby S, Jamieson CW. A comparison of a reactive hyperaemia test with a standard exercise test in the evaluation of peripheral vascular disease. *Br J Surg* 1977; 64:290
- Froelicher VF, Thompson AJ, Wolthius R, Fuchs R, Balusek R, Longo MR, Triebwasser JH, Lancaster MC. Angiographic findings in asymptomatic aircrewmen with electrocardiographic abnormalities. *Am J Cardiol.* 1977; 39:32-8
- Fry DL: Acute vascular endothelial changes associated with increased blood velocity gradients. *Circ Res* 1968; 22: 165-197



- Fryer JA, Myers PC, and Appleberg M.: Carotid intraplaque hemorrhage: The significance of neovascularity. *J. Vasc. Surg.* 1987; 6:341-9
- Galton F. *Inquiries into human faculty and development.* London MacMillan 1883
- Gautier JC, Rosa A and Lhermitte F.: Auscultation carotidienne. Correlations chez 200 patients avec 332 angiographies. *Rev. Neurol. (Paris)* 1975; 131:175-84
- Gelabert HA, Moore WS. Reducing the risk of stroke: identifying patients to refer for carotid endarterectomy. *Geriatrics* 1991; 46(9): 22-26
- Giddens DP, Mabon RF, and Cassanove RA: Measurements of disordered flow distal to subtotal vascular stenoses in the thoracic aorta of dogs. *Circulation* 1976; 39(1):112-19
- Gill JS, Zezulka AV, Shipley MJ, Gill SK, Beevers DG: Stroke and alcohol consumption. *N. Engl J. Med.* 1986; 315:1041-6
- Gill RW, Kossoff G, Warren PS, Garrett WJ: Umbilical venous flow in normal and complicated pregnancy. *Ultrasound Med. Biol.*, 1984; 10: 349-363
- Ginsberg HN: Lipoprotein physiology in non-diabetic and diabetic states. Relationship to atherogenesis. *Diabetes care* 1991;14:839-55
- Giral P, Pithois-Merli I, Filitti V, Levenson J, Plainfosse MC, Mainardi C, Simon AC: Risk factors and early extracoronary atherosclerotic plaques detected by three-site ultrasound imaging in hypercholesterolaemic men. *Arch Intern Med.* 1991;151:950-6
- Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ Compensatory enlargement of human atherosclerotic coronary arteries. *N Eng J Med* 1987; 316:1371-5
- Glagov S. and Zarins CK.: Is intimal hyperplasia an adaptive response or a pathological process. *J Vasc Surg*, 1989; 10:571-573
- Glagov S. Zarins CK: Natural history of human atherosclerotic lesions: Changes in luminal configuration. in Budinger et al. (eds): *Noninvasive techniques for assessment of atherosclerosis in peripheral, carotid, and coronary arteries.* New York Raven Press
- Glagov S, Zarins CL, Bassiouny H and Giddens DP. Haemodynamic determinants of intimal thickening. Abstract presented at the 1990 Milan International workshop on ultrasound methods for atherosclerosis research and prevention.
- Goes E, Janssens W, Maillet B, Freson M, Steyaert L, Osteaux M: Tissue characterization of atheromatous plaques: Correlation between ultrasound image and histological findings. *J. Clin Ultrasound* 1990; 18:611-17
- Gordon D. :Echoencephalography *Brit Med J.* 1959 i:1500
- Gorelick PB, Rodin MB, Langenberg P, Hier DB, Costigan J, Gomez I, Spontak S. Is acute alcohol ingestion a risk factor for ischaemic stroke? *Stroke* 1987;18:359-64
- Gosling RG, Hayes JA, Segre-Mackay W.: Induction of atheroma in cockerels as a model for studying alterations in blood flow. *J. Atherosclerosis Res.* 1969; 9:47-56
- Gostomzyk J-G, Heller W-D, Gerhardt P, Lee PN, Keil U: B-scan ultrasound examination of the carotid arteries within a representative population (MONICA Project Augsburg) *Klin Wochenschr* 1988 66(suppl 11):58-65

- Gray-Weale AC, Graham JC, Burnett JR, Byrne K and Lusby RJ.: Carotid artery atheroma: Comparison of preoperative B-mode ultrasound appearance with carotid endarterectomy specimen pathology. *J. Cardiovasc. Surg* 1988; 29:676-681
- Green PS: Real time, high resolution ultrasonic carotid arteriography system. In: Bernstein EF (ed). *Noninvasive diagnostic techniques in vascular disease*. pp 29-39 1978 CV Mosby Co. St. Louis.
- Greenhalgh RM, Laing S, Ellis MR. How can we detect early subclinical disease in vascular surgery. In: *Issues in current practice*, Greenhalgh R.M., Jamieson C.W., Nicolaides A.N. Grune and Stratton 1986:57
- Grigg MJ, Wolfe JHN, Tovar A and Nicolaides AN.: The reliability of duplex derived haemodynamic measurements in the assessment of femoro-distal grafts. *Eur. J. Vasc. Surg.* 1988; 2: 171-181
- Grottum P, Svindland A, Walloe L.: Localization of early atherosclerotic lesions in the right carotid bifurcation in humans. *Acta Patol Microbiol Immunol Scand (A)*1983; 91:65-70
- Haapanen A, Koskenvuo M, Kaprio J, Kesaniemi YA, Heikkila K: Carotid atherosclerosis in identical twins discordant for cigarette smoking *Circulation* 1989;8:10-16
- Hannan LA, Stedje KJ, Skorcz MJ.: Venous imaging of the extremities: our first twenty-five hundred cases. *Bruit*, 1986; 10: 29-32
- Harrison MJG and Wilson LA: Effect of blood pressure on prevalence of carotid bifurcation atheroma. *Stroke* 1983;14:550-1
- Hartung HP, Kladetzky RG, Melnik B, Hennerici M: Stimulation of scavenger receptor on monocyte-macrophage evokes release of arachidonic acid metabolites and reduced oxygen species. *Lab Invest.* 1986; 55:209-216
- Hartung HP and Hennerici M.: Role of macrophages in atherosclerosis. In *Carotid artery plaques* Eds Hennerici M., Sitzer G., Weger H-D. 1988 pp47-63
- Hata Y, Hower J, Insull W.: Cholesterol Ester-rich inclusions from human aortic fatty streak and fibrous plaque lesions of atherosclerosis. *Am J Pathol.* 1974; 75:423-56
- Haust MD. The morphogenesis and fate of potential and early atherosclerotic lesions in man. *Human Pathol* 1971;2:1-29
- Heath D, Smith P, Harris P. The atherosclerotic human carotid sinus. *J Pathol* 1973; 110:48-59
- Heiss G, Sharrett AR, Barnes R, Chambless LE, Szklo M, Alzola C: Carotid atherosclerosis measured by B-mode ultrasound in populations: associations with cardiovascular risk factors in the ARIC study. *Am. J. Epidemiol.* 1991;134:250-6
- Hennerici M, Reifscheinder G, Trockel U and Aulich A.: Detection of early atherosclerotic lesions by duplex scanning of the carotid artery. *J. Clin Ultrasound* 1984; 12:455-64
- Hennerici M, Rautenberg W, Trockel U and Kladetzky KC: Spontaneous progression and regression of small carotid atheroma. *Lancet* 1985;1:1415-19

- Hennerici M, Hulsbomer H-B, Hefter H, Lammers D and Rautenberg W.: Natural history of asymptomatic extracranial arterial disease: Results of a long term prospective study. *Brain* 1987; 110:777-791
- Hennerici M, Kleophas W and Gries FA. Regression of carotid plaques during low density lipoprotein cholesterol elimination. *Stroke* 1991; 22:989-92
- Hertzer NR, Beven EG, Young JR, O'Hara PJ, Graor RA, Ruschhaupt WF: Incidental asymptomatic carotid bruits in patients scheduled for peripheral vascular reconstruction: Results of cerebral and coronary angiography. *Surgery* 1984;96:535-44
- Hertzer NR, Flanagan RA, O'Hara and Bevan EG: Surgical versus non-operative treatment of asymptomatic carotid stenosis: 290 patients documented by intravenous angiography. *Ann. Surg.* 1986;204:163-71
- Heyman A, Wilkinson WE, Heyden S, Helms MJ, Bartel AG, Karp HR, Tyroler- HA, Hames CG.: Risk of stroke in asymptomatic persons with cervical arterial bruits: a population study in Evans County, Georgia. *New. Eng. J. Med.* 1980; 302:838-41
- Hillbom M and Kaste M.: Ethanol intoxication : A risk factor in ischaemic brain infarction *Stroke* 1983;114:694-99
- Hiyashi K, Sato M, Handa H and Moritake K.: Biochemical study of the constitutive laws of vascular walls. *Experimental mechanics* 1974;14:440-4
- Hiyashi K, Handa H, Nagasawa S, Okumara A and Moritake K.: Stiffness and elastic behaviour of human intracranial and extracranial arteries. *J Biomechanics.* 1980;13:175-84
- Hobson RW, Silvia MD, Katocs AS: Pulsed Doppler and real-time B-mode echoarteriography for non-invasive imaging of the extracranial arteries. *Surgery* 1980; 87:286-293.
- Hobson RW. II. : Carotid endarterectomy for asymptomatic carotid stenosis: report of the VA cooperative trial. In *Cerebral revascularization*. Eds. Bernstein EF, Callow AD, Nicolaides AN and Shifrin EG. Med-Orion Publishing Co. 1993 pp 551-558
- Hoeks APG: On the development of a multigate pulsed Doppler system with serial data processing; thesis University of Limburg, Maastricht 1982
- Hokanson DE, Mozersky DJ, Sumner DS and Strandness DE: Ultrasonic arteriography: a new approach to arterial visualisation. *Biomed Eng.* 1971; 6:420
- Holmes JH, Wright W, Meyer EP.: Ultrasonic contact scanner for diagnostic application. *Am J. Med. Electron.* 1965; 4:147
- Homer D., Ingall T., Baker H. O'Fallon WM, Kottle BA, Whisnant JP: Serum lipids and lipoproteins are less powerful predictors of extracranial carotid artery atherosclerosis than are cigarette smoking and hypertension. *Mayo Clin Proc.* 1991; 66:259-67
- Howard BV, Savage PJ, Bennion LJ, Bennett PH.: Lipoprotein metabolism in diabetes mellitus. *Atherosclerosis* 1978; 30: 153-162
- Howry DH. and Bliss WR.: Ultrasonic visualisation of soft tissue structures of the body. *J. Lab Clin Med* 1947; 40:579

Humber PR, Leopold GR, Wickbom IG and Bernstein EF.: Ultrasonic imaging of the carotid arterial system. *Am J. Surgery.* 1980;140:199-202

Hummel BW, Hummel BA, Mowbry A, Maxiner-W, Barnes RW. Reactive hyperaemia vs treadmill exercise testing in arterial disease. *Arch Surg* 1978; 113:95-98

Humphries AW, Young JR, Santilli PH, Beven EG, and de Wolfe VG.: Unoperated asymptomatic significant internal carotid artery stenosis: A review of 182 instances. *Surgery* 1976;80:695-8

Imparato AM, Riles TS, Mintzer R, Baumann FG.: The importance of haemorrhage in the relationship between gross morphological characteristics and cerebral symptoms in 376 carotid artery plaques. *Ann Surg* 1983; 197:195-203

Insull W, Bond MG, Wilmoth SK, Fischel J, Herson J.: Ultrasound lesions of the carotid artery and risk factors in men. In :Evolution of the human atherosclerotic plaque. Eds Glagov S., Newman W.P., Schaffer S. New York Springer-Verlag 1989; 647-653

Inzitari D, Bianchi F, Pracucci G. Albanese V, Argentino C, Bono G, Brambilla GL: The Italian multicentre study of reversible cerebral ischaemic attacks:4 Blood Pressure components and atherosclerotic lesions. *Stroke* 1986;17:185-92

James EM, Earnest F, Forbes GS, Reese DF, Houser OW, Folger WN: High resolution dynamic ultrasound imaging of the carotid bifurcation: a prospective evaluation. *Radiology* 1982;144:853-8

Javid H, Ostermiller WE, Hengesh JW, Dye WS, Najafi H, Julian OC: Natural history of carotid bifurcation atheroma. *Surgery* 1970 ; 67:80-6

Johnson JM, Kennelly MM, Descare D, Morgan S, Sparrow A.: Natural history of asymptomatic carotid plaque. *Arch Surg.*1985; 120:1010-2

Johnson N, Burnham SJ, Flanagan DP, Goodreau JJ, Yao JS, Bergan JJ: Carotid endarterectomy:A follow-up study of the contralateral unoperated carotid artery. *Ann. Surg.*1978;188:748-52

Johnston WC. Doppler ankle pressure and reactive hyperaemia in the diagnosis of arterial insufficiency. *J Surg Res.* 1975; 18:177-180

Joy M and Trump DW.: Significance of minor ST segment and T-wave changes in the resting electrocardiogram of asymptomatic subjects. *Br Heart J.* 1981; 45:48-51

Jungquist G, Hanson BS, Isacson S-O, Janzon L, Steen B, Lindell S-E. Risk factors for carotid artery stenosis: an epidemiological study of men over 69 years. *Clin J Epidemiol* 1991;44:347-53

Jurgens G, Koltringer P: Lipoprotein (a) in ischaemic cerebrovascular disease: a new approach to the assessment of risk for stroke. *Neurology* 1987; 37:513-515

Kalmus HP.: Electronic flowmeter system. *Rev. Scient. Instrum.* 1954; 25:201

Kannel WB and McGee DL. Diabetes and cardiovascular risk factors: The Framingham study. *Circulation* 1979; 59:8-13

Kannel WB. and Sytkowski PA.: Atherosclerosis risk factors. *Pharmac. Ther* 1987; 32: 207-35

- Karino T. and Goldsmith HL. Adhesion of human platelets to collagen on the walls distal to a tubular expansion. *Microvasc Res* 1979; 17:238-262
- Karino T, Goldsmith HL: Particle flow behaviour in models of bending vessels. I Effects of branching angle and diameter ratio on flow pattern. *Biorheology* 1985;22:87-105
- Kartchner MM and McRae LP.: Noninvasive evaluation and management of the asymptomatic carotid bruit. *Surgery* 1977; 82:840-847
- Kawasaki T, Sasayama S, Yagi S, Asakawa T and Hirai T.: Non-invasive assessment of the age related changes in stiffness of major branches of the human arteries. *Cardiovascular research*. 1987;21:678-687
- Kazner E, St Kunze E and Schiefer W.: Die Bedeutung der Echoencephalographie für die Erkennung epiduraler Haematome. *Arch klin Chir* 1965; 310:267
- Kishida H, Cole JS, Surawicz B. Negative U-wave: a highly specific but poorly understood sign of heart disease.. *Am J Cardiol*. 1982; 49:2030-6
- Klop RB., Eikelboom BC. Taks AC. Screening of the internal carotid arteries in patients with peripheral vascular disease by colour flow scanning. *Eur J Vasc Surg* 1991;5:41-5
- Kohler TR, Zierler RE, Martin RL, Nicholls SC, Bergelin RO, Kazmers A, Beach KW Strandness DE: Non-invasive diagnosis of renal artery stenosis by ultrasonic duplex scanning. *J. Vasc. Surg.* 1986; 4:450-6
- Koltringer P and Jurgens G.: A dominant role of Lipoprotein (a) in the investigation and evaluation of parameters indicating the development of cervical atherosclerosis. *Atherosclerosis* 1985; 58:187-98
- Kossoff G. and Garrett WJ: Ultrasonic film echoscopy for placental localisation. *Aust. N.Z. J. Obst. Gynaecolog.* 1972; 12:117-21
- Kritchevski D. Atherosclerosis and nutrition. *Nutrition Int.* 1986; 2:290
- Krolewski AS, Kosinski EJ, Warram JH, Leland OS, Busick EJ, Asmal AC, Rand LI, Christlieb AR, Bradley RF, Kahn CR. Magnitude and determinants of coronary artery disease in juvenile onset, insulin dependent diabetes mellitus. *Am J. Cardiol* 1987; 59:750-755
- Ku DN and Giddens DP: Pulsatile flow in a model carotid bifurcation. *Arteriosclerosis*, 1983;3:31-39
- Laakso M, Pyroala K, Voutilainen E, and Marniemi J.: Plasma insulin and serum lipids and lipoproteins in middle-aged non-insulin dependent diabetic and non-diabetic subjects. *Am. J. Epidemiology* 1987; 125: 11-21
- Laing S. and Greenhalgh RM.: The detection and progression of asymptomatic peripheral arterial disease. *Br. J. Surg* 1983; 70:628-30
- Laing S. The detection of occult peripheral arterial disease using the one-minute exercise test. In *Occult atherosclerotic disease. Diagnosis, assessment and management.* Eds Salmasi A-M and Nicolaidis A.N. Kluwer Academic Publishers. Dordrecht 1991, p227

- Langlois Y, Roederer GO, Chan A, Phillips DJ, Beach KW, Martin D, Chikos PM, Strandness DE: Evaluating carotid artery disease concordance between pulsed Doppler spectrum analysis and angiography. *Ultrasound in Medicine and Biology* 1983; 9:51-63
- Langsfeld M, Grey-Weale AC, and Lusby RJ. The role of plaque morphology and diameter reduction in the development of new symptoms in asymptomatic carotid arteries. *J. Vasc. Surg* 1989; 9:548-57
- Laogun A, and Gosling RG. In vivo arterial compliance in man. *Clin Phys Physiol Meas.* 1982; 3: 201-212
- Legemate DA, Teeuwen C, Hoeneveld H, Ackerstaff RGA, and Eikelboom BC.: The potential of duplex scanning to replace aorto-iliac and femoro-popliteal angiography. *Eur J Vasc Surg* 1989; 3:49-54
- Lehmann ED (i), Watts G.F. and Gosling R.G. Aortic distensibility and hypercholesterolaemia. *Lancet* 1992; 340:1171-2
- Lehmann ED (ii) Gosling R.G. and Sonsken P.H. Arterial wall compliance in diabetics. *Diabetic Medicine*, 1992 ;9:114-119
- Leskell L.: Echo-encephalography I. Detection of intracranial complications following head injury. *Acta Chir Scand* 1956; 110:301
- Leskell L.: Echo-encephalography II. Midline echo from the pineal body as an index of pineal displacement. *Acta Chir Scand* 1958; 115:255
- Letourneau JG, Day DL, Ascher NL, Snover DC, Steely JW, Crass JR, Najarian JS, Feinberg SB : Abdominal ultrasonography after hepatic transplantation : results in 36 patients. *AJR* 1987 ;149:299-303
- Levin SM, Sondheimer FK, Levin JM.: The contralateral diseased but asymptomatic artery: to operate or not? An update. *Am. J Surg.* 1980; 140:203-5
- Lewis RR, Beasley MG, Gosling RG.: Detection of disease at the carotid bifurcation using ultrasound - including an imaging system. *J. Royal Soc. of Med.* 1980; 74:172-179
- Libber SM, Plotnick LP, Johanson A. Long term follow-up of hypopituitary patients treated with growth hormone. *Medicine* 1990; 69:46-55
- Lisch HJ, and Sailer S. : Lipoprotein patterns in diet, sulphonylurea and insulin-treated diabetics. *Diabetologia* 1981; 20:118-122
- Lo CS, Relf IRN, Myers KA and Wahlqvist ML. Doppler ultrasound recognition of preclinical changes in the arterial wall in diabetic subjects : compliance and pulse wave damping *Diabetes care*, 1986; 9:27-31
- Ludbrook J, Clarke AM, McKenzie JK. Significance of absent ankle pulse. *Br Med J* 1962; 1:1724
- Lusby RJ, Ferrell LD, Ehrenfeld WK. Carotid plaque haemorrhage. Its role in the production of cerebral ischaemia. *Arch Surg* 1982;117:1479-88
- Lusby RJ, Ferrell LD, Reilly LM, Kersh R, Ehrenfeld WK, Stoney RJ Stroke: the significance of carotid plaque haemorrhage with ulceration. *Stroke*, 1983; 14:133.
- Lusiani I, Visona A, Pagnan A: Noninvasive study of arterial hypertension and carotid atherosclerosis. *Stroke* 1990;21(3):410-4

- Lynn JG and Putnam TJ: Histology of cerebral lesions produced by focussed ultrasound. *Am J Path.* 1944; 20:637
- Malinow MR: Atherosclerosis : Progression, regression and resolution. *AnnHeart J* 1984; 108:1523-1537
- Marks H., Krall LP. Onset course prognosis and mortality in diabetes mellitus. In *Joslin's Diabetes Mellitus*. Eds Marble A., White P., Bradley R.F., and Krall L. Philadelphia: Lea and Feabiger, 1971: p209
- Matalanis G, Lusby RJ.: Is there still a place for carotid endarterectomy. *Clin Exp Neurol* 1988;25: 17-26
- MBewu AD, Durrington PN.: Lipoprotein (a): structure, properties and possible involvement in thrombogenesis and atherogenesis. *Atherosclerosis* 1990; 85:1-14
- McDicken WN, Bruff K, and Paton J.: An ultrasonic instrument for rapid B-scanning of the heart. *Ultrasonics* 1974; 12:269-72
- Meade TW, Gardner MJ, Cannon P, Richardson PC: Observer variability in reading the peripheral pulses. *Br Heart J.* 1968; 30:661-5
- Merimee TJ, Fineberg SE, Hollander W. Vascular disease in the chronic GHG-deficiency state. *Diabetes* 1973; 22:813-19
- Messerli FH, Ventura H, Aristimuno GG, Suarez DH, Dreslinski GR, Frohlich ED. Arterial compliance in systolic hypertension. *Clin Exp Hyperten* 1982; 4(a):1037-44
- Meyer WW and Noll M. : Gross patterns of early lipid deposits in the carotid artery and their relation to preformed arterial structures. *Artery* 1974; 1:31-45
- Miles LA, Plow EF: Lipoprotein (a): an interloper into the fibrinolytic system? *Thromb Haemost* 1990; 63:331-335
- Modrak JB and Langer RO. Possible relationships of cholesterol accumulation and collagen synthesis in rabbit aortic tissue. *Atherosclerosis* 1980; 37:211-8
- Mody FV, Nademanee K, Intarachot V, Josephson M, Robertson HA, Singh BT. Severity of silent myocardial ischaemia on ambulatory ECG monitoring in patients with stable angina pectoris. *JACCV* 1988; 12:1169-76
- Moneta GL, Taylor DC, Nicholls SC.: Operative versus non-operative management of asymptomatic high-grade internal carotid artery stenosis: Improved results with endarterectomy. *Stroke* 1987;18:1005-1010
- Moneta GL, Taylor DC, Zieler RE, Kazmers A, Beach K, and Strandness DE Jr. Asymptomatic high-grade internal carotid stenosis : Is stratification according to risk factors or duplex spectral analysis possible. *J Vasc Surg* 1989; 10:475-483
- Moneta G.L. Basic data concerning noninvasive vascular testing. In *Basic data underlying clinical decision making in vascular surgery*. Ed John D Porter Quality Medical Publishing St.Louis, Missouri 1991 11-13
- Moore DJ, Sheehan MP, Kolm P, Russell JB, Sumner DS. Are strokes predictable by non-invasive methods: a five year follow-up of 303 unoperated patients. *J. Vasc Surg.* 1985;2:654-660
- Moore WS. The asymptomatic carotid artery stenosis study (ACAS). In *Cerebral revascularization*. Eds. Bernstein EF, Callow AD, Nicolaidis AN and Shifrin EG. Med-Orion Publishing Co. 1993 pp 559-564.

- Moss PRL, Campbell GR, Campbell JH. Smooth muscle phenotype expression in human carotid arteries. *Arteriosclerosis* 1968;6:664
- Motomiya M, Karino T : Flow patterns in the human carotid artery bifurcation. *Stroke* 1984; 15:50-56
- Mozerky DJ, Hokanson DE, Sumner DS and Strandness DE: Ultrasonic visualisation of the arterial lumen. *Surgery* 1972; 72:253-9
- Murai A, Miyahara T, Fujimoto N, Matsuda M, Kameyama M: Lp (a) lipoprotein as risk factor for coronary heart disease and cerebral infarction. *Atherosclerosis* 1986; 59:199-204
- Murphy EA, Rowsell HC, Downie HG. Encrustation and atherosclerosis: the analogy between early in vivo lesions and deposits which occur in extracorporeal circulations. *Can Med Ass J.* 1962; 87:259-274
- National Institute of Neurological Disorder and Stroke. Clinical alert: Benefit of carotid endarterectomy for patients with a high grade stenosis of the internal carotid artery. *Stroke* 1991; 22:816-7
- Naumann A.: *Stromungsfragen der medizin.* Arb Gem Forsch Nordrh Westf 1969;203
- Naumann A, Schmid-Schonbein H. A fluid dynamicists and a physiologists look at arterial flow and arteriosclerosis;in *Fluid dynamics as a localising factor for atherosclerosis.* Eds Schettler G., Nerem R.M., Schmid-Schonbein H.Berlin Springer, 1983 pp 9-25
- Newman DL, Gosling RG, Bowden NLR. Changes in aortic distensibility and area ratio with the development of atherosclerosis. *Atherosclerosis* 1971;14:231-240
- Nicolaidis A., Christopoulos D., and Vasdekis S. Progress in the investigation of chronic venous insufficiency. *Ann. Vasc.Surg.* 1989 3:278-292
- Niederhorn K. Asymptomatic carotid artery disease detected by duplex scanning, incidence and correlation with risk factors, cerebral blood flow and CT findings. *Eur Neurol* 1990;30:61-6
- Nolsoe CP, Engel U, Karstrup S, Torp-Pederson S, Garre K, Holm HH. The aortic wall: an in-vitro study of the double lumen pattern in high resolution ultrasound. *Radiology* 1990; 175: 387-390.
- Nomura A, Comstock GW, Kuller JA and Tonascia JA: Cigarette smoking and strokes. *Stroke*1974; 5:483-6
- Norris CS, Pfeiffer JS, Rittgers SE, Barnes RW.: Non-invasive evaluation of renal artery stenosis and renovascular resistance: Experimental and clinical studies. *J. Vasc. Surg.*, 1984; 1: 192-201
- Norris JW Bornstein NM. Progression and regression of carotid stenosis. *Stroke* 1986 17:755-57
- Norris JW, Zhu CZ, Bornstein NM, Chambers BR. Vascular risks of asymptomatic carotid stenosis. *Stroke* 1990; 22:1485-90
- Norris JW. Natural history of asymptomatic carotid stenosis. In: Bernstein EF ed. *Noninvasive Diagnostic Techniques in Vascular Disease.* The CV Mosby Co. St Louis 1990; 119



North American Symptomatic Carotid Endarterectomy Trial Collaborators :  
Beneficial effect of carotid endarterectomy in symptomatic patients with high grade  
carotid stenosis. *N Eng J Med* 1991 325:445-53

O'Donnell TF, Erdoes L, Mackey WC, McCullough J, Shephard A, Heggerick P,  
Isner J, Callow AD: Correlation of B-mode ultrasound imaging and arteriography  
with pathological findings at carotid endarterectomy. *Arch Surg.* 1985 120:443-9

O'Leary DH, Polak JF, Wolfson SK, Bond MG, Bommer W, Sheth S: Use of  
sonography to evaluate carotid atherosclerosis in the elderly. The Cardiovascular  
Health study. CHS collaborative research group. *Stroke* 1991; 22:1155-63

Olinger CP, Ultrasonic carotid echoarteriography. *Am.J. Roentgenol. Radium Ther  
Nucl Med* 106:2822-295

Osmundson PJ, Chesbero JH, O'Fallon WM, Zimmerman BR, Kazmier FJ Palumbo  
PJ. A prospective study of peripheral occlusive arterial disease in diabetes ; II  
Vascular laboratory assessment. *Mayo Clin Proc* 1981; 56 (4):223-232

Panzram G. Mortality and survival in type2 (non-insulin dependent) diabetes mellitus.  
*Diabetologia* 1987; 30:123-131

Passero S, Rossi G, Nardini M, Bonelli G, D'Ettorre M, Martini A, Battistini N,  
Albanese V, Bono G, Brambilla G Italian multicentre study of reversible cerebral  
ischaemic attacks: Risk factors and cerebral atherosclerosis. *Atherosclerosis* 1987;  
63:211-224

Persson AV, Robichaux WT. The natural history of carotid plaque development.  
*Arch Surg.* 1983; 118:1049-52

Persson AV. Use of ultrasonic colour imaging in the evaluation of the venous system.  
In : *Cardiovascular applications of Doppler ultrasound.* Ed: Salmasi A.M., Nicolaides  
A.N. Churchill Livingstone, 1989:366-62

Persson J, Stavenow L, Wikstrand J. Noninvasive quantification of atherosclerotic  
lesions. Reproducibility of ultrasonographic measurement of arterial wall thickness  
and plaque size. *Arteriosclerosis and Thrombosis* 1992; 12(2): 261-6

Picano E, Landini L, Lattanzi F, Michelassi C, Salvadori M, Santarelli F, Distante A,  
L'Abbate A. Ultrasonic tissue characterization of atherosclerosis: state of the art  
1988. *J Nuclear Med & allied Sci* 1988;12:174-84

Pignoli P. Ultrasound B-mode imaging for arterial wall thickness measurement.  
*Atherosclerosis Rev.* 1984 12:177-184

Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. :Intimal plus medial thickness of  
the arterial wall: a direct measurement with ultrasound imaging. *Circulation* 1986  
74:1399-1406

Pignoli P, Longi T. Evaluation of atherosclerosis with B-mode ultrasound imaging. *J.  
Nuc Med and Allied Sciences* 1988 32(3):166-173

Pilsbury HC, Hung W, Kyle MC and Freis ED. Arterial pulse waves and velocity and  
systolic time intervals in diabetic children. *Am Heart J.* 1974;87:783-90

Podore PC, DeWeese JA, May AG, Rob CG. Asymptomatic contralateral carotid  
artery stenosis: A Five year follow-up study following endarterectomy. *Surgery*  
1980;88:748-52

Poli A, Tremoli E, Colombo A, Sirtori M, Pignoli P, Paoletti R.: Ultrasonographic measurement of the common carotid wall thickness in hypercholesterolaemic patients. *Atherosclerosis* 1988; 70(3):253-61

Pujia A., Rubba P., Spencer MP. Prevalence of extracranial carotid artery disease detectable by echo-doppler in an elderly population. *Stroke* 1992;23:818-22

Pyorala K, Laakso M. Macrovascular disease in diabetes mellitus. In Mann JJ, Pyorala K, Teuscher A. eds. *Diabetes in Epidemiological Perspective*. Edinburgh: Churchill Livingstone, 1983: 183-247

Qamar MI, Reed AE, Skidmore R, Evans JM, Wells PNT. Transcutaneous Doppler ultrasound measurement of superior mesenteric artery blood flow in man. *Gut*, 1986; 27:100-5

Ramsey DE, Miles RD, Lambeth A, Sumner DS: Prevalence of extracranial carotid artery disease: A survey of an asymptomatic population with non-invasive techniques. *J. Vasc Surg* 1987;5:584-8

Randall OS. Effect of arterial compliance on systolic blood pressure and cardiac function. *Clin Exp Hyperten*. 1982; 4(a) 1045-57

Reckless JPD, Betteridge DJ, Wu P, Payne B, Galton DJ. High density and low density lipoproteins and prevalence of vascular disease in diabetes mellitus. *Br Med J* 1978; i: 883-886

Reid JM and Spencer MP Ultrasonic Doppler technique for imaging blood vessels. *Science* 1972; 176:1235-6

Reilly LM, Lusby RJ, Hughes L, Ferrell LD, Stoney RJ, Ehrenfeld WK.: Carotid plaque histology using real time ultrasonography. *Clinical and Therapeutic Implications*. *Am. J. Surg.* 1983; 146: 188-193

Reinertson JE and Barnes RW: Carotid flow velocity scattering: diagnostic value in Doppler ultrasonic arteriography (abst) *Clin. Res.* 1976; 24:594

Reneman RS, van Merode T, Hick P, and Hoeks APG: Flow velocity patterns in and distensibility of the carotid artery bulb in subjects of various ages. *Circulation*. 1985; 71:500-509

Reneman RS, van Merode T, Smeets FAM and Hoeks APG.: Velocity patterns and vessel wall properties in the carotid artery bulb in man - their relation to atherogenesis. In *Carotid artery plaques* Eds Hennerici M., Sitzer G., Weger H-D. 1988 pp143-162

Reneman RS, Van Merode T, Smeets FAM, Hoeks APG. Velocity patterns and vessel wall properties in the carotid artery bulb in man - their relation to atherogenesis. In *Carotid artery plaques* Eds Hennerici M., Sitzer G., Weger H-D. 1988 pp 143-162

Ricotta JJ, Bryan FA, Bond MG. Multicentre validation study of real time (B-mode) ultrasound, arteriography, and pathology. In: *Pathobiology of human atherosclerotic plaque*. Glasgow S, Newman WP, and Schaffer SA Springer-Verlag New York 1990

Rigsby CM, Burns PN, Weltin GG, Chen B, Bia M, Taylor KJ. Doppler signal quantification in renal allografts: Comparison in normal and rejecting transplants with pathological correlation. *Radiology*, 1987; 162: 39-42

Riley WA , Freedman DS, Higgs NA, Barnes RW, Stephen A, Zinkgraf SA, and Berenson GS. Decreased arterial wall elasticity associated with cardiovascular disease risk factors in the young. *Arteriosclerosis* 1986;6:378-386.

Riley WA Ultrasonic evaluation of arterial wall dynamics. In: *Pathobiology of human atherosclerotic plaque*. Eds: Glagov S., Newman WP II and Schaffer SA. Springer-Verlag New York 1990 pp687-696

Rindt CCM, van der Vosse FN, van Steenhoven AA, Janssen JD, Reneman RS.: A numerical and experimental analysis of the flow field in a 2 dimensional model of the human carotid artery bifurcation. *J Biomech.* 1987; 20:499-509

Rocco MB, Nabel EG, Campbell S, Goldman L, Barry J, Mead K, Selwyn AF: Prognostic importance of myocardial ischaemia detected by ambulatory monitoring in patients with coronary artery disease. *Circulation.* 1988; 78:877-84

Roederer GO, Langlois Y, Strandness DE Jr.: Ultrasonic duplex scanning of the extracranial carotid arteries: improved accuracy using new features from the common carotid artery. *J. Cardiovasc Ultrasonography.* 1982 1:373-9

Roederer GO(i), Strandness DE Jr.: A simple spectral parameter for the classification of severe carotid disease. *Bruit,* 1984; 8: 174-178

Roederer GO.(ii), Langois YE, Jager KA Primzich JF, Beach KW, Phillips DJ, Strandness DE: The natural history of carotid arterial disease in asymptomatic patients with cervical bruits. *Stroke* 1984 15:605-613

Roederer GO(iii), Langois YE, Lusiani L, Jager KA, Primovzich JF, Lawrence RJ, Phillips DJ, Strandness DE Jr. Natural history of carotid artery disease on the side contralateral to endarterectomy. *J. Vasc Surg* 1984; 1: 62-72

Rokitansky C. A manual of pathological anatomy. 4th vol. Translated by Dan G.E., The Sidenham Society, London, 1852

Rose GA, Blackurn H. Cardiovascular survey methods 1968; WHO monograph, Series no 56, Geneva.

Rosen T, Bengtsson B. Premature mortality due to cardiovascular disease in hypopituitarism. *Lancet* 1990; 336:285-88

Ross R and Glomset JA. The pathogenesis of atherosclerosis. *New Eng J Medicine.* 1976; 295: 369-77

Ross R, Wight TN, Strandness E, Thiele B. Human Atherosclerosis, I Cell constitution and characteristics of advanced lesions of the superficial femoral artery. *Am J Pathol* 1984, 114:79-93

Ross RT and Lye CR. The rate of progression of carotid atherosclerosis. *Stroke* 1984; 15 (1):178-9

Ross R. The pathogenesis of atherosclerosis- An update. *New Eng J Medicine.* 1986; 314:488-500

Ross R, Raines EW, Bowen-Pope DF. The biology of platelet derived growth factor. *Cell* 1986; 46:155

Rubens J, Espeland MA, Ryu JHarpold G, McKinney WM, Kahl FR, Toole JF, Crouse JR: Individual variation in susceptibility to extracranial carotid atherosclerosis. *Arteriosclerosis* 1988; 8:389-97

Rubin JR, Bondi JA, Rhodes RS. Duplex scanning versus conventional arteriography for the evaluation of carotid artery plaque morphology. *Surgery* 1987; 102:749-55

Rudofsky G. and Hirche H. Verlauf praklinischer Arteriosklerose bei Risikopatienten. *NGM* 1989; 3:64-8

Safar ME, and London GM. Arterial and venous compliance in sustained essential hypertension. *Hypertension*. 1987; 10: 133-139

Salmasi AM, Nicolaides AN, Vecht RJ, Hendry WG, Salmasi SNA, Nicolaides EP, Kidner PH, Besterman EMM. Electrocardiographic chest wall mapping in the diagnosis of coronary artery disease. *BMJ* 1983; 287:9-12

Salmasi A-M, Salmasi S, Nicolaides AN, Dore C, Hendry WG, Kidner HP: Non-invasive assessment of left ventricular function in coronary artery disease by Doppler stress testing. *J Cardiovasc Surg* 1987; 28:313-20

Salonen JT, Puska P, Tuomilehto J, Homan K.: Relation of blood pressure, serum lipids and smoking to the risk of cerebral stroke. *Stroke* 1982; 13:334-9

Salonen JT, Salonen R, Seppanen K, Kantola M, Suntioinen S, Korpela H: Interactions of serum copper, selenium, and low density lipoprotein cholesterol in atherogenesis. *Br. Med. J.* 1991;302:756-60

Salonen R., Seppanen K., Rauramaa R., and Salonen J.T. Prevalence of carotid atherosclerosis and serum cholesterol levels in Eastern Finland. *Arteriosclerosis* 1988; 8:788-92

Salonen R and Salonen JT. Progression of carotid atherosclerosis and its determinants: a population based ultrasonography study. *Atherosclerosis* 1990; 81:33-40

Salonen R and Salonen JT(i). Determinants of carotid intima-media thickness: a population based ultrasonography study in Eastern Finnish men. *Int J Med* 1991; 229:225-231

Salonen R and Salonen JT(ii). Carotid atherosclerosis in relation to systolic and diastolic blood pressure: Kuopio ischaemic heart disease risk factor study *Ann Med* 1991; 23:23-27

Santen RJ, Willis PW, Fajans SS. Atherosclerosis in diabetes mellitus. Correlation between serum lipid levels, adiposity and serum insulin level. *Arch Intern Med.* 1972; 130: 833-843

Satomura S: Study of flow patterns in peripheral arteries by ultrasonics, *J Acoust. Science. Jpn* 1959; 15:151

Scarpello JHB, Martin TRP, Ward DJ. Ultrasound measurement of pulse wave velocity in the peripheral arteries of diabetic subjects. *Clin Sci* 1980; 58:53-57

Schang SJ, Pepine CJ. Transient asymptomatic S-T segment depression during daily activity. *Am J Cardiol.* 1977; 39(3)396-402

Schmid-Schonbein H, Wurzinger LJ. Vortex transport phenomena of the carotid trifurcation: Interaction between fluid-dynamic transport phenomena and haemostatic reactions. In *Carotid artery plaques* Eds Hennerici M., Sitzer G., Weger H-D. 1988 pp 64-91

- Schneidau A, Harrison MJG, Hurst C, Wilkes HC, Meade TW: Arterial disease risk factors and angiographic evidence of atheroma of the carotid artery. *Stroke* 1989; 20:146-71
- Schroeder T, Helgstrand UJV, Egeblad MR and Engell HC. Asymptomatic carotid lesions after endarterectomy of the contralateral carotid artery. *Arch Surg.* 1987 122:795-801
- Schroll M and Munck O. Estimation of peripheral atherosclerotic disease by ankle pressure measurement in a population study of 60 year old men and women. *J Chronic Dis.* 1981; 34:261-9
- Schuster HM, Kroner KK, Keller C, Spengel FA, Wolfram G, Zollner N. Atherosclerosis of the carotid arteries documented by duplex scan as a predictor of coronary artery disease in familial hypercholesterolaemia. *Klin Wochenschr* 1987; 65:34-39
- Schwartz SM, Reidy MA, Hansson GK.: Injury at the vascular surface; in *Fluid dynamics as a localising factor for atherosclerosis.* Eds Schettler G, Nerem RM, Schmid-Schonbein H. Berlin, Springer, 1983, pp188-197
- Scmid-Schonbein H. and Wurzinger LJ. Vortex transport phenomena of the carotid trifurcation: Interaction between fluid-dynamic transport phenomena and hemostatic reactions. In *Carotid artery plaques* Eds Hennerici M., Sitzer G., Weger H-D. 1988 pp64-91
- Semrow CM, Friedell M, Buchbinder D, Rollins DL Characterisation of lower extremity venous disease using real time B-mode ultrasonic imaging. *J.Vasc. Technol.*, 1987; 11: 187-191
- Sequeira RF, Light LH, Cross G, Rafferty EB: Transcutaneous aortovelocity: A quantitative evaluation *Br Heart J.* 1976; 38:443-50
- Seviour PW, Teal TK, Richmond W, Elkeles RS. Serum lipids, lipoproteins and macrovascular disease in non-insulin dependent diabetics: A possible new approach to prevention. *Diabetic Med* 1988; 5: 166-171
- Shinton R, Beevers G: Meta-analysis of relation between cigarette smoking and stroke. *Br. Med J.* 1989; 298: 789-794
- Simpson RW, Mann JI, Hockaday TDR, Hockaday JM, Turner RC, Jelfs R. Lipid abnormalities in untreated maturity-onset diabetes and the effect of treatment. *Diabetologia* 1979; 16:101-106
- Smith EB, Staples EM. Intimal and medial plasma protein concentrations and endothelial function. *Atherosclerosis* 1982;41:295-308
- Smith EB. Fibrin in the arterial wall. *Atherosclerosis* 1988; fd70: 186-93
- Solberg L. and Eggen D.: Localisation and sequence of development of atherosclerotic lesions in carotid and vertebral arteries. *Circulation* 1971; 43:711-724
- Somer JC. Electronic sector scanning for ultrasonic diagnosis. *Ultrasonics* 1968; 6:153-9
- Stary HC: What is the nature of coronary atherosclerotic lesions that have been shown to regress in experiments with non-human primates and by angiography in man. *Vasa* 1984;13:298-304

- Steinberg D, Pittman RC, Garew TE. Mechanisms involved in the uptake and degradation of low density lipoprotein by the artery wall in vivo. *Ann NY Acad Sci.* 1985;454:195-206
- Stender S and Zilversmit DB. Transfer of lipoprotein components and of plasma proteins into the aortas of cholesterol fed rabbits. *Atherosclerosis* 1981; 39:97-109
- Stern S, Tzivoni D: Early detection of silent ischaemic heart disease by 24 hour electrocardiographic monitoring of active subjects. *Br Heart J.* 1974; 36: 481-6
- Sullen KC, Wolfson SK, Kuller LH. Carotid and lower extremity arterial disease in elderly patients with isolated systolic hypertension. *Stroke* 1987 ; 18:817-22
- Taylor KJW, Ramos I, Morse SS, Fortune KL, Hammers L, Taylor CR: Focal liver masses: differential diagnosis with pulsed Doppler US. *Radiology* 1987 164:643-647
- Taylor KJW: Gastrointestinal Doppler ultrasound. In: *Clinical Applications of Doppler Ultrasound.* Ed: Taylor K.J.W., Burns P.N., Wells P.N.T. Raven Press, New York, 1988.
- Tell GS. Crouse J.R., and Furberg C.D.: Relation between blood lipids, lipoproteins, and cerebrovascular disease. *Stroke.* 1988;19:423-430
- Tell GS. Howard G. and Mc Kinney W. Risk factors for site specific extracranial carotid artery plaque distribution as measured by B-mode ultrasound. *J. Clin. Epidemiol.* 1989; 42:551-559
- Tell GS, Howard G, Evans GW, Smith ML, McKinney WM, Toole JF. Cigarette smoking and extracranial carotid atherosclerosis. *Adv Exp Med Bio* 1990; 273:39-49
- The ARIC Investigators: The atherosclerosis risk in communities (ARIC) study, design and objectives. *Am J Epidemiol* 1989; 129:687-702
- The MIDAS research group: Multicentre israpidine diuretic atherosclerosis study (MIDAS) *Am J Med* 1989; 86:37-39
- Thompson JE, Kartchner MM. Austin DJ, Wheeler CG, Patman RD. Carotid endarterectomy for cerebrovascular insufficiency (stroke): follow-up of 359 cases. *Ann. Surg.* 1966 163:751-63
- Thompson JE, Patman RD, and Talkington CM. Asymptomatic carotid bruit: long term outcome of patients having carotid endarterectomy compared with unoperated controls. *Ann. Surg* 1978; 188:308-16
- Toto-Moukoko JJ, Achimastos A., Asmar RG, Hugues CJ, Safar ME. Pulse wave velocity in patients with obesity and hypertension. *Am Heart J.* , 1986; 112:136-40
- Trudinger BJ. Giles WB, and Cook CM. Utero-placental blood flow velocity time waveforms in normal and complicated pregnancy. *Br. J. Obstetrics and gynaecology.* 1985 92:39-45
- Turner RC In: Annual report of the British Empire Cancer Campaign 1952 p72
- US Atomic Energy Commission: Studies in methods and instruments to improve the localisation of radioactive materials in the body with special reference to the diagnosis of brain tumours and the use of ultrasonic techniques. 1955 Report AECU-3012 (Universi

- Uusitupa M, Siitonen O, Voutilainen E, Aro A, Hersio K, Pyrola K, Ehnholm C. Serum lipids and lipoproteins in newly-diagnosed NIDDM patients with special reference to factors influencing HDL-cholesterol and triglyceride levels. *Diabetes Care* 1986; 9 :17-22
- Van Merode T., Hick PJJ, Hoeks APG, Rahn KH, and Reneman RS. Carotid wall properties in normotensive and borderline hypertensive subjects of various ages. *Ultrasound in Med & Biol.* 1988;14:563-9
- Van Merode T., Hick P.J.J., Hoeks A.P.G., and Reneman R.S. Noninvasive assessment of artery wall properties in children aged 4-19. *Paed. Res.* 1989;25:94-96
- Van Merode T., Lodder J., Smeets F.A., and Hoeks A.P.G. An accurate noninvasive assessment method to diagnose minor atherosclerotic lesions in the carotid artery bulb. *Stroke* 1989; 20:1336-41
- Vasdekis S. Doppler ultrasound in chronic venous insufficiency. In: *Cardiovascular applications of Doppler ultrasound.* Ed: Salmasi AM Nicolaidis AN Churchill Livingstone, 1989
- Veterans Administration Cooperative Study Group. Role of carotid endarterectomy in asymptomatic carotid stenosis. *Stroke* 1986; 17:534
- Visona A. Lusiani L, Castellani V, Ronsisvalle G, Bonanome A, Pagnan A: The echo-Doppler (duplex) system for the detection of vertebral occlusive disease: comparison with angiography. *J. Ultrasound Med* 1986; 5:247-50
- Wahlqvist ML, Relf IRN, Myers KA, and Lo CS. Diabetes and macrovascular disease: risk factors for atherogenesis and non-invasive investigation of arterial wall disease. *Hum Nut Clin Nutr* 1984;38c:175-84
- Walsh Jr JJ, Cofelice M, Lumpkin D, Kerstein MD: Is screening for vascular disease a valuable proposition? *J Cardiovasc Surg* 1988;29:306-9
- Weber G. Biancardi G. Bussani R. Atherosclerosis and aging. A morphometric study on arterial wall lesions of elderly and very elderly necropsy subjects. *Arch Pathol Lab Med.* 1988; 112:1066-70
- Weisgraber KH, Innerarity TL, Rall SC, Mahley RW. Receptor interactions controlling lipoprotein metabolism. *Can J Biochem Cell Biol* 1985; 63:898-905
- Welin L, Svardsudd K, Wilhelmsen L, Larsson B, Tibblin G. Analysis of risk factors for stroke in a cohort of men born in 1913. *N. Engl J. Med.* 1987; 317:521-6
- Wells PNT. Developments in medical ultrasonics. *World Med Electron* 1966; 4:272
- Wendelhag I. Wiklund O. Wikstrand J. Arterial wall thickness in familial hypercholesterolaemia. Ultrasound measurement of intima media thickness in the common carotid artery. *Arteriosclerosis and Thrombosis* 1992; 12(1):70-7
- West KM, Ahuja MMS, Bennett PH, Czyk A, De-Acosta OM, Fuller JH, Grab B, Grabauskas V, Jarrett RJ, Kosaka K. The role of circulating glucose and triglyceride concentrations and their interactions with other risk factors as determinants of arterial disease in nine diabetic population samples from the WHO Multinational Study. *Diabetes Care* 1983 6:361-9
- Whisnant JP, Homer D, Ingall TJ, Baker HL, O'Fallon WM, Wievers DO: Duration of cigarette smoking is the strongest predictor of severe extracranial carotid artery atherosclerosis. *Stroke* 1990; 21:707-14

- WHO study group. Diabetes mellitus. WHO Technical Report Series 727. Geneva; WHO 1985
- Wiebers DO, Whisnatt JP, Sandok BA, O'Fallon WM. Prospective comparison of a cohort with asymptomatic bruit and a population based cohort without a bruit. *Stroke* 1990; 21:984-988
- Wild JJ and Read JM: Application of echo ranging techniques to the determination of structure of biological tissues. *Science* 1952; 115:226
- Williams MA Deacon DFS Szendro G Belcaro G Nicolaides AN: Duplex scanning in the detection and grading of internal carotid artery stenosis In: Cardiovascular applications of Doppler ultrasound. Ed: Salmasi AM Nicolaides AN Churchill Livingstone, 1989
- Winternitz MC, Thomas RM, Le Comte PM. The biology of arteriosclerosis. Springfield, Thomas 1938
- Wolf PA, D'Agostino RB, Kannel WB, Bonita R, Belanger AJ: Cigarette smoking as a risk factor for stroke: The Framingham Study *JAMA* 1988; 259:1025-9
- Wolf PA, Kannel WB, Sorlie P, McNamara P. Asymptomatic carotid bruit and the risk of stroke. The Framingham study. *JAMA* 1981 245:1442-5
- Woo J, Lau E, Lam CWK Kay R, Teoh R, Wong HY, Prall WY, Kreel L: Hypertension, lipoprotein (a), and apolipoprotein A-I as risk factors for stroke in the Chinese. *Stroke* 1991; 22:203-208
- Woodcock J.: Morphology and dynamics of carotid plaque development. In Carotid artery plaques Eds Hennerici M., Sitzer G., Weger H-D. 1988 pp133-142
- Wozney P, Zajko AB, Bron KM, Point S, Starzi TE. Vascular complications after liver transplantation, a five year experience., *AJR* 1986 147:657-63
- Wuster C, Slenczka E, Ziegler R. Erhohte prevalenz von osteoporose und arteriosklerose bei konventionell substituierter hypophysenvorderlappeninsuffizienz: Bedarf einer zusatzlichen wachstumshormonsubstitution? *Klin Wochenschr* 1991; 69:769-773
- Yao JST. Haemodynamic studies in peripheral arterial disease. *Br J Surg* 1970; 57:761
- Zarins CK, Giddens DP, Bharadvaj BK, Sottiurai VS, Mabon RF, Glagov S: Carotid bifurcation atherosclerosis. Quantitative correlation of plaque localization with flow velocity profiles and wall shear stress. *Circ Res* 1983; 53:502-514
- Zenker G, Koltringer P, Bone G, Neiderkorn K, Pfeiffer K, Jurgens G. Lipoprotein (a) as a strong indicator for cerebrovascular disease. *Stroke* 1986; 17:942-5
- Zimmerman BR, Palumbo PJ, O'Fallon WM, Ellefson RD, Osmundson PJ, Kazmier FJ A prospective study of peripheral occlusive arterial disease in diabetes. *Mayo Clinic Proc* 1981; 56:233-42
- Zioncheck TF, Powell LM, Rice GC, Eaton DL, Lawn RM: Interaction of recombinant apolipoprotein (a) and lipoprotein(a) with macrophages. *J Clin Invest* 1991; 87(3):767-71



## Appendix 1

**Table A1 Basic Descriptive Statistics for the wall thickness measurements taken in the anterior longitudinal view**

	Near wall					Far wall				
		Regions					Regions			
	Bulb origin	I	II	III	IV	Bulb origin	I	II	III	IV
n	47	63	60	62	61	47	63	61	61	60
Mean	1.5	1.3	1.4	1	0.9	1.5	1.4	1.4	1	1
Variance	0.4	0.3	0.5	0.2	0.2	0.5	0.4	0.7	0.2	0.3
SD	0.6	0.5	0.7	0.4	0.4	0.7	0.6	0.8	0.5	0.5
SEM	0.08	0.06	0.09	0.05	0.05	0.1	0.08	0.1	0.06	0.07
95% CL (lower)	1.4	1.2	1.2	0.9	0.8	1.3	1.2	1.2	0.9	0.8
95% CL (upper)	1.7	1.4	1.6	1.1	1	1.7	1.5	1.6	1.2	1.1
Geometric mean	1.4	1.2	1.3	0.9	0.9	1.3	1.2	1.2	0.9	0.9
Skewness	0.9	0.9	0.9	1.5	2.5	1.7	0.9	1.3	1.4	1.5
Kurtosis	0.04	0.7	0	2.5	8.1	2.8	0.4	1.2	1.7	2.1
Maximum	3.1	2.9	3.1	2.4	2.8	4	3.3	4.2	2.7	2.8
Upper quartile	1.9	1.6	1.8	1.1	1	1.6	1.7	1.9	1.3	1.2
Median	1.4	1.2	1.2	0.9	0.9	1.3	1.3	1.1	0.9	0.8
Lower quartile	1.1	1	0.9	0.7	0.8	1.1	0.9	0.9	0.7	0.6
Minimum	0.7	0.4	0.4	0.5	0.4	0.6	0.5	0.4	0.4	0.5
Range	2.4	2.5	2.7	1.9	2.4	3.5	2.8	3.8	2.3	2.4

**Table A2 Basic Descriptive Statistics for the wall thickness measurements taken in the lateral longitudinal view**

	Near wall					Far wall				
		Regions					Regions			
	Bulb origin	I	II	III	IV	Bulb origin	I	II	III	IV
n	46	63	61	63	62	45	63	60	62	62
Mean	1.8	1.5	1.5	1.2	1	1.5	1.4	1.5	1.1	0.9
Variance	0.6	0.7	0.6	0.4	0.2	0.5	0.7	0.7	0.4	0.2
SD	0.8	0.8	0.8	0.6	0.5	0.7	0.9	0.9	0.7	0.4
SEM	0.1	0.1	0.1	0.07	0.06	0.1	0.1	0.1	0.08	0.06
95% CL (lower)	1.5	1.3	1.3	1.1	0.9	1.3	1.2	1.3	0.9	0.8
95% CL (upper)	2	1.7	1.7	1.4	1.1	1.7	1.6	1.7	1.3	1
Geometric mean	1.6	1.3	1.3	1.1	0.9	1.3	1.2	1.3	1	0.8
Skewness	1.2	1.2	1.2	1.1	1.7	1.9	1.6	1.3	1.8	2
Kurtosis	1.7	1.4	1.4	0.9	5	4.6	1.8	0.9	2.4	4.6
Maximum	4.5	4.5	4.5	3.2	3	4.3	4	4	3	2.7
Upper quartile	2	2	2	1.6	1.2	1.8	1.7	1.8	1.3	1
Median	1.6	1.2	1.2	1	1	1.4	1	1.3	0.9	0.8
Lower quartile	1.1	1	1	0.7	0.7	1	0.8	0.9	0.7	0.6
Minimum	0.6	0.4	0.4	0.3	0.3	0.6	0.5	0.5	0.4	0.4
Range	3.9	4.1	4.1	2.9	2.7	3.7	3.5	3.5	2.6	2.3

**Table A3 Basic Descriptive Statistics for the wall thickness measurements taken in the transverse view**

	Near wall - views					Far wall - views				
	1	2	3	4	5	1	2	3	4	5
n	61	62	61	57	38	63	63	63	58	43
Mean	0.9	1.1	1.6	1.3	1.1	0.9	1.2	1.7	1.3	1
Variance	0.07	0.3	0.8	0.5	0.3	0.08	0.3	0.9	0.4	0.3
SD	0.3	0.6	0.9	0.7	0.6	0.3	0.6	0.9	0.6	0.6
SEM	0.04	0.07	0.1	0.1	0.09	0.04	0.07	0.1	0.08	0.09
95% CL (lower)	0.8	0.9	1.4	1.1	0.9	0.8	1.1	1.5	1.1	0.9
95% CL (upper)	1	1.2	1.8	1.5	1.3	1	1.3	1.9	1.5	1.2
Geometric mean	0.9	1	1.4	1.2	0.9	0.9	1.1	1.5	1.2	1
Skewness	2	2.1	1.1	2.9	2	2.6	1.5	1.2	1.3	2.5
Kurtosis	3.5	4.8	1	12	3.3	8.5	1.6	0.8	1.4	6.2
Maximum	1.8	3.5	4.8	5.2	3	2.3	3.2	4.5	3.2	3.4
Upper quartile	1	1.2	2.2	1.7	1	1	1.5	2.3	1.7	1
Median	0.8	0.8	1.3	1.2	0.8	0.8	1	1.4	1.1	0.8
Lower quartile	0.8	0.8	0.8	0.8	0.8	0.8	0.8	1	0.8	0.8
Minimum	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.6
Range	1.2	2.9	4.2	4.6	2.4	1.7	2.6	3.9	2.5	2.8

**Table A4 Comparison between the number of plaques on each wall in each of the longitudinal views**

**a) Region I**

	Plaque	No plaque	Total
Anterior view, near wall	25	35	60
Anterior view, far wall	41	22	63
Lateral view, near wall	34	29	63
Lateral view, far wall	34	29	63

$\chi^2=6.99$  NS

**b) Region II**

	Plaque	No plaque	Total
Anterior view, near wall	27	30	57
Anterior view, far wall	30	31	61
Lateral view, near wall	34	28	62
Lateral view, far wall	32	28	60

$\chi^2 = 0.878$  NS

**c) Region III**

	Plaque	No plaque	Total
Anterior view, near wall	9	50	59
Anterior view, far wall	20	41	61
Lateral view, near wall	23	40	63
Lateral view, far wall	18	44	62

$\chi^2 = 7.609$   $p < 0.05$

**d) Region IV**

	Plaque	No plaque	Total
Anterior view, near wall	3	55	58
Anterior view, far wall	15	46	61
Lateral view, near wall	12	50	62
Lateral view, far wall	8	55	63

$\chi^2 = 9.61$        $p < 0.05$

**e) Bulb origin**

	Plaque	No plaque	Total
Anterior view, near wall	29	18	47
Anterior view, far wall	27	20	47
Lateral view, near wall	34	12	46
Lateral view, far wall	27	19	46

$\chi^2 = 3.36$       NS

**Table A5**      **Comparison between the number of plaques on each wall in each of the transverse views**

	Near wall	Far wall
View 1	7	7
View 2	14	20
View 3	34	38
View 4	26	26
View 5	4	9

$\chi^2 = 2.0$  to 4 DF, NS

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