UNIVERSITY OF SOUTHAMPTON

The Risk Factors Associated with Carotid Artery Disease

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Statement

This thesis was submitted for examination in July 2002. The author certifies that this thesis, entitled "The risk factors associated with carotid artery disease", is the result of work done mainly while I was a registered candidate.

In Chapter 2 "The potential of dietary lipid supplements to benefit patients with carotid artery disease" the author worked jointly with Dr. J.M.C. Garry, who was awarded a PhD in nutrition by the University of Southampton in 2001. However this thesis focuses mainly on the author's own work about the effects of fish oil supplementation on the morphology and inflammatory process in carotid plaque, most of which has not been submitted for examination before.

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ABSTRACT

FACULATY OF MEDICINE, HEALTH AND BIOLOGICAL SCIENCES

DEPARTMENT OF VASCULAR SURGERY

Doctor of Philosophy

THE RISK FACTORS ASSOCIATED WITH CAROTID ARTERY DISEASE

By Kittipan Rerkasem

Atherosclerosis plays a primary role in ischaemic heart disease, the most common cause of death in the developed world. Inflammation, the fundamental pathological process in atherogenesis and formation of plaque, was investigated in a randomised controlled trial conducted on 189 patients awaiting carotid endarterectomy (CEA). The main objective of this study was to compare the effect of n-3 polyunsaturated fatty acid (PUFA) supplements on the inflammatory process in carotid plaques compared with n-6 PUFA supplements and a placebo. It was found that the number of macrophages in the plaque was significantly lower in patients taking n-3 PUFA supplements than in those of the other groups, which may make these plaques more stable and less likely to rupture. The level of high sensitivity C-reactive protein was significantly higher in patients with symptomatic carotid stenosis than in those without symptoms, which may help to identify carotid stenosis patients at a high risk of developing neurological deficits. Another objective of this study was to assess the extent to which physicians recognised the importance of control of hyperlipidaemia in patients with severe atherosclerosis. It was found that 32.1% of these patients had higher levels of plasma cholesterol than the recommended value and 22% of them had never had any tests for blood lipids.

Many large randomised controlled trials have found that CEA in patients with symptomatic severe carotid stenosis has clearly reduced the incidence of stroke, compared with medical treatment only. From a systematic review of the literature of carotid endarterectomy in the period 1960 – 2000 and a meta-analysis, it was found that the risk of stroke and death in patients with symptomatic carotid stenosis was 4.6% and has not changed significantly over the last 15 years. Presentation, such as stroke, transient ischaemic attacks and amaurosis fugax, strongly influences stroke / death rates and therefore, reports of the outcome of surgery should always be stratified accordingly. Furthermore this figure for risk varied enormously and depended on research design and reporting. One should take the quality of study design and reporting into account before judging the performance of the surgeons in these studies.

Another controversial issue is whether CEA is best performed under local anaesthetic (LA) or general anaesthetic (GA). A systematic review of the literature for the period 1966 – 2001 and a meta-analysis were done. In the non-randomised group of papers, LA seemed to be better than GA, but in the randomised group of papers, it was difficult to draw any solid conclusions because of the small samples. More randomised controlled trials are needed.

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Preface

The research for this thesis was carried out from May 1998 to May 2002 while I was a research fellow with Professor Clifford Shearman in the Department of Vascular Surgery at the Southampton General Hospital in Southampton. In 1998 I began a trial entitled "A randomised study to identify factors influencing behaviour of carotid atherosclerotic plaque". For this trial I was responsible for the recruitment of patients, collection of samples, and recording of data, which required the collation of information from patients, review of hospital medical records, and drafting of various summaries of data. I visited patients not only at times samples were taken, but also on pre-operative days and on post-operative days until discharge from hospital. I collected, processed, and stored most samples by myself, although I would like to acknowledge the help and advice of Mr. Adisabandh Chulakadabba and Mrs Jenny Williams. I was also in charge of the histological examination of carotid plaques and analysed the data from the trial.

Since October 2000 I have collaborated with Dr. Peter Rothwell of the Oxford Stroke Prevention Research Unit, Department of Clinical Neurology, University of Oxford on the identification of several clinical risk factors for stroke and death following carotid endarterectomy. This thesis contains a number of systematic reviews of the literature, including one Cochrane review. I searched the literature and assessed papers, although Mr. Richard Bond repeated my assessment for accuracy and reproductivity. I also located the majority of the papers that required a manual search and analysed the data from the systematic reviews.

Acknowledgments

I would like to thank my supervisors, Professor Clifford Shearman, for giving me the opportunity to carry out many projects and for his endless support and encouragement in many difficult situations. I would also like to thank Dr Peter Rothwell for teaching me many epidemiological techniques and always stimulating my interest.

The atherosclerotic work described in this thesis was supported by the Ministry of Agriculture, Fisheries and Food. I would like to thank everyone involved in this project, especially Professor Robert Grimble for their cooperation, Professor Philip Calder for supporting and permitting me to use his diagrams for this thesis, Dr Patrick Gallagher for teaching me techniques for histological analysis of carotid plaques and permitting me to use his images in my thesis, Mr Adisabandh Chulakadabba and Mrs Jenny Williams for their advice and support in recruiting patients and collecting samples, Dr Jennifer Garry and Dr Frank Thies for their help with lipid tests and immunohistochemistry, Drs Raphael Uwechwe, Simon Boyles and Tim Chan for help in histomorphometry, and Drs David Rowe, Jonathan Powell and Tina Hurst for help with inflammatory mediators. I would also like to thank all the patients who took part in this study, without whose help it would have been impossible.

The systematic review and meta-analysis of carotid endarterectomy in this thesis was supported by the Oxford Stroke Prevention Research Unit, Department of Clinical Neurology, University of Oxford. I would like to thank Mr Richard Bond, who was a very good partner in the systematic review, Dr Ursula Schulz for translation of many foreign language papers and several forest plot graphs in this thesis were created using programs initially developed by Dr Sergei Gutnikov. I also would like to thank Dr Chanpong Tangkanakul and Professor Charles Warlow for their support in helping me to carry out the Cochrane review.

I would like to thank my funding organizations, the Royal Thai Government and the Faculty of Medicine at Chiang Mai University. I would also like to thank many friends for their help and encouragement along the way, especially Dr. Watana Kattipathanapong for saving me from many dire situations, including a lack of funding, since Thailand has been suffering from an economic crisis; Mr. Edward Rose for amending my thesis, as without him none of this work would have been in proper English; and Mr. Matthew Farey, my best friend for giving me the strength and courage to believe in myself. I also would like to thank my family for their support.

However my most profound thanks are for Dr Amaroporn Kamnounkija for her support and patience in waiting for me in Thailand for four years and for making my life so much more meaningful. I could not have achieved anything without her.

Non-standard abbreviations

ACAS	Asymptomatic Carotid Atherosclerosis Study
ACST	Asymptomatic Carotid Surgery Trial
AHA	American Heart Association
ANOVA	Analysis of Variance
BMI	Body Mass Index
CABG	Coronary Artery Bypass Grafting
CAD	Coronary Artery Disease
CARE	Cholesterol And Recurrent Event
CASANOVA	Carotid Artery Stenosis Asymptomatic Narrowing Operation Versus Aspirin
CAVATAS	Carotid and Vertebral Artery Transluminal Angioplasty Study
CD	Cluster of Differentiation
CE	Cholesterol Ester
CEA	Carotid Endarterectomy
CI	Confidence Interval
CONSORT	Consolidated Standards of Reporting Trials
CRP	C-Reactive Protein
CT	Computerized Tomography
CTIA	Crescendo Transient Ischaemic Attack
DART	Diet And Re-infarction Trial
DHA	Docosahexaenoic Acid
DPA	Docosapentaenoic Acid
eCEA	Eversion carotid endarterectomy
ECST	European Carotid Surgery Trial
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Eicopentaenoic acid
E-selectin	Endothelial leukocyte adhesion molecules
GA	General Anaesthetic

GALA	General anaesthetic versus local anaesthetic for carotid surgery trial
HPS	Heart Protection Study
hs-CRP	High sensitivity CRP
ICA	Internal Carotid Artery
ICAM	Intercellular Adhesion Molecule
IHD	Ischaemic Heart Disease
IL	Interleukin
KR	Kittipan Rerkasem
LA	Local Anaesthetic
LDL	Low Density Lipoprotein
LT	Leukotrienes
MACE	Mayo Asymptomatic Carotid Endarterectomy
MH	Mantel Haenszel
MI	Myocardial Infarction
Min	Minutes
MMP	Matrix Metalloproteinases
NASCET	North American Symptomatic Carotid Endarterectomy Trial
NO	Nitric Oxide
NQMI	Non-Q Wave Myocardial Infarction
OR	Odd Ratio
PDGF	Platelet-Derived Growth Factor
PET	Positron Emission Tomography
PG	Prostaglandins
p(het)	p value for heterogeneity
PL	Phospholipid
PMR	Peter M Rothwell
PUFA	Polyunsaturated Fatty Acid
RB	Richard Bond
RCT	Randomised Controlled Trial

RIND	Reversible Ischaemic Neurological Deficit
rpm	Revolutions Per Minute
s	Soluble
S/D rate	Stroke and Death rate
Sec	Second
SIE	Stroke In Evolution
Sig	Significance (Statistical)
SPSS	Statistical Package for the Social Science
TAG	Triacylglycerol
TBS	Tris-Buffered Saline
TCD	Transcranial Doppler
T-cell	T-lymphocytes
TFN	Interferon
TIA	Transient Ischaemic Attack
TNF	Tumor Necrosis Factor
TX	Thromboxanes
V1	Visit before supplementation
V2	Visit after supplementation
VAAST	Veterans Administration Asymptomatic Stenosis Trial
VASST	Veterans Administration Symptomatic Stenosis Trial
VCAM	Vascular Cell Adhesion Molecule
VSMC	Vascular Smooth Muscle Cell
WOSCOPS	West of Scotland Coronary Prevention Study
Yrs	Years

Section 1

Risk factors associated with atherosclerotic lesions

Chapter 1

Introduction to atherosclerosis and plaque stability

1.1 Summary

In this chapter recent concepts of atherosclerosis are reviewed. The theory of atherogenesis, the role of major components of atherosclerotic plaques, and the concept of plaque instability are covered. In addition the role of risk factors is reviewed.

1.2 Introduction

In most industrial countries, ischaemic heart disease (IHD) is the most common cause of death and it is well recognised that atherosclerosis in the coronary arteries is the main pathology of IHD. 30 percent of all deaths among men and 22 percent of all deaths among women in England and Wales are the result of IHD (Marmot & Mann 1996) and about 156,000 people die every year in England and Wales with a diagnosis of IHD. It is therefore of paramount importance to identify and control atherosclerotic lesions.

1.3 Theories of atherogenesis

Many theories have been proposed as mechanisms for atherogenesis. In 1865 the response-to-injury hypothesis was introduced by Virchow, who suggested that atherosclerosis occurred as part of the healing process in which the arterial intima responded to stimuli such as prior mechanical injury (Rudd & Weissberg 2001). In 1913 Anitschkow proposed that atherosclerosis was caused by gradual deposition of lipids on the vascular wall, the lipidic theory, which was supported by the association of hyperlipidaemia with atherogenesis (Berliner *et al.* 1995). In the thrombogenic theory, it was proposed that microthrombi were incorporated into arterial walls, which has been supported by the finding of fibrin in atherosclerotic lesions (Bini *et al.* 1989). In 1973 Ross (1973) proposed a modification to the response-to-injury hypothesis of Virchow in which he claimed that an atherosclerotic lesion was largely due to excessive proliferation of reparative vascular smooth muscle cells (VSMCs) in response to endothelial injury. This theory was later revised and Ross (1986) proposed the concept of endothelial dysfunction caused by anything (e.g. hyperlipidaemia) rather than endothelial injury alone (e.g. mechanical injury). Most recently Ross (1999) proposed an inflammatory theory, the current theory, in which endothelial dysfunction is the

origin of atherogenesis and in which the role of inflammation is emphasized in every step of atherogenesis.

1.4 Haemodynamics and atherosclerosis

Although atherosclerosis is a systemic disease involving especially large and medium size arteries, atherosclerotic plaque tends to develop in certain places such as the carotid artery, the infra-renal aorta, and the arteries of the lower extremities, in particular at the sites of bifurcation, the ostia, the branchings, and the bends. This suggests that haemodynamic forces play a role in atherogenesis; many hypotheses have been proposed to explain this unique focal pattern.

In the carotid artery, plaque usually develops at the outer wall of the carotid sinus, which is an area of low shear stress. Flow visualisation shows that the flow at the inner wall is rapid with high shear stress, whereas at the outer wall the flow is slow, reversed, and even turbulent. Therefore at the outer wall there is more time for interaction of lipids with the vessel wall and there are more localizing factors for atherogenesis (Tropea, Glagov & Zarins 1997). Some evidence has also showed that shear stress can influence the expression of some endothelial genes, especially when flow is slow. Toppler *et al.* (1996) found furthermore that laminar flow can increase the expression of anti-atherogenic genes, superoxide dimutase, cyclooxygenase-2, and the endothelial isoform of nitric oxide synthetase, whereas turbulent flow at the outer wall does not increase the expression of these genes. Similarly the infra-renal aorta tends to have a higher incidence of atherosclerosis than the suprarenal aorta. As one fourth of the cardiac output perfuses the kidneys, the flow in the infra-renal aorta is relatively low velocity. There also are vortices, increased residence times for particles, flow reversals, and stagnation in the infra-renal aorta, especially at the posterior wall (Tropea, Glagov & Zarins 1997). Plaque is therefore likely to be found in this area. Interestingly Taylor *et al.* (1999) found that simulated moderate exercise of the lower extremities increased flow significantly and the complex flow patterns were replaced with uniform laminar

flow. This may partly explain the benefit of exercise by modification of haemodynamics.

A lot of evidence has shown that variations in anatomy affect hemodynamics; they may affect plaque formation as well. For example, 161 coronary angiograms were reviewed for the relationship between the anatomical variation of the coronary artery and distribution of stenotic lesions (Saltissi, Webb-Peploe & Coltart 1979). It was found that there were more proximal athersclerotic lesions in shorter left main coronary arteries. Clearly more proximal lesions in the coronary artery will increase the area for ischaemia. Sharp (1982) and colleagues reviewed one hundred abdominal angiograms and found that the average bifurcation angle of the distal aorta in occlusive disease was 38°, whereas in normal or aneurysmal disease it was 52°. They also studied haemodynamics using glass models and showed that a more acute angle causes greater shear stress on the inner wall of the bifurcation and less shear stress on the outer wall. All of this demonstrates that haemodynamic forces have some effect on atherogenesis.

1.5 Atherogenesis

Detailed postmortem studies of patients of different ages show that atheromatous lesions progress from small and inconsequential fatty streaks to fibrolipid plaques and complicated lesions which are the cause of many different clinical disorder such as myocardial infarction or stroke (Figure 1.1a-c) (Gallagher 2000).

Atherosclerotic plaques begin with the subendothelial accumulation of lipid-laden foamy macrophages and T-lymphocytes (T-cells), which form a non-stenotic fatty streak (Weissberg 2000). This progresses to the formation of an acellular core of lipid cholesterol, bound by a fibrous cap that contains VSMCs and inflammatory cells, especially macrophages, mast cells and T-cells (Figure 1.2) (Weissberg 2000). In an advanced lesion, new blood vessels and calcium hydroxyapatite are present.

Figure 1.1 Lesion of atherosclerosis. Figure 1.1a Multiple fatty streaks in the carotid artery of a 39-year-old male. Figure 1.1b Large fibrolipid plaques in the abdominal aorta of a 64-year-old male with extensive atherosclerosis. Figure 1.1c Complicated carotid atherosclerosis.

Figure 1.1a

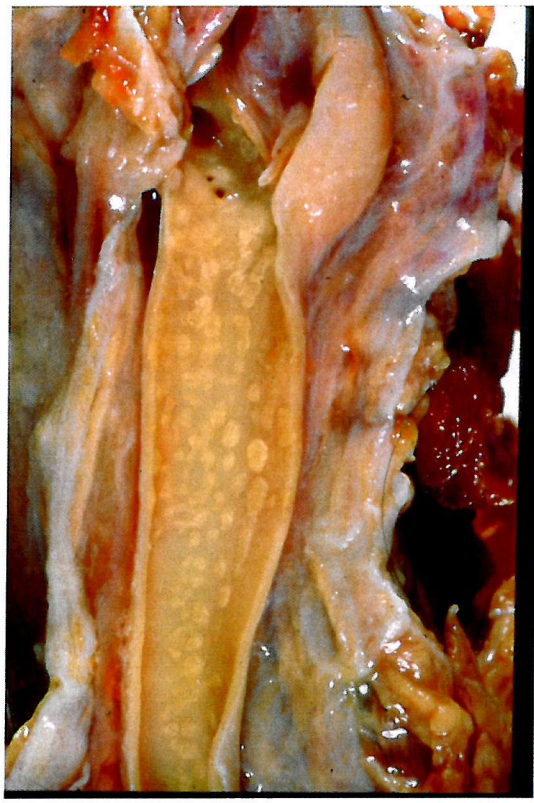


Figure 1.1b

Figure 1.1c

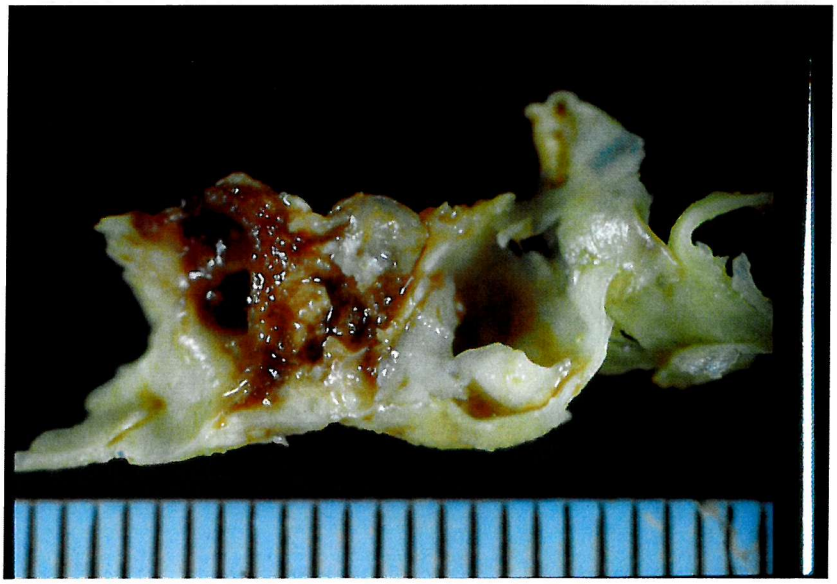
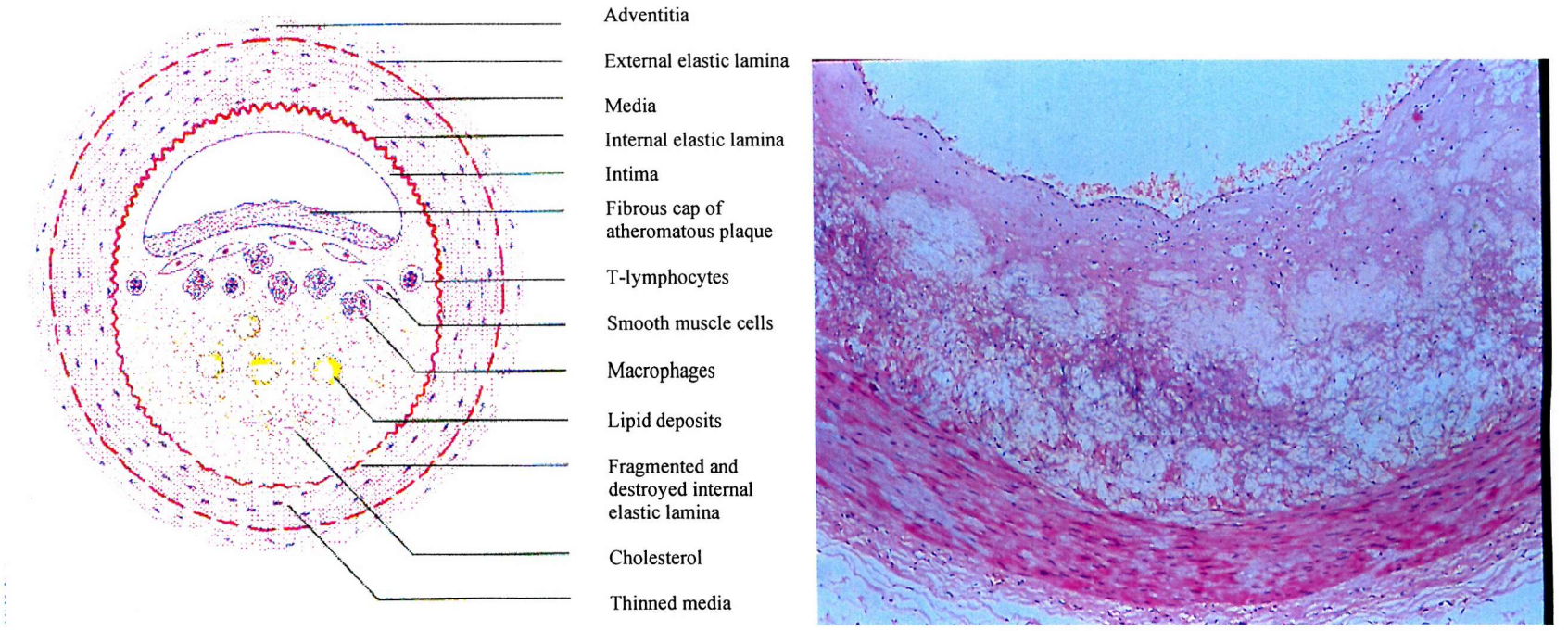


Figure 1.2 Atheromatous plaque. Diagram of an atheromatous plaque. Some of the features can be seen in the photomicrograph (right) from a 72-year-old male. (from Gallagher 2000, with permission).



Cellular roles in atherogenesis

1.5.1 The endothelium

Recently the role of endothelium as the pivotal factor in atherosclerosis has become more clear. Endothelium produces nitric oxide (NO), which has been reported to have many roles in atherosclerotic lesions including the modulation of vascular tone, the inhibition of VSMC proliferation, and the destruction of free radicals (Weissberg 2000). Abnormal vascular tone in atherosclerotic vessels has been assumed to indicate abnormality of NO production by the overlying endothelium. In the early stage of atherosclerosis, decreased production of NO was found, based on pharmacological or haemodynamic stimuli in children with hypercholesterolaemia (Sorensen *et al.* 1994). This resulted in the hypothesis that high concentrations of atherogenic lipoproteins in the circulation led to endothelial dysfunction and subendothelial accumulation of lipids. However it remains unclear whether this manifestation of endothelial dysfunction is a cause or an effect of modified lipid accumulation, in particular oxidised LDL. Interestingly this reduced production of NO has been associated with coronary risk factors such as smoking, old age and male sex (Celermajer *et al.* 1994). Dysfunction of the endothelium also results in the production of other adhesion molecules such as the intercellular adhesion molecule-1 (ICAM-1) and the vascular cell adhesion molecule-1 (VCAM-1), which are important for the recruitment of leukocytes into atherosclerotic lesions. In summary, endothelial cells can be damaged by various agents and this damage leads to endothelial dysfunction. The damaged endothelial cells then become more adhesive to inflammatory cells.

1.5.2 Inflammatory cells

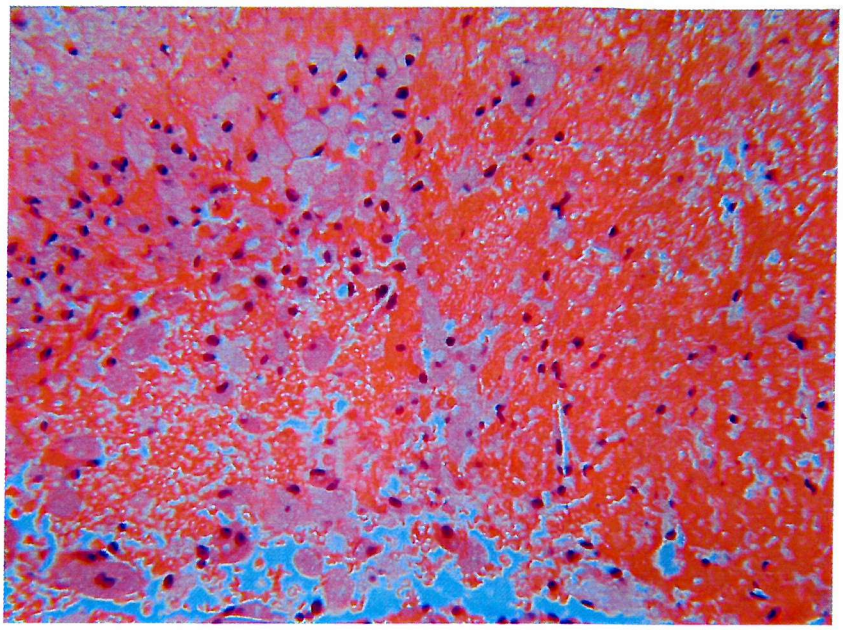
Leukocyte recruitment is dependent on the expression of adhesion molecules. These adhesion molecules attract inflammatory cells and facilitate their migration to the subendothelial area (Nakashima *et al.* 1994; Ross 1999). In fact it is unclear by which route leukocytes enter plaque. There are three possibilities: first, via the intima lining the lumen; second, via the vasa vasorum; and third, via new vessels appearing often in the intima of complex plaques (Golledge, Greenhalgh & Davies, A.H. 2000). Once

monocytes move into the subendothelial space, they become macrophages and express the necessary scavenger receptors to ingest oxidised LDL, a highly inflammatory substance, and become foamy macrophages (Figure 1.3) (Weissberg 2000). Although macrophages are believed to have a beneficial role in neutralizing potentially harmful lipid components in vessel walls, activated macrophages also produce several inflammatory mediators and growth factors that are both beneficial and detrimental for the development of atheroscleorotic plaques (Weissberg 2000). Some of these cytokines induce the migration of VSMCs from media to intima, where they produce the matrix for the fibrous cap. On the other hand, mediators of inflammation such as tumor necrosis factor-α (TNF-α), interleukin-1, and macrophage stimulating factor increase the binding of LDL to endothelium and increase transcription of the LDL receptor (Farzaneh-Far, Rudd & Weissberg 2001). Therefore a vicious cycle is activated by the presence of modified LDL in arterial walls that results in inflammation, which itself causes further modification of lipoproteins, in turn increasing the inflammatory response.

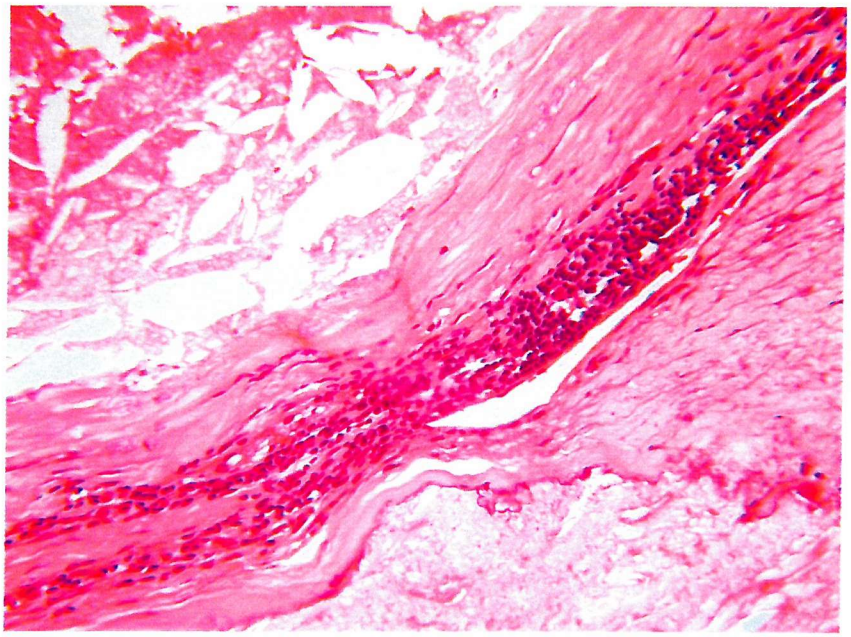
There is also a high incidence of dead and dying macrophages (apoptosis), which release their lipid contents into the lipid core (Figure 1.2) (Weissberg 2000). These dead cells are also a source of tissue factors in the lipid core, which may result in thrombosis if the lipid core is exposed to circulating platelets.

T-cells are also involved in atheromatous plaques (Figure 1.4). They produce several mediators such as interleukin-2 or interferon-gamma (IFN-γ), which play a part in the regulation of adhesion molecules and recruit more cells to join the inflammatory process (Rudd & Weissberg 2001).

Figures 1.3 Microscopic image of foamy macrophages on a carotid plaque.



Figures 1.4 Microscopic image of lymphocytes on a carotid plaque.



1.5.3 Vascular smooth muscle cells (VSMCs)

VSMCs are normally located in the media of vessel walls and contain large amounts of contractile protein. When cytokines are produced by activated macrophages, VSMCs migrate from the media to the intima (Figure 1.2) towards the site of inflammation in response to the inflammatory cell cytokines. When VSMCs are in the intima, they change their function and produce a predominantly synthetic protein rather than the usual contractile protein (Weissberg 2000). Several matrix proteins such as collagen isoform 1 or 3, glycosaminoglycan, and elastin are necessary for the formation of a fibrous cap over the lipid core from circulating platelets and the proteins of the coagulation cascade (Rudd & Weissberg 2001). VSMCs are the only cells that produce the matrix protein in the fibrous cap, which separates the very thrombogenic lipid core from circulating platelets and clotting factors, thereby playing a major anti-thrombotic role for plaque. Consequently VSMCs are now considered as guardians of the integrity of the fibrous caps of plaques (Libby 1995), whereas previously they were thought to be a key initiating factor in the pathogenesis of an atherosclerotic lesion (Ross & Glomset 1973). Plaques with thin fibrous caps over large lipid cores are prone to rupture and cause thrombosis, so-called "unstable plaques" (Figure 1.1c).

1.6 Atherosclerosis and symptomatology

There are two mechanisms by which symptoms of atherosclerotic lesions can develop. Firstly by the time that an atherosclerotic lesion has increased in size with the deposition of a necrotic core, inflammatory cells and a fibrous cap, the lesion is so large that the downstream blood supply is not sufficient. Initially this usually occurs because of exertion associated with increased blood flow such as in stable angina or intermittent claudication of the calf. The second mechanism begins with erosion or rupture of plaque, resulting in exposure of the blood to thrombogenic lipid cores. Consequently a rapid accumulation of platelets, deposition of fibrin, and occlusion of the vessel by thrombi or distal embolisation of thrombotic material occur, which may lead to very severe problems such as acute myocardial infarction. Anti-platelets can therefore inhibit the formation of thrombi and attenuate the complications of plaque rupture.

There are two ways in which thrombosis is associated with plaque (Davies, M.J. 1996). Firstly a plaque erodes, and a thrombus forms on the surface of the lesion, which comes from the denudation and erosion of the endothelial surface. This is common in young women (Farb *et al.* 1996). With disruption of the plaque, a thrombus is formed on the ruptured or torn fibrous cap by blood from the lumen that contacts the lipid core of plaque. In general plaque disruption in coronary artery is thought to be about three times more common than plaque erosion (Davies, M.J.1996).

There is a broad spectrum of pathology of plaques (Davies, M.J. 1996). In patients with endothelial erosion, microscopic platelet thrombi have no clinical significance besides the stimulation of growth of smooth muscle. However, denudation of large areas of endothelial cells can result in bigger thrombi that obstruct the lumen sufficiently to produce symptoms. At the other end of the spectrum, in plaque disruption if the break in the fibrous cap is small, the overall shape of the plaque remains unchanged, although the plaque volume is increased by blood within the core. However bigger tears allow more blood into the plaque, and the lumen is reduced. Major episodes may involve multiple tears in the plaque with the extrusion of the contents of the plaque into the lumen.

If a thrombus does not completely obstruct the lumen and stop the flow of blood, there will be antegrade blood flow in the artery allowing distal embolisation (Davies, M.J. *et al.* 1986; Falk 1985). Falk (1985) conducted an extensive microscopic examination of epicardial arteries and myocardium in 25 patients who died suddenly from acute coronary thrombosis. He found that 73% of the cases, small fragments of thrombus material and recent microinfarcts were found in the myocardium distal to evolving coronary thrombi indicating that thrombus material may break off and be carried away with the blood (peripheral embolisation). Once a plaque ruptures, a thrombus forms as a result of the exposure of the blood to the lipid core. This thrombus may cause either complete occlusion, such as that which occurs in acute myocardial infarction, or a

marked reduction of the lumen. Incomplete occlusions occur quite commonly in patients with plaque rupture in coronary arteries. Davies *et al.* (Davies, M.J.1995) found that up to 70% of plaques causing severe stenosis (> 50%) contained histological evidence of previous ruptures, non-occluding thrombi, and subsequent repair. After formation of the thrombus, the healing process begins and VSMCs move from the media into the thrombus. Non-occluding lesions thus induce formation of a new fibrous cap over the organizing thrombus.

1.7 Inflammation and instability of atherosclerotic plaques

Plaques in the coronary arteries and human aorta that have ruptured, has been studied in an attempt to characterise plaques that are at risk of rupture. Various investigators have identified several different characteristics of atherosclerotic plaques. Davies *et al.* (Davies, M.J.1996) have proposed that plaques with a high risk of rupture are those with thin fibrous caps, an increased number of macrophages, lipid cores occupying a high proportion of overall plaque volume, a reduced number of smooth muscle cells, and a high tissue factor content. Plutzky *et al.* (1999) has indicated that plaques with thin fibrous caps, high incidences of inflammatory cells, and a paucity of SMVCs have a high risk of rupture. Felton *et al.* (1997) studied about 300 aortic plaques and found that ruptured plaques have more inflammatory cells than intact plaques. It is interesting to note that in every series, inflammatory cells play a leading role in plaque instability. There are several mechanisms by which the two main types of inflammatory cells, lymphocytes and macrophages, affect plaque vulnerability.

1.7.1 T-lymphocytes

Activated T-lymphocytes produce pro-inflammatory cytokines, for example IFN-γ, that directly inhibit proliferation of VSMCs or almost completely shut down collagen synthesis (Libby 1995). IFN-γ also activates a function of macrophages related to plaque vulnerability. The production of matrix protein in fibrous caps by VSMCs, is consequently poor in the presence of activated T-lymphocytes.

1.7.2 Macrophages

Macrophages have many effects on plaque stability. Firstly they produce cytokines such as interleukin (IL-1β) and tumor necrosis factor (TNF-α). When these two cytokines work together with IFN-γ produced by T-lymphocytes, they are cytotoxic for VSMCs. Geng *et al.* (1996) found that when combined, the cytokines IFN-γ, TNF-α, and IL-1β induce apoptosis of cultured human and rat VSMCs.

Secondly, activated macrophages can induce apoptosis by cell-to-cell contact. Boyle *et al.* (2001) found that macrophages induced apoptosis of VSMCs by direct cell-to-cell contact or proximity via Fas-Fas-L interactions. On attachment of ligands of Fas-L, apoptosis is induced by a cascade of cysteine protease (caspases), especially caspases-8 and caspases-3.

Thirdly macrophages in what is perhaps the most important mechanism causing plaque instability produce a variety of matrix metalloproteinases (MMPs) that degrade the matrix component of the fibrous cap by proteolytic cleavage of its protein component. Libby *et al.* (1995) found that lipid-laden macrophages, isolated from atherosclerosis produced experimentally by diet and balloon injury in the rabbit aorta, expressed MMP, namely stromelysin and interstitial collagenase, both in situ and in vitro. In contrast, alveolar macrophages from the same animals exposed to the same degree of hyperlipidaemia did not express these MMPs. These macrophages may be stimulated by many likely cytokine candidates such as IFN-γ, tumor necrosis factor, interleukin-1, or macrophage colony stimulating factors (Libby 1995).

Lastly macrophages are the principle source of tissue factors within atherosclerotic plaques that are potent initiators of thrombosis (Wilcox *et al.* 1989). Macrophages may not only influence integrity of the fibrous cap and the tendency of a plaque to rupture, but also promote thrombosis after plaque rupture.

In summary, in the presence of the threat of a large number of activated inflammatory cells, VSMCs proliferate poorly and become more susceptible to apoptosis. A large number of activated inflammatory cells are also associated with the destruction of the fabric component of the fibrous cap, and the remaining VSMCs compensate poorly because of a high level of inflammatory mediators.

1.8 The balance of atherosclerosis

It used to be believed that atherosclerotic plaques could not be modified, so therapy was designed only to improve flow distal to stenotic lesions. This was achieved by either percutaneous balloon angioplasty or surgical reconstruction. However in lipid-lowering drug (statin) trials in patients with coronary atherosclerosis, patients taking statins had lower myocardial infarction rates than those in a control group (Shepherd *et al.* 1995; Sacks *et al.* 1996). This demonstrated that atherosclerotic lesions are modifiable, especially plaque stability. Moreover statins have been shown to have other effects on plaque, such as reducing plaque inflammation and thrombus formation, promotion of repair (VSMC proliferation), improved endothelial function, and beneficial modulation of immune function (Weissberg 1999; Golledge, Greenhalgh & Davies, A.H. 2000). It is now well recognised that atherosclerotic plaque is dynamic and modifiable.

Atherosclerosis is a dynamic process in which a balance between the destructive influence of inflammatory cells and the reactive, stabilizing (repair) effects of VSMCs determines the outcome. For example, a correlation has also been reported between coronary plaque progression and polymorphism in the stromelysin-1 gene promoter (Ye *et al.* 1995). As stromelysin is a member of the family of MMPs that degrade extracellular matrix, this polymorphism has some effect in making plaque more unstable. There would be an obvious clinical benefit if the balance was altered and plaque stability improved by decreasing inflammation. An understanding of plaque stability may lead to the prospect of developing therapies targeted at the specific events in the pathogenesis of atheroma.

Chlamydia pneumoniae, which can be found in plaque and vascular walls, may evoke a cell-mediated cytokine response in plaques (Gibbs, Carey & Davies, A.H.1998) and tip the balance in favour of plaque rupture. However the relationship between chlamydia infection and the severity and extent of atherosclerotic lesions is not clear (Thomas *et al.* 1999; Wong, Gallagher & Ward 1999). In fact, mild atherosclerotic lesions in the coronary artery have been as likely to be positive for Chlamydia pneumoniae as severe lesions (Thomas *et al.* 1999). Similarly, in a carotid plaque study Chlamydia pneumoniae was found in 25% of symptomatic carotid plaques, but it was not associated with cerebral embolisation when transcranial Doppler imaging was used or ipsilateral hemispheric infarcts in the region of the middle cerebral artery when computerized tomographic (CT) scans of the brain were used (Gibbs *et al.* 2000).

1.9 Influence of the degree of stenosis and the plaque morphology on clinical events

The degree of stenosis caused by atherosclerotic lesions has been using as an indication of the need for intervention for a long time because severe stenosis is believed to cause insufficiency of the blood supply. Lesions with lesser degrees of stenosis have often not been treated. Reviewing angiograms after thrombolysis for acute myocardial infarction, Falk *et al.* (1995) found that in 70% of patients, the stenosis of coronary arteries was less than 50%. The degree of stenosis alone is therefore not the only factor. It makes sense to look at the differences in plaque to try to establish the relationship with vascular events such as stroke. In the morphology of carotid plaques, this problem has been investigated for some 30 years now, especially by considering echolucency on ultrasound B-mode images. In a large cohort study of 4886 patients with asymptomatic carotid stenosis, echolucent plaques were associated with an increased risk of stroke. When adjusted, however, for confounders in a Cox regression model, a degree of stenosis greater than 50% had a more prominent relative risk than echolucency (Polak *et al.* 1998). Holdsworth *et al.* (1995) also suggested that plaque morphology does not increase the sensitivity of stenosis in predicting the presence of symptoms. The use of plaque morphology as a discriminating factor for carotid surgery has not been supported by other groups either (Hayward, Davies, A.H. & Lamont 1995).

This discrepancy might be explained partially by the difference in the haemodynamics of coronary and carotid circulation.

1.10 The risk of vascular events for carotid plaques

1.10.1 Inflammation

As mentioned above, inflammation is the fundamental pathology of atherosclerosis (Ross 1999). Many studies have shown that carotid plaques from symptomatic patients have more inflammation than those from asymptomatic patients. Using immunohistochemistry, Husain *et al.* (1999) found that carotid plaques of patients with symptomatic neurological deficits have more macrophages than those of patients in an asymptomatic group. Golledge *et al.* (2000) reviewed 21 studies that compared carotid plaque histology. They proposed that unstable carotid plaques are characterised by surface ulceration/plaque rupture, thinning fibrous caps, and infiltration of the caps by a large number of macrophages and T-cells.

Statins have shown a clear benefit in reducing coronary morbidity and mortality in both primary and secondary prevention. This benefit occurs within a relatively short period after statins are taken, presumably too short a period to achieve to a significant reduction in atheroma (Palinski 2001). One of the hypotheses is that lipid-lowering therapy has an effect of decreasing inflammation in plaque. Crisby *et al.* (2001) have compared carotid plaques of patients taking pravastatin 40 mg/d for three months with those of patients with no lipid-lowering therapy. They found that plaque of patients taking pravastatin had fewer macrophages, fewer T-cells, a lower level of MMP-2 and a higher collagen content. This indicated that lipid-lowering therapy decreases inflammation and results in substantial accumulation of collagen, a feature of stable plaque. Therefore it makes sense to look for any medications or supplements which have lipid-lowering and anti-inflammatory effects on atherosclerotic plaques, as statins do. Fish oil supplements, which are rich in n-3 polyunsaturated fatty acid, is a candidate. Fish oil has been shown to have lower levels of all classes of lipids in many epidemiological studies (Bang, Dyerberg & Nielsen 1971). In an animal study, fish oil

also decresased the number of macrophages in plaque (Davies, H.R.1987). Many randomised controlled trials have found that fish oil (n-3 PUFA supplements) decreased cardiovascular deaths (GISSI-P 1999; Singh *et al.* 1997). No studies, however, have been conducted by others on its anti-inflammatory effects on atherosclerotic plaque in clinical practice, so this was investigated and is a major subject in this thesis (Chapter 2).

1.10.2 Surgery and carotid plaques

As mentioned earlier, atherosclerotic plaques can rupture and lead to thrombosis, which can cause stroke if it occurs in a carotid artery. In the last decade, surgical removal of this plaque, carotid endarterectomy, has been proven to reduce the risk of stroke in certain groups of patients. A large randomised controlled trial in the USA has shown that the 2-year risk of stroke in patients with severe symptomatic carotid stenosis was 26% with medical treatment, whereas it was only 9% in a carotid endarterectomy group (NASCET 1991). However carotid endarterectomy can also cause stroke or even death. To achieve the maximum benefit of this operation, surgeons try to identify the characteristics of patients or techniques that are associated with high stroke and death rate. This has been investigated in this thesis using a systematic review of the literature and meta-analysis (Chapters 7-9).

1.11 Risk factors for atherosclerosis

The Framingham study has shown for over 30 years that hyperlipidaemia, male gender, hypertension, diabetes, cigarette smoking, excess body weight, elevated blood sugar, lack of exercise, stress and electrocardiographic abnormalities are associated with coronary heart disease (Castelli 1984). This study proposed the idea of risk factor management in order to change the course of coronary artery disease. Since then risk factor management has received much more emphasis.

1.11.1 Hyperlipidaemia

Hyperlipidaemia is one of the most interesting areas of investigation because it is a modifiable factor. Many randomised controlled trials in either healthy or patients with a recent history of myocardial infarction have found that participants who are aggressively treated with statins for hyperlipidaemia have a considerably reduced incidence of coronary events (Shepherd *et al.* 1995; Sacks *et al.* 1996; HPS 2002). Although several large trials and many guidelines have stressed the importance of detection and treatment of hyperlipidaemia, it is not known to what extent these have been applied to patients with peripheral vascular disease. This thesis has approached this issue in patients with carotid stenosis (Chapter 3).

1.11.2 Hypertension

Hypertension is a strong predictor of cardiovascular disease, especially stroke and ischaemic heart disease. The association between atherosclerosis and hypertension is partly due to endothelial dysfuction (Celermajer *et al.* 1994). A clear benefit of treating hypertension has been demonstrated in several studies: stroke rate has been reduced by 38%, cardiovascular deaths by 14%, and other vascular deaths by 14% (Shearman & Chulakadabba 1999). Hypertension should be treated aggressively following accepted guidelines such as those of the British Hypertension Society, which suggest that systolic pressure should be less than 140 mmHg and diastolic pressure less than 85 mmHg (Ramsay *et al.* 1999).

1.11.3 Diabetes

80% of patients with diabetes die from atherosclerosis. Three quarters of these deaths are due to coronary events; the remainder are due to carotid disease or peripheral arterial disease (McCormick *et al.* 2001). Advanced accumulation of end products of glycosylation leads to stiffness of vessel walls, binding of lipopoteins, and proliferation of vascular smooth muscle cells (McCormick *et al.* 2001). Intensive control of blood sugar reduces microvascular diabetic complications, and adequate management of other

risk factors in type II diabetics such as hypertension decreases cardiovascular events (UKPDS 38 1998).

1.11.4 Smoking

30% of coronary heart disease is attributed to smoking, which doubles the risk of cerebral infarction (Jonas *et al.* 1992). Nicotine and carbon monoxide in cigarette smoke appear to cause harm in several ways. Smoking increases serum cholesterol and blood pressure, reduces oxygen transport, increases vascular resistance and vasospasm, and adversely affects vascular endothelium and platelet and thrombotic mechanisms (Shearman & Chulakadabba 1999). Cessation of smoking rapidly decreases the risk of ischaemic stroke and myocardial infarction by 50%, compared to those who continue to smoke (Ockene & Miller 1997).

1.11.5 Gene polymorphism

Many case-control studies have identified several gene polymorphisms, which are associated with atherosclerotic disease. These include adverse alleles, for example ApoE4 and protective alleles, for example FXIIIVal34 Leu. Apolipoprotein (ApoE), which was one of the first genes to be identified and which plays a primary role in the regulation of lipid metabolism. Three alleles have been identified: E2, E3, and E4. The E2 allele is associated with low LDL, high triglyceride and low LDL, but the E4 allele is associated with high LDL, high triglycerides and low HDL (Nicolaides *et al.* 2000). However the association between polymorphism and atherosclerosis is not straightforward for several reasons (Nicolaides *et al.* 2000). Atherosclerotic disease is polygenic, so a number of genes determine the characteristics of atherosclerosis and each polygene has only a slight effect. Some gene mutations also have incomplete penetrance. Expression is modulated by external factors, the environment and other genes.

On the whole, atherosclerosis seems to be the result an interaction between different gene polymorphisms and exogenous risk factors including smoking and hypertension.

Some people with low risk polymorphism might have a low chance of heart attack, although they are heavy smokers. For instance the relative risk of myocardial infarction is 2 in smokers of RQ353 and QQ353 genotype of factor VII polymorphism, but it is 6 in RR353 genotype (Nicolaides *et al.* 2000).

1.11.6 Emerging risk factors in cardiovascular disease

Many models based on the traditional risk factors in the Framingham study have been developed to estimate the risk of IHD in populations. Even though evaluation of traditional factors produces reasonably accurate estimates of risks for a population, it fails to predict about 40%-50% of the variation in the absolute risk of an event for an individual in that population (Farzaneh-Far, Rudd & Weissberg 2001). Thus, the use of other risk factors may improve predictive power and will probably result in better decisions for proven therapies for prevention.

As is well known, the basic pathological process of atherosclerosis is inflammation. This has prompted a great deal of research about identification of measurable biochemical markers of plaque inflammation, the assumption being that the level of inflammatory markers in the blood reflects the degree of plaque inflammatory activity and perhaps plaque instability. Some of these markers may reflect the degree of activation of plaque macrophages. Such markers include C-reactive protein (CRP), serum amyloid A (SAA), and tumor necrosis factor-α (TNF-α). Recently in a large cohort study of healthy women, it was found that women in the highest quartile with respect to baseline CRP had a cardiovascular risk 4.4 times greater than women in the lowest quartile (Ridker *et al.* 2000).

In addition, as discussed above, adhesion of circulating leukocytes to vascular endothelial cells and their subsequent trans-endothelial migration has been proposed as an important step in the initiation of atherogenesis, a process mediated by cellular adhesion molecules in response to sub-endothelial inflammation. Clayton *et al.* (2002) found the levels of ICAM-1 and P-Selectin were higher in patients with symptomatic

carotid plaques than in those with asymptomatic plaques. The production of proteolytic cellular adhesion molecules, which are up-regulated during atherogenesis, can be found in plasma. This raises the possibility that the plasma concentration of cellular adhesion molecules may be high in patients with atherosclerosis. Ridker *et al.* (1998b) conducted a large cohort study to examine the relationship between baseline levels of ICAM-1 and the risk of MI. They found that patients with high levels of ICAM-1 had a higher risk of MI (relative risk 1.4) than those with low levels. However few studies have been conducted on the relationship between the levels of these inflammatory markers, including CRP, in patients with carotid stenosis. This subject is investigated in this thesis (Chapter 4).

1.12 Conclusion

In conclusion, there is a dynamic balance in atherosclerosis between the natural stabilizing properties of surrounding VSMCs and the inflammatory cells and the cytokines within plaques. The composition of plaque is considered to be increasingly important. In the next three chapters a trial is reported about the effects of fish oil capsules, rich in n-3 PUFA, on inflammation in atherosclerotic plaques. Another study is reported about awareness of the importance of control of hyperlipidaemia by clinicians treating patients with carotid atheroma. Then a study is reported in which the role of inflammatory mediators identified in coronary artery disease were evaluated in patients with cerebrovascular diseases. Finally, in the next five chapters, the risks and risk factors of stroke and death following carotid endarterectomy are investigated.

Chapter 2

The potential of dietary lipid supplements to benefit patients with carotid artery disease

2.1 Summary

Objective: Many epidemiological studies and randomised controlled trials have shown that individuals taking fish oil have had a lower risk of coronary events than those not taking it. To date, however, the mechanism of this benefit has not been clear. The objective of this study was to investigate the anti-inflammatory effects of supplements of fish oil (n-3 polyunsaturated fatty acid (PUFA)) on carotid plaques, compared with the effects of supplements of n-6 PUFA and a placebo.

Design: A randomised, controlled, double blind study.

Methods: 189 patients awaiting carotid endarterectomy (CEA) were randomised and given one of three types of capsules containing n-3 PUFA, n-6 PUFA, or a placebo. Plasma lipid profiles were determined before and after supplementation. Carotid plaques were examined by histomorphometry and scored using the American Heart Association (AHA) criteria and its modification and fatty acid composition determined. The number of T-lymphocytes and macrophages in the plaque was measured using immunohistochemistry techniques.

Results: The median duration of supplementation was 50 days and was the same for all groups. The proportions of eicosapentaenoic (EPA) and docosahexaenoic (DHA) increased significantly in plasma LDL lipid fractions in patients receiving fish oil. The proportions of EPA and DHA were higher in carotid plaque phospholipids, cholesterol esters, and triacylglycerols from patients treated with fish oil compared with patients in the control group. The number of monocyte/macrophages in the plaques from patients receiving fish oil was lower than in those from patients in the other two groups.

Conclusions: Incorporation of n-3 PUFA into carotid plaques is associated with a reduced number of macrophages. As macrophages play a primary role in plaque instability, this study suggests n-3 PUFA supplements might have a role in plaque stability.

2.2 Introduction

2.2.1 Ischaemic heart disease and fish oil in Eskimos

Thirty years ago scientists were surprised by the very low rate of myocardial infarction (MI) in Eskimos, despite the fact that most Eskimos were heavy cigarette smokers and existed on a diet extremely rich in animal fats (Bang, Dyerberg & Nielsen 1971; Bang, Dyerberg & Sinclair 1980). Deaths of Eskimos in Greenland from ischaemic heart disease (IHD) were 3.5% of all deaths, a very low percentage compared with the percentage in Denmark during the years 1963 to 1967. As hypercholesterolaemia is associated with MI (Castelli 1984), many scientists have been interested in the profiles of plasma lipids of Eskimos. Bang *et al.* found that Eskimos had significantly lower levels of all types of plasma lipids (total cholesterol and triacylglycerol). This might be explained by genetics or the Eskimos' way of life, including their diet. Bang *et al.* proceeded and compared the lipid levels of female Eskimos in Greenland with female Eskimos in Denmark. They found that female Eskimos in Denmark had lipid concentrations comparable to Danish people rather than female Eskimos in Greenland. This suggested that the differences in lipid profiles were exogenous. Diet has a considerable influence on plasma lipids, so scientists began to investigate the diet and lifestyles of Eskimos. Most of them still depended on hunting and fishing for their food, so their diet consisted largely of fish such as capelin and some kinds of salmon, which are rich in n-3 PUFA. As is well known, polyunsaturated fatty tissue in these fish can decrease the plasma cholesterol level (Harris *et al.* 1990). These two facts suggest that the lower levels of plasma lipids in Eskimos might be due to their high consumption of fish. In 1976 Bang *et al.* (1980) compared the diet of Eskimos in northwestern Greenland with the typical Danish diet. They found that the Eskimo diet had a higher ratio of PUFA to saturated fatty acids and more PUFA than did that of the Danes: the ratio of PUFA to saturated fatty acids was 0.84 for Eskimos and 0.24 for Danes. Furthermore PUFA in Eskimos was predominantly of the α- linolenic class (n-3 PUFA), whereas that in Danes was of the linoleic class (n-6 PUFA). They proposed that the rarity of IHD in Eskimos might be partially explained by the anti-thrombotic effect of

long-chain PUFA, in particular n-3 PUFA (Bang, Dyerberg & Sinclair 1980) (Dyerberg *et al.* 1978). It was therefore suggested that Eskimos had a low incidence of MI because of a high consumption of oily fish, which were rich in n-3 PUFA.

Many other epidemiological studies have confirmed this finding in Greenland. One study was carried out in Upernavik Disrict in northwest Greenland. 1300-2000 people lived there, most of whom depended on the traditional occupations of sealing and whaling for a living. The investigators in this study reviewed the patients' histories (chart) for the previous 25 years. Interestingly, only 3 people in that interval had had MIs, which was very unusual. In a group of typical Danes of the same size, 40 people could have been expected to have MIs in the same interval (Kromann & Green 1980). Bjerregaard *et al.* (1988) compared the causes of death of Greenlanders to those of people in Denmark and found that Greenlanders died from IHD relatively less often than people in Denmark. In addition Newman *et al.* (1993) graded pathological specimens from necropsy of abdominal aortas and coronary arteries of Alaska natives and non-Alaska natives. After adjustment for age and sex, they found that Alaska natives had a consistently lower incidence of advanced atherosclerotic lesions than non-Alaska natives.

2.2.2 Studies of fish oil supplements

The results from epidemiological data have prompted a great deal of research in this area. In animal models dietary fish oil has been demonstrated to decrease atherosclerosis. A study at Southampton found that rabbits taking fish oil had significantly smaller areas of aortic atherosclerosis than those taking coconut oil and corn oil (Bolton-Smith *et al.* 1988). Weiner *et al.* (1986) studied balloon abrasion-induced atherosclerostic lesions in swine. They found that the swine that had been fed cod liver oil for eight months had fewer arterial stenotic lesions than those in a control group. Renier *et al.* (1993) found that mice receiving fish oil had less plaque progression and less production of cytokines that are believed to play a role in atherogenesis (TNF-α and IL-1β) than those in a control group. Davies *et al.* (Davies,

H.R.1987) studied rhesus monkeys, whose physiology is close to that of humans. Following 12 months of fish oil supplementation, the monkeys had smaller atherosclerotic lesions and fewer macrophages in plaques than those in a group, which received coconut oil supplements.

The benefits of a high consumption of fish and fish oil have appeared in many randomised controlled trials. In Wales Burr *et al.* (1989) reported the results of the Diet And Re-infarction Trial (DART) in which they studied myocardial re-infarction and deaths in male patients who had already suffered a MI. The results suggested that high consumption of fatty fish (at least two weekly portions of 200-400 g) reduced overall mortality by 29% during the first two years. In the GISSI-P (1999) trial in Italy 11,324 post-MI patients were studied. Clinical outcomes of patients with diets supplemented by n-3 PUFA were compared to those of patients with diets using vitamin E supplements and a placebo. It was found that patients taking 1 g/day of n-3 PUFA for 2 years had a significantly lower risk of death, non-fatal MI, and non-fatal stroke than those in a control group. In particular the risk of cardiovascular death for patients in the n-3 PUFA supplement group was 17% less than that for patients in the control group. In a study of MI in India, Singh *et al.* (1997) found that 24.5% of the patients taking 1.08 g/day of n-3 PUFA had cardiac events (cardiac death and non-fatal MI) in one year, whereas 34.7% of the patients in a control group had cardiac events during the same time period. In addition a coronary angiographic study showed significant regression of atherosclerotic plaques in patients who had taken 6 g/d of fish oil concentrate for 2 years (von Schacky *et al.* 1999).

2.2.3 Basic biochemistry of fish oil and n-3 PUFA

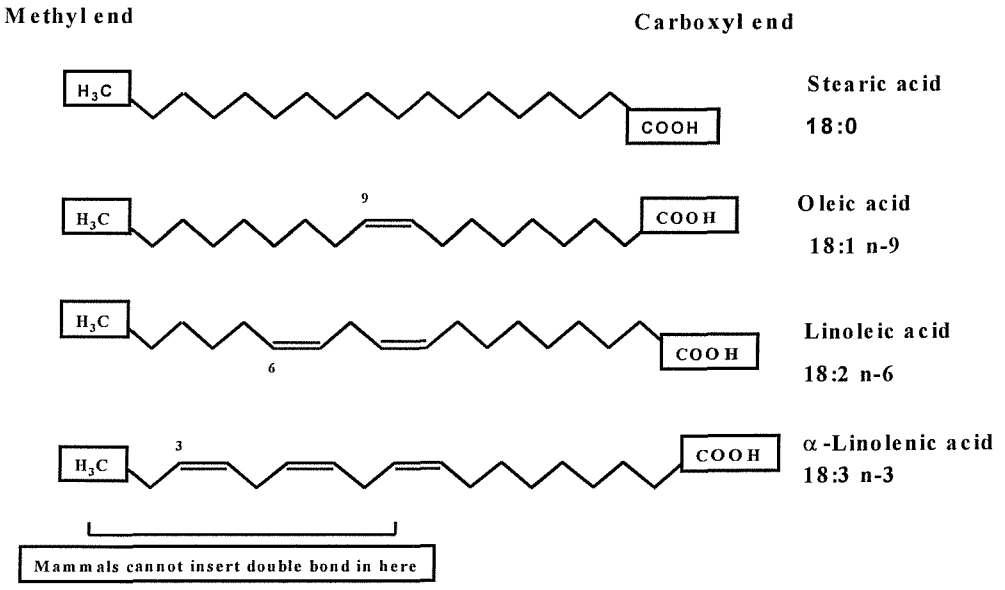
2.2.3.1 Fatty acids in the human diet

Fatty acids are part of many types of lipids. For example the molecule of triacylglycerol is composed of three fatty acids esterified to glycerol. The molecule of phosphoacylglycerol, like triacylglycerol, has three ester links to the glycerol molecule, but only two of the three involve fatty acids, the remaining link being esterified to

phosphoric acid, usually with an alcohol molecule attached. The general term to describe a lipid containing phosphorus is phospholipid (Gurr 1998). Cholesterol ester contains one fatty acid esterified to one of the rings of cholesterol. In general triacylglycerol is the main lipid component of the human diet, constituting more than 95% of dietary fat (Calder PC 2001).

There are various types of fatty acids, which are composed of even numbers of carbon atoms. They range from acids with four carbon atoms to acids with 30 carbon atoms in fish oil. Fatty acids can be subdivided into saturated fatty acids and unsaturated fatty acids. Saturated fatty acids do not have any double bonds between carbon atoms, whereas unsaturated fatty acids have at least one double bond between carbon atoms (Figure 2.1) (Calder PC 2001). PUFAs are unsaturated fatty acids that have more than one double bond. Not only do saturated and unsaturated fatty acids have different structures, but they also have different physical properties. PUFAs such as those in sunflower oil and corn oil are liquid at room temperature, whereas saturated fatty acids such as those in butter are solid.

Figure 2.1 Structure of some fatty acids (from Calder 2001 with permission).



Several systems of nomenclature have been used to describe fatty acids (Table 2.1) (Calder PC 2001). In general, the notation uses the number of carbon atoms, the number of the double bonds, and the position of the first double bond with respect to the methyl group at the end of the chain. For example, 20:5 n-3 means the fatty acid molecule has twenty carbon atoms and five double bonds and the first double bond is at the third carbon atom from the methyl end of the chain. The n notation is sometimes referred to as omega (Calder PC 2001).

Table 2.1 Fatty acid nomenclature (from Calder 2001 with permission).

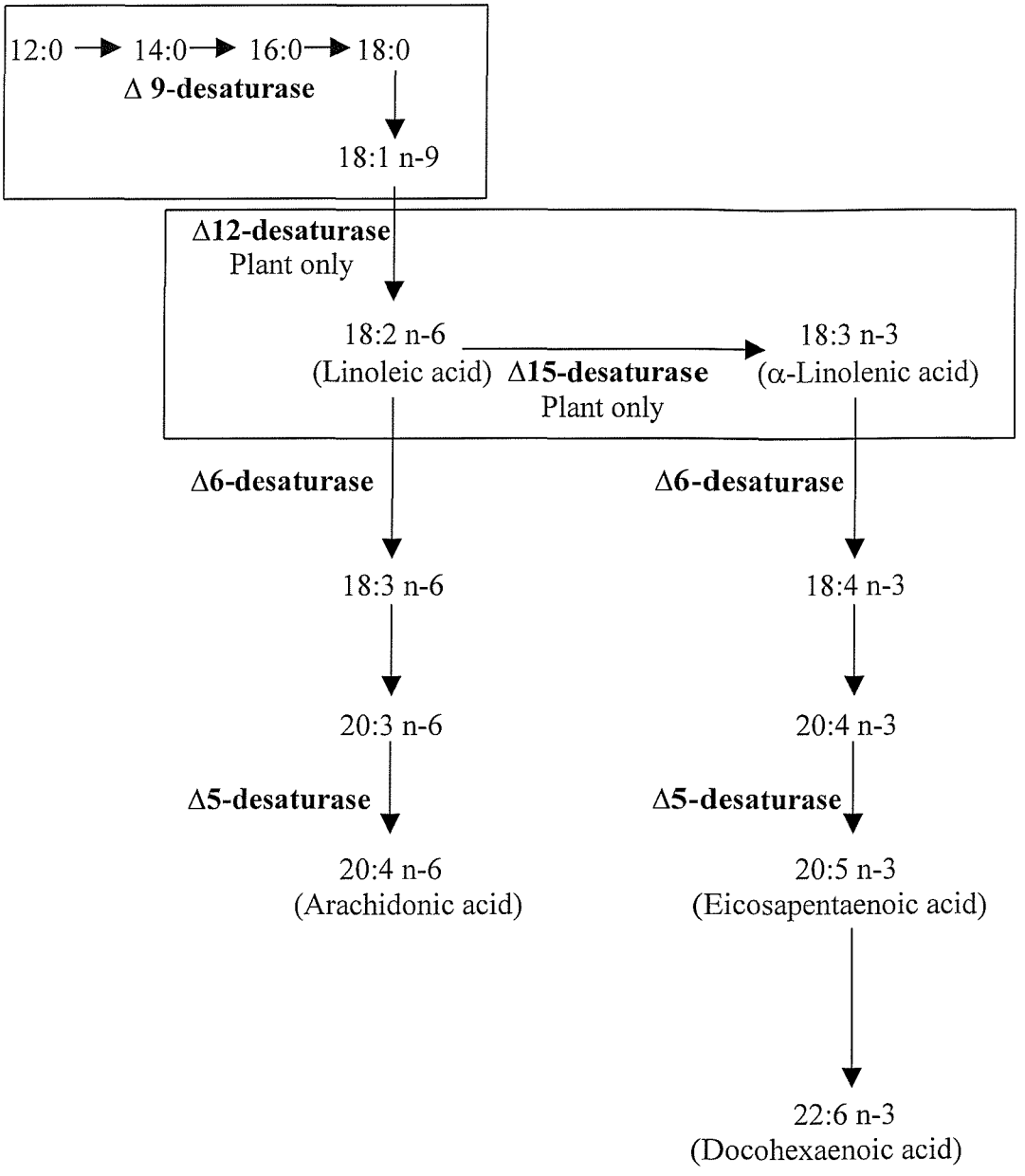
Systematic name	Trivial name	Notation
Decanoic	Capric	10:0
Dodecanoic	Lauric	12:0
Tetradecanoic	Myrsitic	14:0
Hexadecanoic	Palmitic	16:0
Octadecanoic	Stearic	18:0
9-Hexadecenoic	Palmitoleic	16:1 n-7
9-Octadecenoic	Oleic	18:1 n-9
9,12-Octadecadienoic	Linoleic	18:2 n-6
9,12,15-Octadecatrienoic	α-Linolenic	18:3 n-3
6,9,12-Octadecatrienoic	γ-Linolenic	18:3 n-6
5,8,11-Eicosatrienoic	Mead	20:3 n-9
8,11,14- Eicosatrienoic	Dihomo-γ-linolenic	20:3 n-6
5,8,11,14-Eicosatetraenoic	Arachidonic	20:4 n-6
5,8,11,14,17-Eicosapentaenoic	Eicosapentaenoic (EPA)	20:5 n-3
7,10,13,16,19-Docosapentaenoic	Docosapentaenoic (DPA)	22:5 n-3
4,7,10,13,16,19-Docosahexaenoic	Docosahexaenoic (DHA)	22:6 n-3

In mammalian tissues saturated fatty acids and most monounsaturated fatty acids can be synthesized from non-fat precursors such as glucose and protein. However in mammals a double bond cannot be inserted between the terminal methyl group and the ninth carbon atom from the methyl end of the chain (Figure 2.1). Therefore in mammals oleic acid (18:1 n-9) cannot be converted to linoleic acid (18:2 n-6); an enzyme mediating this process is Δ 12-desaturase, which is found only in plants (Figure 2.2). Similarly in mammals linoleic acid (18:2 n-6) cannot be converted to α-linolenic acid (18:3 n-3), which is mediated by the enzyme Δ 15-desaturase, again found only plants. Some plants and plant oils (e.g. corn oil) are rich in either linoleic acid or α-linolenic acid (Grimm *et al.* 2002).

2.2.3.2 Synthesis of long chain PUFA

Although a double bond cannot be inserted at the carbon atoms number 3 or 6 in mammals, once they consume PUFA they can make longer chain fatty acids from them (Grimm *et al.* 2002). In mammals linoleic acid can be coverted via γ-linoleic acid (18:3 n-6) and dihomo-γ-linoleic acid (20:3 n-6) to arachidonic acid (20:4 n-6) (Figure 2.2) (Calder PC 2001; Holub 2002). Using the same pathway, α-linolenic acid can be converted to eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) (Holub 2002). There is competition between these two pathways since they use the same enzymes (Holub 2002). The tissues of so-called oily fish such as fresh tuna and sardines are rich in EPA and DHA, as are the commercial preparations called fish oils, which comes from body oils of oily fish. EPA and DHA are also found in high concentrations in the livers of warm water fish such as cod.

Figure 2.2 Metabolic pathways for the synthesis of PUFA (from Calder 2001 with permission).



2.2.3.3 Change in the pattern of consumption of fatty acids

In the last forty years the consumption of fatty acids by western people has changed considerably. For example the proportions of saturated fatty acids and monounsaturated fatty acids in the diet of people in the UK have decreased by 40% and 30%, respectively, in contrast to the proportion of PUFA, which has increased by 25% (Calder PC 2001). The reason for these changes is that consumption of saturated fatty acids has been associated with hypercholesterolaemia, which in turn has been associated with a high incidence of heart attacks (MI) (Calder PC 2001), so people have consumed PUFA, especially n-6 PUFA, from margarine and cooking oils. It has also been reported that n-6 PUFA such as linoleic acid, common in margarine and cooking oils, decreases the level of cholesterol (Calder PC 2001; Holub 2002).

Inflammatory and immune cells have high concentrations of n-6 PUFA and low concentrations of n-3 PUFA. For example the phospholipids in human mononuclear cells are 6-10% linoleic acid and 15-25% arachidonic acid, whereas α-linolenic acid is rare and the proportions of EPA and DHA are low, being 0.1-0.8% and 2-4%, respectively (Calder PC 2001; Yaqoob *et al.* 2000).

In animal studies it has been found that an increase in dietary fish oil has resulted in a decrease in the concentration of arachidonic acid and an increase in the concentration of EPA in phospholipids of immune cells and platelets (Bolton-Smith *et al.* 1988; Calder PC 2001). Similarly in human studies an increase in dietary fish oil has resulted in an elevation of EPA and DHA concentrations in the peripheral blood mononuclear cells (Grimm *et al.* 2002; Yaqoob *et al.* 2000).

2.2.3.4 Eicosanoids, inflammation, and immunity

The fatty acid precusor for eicosanoid synthesis is released from cell membrane phopoholipids, usually by the action of phopholipase A_2 activated in response to particular stimuli (e.g. cytokines, growth factors, oxygen free radicals) (Calder 1997). The membranes of most immune cells such as monocytes, macrophages, and

neutrophils contain a large amount of arachidonic acid. Its main function is as a substrate for the synthesis of bioactive mediators known as eicosanoids e.g. prostaglandins (PG), thromboxanes (TX), leukotrienes (LT), and hydroxyeicosatetraenoic acids (Calder PC 2001). Free arachidonic acid can be acted upon by the enzymes cyclooxygenase or lipoxygenase to produce various types of eicosanoids (Grimm *et al.* 2002). In summary, arachidonic acid can be converted to produce 2-series PG (e.g. PGE_2) and 4-series LT (e.g. LTB_4, LTC_4, LTD_4, LTE_4), whereas EPA can be converted to produce 3-series PG and 5-series LT.

The effects of prostaglandin E_2 (PGE_2) and leukotrieneB_4 (LTB_4) have been investigated extensively. PGE_2 has a number of inflammatory effects including fever, vasodilatation, and increased vascular permeability (Calder PC 2001). It is also immunosuppressive and anti-inflammatory, suppressing the proliferation of lymphocytes and the activity of natural killer cells and inhibiting the production of tumor necrosis factor (TNF-α), interleukin (IL) -1, IL-2 and IL-6, and interferon (IFN-γ). LTB_4 increases vascular permeability and enhances local blood flow. It is also a chemotactic agent for leukocytes, induces release of lysosomal enzyme, enhances generation of species of reactive oxygen, inhibits proliferation of lymphocytes, and promotes the activity of natural killer cells. 4-series LT also regulates the production of TNF- α, IL-1, IL-2, and IFN- γ. However, eicosanoids derived from EPA are often less biologically potent than the analogous eicosanoids synthesized from arachidonic acid. For example LTB_5 is less than 10% as potent as LTB_4 as a chemotactic agent and in promoting the release of lysomal enzymes (Calder PC 2001). The range of biological effects of EPA derived eicosanoids has not been thoroughly studied, but EPA consumption can reduce the production of potent inflammatory mediators from arachidonic acid (Grimm *et al.* 2002).

In both animal and human studies it has been shown that an increase in the amount of fish oil consumed results in a decrease in the amount of arachidonic acid in the membranes of several types of cells in the body, including inflammatory cells such as

monocytes, macrophages, and lymphocytes (Bolton-Smith *et al.* 1988; Calder PC 2001). In other words, consumption of large amounts of fish oil reduces the substrate for the synthesis of eicosanoids from arachidonic acid. In this process both arachidonic acid and EPA compete for action from the same enzyme (Figure 2.2). With an increased consumption of fish oil, this competition therefore inhibits the oxygenation of arachidonic acid by cyclooxygenase. Because of its reduction of mediators derived from arachidonic acid, a high consumption of fish oil has been considered as having anti-inflammatory effects and possibly enhancing the functioning of the immune system (Calder PC 2001; Grimm *et al.* 2002).

2.2.4 Mechanisms proposed to explain the cardiovascular benefits of n-3 PUFA supplementation

Although many epidemiological and randomised controlled trials have found that fish oil offers cardiovascular protection, to date the precise mechanism of this protection is unknown. Four main mechanisms have been proposed.

Firstly, n-3 PUFAs decrease the level of lipids, so clearly this results in less deposition of lipids in atherosclerotic plaques. This has been confirmed by the findings of Bang (1971) and Harris (1996) in plasma lipids. However the GISSI-P study (1999) found a cardiovascular benefit without any clinically important reduction of lipids.

Secondly n-3 PUFA is anti-thrombotic, as Dyerberg *et al.* (1978) proposed. Therefore arteries will be less likely to become occluded (Dyerberg *et al.* 1978). Dyerberg *et al.* studied the effects of arachidonic acid and EPA on the aggregation of platelets in human platelet-rich plasma and found that EPA did not induce aggregation, whereas arachidonic acid did, and EPA inhibited adenosine-5-diphosphate -induced aggregation of platelets.

Thirdly there is evidence that fish oil supplements reduce cardiac arrhythmias, at least in animal studies, including sudden cardiac death models. Investigators ligated the left

main coronary artery and placed an inflatable cuff around the left circumflex artery in dogs, after which arrhythmia was induced while the dogs were running on treadmills (Leaf *et al.* 1998). It was found that only 10 out of 13 dogs infused with n-3 PUFA prior to the exercise test had fatal ventricular arrhythmias, whereas all dogs in the control group had ischaemia-induced ventricular arrhythmias. The same investigators also studied the effect of fish oil on contraction in neonatal rat cardiac myocytes (Leaf *et al.* 1998) and found that n-3 PUFA, especially EPA and DHA, profoundly reduced the rate of contraction of the myocytes without a significant change in the amplitude of the contraction. They proposed that n-3 PUFA supplements elevated the threshold and prolonged refractory periods. Furthermore Siscovick *et al.* (1995) assessed the association between consumption of n-3 PUFA in seafood and risk of cardiac arrest in patients and found that patients consuming 5.5 grams of n-3 PUFA per month had a 50% reduction in the risk of primary cardiac arrest.

Fourthly, as stated above, EPA-derived eicosanoids are far less potent than arachidonic acid-derived eicosanoids, so there tends to be less inflammation in individuals taking n-3 PUFA. As is well known, inflammation is the fundamental pathology of atherogenesis (Ross 1999). This has been supported by a decrease in many inflammatory mediators that are needed for atherogenesis in humans taking fish oil supplements. Endres *et al.* found that fish oil supplements reduced the production of IL-1 and TNF by mononuclear cells (Endres *et al.* 1989; Marx 1988). Another study showed that the platelet-derived growth factor (PDGF) mRNA level was significantly lower in patients taking n-3 PUFA (Baumann *et al.* 1999). Lee *et al.* (1985) found that monocytes and neutrophils from subjects taking fish oil produced lower amounts of leukotriene B_4 than those from subjects in a control group. These impair endothelial cell adhesion and migration of leukocytes, which is very important in the early stage of atherogenesis. Moreover rhesus monkeys fed fish oil had a lower infiltration of macrophages into arterial plaque than a control group (Davies, H.R.1987). In addition von Schacky *et al.* (1999) found plaque regression in patients taking n-3 PUFA for 2 years. N-3 PUFA may therefore have some direct effect on plaque. This has been

supported by Rapp's study (1991), which indicated that n-3 PUFA was incorporated into plaque. 11 patients with severe atherosclerotic plaque consumed fish oil (16.0-21.3 g/day of n-3 PUFA) and 18 patients with the same disease took non-fish oil supplements. It was found that the level of n-3 PUFA in atherosclerotic plaque, especially DHA, was significantly higher in patients taking n-3 PUFA than in those of a control group.

2.2.5 Hypothesis

Although to date the precise mechanism explaining the cardiovascular benefit of n-3 PUFA remains unknown, n-3 PUFA supplements appear to have an effect on atherosclerotic plaque. This has been emphasised by the finding of von Schacky *et al.* (1999) of plaque regression. As is well known, inflammation is the basic pathology of atherosclerosis (Ross 1999). It has been suggested that n-3 PUFA may have benefits for the inflammation involved in atherosclerotic plaques, but the author was not aware of any studies that have been carried out about the effect of n-3 PUFA on atherosclerotic plaque that focus on inflammatory cells or inflammatory markers.

2.2.6 Objectives

The main objective of this study was to determine whether the composition of adipose tissue and plaque n-3 PUFA can be changed by short-term changes in dietary n-3 PUFA. A secondary objective of this stidy was to investigate the pathology of plaque removed from patients in the different treatment groups in order to to determine whether the incorporation of n-3 PUFA into plaque alters the morphology or structure of plaque and signs of inflammation within plaque.

2.3 Design of study and methods

2.3.1 Design of study

This study was designed as a randomised, controlled, double blind study. Initially it was considered desirable to look at the effect of n-3 PUFA supplements on plaques compared with a placebo. Since, however, the intervention capsules rich in n-3 PUFA in

this study also contained n-6 PUFA (Table 2.2), and many studies have reported the cardioprotective benefits of n-6 PUFA (Calder PC 2001), this study was modified to incorporate a third arm (n-6 PUFA) in order to isolate the effect of n-3 PUFA supplements alone. This three-arm trial compared the effects of fish oil capsules rich in n-3 PUFA, sunflower oil capsules rich n-6 PUFA, and placebo capsules with the average UK fatty acid intake. The study was approved by the Southampton and South West Hampshire Research Ethics Committee and supported financially by the Ministry of Agriculture, Fisheries and Food (Grant no. ANO238).

Table 2.2 Fatty acid composition of capsules used.

Fatty acid	Fatty acid (g/100 g total fatty acids)		
	Group A (Capsule A)	Group B (Capsule B)	Group C (Capsule C)
Lauric (12:0)	-	-	0.9
Myristic (14:0)	6.2	1.5	2.1
Palmitic (16:0)	20.4	8.6	34.9
Palmitoleic (16:1n-7)	12.3	1.0	2.0
Stearic (18:0)	5.7	3.5	3.7
Oleic (18:1n-9)	10.1	18.6	33.8
Linoleic (18:2n-6)	2.3	62.8	18.9
α-Linolenic (18:3n-3)	4.6	1.4	1.8
Arachidonic (20:4n-6)	1.1	1.2	-
EPA (20:5n-3)	14.3	-	-
DPA (22:5n-3)	1.5	-	-
DHA (22:6n-3)	8.3	-	-

2.3.2 Participants

Inclusion criteria

All patients who were awaiting elective carotid endarterectomy at Southampton General Hospital in the period December 1997-Febuary 2000 were asked to take part.

Exclusion criteria

Patients were excluded if they

1. Required emergency surgery or a carotid endarterectomy combined with another operation such as a coronary artery bypass graft.
2. Were consuming fish oil, cod liver oil or evening primrose oil capsules.
3. Unwilling to give their written informed consent.

2.3.3 Randomisation and blinding (masking)

To reduce bias that might have distorted results, this study was conducted in a randomised and double blind fashion. To achieve a balance of the numbers in each arm, the technique of block randomisation was applied. Capsules were labelled A, B, or C and the explanations of these codes placed in a thick opaque envelope. At the end of the study, it was disclosed that capsules labeled A contained fish oil, capsules labeled B contained sunflower oil, and capsules labeled C contained the placebo. There were 3 groups in our study: group A taking capsule A, group B taking capsule B, and group C taking capsule C.

All three types of capsules had the same physical appearance. As a result of this masking technique it was impossible for the patients, health personnel, or the investigators of this study to know what the capsules contained while the study was being conducted.

2.3.4 Sample size

The sample size was estimated from the Rapp study (1991), in which the level of EPA in plaque phospholipids increased about ten times after supplements: the EPA level there was 0.2% in control patients and 2.1% after fish oil supplementation (standard deviation 0.4). It was expected that levels would respond as they had in Rapp's study, although daily intakes of n-3 PUFA supplements by participants were about one-tenth of those of patients in the Rapp study (1.7 g/day vs 16-21.3 g/day). On this basis it was

calculated that a sample size of 50 would be required to detect a 1-fold increase in EPA in plaque phospholipid at $p < 0.05$. To allow for a drop-out rate of 20%, 189 patients were recruited into the study.

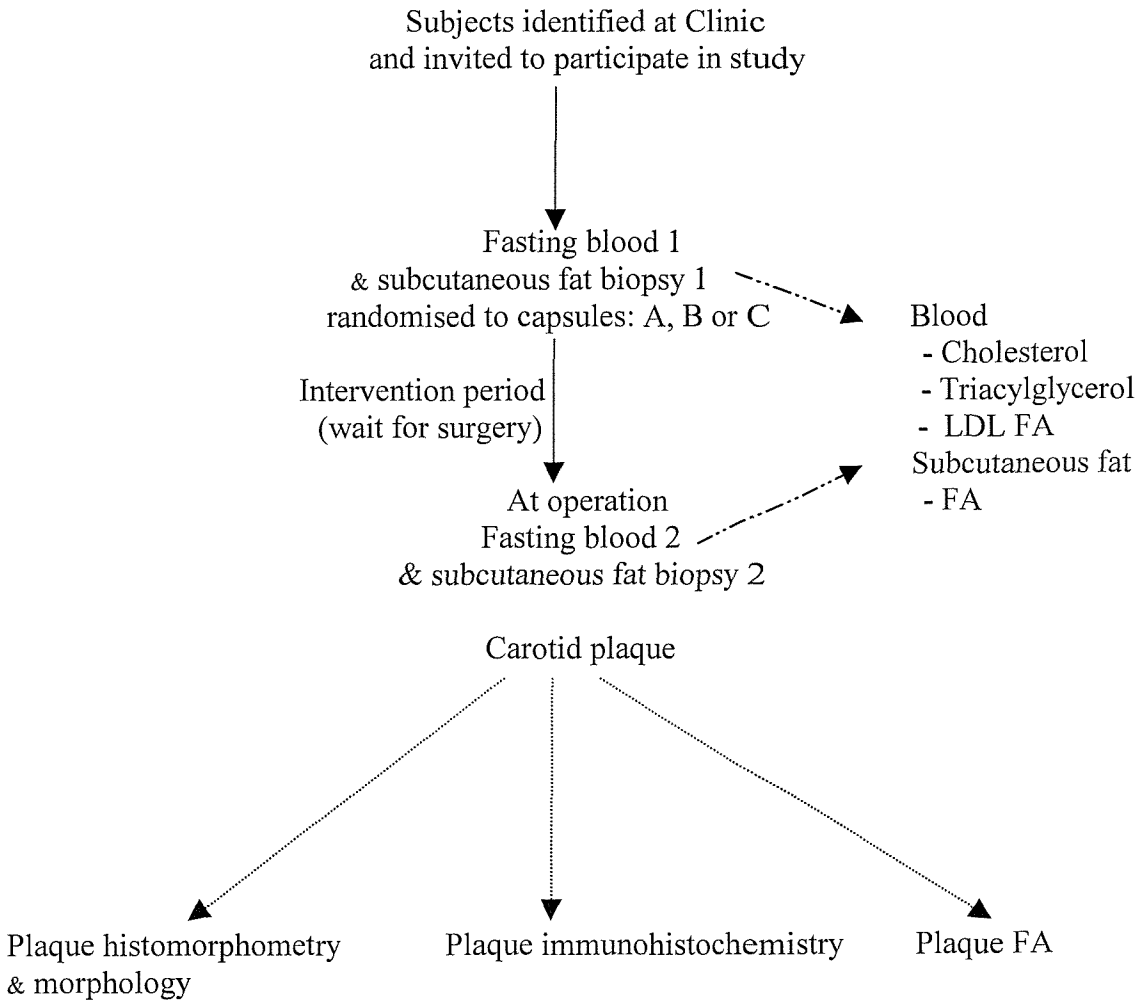
2.3.5 Intervention and composition of capsules

Alterations of the dietary intake of n-3 and n-6 PUFA of a magnitude likely to occur under normal dietary conditions were achieved by supplementing the patients' normal diet with capsules A and B (Table 2.2). Subjects consumed two capsules containing 1 g each of oil three times a day with food, a total of 6 capsules per day. Therefore supplementation of the diet was done with 6 g fish oil (capsule A) or sunflower oil (capsule B) per day. The capsule C consumed by the control group contained the same amount of fat as the test capsules and were an 80: 20 mixture of palm oil and soya bean oil, the fatty acid composition of which was very similar to that of the average UK diet, as shown in Table 2.2, (British Nutrition Foundation.1992). All capsules contained the same levels of vitamin E (300 mg/g oil).

2.3.6 Collection of data and samples

At the beginning of the study, fasting blood samples were taken (Figure 2.3). To obtain subcutaneous adipose tissue a biopsy was done on the abdominal wall. Firstly the skin was infiltrated with 0.5% Xylocaine, after which a GA19 needle attached to a 20 ml syringe was introduced into the subcutaneous tissue. Maximum aspiration was exerted on the subcutaneous fat until a plug of fat had been drawn into the needle. Then the negative pressure was released and the needle removed from the subcutaneous tissue. Lastly the fat collected in needle was stored in 0.5 ml aliquot at –20°C.

Figure 2.3 Study protocol.



Capsule A= Fish oil

Capsule B= Sunflower oil

Capsule C= Placebo

FA = Fatty acid composition

LDL FA=LDL fatty acid composition

Patients were then given a diary to record their diet and supplements and scales to weigh their food. They were asked to record their 7- day weighed food intake in a diary. It was suggested that patients record only 7 consecutive diary of typical day. If patients were going to have a special party it was suggested that they not record that period, but begin their record after the party. They were asked to record and weigh everything they consumed except water, separating the ingredients. For example, for fish and chips the weights of fish and potatoes were recorded, including the approximate amount of oil used. If patients normally went out for dinner, they were asked to record what they ate along with the name and address of the restaurant. For frozen food, they were asked to record what they ate together with the brand name and any nutritional information on the package, such as ingredients and their content. They were also asked to record the amount of food at the beginning of a meal or snack and the amount remaining after they had finished. For fruit with edible skin, patients were asked whether or not they removed the skin. On the whole, patients were asked to record as much detail as possible. Normally researchers checked with patients on the day that they started their diaries to ensure that patients understood everything. Patients were also encouraged to contact rsearchers whenever they had any further questions. See Appendix 1 for a sample of entries in a diary for one day. Patients began consumption of dietary supplementary capsules immediately and continued until the day of their operation. Compliance with the dietary regimens was checked verbally, from diaries, and by reviewing the number of capsules remaining. Carotid plaques were removed surgically from 1 to 27 weeks later, corresponding to current clinical practice, so supplements were taken for various lengths of time in this study, allowing an assessment of the speed of onset of any change. A second set of fasting blood and adipose tissue samples was taken just before plaques were removed surgically.

The following were measured or collected for all patients consuming the three different types of supplements:

A. The daily intake of nutrients.

B. For plasma, total cholesterol level and triacylglycerol concentrations was measured. Fatty acid compositions of LDL in three major types of lipid (cholesterol ester (CE), phospholipid (PL) and triacylglycerol (TAG)) fractions before and after intervention was determined.

C. Composition of adipose tissue fatty acids before and after intervention.

D. Carotid plaque.

- D1 Gross pathological specimens.
- D2 Fatty acid composition in three major types of lipid was measured (CE, PL, TAG).
- D3 Histomorphometry.
- D4 Grading of plaque histology following the criteria of the American Heart Association and its modification.
- D5 Immunohistochemistry.

2.3.7 Laboratory methods

2.3.7.1 Analysis of daily intake of nutrients

After patients had completed their 7-day weighed-nutrient intake diaries, the daily intake of nutrient data was analysed by Dr. B. M. Margetts of the Institute of Human Nutrition, University of Southampton by using a modification of FOODBASE software (Institute of Brain Chemistry, London, UK) with values for nutritional components taken from the database of the Royal Society of Chemistry (McCance *et al.* 1991).

2.3.7.2 Plasma lipids analyses

Plasma total cholesterol and TAG concentrations were determined by Dr. J. M. C. Garry of the Institute of Human Nutrition, University of Southampton by using commercially available colorimetric assays (Sigma Chemical Co., Poole, UK) (Detail in Appendix 2).

2.3.7.3 Fatty acid composition analyses

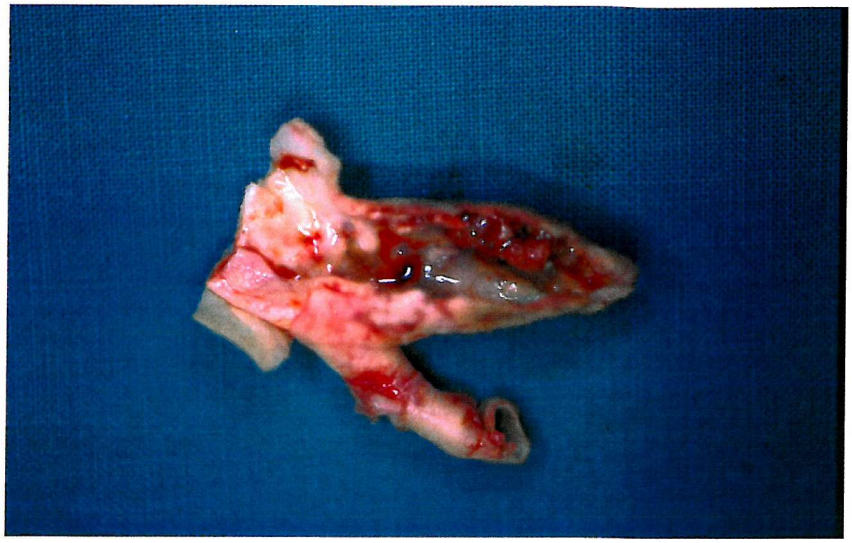
The fatty acid compositions of the PL, CE and TAG fractions were determined in plasma LDL (LDL PL, LDL CE, LDL TAG), adipose tissue and the frozen section close to the bifurcation of the carotid plaque. Total lipid was extracted, lipid fractions separated by thin layer chromatography and the fatty acid composition of each fraction determined by gas chromatography as in Appendix 3.

2.3.7.4. Plaque studies

2.3.7.4.1 Carotid plaque preparation

After specimens were obtained (Figure 2.4), carotid plaques were cleaned with saline, and the lengths of the carotid plaques were measured from the common carotid artery to the internal carotid artery. Plaques from the external carotid artery segment were removed, after which they were examined for evidence of haemorrhage and rupture. Lastly all plaques were photographed. Serial transverse 2 mm sections were taken. (Figure 2.5). These sections were then labeled alphabetically starting from the distal end of the internal carotid artery plaque and ending at the common carotid artery plaque. Alternate sections of the plaque were used for plaque histology (stored in formalin solution) and biochemical analysis (snap frozen in liquid nitrogen and stored at -70°C). The section closest to the bifurcation was embedded in paraffin (OCT compound, Agar Scientific Ltd) and frozen (-70°C) for later sectioning by a cryostat for immunohistochemistry (Figure 2.5).

Figure 2.4 Carotid plaque specimen.



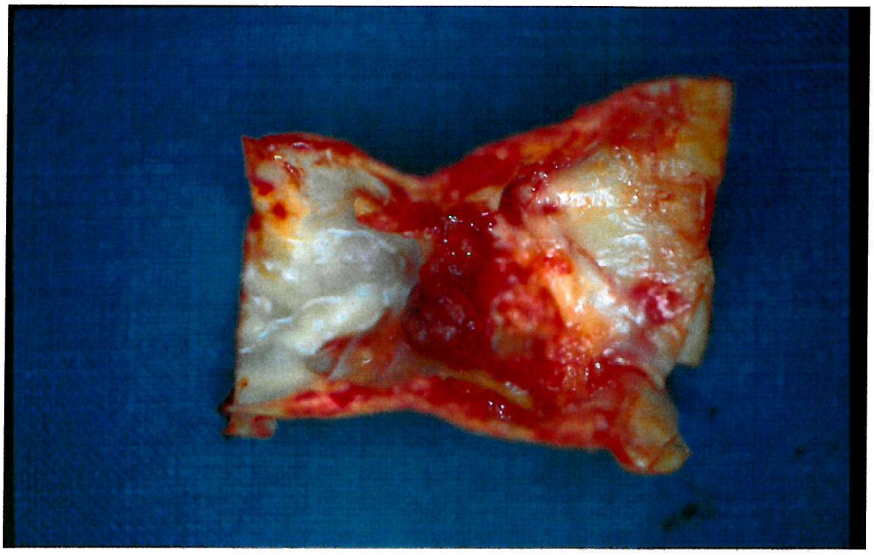
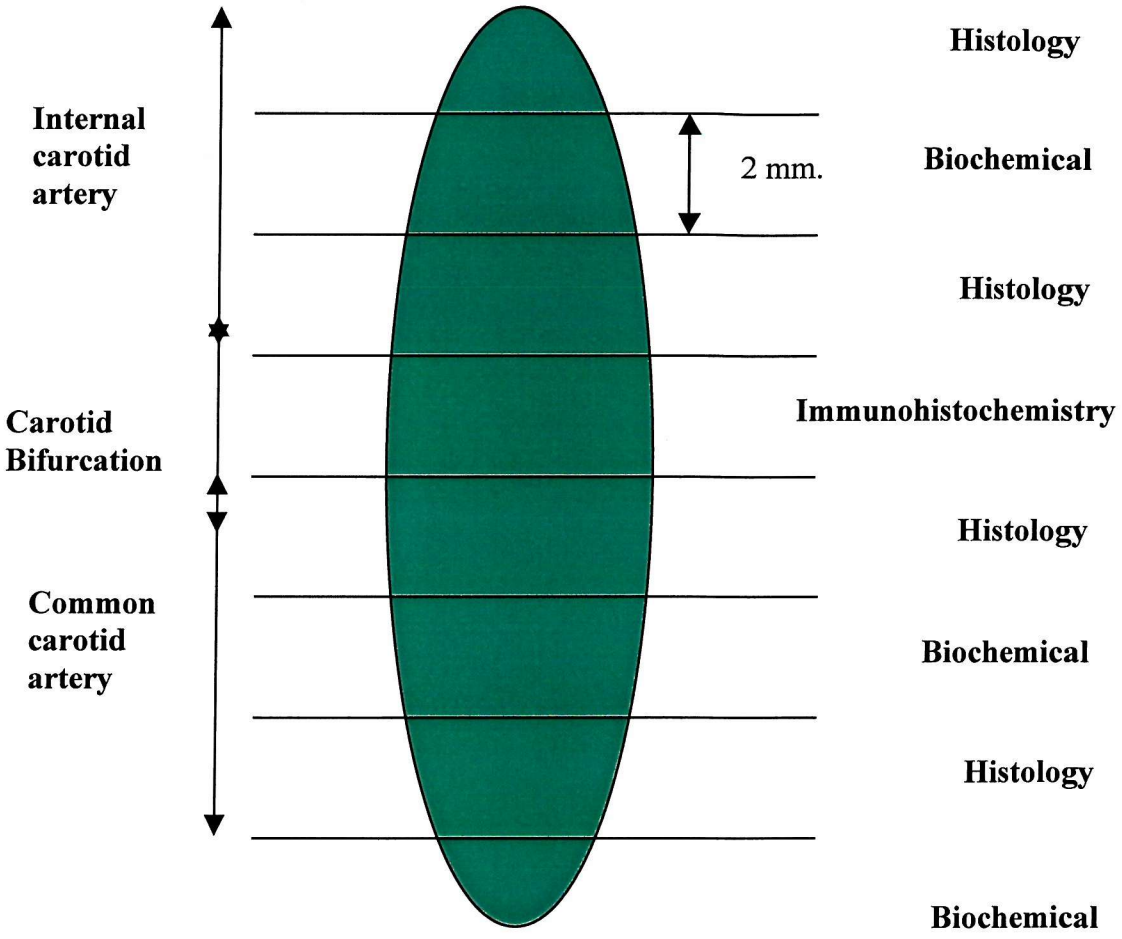


Figure 2.5 The preparation of carotid plaques for histological and biochemical studies.



2.3.7.4.3 Histological analysis of carotid plaques

2.3.7.4.3.1 Histomorphometry

A section was cut from each block and stained with haematoxylin and eosin (H & E). A method was used that was developed at Southampton for a previous European Union study of carotid atherosclerosis (European Carotid Plaque Study Group 1995). The amount of fibrous intimal thickening, lipid, haemorrhage, and calcification (Figure 1.3 - 1.4 & Figure 2.6a - c) were measured volumetrically with a standard light microscope that had a graticule, which imposed a grid of many equally-sized squares in the eyepiece (Figure 2.6d). For each histological section the areas of the following were estimated:

A) Fibrous intimal thickening.
B) Intramural haemorrhage.
C) Calcification.
D) Lymphocytes.
E) Foam cells/ macrophages.
F) Lipid deposits / cholesterol clefts.
G) Old haemorrhage.
H) Small blood vessels (neovascularisation).
I) Soft lipids.

This was done by identifying and recording in each field of view the type of tissue at the 36 graticule points (intersections of two graticule lines) (Figure 2.6d). The field of view was shifted carefully to ensure that no areas of the section were missed or counted more than once. Between 6 and 20 fields were examined in order to cover the surface area of each section. After analysis of each section a data sheet was prepared. The total number of graticule points and the number for each tissue type were recorded. Then the percentage of each constituent was calculated by dividing the graticule points for that type of tissue by the total number.

Figure 2.6a Microscopic image of fibrous cap (thin arrow) and amorphous lipid (thick arrow) in a carotid plaque.

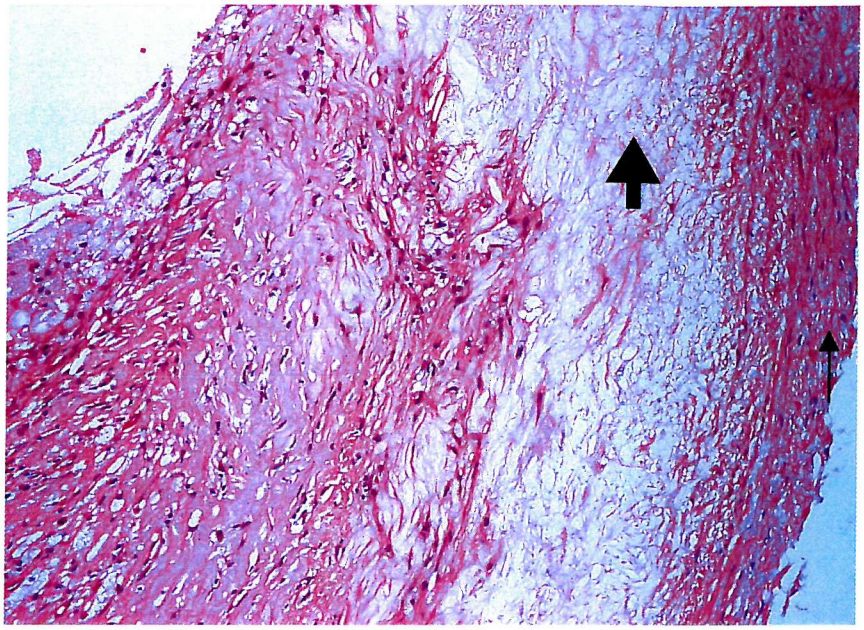


Figure 2.6b Microscopic image of crystalline lipid (lipid cleft) in a carotid plaque.

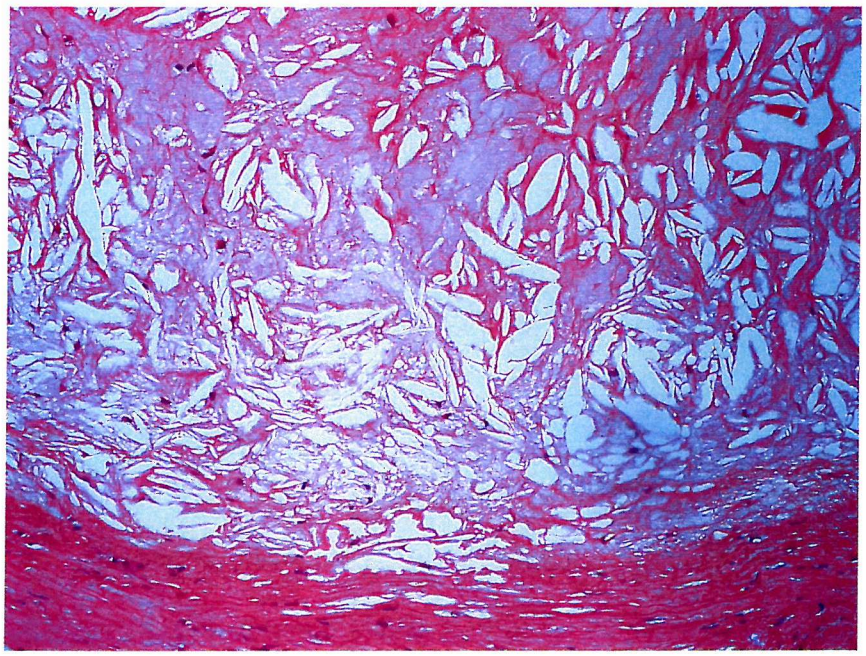


Figure 2.6c Microscopic image of calcification in a carotid plaque.

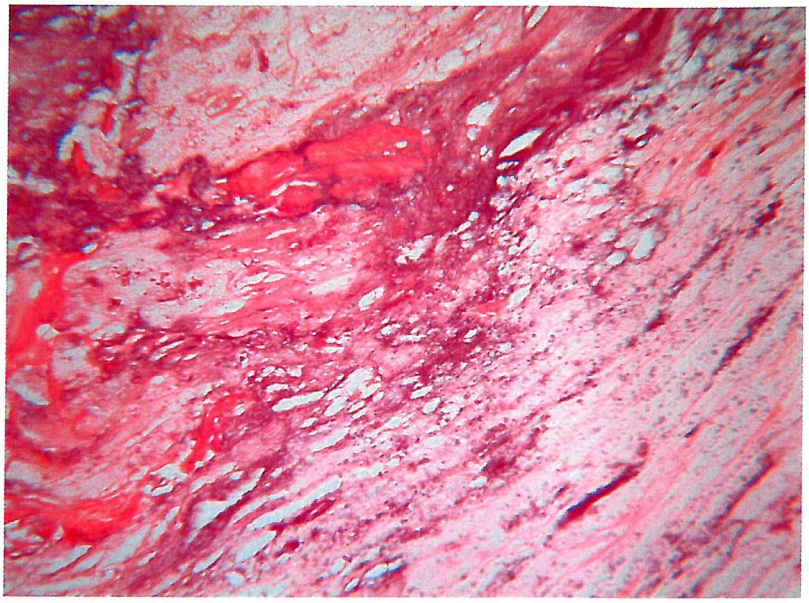
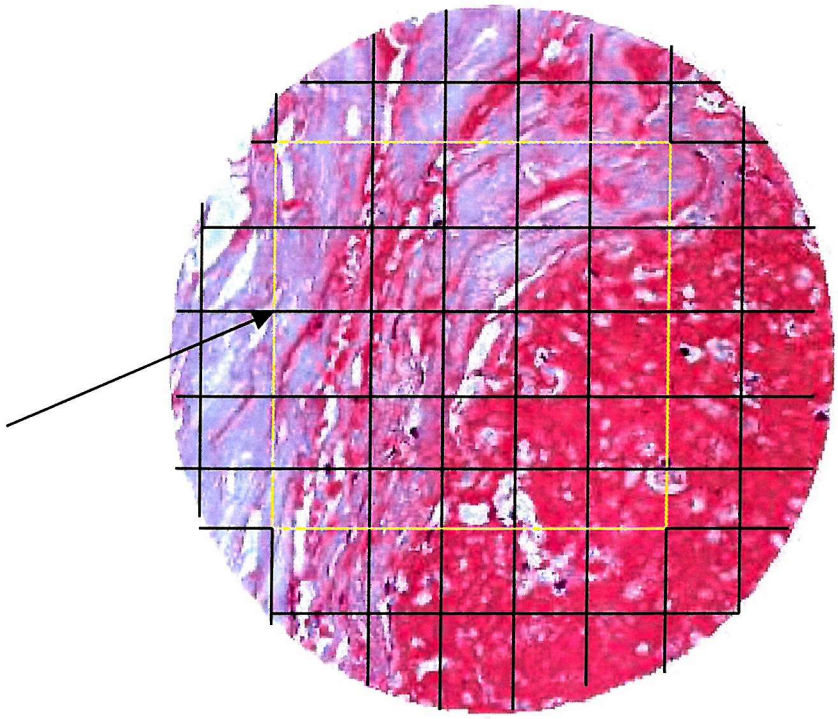


Figure 2.6d Microscopic field with graticule in eyepiece. Researchers identified and recorded the type of tissue or substance at the 36 intersections of the graticule lines inside the yellow border. Amorphous lipid (arrow) at the one intersection.



Initially a small pilot study was done on 17 plaques to determine whether there were any differences between sections by using a paired t-test. None were found in major components between the bifurcation and the rest of the plaques (Table 2.3). Therefore the bifurcation sections were used to compare components for all three arms of the study. The histomorphometry was done by Dr. P. J. Gallagher of the Department of Chemical Pathology Southampton General Hospital and Mr. T. Chan, Mr S. Boyles Department of Vascular Surgery and the author.

Table 2.3 Histomorphometry of bifurcation sections and remaining bifurcation sections from 17 carotid plaques (under 40 x power of magnification).

Component	Mean percentage (± SE)		P-Value*
	Bifurcation Section	Remaining Section	
Fibrous	58.0 ± 5.2	57.3 ± 4.2	0.83
Amorphous lipid	8.3 ± 2.5	8.6 ± 2.1	0.84
Macrophage	0.5 ± 0.2	0.5 ± 0.2	0.92
Haemorrhage	4.7 ± 1.8	2.8 ± 0.9	0.24
Calcification	6.13 ± 2.3	2.6 ± 0.7	0.10
Lymphocyte	1.8 ± 0.7	2.1 ± 0.5	0.75
Crystalline lipid	10.4 ± 3.2	7.4 ± 1.5	0.23
Old haemorrhage	0.9 ± 0.6	0.2 ± 0.1	0.28
New blood vessels	0.44 ± 0.2	0.41 ± 0.2	0.91

Data are presented as mean ± SE * The data were analysed with a paired sample t-test.

2.3.7.4.3.2 Scoring using criteria of the American Heart Association (AHA)

The plaque histology (H & E sections) was also scored using criteria of the AHA. (Figure 2.7a-d) (Stary *et al.* 1995). Since AHA score is difficult to use because of a long list of Roman numerals and limitations in the pattern of plaque progression, a modification of the AHA score was also used (Virmani *et al.* 2000). Stained sections were viewed with a microscope under 10 x magnification in random order and grading was performed blindly by Dr. P.J. Gallagher.

Figure 2.7a AHA type 4: fibrous cap atheroma.

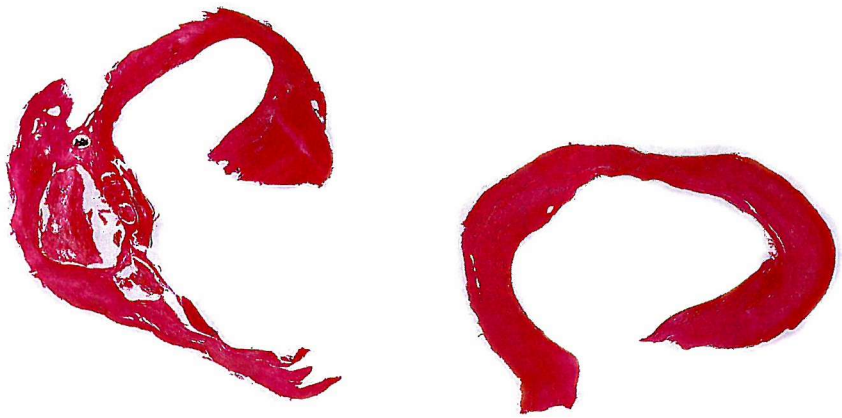


Figure 2.7b AHA type 4: fibrous cap atheroma.

Figure 2.7c AHA type5a: thin fibrous cap atheroma (arrow).

Figure 2.7d AHA type 6: plaque rupture.

2.3.7.4.4 Immunohistochemistry of carotid plaques

The plaque section from the bifurcation was used for immunohistochemistry (Figure 2.5). Cryostat sections of frozen plaque were mounted on organosilan-coated microscope slides (see Appendix 4 for more details). Endogenous peroxidase activity was blocked and then the sections were successively incubated with optimal dilutions of the different anti-human antibodies (Table 2.4), biotinylated goat anti-mouse (except swine anti-goat for staining of vascular cell adhesion molecule-1; VCAM-1), immunoglobulin G (DAKO, Ely, UK), and streptavadin-horseradish peroxidase (DAKO, Ely, UK). Finally, peroxidase activity was visualised using hyrogen peroxide as a substrate and 3-amino-9-ethyl carbazole (Sigma Chemical Co., Poole, UK) as a chromogen. Stained sections were fixed using formalin, counterstained with Harris hematoxylin, and viewed with a microscope under 10 x magnification. Primary antibodies used were mouse anti-human CD3 (Leu 4; Becton Dickinson, Oxford, UK), mouse anti-human CD68 (KP1; DAKO, Ely, UK), mouse anti-human intercellular adhesion miolecule-1 (ICAM-1; R & D Systems, Oxford, UK), and goat anti-human VCAM-1 (R & D Systems, Oxford, UK). Staining was graded 0 (0-10 stained cells per section), 1 (moderate staining: 11-50 stained cells per section) or 2 (heavy staining: > 50 stained cells per section) (Figure 2.8a - d). It is interesting to note that although macrophages and lymphocytes can be seen in histological examination of plaque (Figure 2.8e, right), it is difficult to count/ identify them without an immunohistochemical technique (Figure 2.8e, left). Samples were prepared by Dr. F. Thies and viewed in random order and grading was performed blindly by Dr. P. J. Gallagher.

Table 2.4 Description of primary and secondary antibodies used.

Antigen	Primary Antibody	Source of Antibody	Dilution used	Secondary Antibody	Source (dilution)
T-cell (CD3)	Mouse Anti-human, monoclonal Leu-4	BD (347340)	1/20	Goat anti-mouse (biotin)	DAKO (1/200)
VCAM-1 (CD106)	Goat Anti-human, polyclonal	R&D Systems (BBA 19)	1/500	Swine anti-goat (biotin)	DAKO (1/100)
ICAM-1 (CD54)	Mouse Anti-human, monoclonal	R&D Systems (BBA3)	1/500	Goat anti-mouse (biotin)	DAKO (1/200)
Macrophages (CD68)	Monoclonal Mouse, Anti-human KP1	DAKO (M 0814)	1/100	Goat anti-mouse (biotin)	DAKO (1/200)

Figure 2.8a Microscopic image of stained (CD68) macrophage on a carotid plaque.

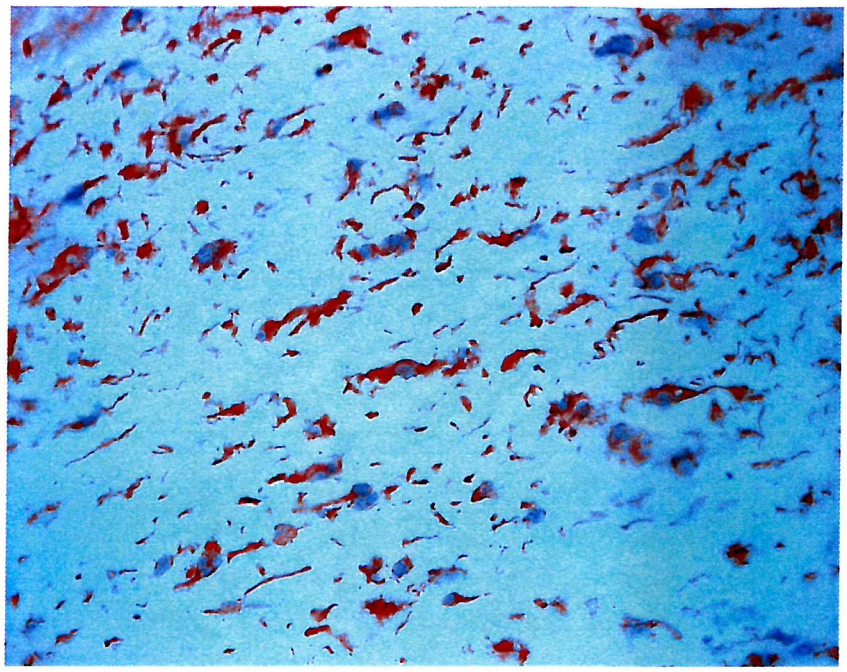


Figure 2.8b Microscopic image of stained (CD3) T-lymphocyte on a carotid plaque.

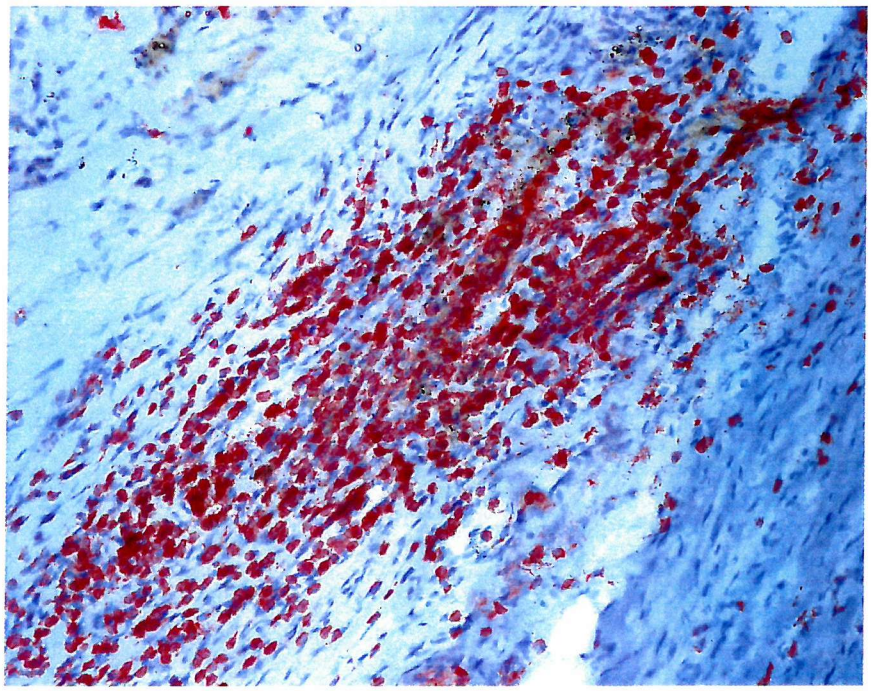


Figure 2.8c Microscopic image of stained ICAM-1 in a carotid plaque (arrow).

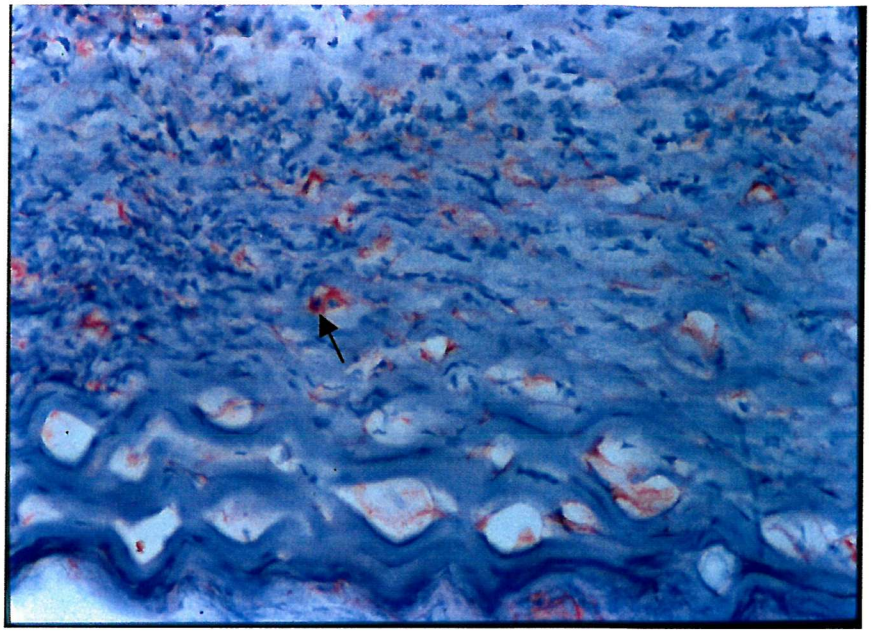


Figure 2.8d Microscopic image of stained VCAM-1 in a carotid plaque.

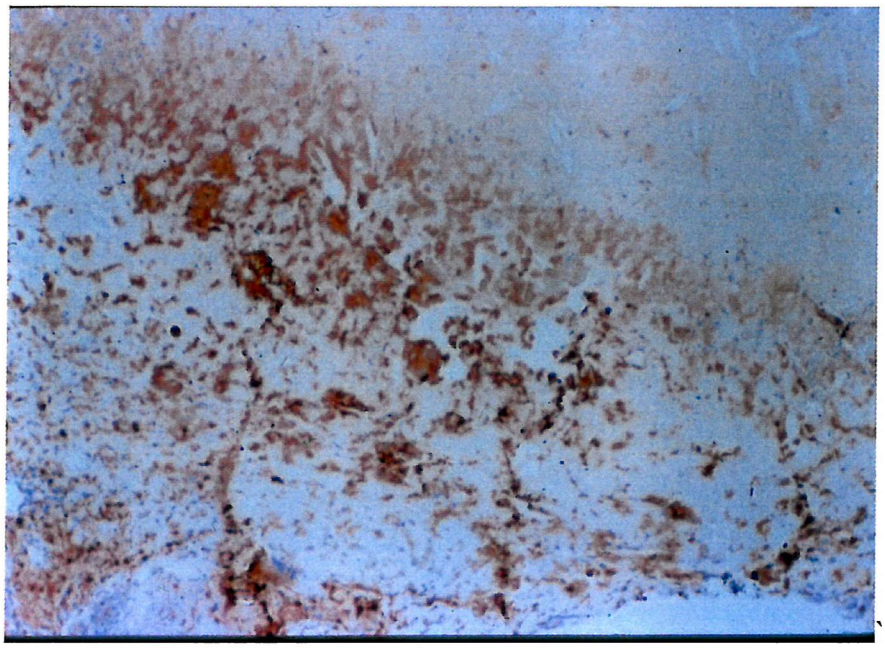
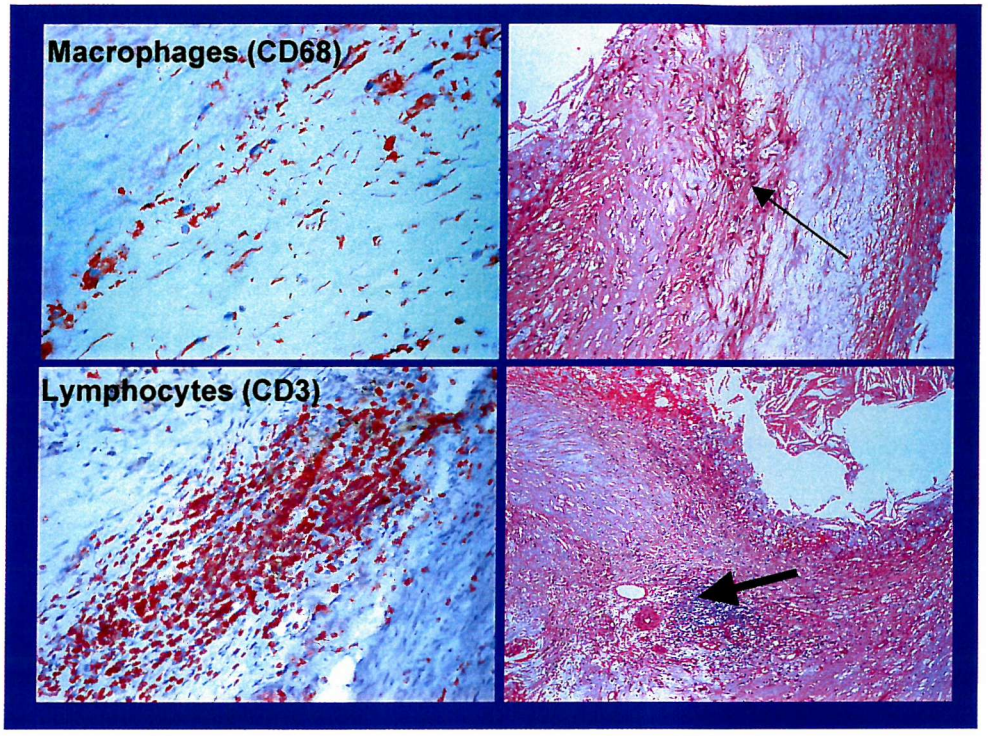


Figure 2.8e Microscopic images of macrophages and lymphocytes using immunohistochemistry (left) and plaque histology (right). The CD-68 macrophages and CD3 T-lymphocytes are stained in red (left). Plaque histology (right) shows the macrophages (thin arrow) and lymphocytes (thick arrow).



2.3.8 Statistical analysis

Data are shown only for patients who completed the study (at least 1 weeks of supplementation). Effects of the supplements on the concentrations of blood lipids and the compositions of fatty acids of LDL lipid fractions, adipose tissue, and carotid plaques, and histomorphometry of carotid plaque were determined by two-way ANOVA. If the ANOVA test showed any significant associations across the three groups, then a Student's t-test was used with a Bonferroni correction. For histomorphometry, the percentage of each histological plaque component was derived from the number of each plaque component with all total plaque component (section 2.3.7.4.3.1). The comparisons of bifurcation sections with other areas were conducted with paired t- tests (Table 2.3), as were comparisons before and after intervention of the plasma lipid and the fatty acid composition in plasma LDL and adipose tissue. The mean ranks of plaque histology (AHA criteria and its modified criteria) and plaque immunohistochemistry were compared by using the Jonckheere-Terpstra test.

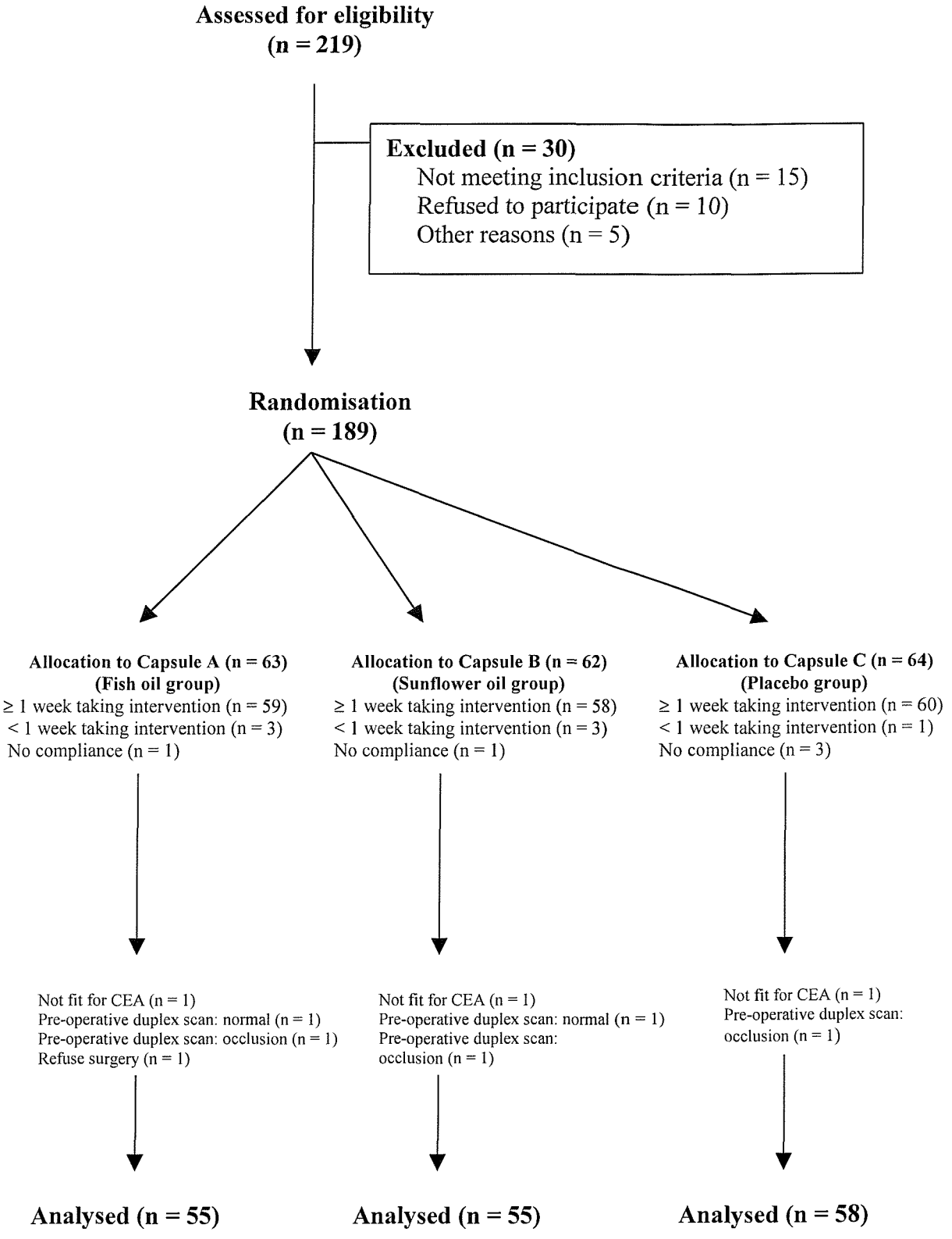
The degree of inter-observer agreement for plaque histology and immunohistochemistry was assessed using simple proportions. Inter- and intra-assay coefficients of variation were determined in the analyses of plasma lipid and fatty acid composition. Analyses were done with SPSS version 10 (SPSS, Chicago, IL USA) and in all cases a value of $p < 0.05$ was taken to indicate a statistically significant difference.

2.4 Results

2.4.1 Recruitment of patients and randomisation

219 patients (Figure 2.9) were initially considered eligible during the study period, 30 patients were excluded, most because they were going to have carotid surgery combined with coronary artery bypass grafts or they refused to participate in the study, the rest because their homes on the island of Guernsey were too far from the study centre. This left 189 patients to be randomised into groups taking one of the three types of capsules. Seven of these were excluded because they took capsules for less than 7 days before surgery. Another five were excluded because of poor compliance: four patients stopped taking capsules because of diarrhoea and one patient had leg pain. Three more patients did not proceed to surgery because of newly diagnosed coronary problems. Five more patients were excluded from the study because duplex scans repeated just before surgery indicated there was no longer a need for carotid endarterectomy. Three had total occlusion of the internal carotid artery and two had normal duplex scans, although previous scans had suggested disease. Another patient was excluded because she refused to have carotid surgery on the day it had been scheduled. After all these exclusions, a total of 168 patients were left for analysis: 55 in group A, 55 in group B, and 58 in group C. It is interesting to note that eight patients (four patients in group A, two patients in group B, and two patients in group C) had supplementation for more than 3 months. The main reasons were long waiting lists, postponement of the date for surgery (especially because of a lack of beds including intensive care beds), and uncertainty about results of carotid stenosis investigations. Two patients had supplementation for about 6 months; surgery for one of these was postponed twice because of illness (cold) and lack of a bed. All 168 patients took more than 80% of the total number capsules supplied from the date of their first visit to the date of their operation, and they took them for at least seven consecutive days before their operation.

Figure 2.9 Flow diagram of the subject progress through the phase of a randomised trial.



2.4.2 Baseline clinical data

Baseline clinical data did not differ for the three groups (Table 2.5), the majority of members of which were male. The median age of patients was about 70. 46% of patients were overweight (BMI 25-30 kg/m^2), while a further 13% were obese (BMI > 30 kg/m^2). The distribution of patients of desirable weight (BMI < 25 kg/m^2), overweight patients, and obese patients was the same in all three-treatment groups. 21 patients were currently smoking and 54 patients (32.1%) had total cholesterol levels greater than 5 mmol/L. Medication was evenly matched among the three groups (Table 2.6). All patients took aspirin, 64.4% taking 75 mg/day and 29.3% taking 150 mg/day.

Table 2.5 Patients Characteristics.

	Group A N=55	**Group B N=55**	**Group C N=58**
Males (%)	35 (63.6)	34 (61.8)	37 (63.8)
Females (%)	20 (36.4)	21 (38.2)	21 (36.2)
Mean age ± SE	69(52-84)	69(44-83)	70(38-85)
Body mass index (kg/m^2) (range)	25.9(17.1-42.6)	25.9(20.1-34.1)	26.4(19.1-37.1)
Hypertension (%)	35 (63.6)	33 (60.0)	44 (75.9)
Diabetes mellitus (%)	11 (20.0)	10 (18.2)	10 (17.2)
Smoking			
- **Current (%)**	5 (9.1)	9 (16.4)	7 (12.1)
- **Ex-smoking (%)**	40 (72.7)	39 (70.9)	42 (72.4)
Severe ipsilateral ICA stenosis (%)	47 (86.0)	44 (80.0)	49 (85.2)
Severe contralateral ICA occlusion (%)	7 (12.7)	11 (19.6)	9 (14.8)
Plasma creatinine level median (range) (mg/dl)	92 (61-195)	92 (64-174)	100 (60-218)
Plasma total cholesterol (mean ± SE)	4.8 ± 0.2	4.7 ± 0.1	4.8 ±0.2
Plasma triacylglycerols (mean ± SE)	1.7 ± 0.2	1.8 ± 0.2	1.7 ± 0.1

Hypertension = medicated or systolic pressure > 160mmHg, Diabetes = controlled diet, oral hypoglycaemic medication or insulin-dependent, ICA = internal carotid artery.

Table 2.6 Use of medication use among patients.

	Group A N=55	Group B N=55	Group C N=58
Length of oil treatment median (range) (days)	53 (7-173)	52 (9-170)	44 (8-189)
Aspirin	55 (100)	55 (100)	58 (100)
β-blocker	12 (21.8)	14 (25.5)	13 (22.4)
ACE inhibitor	9 (16.4)	9 (16.4)	16 (27.6)
Nitrates	11 (20.0)	10 (18.2)	9 (15.5)
Calcium channel blockers	20 (36.4)	15 (27.3)	23 (39.7)
Fibrates	3 (5.5)	1 (1.8)	1 (1.7)
Statins	18 (32.7)	19 (34.6)	21 (36.2)
Insulin	5 (9.1)	1 (1.8)	1 (1.7)
Oral hypoglycaemic drug	6 (10.9)	6 (10.9)	9 (15.5)

Data of medication are presented as number of patients (percentage).

2.4.3 Neurological symptoms and associated cardiovascular disease

Indications for surgery and associated cardiovascular disease were evenly distributed in the 3 groups (Table 2.7). For a majority of patients indications for surgery were recent neurological symptoms, especially transient ischaemic attack. Half of the patients in each group had angina or myocardial infarction.

Table 2.7 Symptoms and associated vascular diseases.

	Group A N=55	Group B N=55	Group C N=58
Indication for surgery			
Symptomatic			
- **Last symptom < 6 months**			
CVA	5 (9.1)	7 (12.7)	7 (12.1)
Amaurosix fugax	9 (16.4)	5 (9.1)	10 (17.2)
TIA	18 (32.7)	21 (38.2)	18 (31.0)
- **Last symptom > 6 months**			
CVA	9 (16.4)	6 (10.9)	7 (12.1)
Amaurosix fugax	1 (1.8)	2 (3.6)	2 (3.5)
TIA	6 (10.9)	9 (16.4)	8 (13.8)
Asymptomatic cases	7 (12.7)	5 (9.1)	6 (10.3)
Associated cardiovascular diseases			
Previous CEA	8 (14.3)	4 (6.9)	6 (9.8)
Abdominal aortic aneurysm	1 (1.8)	3 (5.5)	4 (6.9)
Lower limb ischaemia	12 (22.0)	20 (36.0)	14 (23.6)
Ischaemic heart disease			
Angina pectoralis	18 (32.7)	17 (30.9)	21 (36.2)
Previous MI	11 (20.0)	9 (16.4)	11 (19.0)
Previous CABG	4 (7.3)	4 (7.3)	12 (20.7)

Data are present as number of patients (percentage). Stroke = neurological deficit > 24 hours, TIA (transient ischaemic attack) = neurological deficit < 24 hours, Patients were defined as asymptomatic if they had never had any experience of neurological deficit, either a transient ischaemic attack (TIA) or stroke related to the affected vessel, CEA = carotid endarterectomy, CABG = coronary artery bypass grafting.

2.4.4 Intake of fatty acids

Calculated from food diaries, patients in group A had habitual intakes of EPA and DPA of 0.08 and 0.10 g/day respectively, so capsule A intervention (EPA 0.86 g/day) increased the intake of EPA by more than 10-fold. Patients in group B had a mean habitual intake of linoleic acid of 9.2 g/d (Table 2.8), so capsules B (3.8 g/day) increased the intake of linoleic acid by 41%.

Table 2.8 Habitual fatty acid intake.

Fatty acid (g/day)	Group A	Group B	Group C
Linoleic acid	8.1 ± 0.7	**9.2 ± 0.8**	8.5 ± 0.5
Arachidoic acid	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.1
α-Linolenic acid	0.98 ± 0.08	0.82 ± 0.05	0.91 ± 0.08
EPA	**0.08 ± 0.01**	0.11 ± 0.01	0.11 ± 0.02
DHA	**0.10 ± 0.02**	0.13 ± 0.02	0.15 ± 0.03

Data are present as mean ± SE.

2.4.5 Details and outcomes of surgery

Most patients were administered general anaesthesia, and conventional procedures were used (Table 2.9). The 30-day combined post-operative stroke and death rate was 5.4% This figure was based on the assessment of the surgical team. Two patients died in the post-operative period, one from stroke and the other from myocardial infarction. Both patients had the operation because of pre-operative stroke. Eight patients suffered from post-operative stroke, three of which had disabling strokes (Rankin score > 3) (de Haan 1995). Of these eight patients with post-operative stroke, four required carotid endarterectomy because of a history of preoperative stroke, one because of transient ischaemic attacks, and one because of amaurosis fugax. The remaining two patients were asymptomatic.

Table 2.9 Details of carotid surgery and complication rates.

	Group A N=55	**Group B N=55**	**Group C N=58**
Eversion CEA (%)	**9 (17.0)**	**14 (24.6)**	**11 (18.3)**
Shunt insertion (%)	29 (52.1)	26 (47.2)	30 (50.9)
Primary closure of arteriotomy wound	30 (54.9)	29 (52.7)	33 (56.7)
CEA under local anesthesia (%)	**15 (26.4)**	**13 (24.1)**	**15 (25.0)**
Days in hospital after CEA, median (range)	4 (2-25)	4 (2-15)	4 (1-14)
Cranial nerve injury (%)	7 (12.0)	6 (11.1)	4 (7.3)
Post-operative angina pectoralis or MI (%)	1 (1.8)	0 (0.0)	4 (6.8)
Stroke and death (%)	**3 (5.5)**	**2 (3.6)**	**4 (6.9)**

Data are present as number of patients (percentage).

2.4.6 Concentration of plasma lipids

The levels of plasma total cholesterol decreased significantly in all three groups after intervention (Table 2.10). The decrease of triacylglycerol was much more pronounced in group A than in group C. However no difference in the change of triacylglycerol was found between group B and group C.

Table 2.10 Plasma total cholesterol and triacylglycerol concentrations of patients in the different treatment groups before and after supplementation.

		Group A	Group B	Group C
TC	**V1**	4.8 ± 0.1	4.7 ±0.2	4.8 ± 0.2
	V2	**4.1 ± 0.1***	**4.1 ± 0.1***	**4.4 ± 0.2***
	V2-V1	-0.71 ± 0.14	-0.59 ± 0.12	-0.40 ± 0.10
TAG	**V1**	1.7 ± 0.2	1.7 ± 0.1	1.7 ± 0.1
	V2	1.2 ± 0.1*	1.5 ± 0.1	1.5 ± 0.1*
	V2-V1	**-0.43 ± 0.18[a]**	-0.25 ± 0.09	**-0.18 ± 0.08[b]**

TC = plasma total cholesterol, TAG = triacylglycerol. All values mean ± SE mmol/L before (V1) and after (V2) supplementation. [Superscripts]: Notice the pronounced difference in significance between superscripts a and b. (ANOVA test). [*]: The starred value differs significantly (paired t-test) from V1 value for the same group.

2.4.7 Composition of fatty acids in adipose tissue

The baseline composition of fatty acids in adipose tissue did not differ in the three groups (Table 2.11). In group A the levels of EPA (20:5 n-3) and DHA (22:6 n-3) after intervention increased significantly ($p < 0.001$ and 0.006 respectively) compared to their values before supplementation. The concentrations of EPA and DHA after supplementation in group A were significantly higher than of those of the control group.

Table 2.11 Effect of dietary supplementation on the fatty acid composition of adipose tissue.

	Fatty acid (g/100 g total fatty acids)					
	Group A		Group B		Group C	
	Before	After	Before	After	Before	After
14:0	4.0 ± 0.2	4.0 ± 0.2	4.1 ± 0.2	3.8 ± 0.1	4.0 ± 0.2	3.8 ± 0.2
16:0	25.3 ± 0.4	24.5 ± 0.5	25.7 ± 0.6	24.2 ± 0.4	24.9 ± 0.5	23.8 ± 0.2
16:1 n-7	6.0 ± 0.3	5.3 ± 0.3	6.4 ± 0.4	5.6 ± 0.3	6.6 ± 0.4	6.0 ± 0.4
18:0	3.8 ± 0.2	3.6 ± 0.2	3.8 ± 0.1	3.8 ± 0.2	3.6 ± 0.2	3.5 ± 0.2
18:1 n-9	43.9 ± 0.5	43.9 ± 0.4	43.5 ± 0.6	43.9 ± 0.4	43.6 ± 0.5	43.6 ± 0.4
18:2 n-6	13.6 ± 0.5	13.4 ± 0.5	13.5 ± 0.5	14.0 ± 0.6	14.2 ± 0.6	14.1 ± 0.6
18:3 n-3	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	0.9 ± 0.1
20:1 n-9	0.7 ± 0.1	0.5 ± 0.1	0.9 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
20:4 n-6	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
20:5 n-3	**0.08 ± 0.03**	**0.14 ± 0.02**^{a*}	0.06 ± 0.01	0.12 ± 0.03	0.09 ± 0.02	**0.06 ± 0.01**^{b}
22:6 n-3	**0.20 ± 0.02**	**0.29 ± 0.03**^{a*}	0.24 ± 0.05	0.24 ± 0.03	0.20 ± 0.02	**0.20 ± 0.02**^{b}

Post-treatment values across a row indicated by different superscript letters are significantly different from one another (ANOVA test). Value indicated by * are significantly different from before treatment (paired t- test)

2.4.8 Composition of fatty acids in plasma LDL

The baseline fatty acid composition of LDL in the PL, CE and TAG fractions did not differ in the three groups (Table 2.12). The fatty acid composition of plasma LDL changed considerably in group A. In this group, the levels of EPA (20:5 n-3) and DHA (22:6 n-3) of LDL increased by factors of three and two respectively in the PL, CE, and TAG fractions. Furthermore the levels of EPA and DHA after supplements were significantly higher than the corresponding ones for group B and group C. Moreover the level of DPA (22:5 n-3) in group A increased remarkably in the LDL TAG fraction compared to the other two groups.

The levels of linoleic acid (18:2 n-6) were markedly reduced in the LDL PL and LDL CE fractions in group A after intervention (Table 2.12). These levels after intervention were also significantly lower than those in the other two groups in the LDL PL fraction. In the LDL PL fraction, the level of dihomo-γ linoleic acid (20:3 n-6) also decreased remarkably in for group A, as did the level of arachidonic acid (20:4 n-6), but the difference of arachidonic acid before and after intervention was of marginal significance ($p = 0.054$). In group B the level of linoleic acid (18:2 n-6) increased significantly for LDL PL and LDL CE fractions after intervention. On the whole patients who took fish oil had significantly increased levels of EPA (20:5 n-3) and DHA (22:6 n-3) in plasma LDL fraction with reductions in levels of linoleic acid (18:2 n-6).

Table 2.12 Effect of dietary oil supplementation on the fatty acid composition of LDL lipid fractions.

	Fatty acid (g/100 g total fatty acids)					
	Group A		Group B		Group C	
	Before	After	Before	After	Before	After
PL 18:1n-9	11.7 ± 0.3	10.6 ± 0.2	11.6 ± 0.3	11.0 ± 0.3	11.4 ± 0.3	11.2 ± 0.2
18:2n-6	20.4 ± 0.4	17.7 ± 0.4^{b*}	19.9 ± 0.4	20.8 ± 0.5^{a}	19.5 ± 0.5	20.1 ± 0.4^{a}
20:3n-6	3.4 ± 0.1	**2.3 ± 0.1^{b*}**	3.2 ± 0.1	3.3 ± 0.1^{a}	3.4 ± 0.1	3.4 ± 0.1^{a}
20:4n-6	8.7 ± 0.3	**7.8 ± 0.2**	8.3 ± 0.3	8.6 ± 0.3	8.8 ± 0.3	8.7 ± 0.3
20:5n-3	1.3 ± 0.2	**3.7 ± 0.2^{b*}**	1.3 ± 0.1	1.1 ± 0.1^{a}	1.0 ± 0.1	1.0 ± 0.1^{a}
22:5n-3	1.9 ± 0.1	**2.1 ± 0.1**	2.0 ± 0.2	1.7 ± 0.1	1.8 ± 0.1	1.7 ± 0.1
22:6n-3	3.4 ± 0.2	**6.0 ± 0.2^{b*}**	3.7 ± 0.2	3.7 ± 0.2^{a}	3.3 ± 0.2	3.6 ± 0.2^{a}
CE 18:1n-9	19.2 ± 0.4	20.0 ± 1.2	19.4 ± 0.4	18.0 ± 0.5	19.4 ± 0.8	18.6 ± 0.4
18:2n-6	49.4 ± 0.7	46.7 ± 0.8^{b*}	48.3 ± 0.9	51.3 ± 0.9^{a*}	49.2 ± 1.5	48.7 ± 1.3
20:4n-6	6.7 ± 0.3	5.9 ± 0.3	7.0 ± 0.3	6.6 ± 0.3	6.1 ± 0.2	6.2 ± 0.2
20:5n-3	1.2 ± 0.1	**4.2 ± 0.3^{b*}**	1.3 ± 0.1	1.2 ± 0.1^{a}	1.1 ± 0.1	1.0 ± 0.1^{a}
22:6n-3	1.0 ± 0.1	**2.1 ± 0.2^{b*}**	1.0 ± 0.1	1.2 ± 0.1^{a}	1.0 ± 0.1	1.2 ± 0.1^{a}
TAG18:1n-9	41.4 ± 0.5	37.8 ± 0.6^{b*}	41.6 ± 0.7	39.4 ± 1.0*	41.4 ± 0.5	40.7 ± 0.6^{a}
18:2n-6	15.4 ± 0.6	15.0 ± 0.7	14.7 ± 0.9	16.5 ± 0.9*	15.0 ± 0.7	16.2 ± 0.7
20:4n-6	1.7 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.8 ± 0.1	1.6 ± 0.1	1.7 ± 0.1
20:5n-3	0.5 ± 0.1	**1.7 ± 0.2^{b*}**	0.5 ± 0.1	0.6 ± 0.2^{a}	0.5 ± 0.1	0.4 ± 0.1^{a}
22:5n-3	0.5 ± 0.1	**1.3 ± 0.3^{b*}**	0.5 ± 0.1	0.6 ± 0.1^{a}	0.4 ± 0.1	0.5 ± 0.1^{a}
22:6n-3	0.9 ± 0.1	**2.2 ± 0.2^{b*}**	1.0 ± 0.1	1.1 ± 0.2^{a}	0.9 ± 0.1	1.0 ± 0.1^{a}

Post-treatment values across a row indicated by different superscript letters are significantly different from one another (ANOVA test).

Values indicated by * are significantly different from before treatment (paired t-test).

2.4.9 Composition of fatty acids in plaques

After short term supplementation (median 53 days), the levels of EPA (20:5 n-3) and DHA (22:6 n-3) in plaque were significantly greater in group A than those of the other two groups (Table 2.13), and the level of linoleic acid (18:2 n-6) in PL fractions in plaque was significantly lower in group A than the other two groups.

In group A, the proportion of EPA (20:5 n-3) in plaque PL was linearly correlated with the duration of fish oil supplementation ($r = 0.41$, $p = 0.005$) (Figure 2.10). There was a positive linear correlation between the change in the proportion of plasma EPA in LDL CE and the proportion of EPA in plaque CE in patients consuming fish oil ($r = 0.6$, $p = 0.008$) (Figure 2.11).

Table 2.13 Fatty acid composition of plaque lipid fractions in 3 treatment groups.

		Fatty acid (g/100 g total fatty acids)		
		Group A	Group B	Group C
PL	16:0	39.8 ± 0.7	39.1 ± 0.6	39.1 ± 0.5
	18:0	14.9 ± 0.2	14.9 ± 0.3	14.8 ± 0.3
	18:1n-9	14.2 ± 0.3	13.7 ± 0.3	14.1 ± 0.2
	18:2n-6	**9.9 ± 0.2^{b}**	11.2 ± 0.3^{a}	10.9 ± 0.3^{a}
	20:3n-6	1.9 ± 0.1^{b}	2.0 ± 0.1	2.1 ± 0.1^{a}
	20:4n-6	9.9 ± 0.3	10.1 ± 0.3	10.1 ± 0.3
	20:5n-3	**1.1 ± 0.1^{b}**	0.6 ± 0.1^{a}	0.6 ± 0.1^{a}
	22:5n-3	2.4 ± 0.3	2.9 ± 0.3	2.8 ± 0.3
	22:6n-3	**3.6 ± 0.2^{b}**	2.9 ± 0.2^{a}	3.3 ± 0.2
CE	16:0	14.5 ± 0.3	15.0 ± 0.3	14.5 ± 0.3
	16:1n-7	3.8 ± 0.1	4.1 ± 0.2	3.9 ± 0.2
	18:0	0.7 ± 0.1	0.7 ± 0.1	1.0 ± 0.2
	18:1n-9	26.7 ± 0.5	25.7 ± 0.5^{b}	27.3 ± 0.6^{a}
	18:2n-6	39.5 ± 0.8	40.4 ± 0.8	38.6 ± 0.8
	20:3n-6	2.2 ± 0.2	2.1 ± 0.1	2.4 ± 0.2
	20:4n-6	6.8 ± 0.2	6.6 ± 0.2	6.6 ± 0.2
	20:5n-3	**1.5 ± 0.1^{b}**	1.1 ± 0.1^{a}	1.1 ± 0.1^{a}
	22:5n-3	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
	22:6n-3	**2.0 ± 0.1^{b}**	1.6 ± 0.1^{a}	1.5 ± 0.1^{a}
TAG	16:0	27.4 ± 0.7	28.8 ± 0.5	28.9 ± 0.7
	16:1n-7	3.8 ± 0.4	3.4 ± 0.2	3.7 ± 0.3
	18:0	5.7 ± 0.2	5.8 ± 0.2	5.9 ± 0.3
	18:1n-9	38.8 ± 0.6	37.6 ± 0.5	37.8 ± 0.6
	18:2n-6	15.1 ± 0.4	16.4 ± 0.5	15.9 ± 0.6
	20:4n-6	1.9 ± 0.1	2.2 ± 0.1	2.1 ± 0.1
	20:5n-3	**0.4 ± 0.1^{b}**	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}
	22:5n-3	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
	22:6n-3	**1.2 ± 0.1^{b}**	1.0 ± 0.1	0.9 ± 0.1^{a}

Values across a row indicated by different superscript letters are significantly different from one another (ANOVA test).

Figure 2.10 Relationship between the proportion of plaque 20:5 n-3 (EPA) in PL fraction and the duration of patients' consumption of capsule A, (/days).

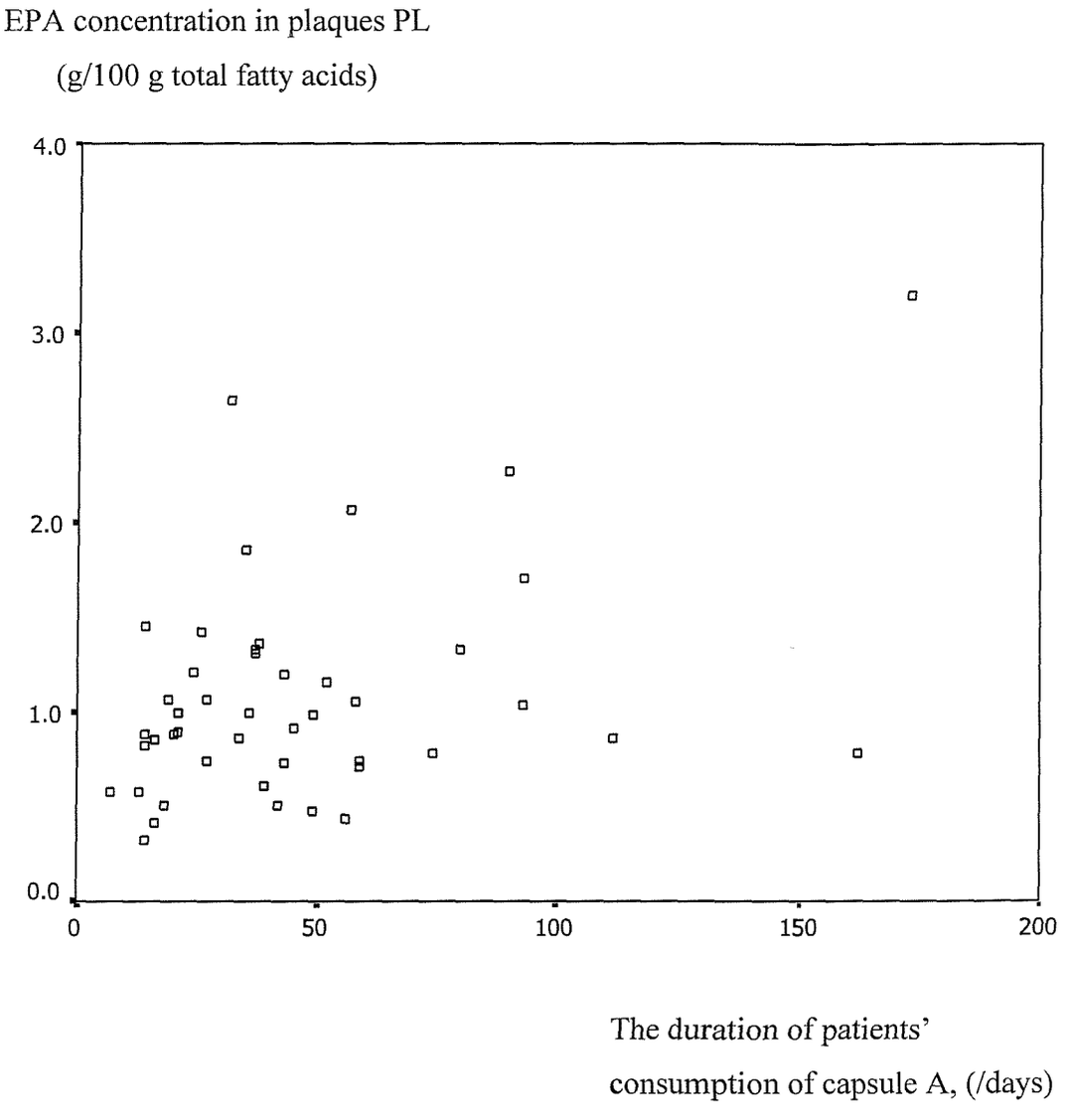
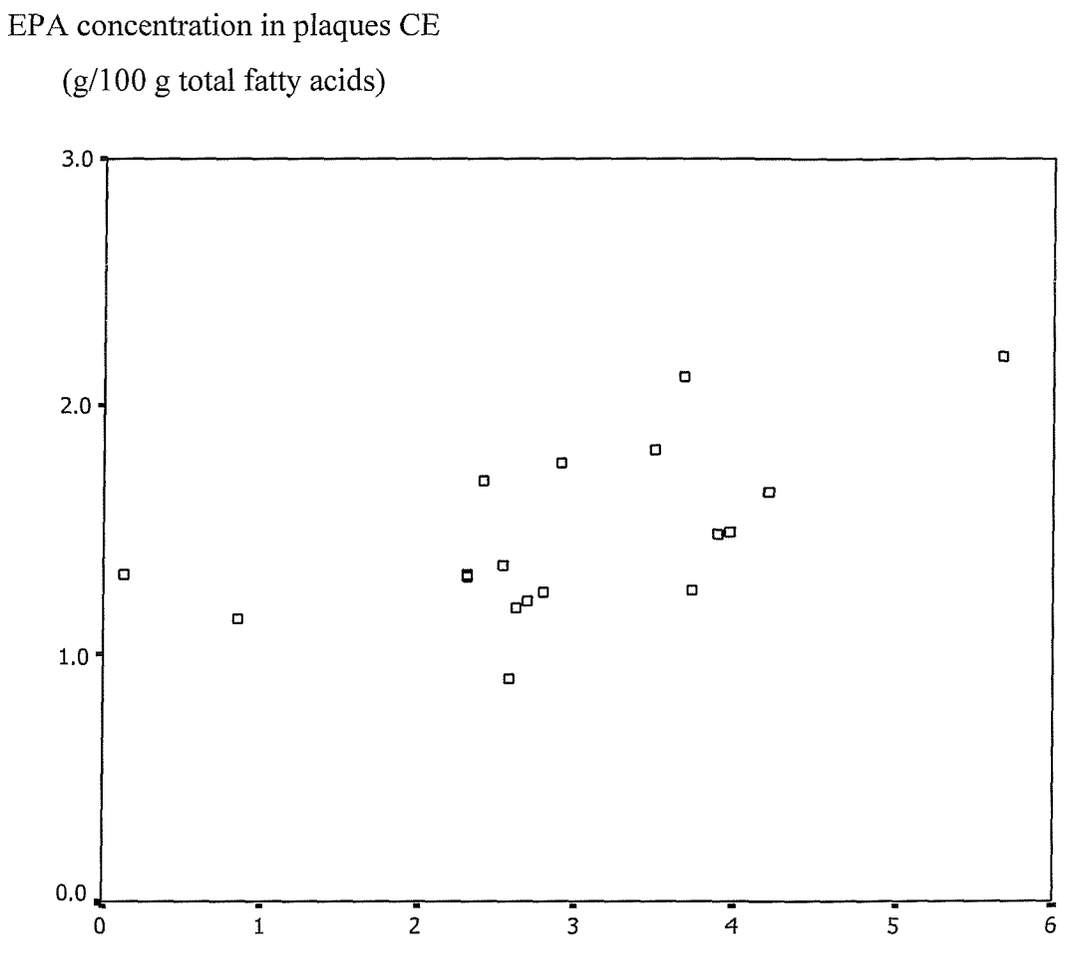


Figure 2.11 Relationship between the change of the concentration of plasma LDL EPA in CE fraction (between 2 visits) and the proportion of plaque 20:5 n-3 (EPA) in CE fraction from patients taking capsule A.



2.4.10 Histology of carotid plaques

2.4.10.1 AHA and modified AHA score

The mean rank of both AHA and modified AHA score was lower in group A than the other groups, but this difference was not significant (Table 2.14). Interestingly with the modified AHA classification patients in group A had the highest incidence of fibrous cap atheromas and the lowest incidence of thin fibrous cap atheromas, a potential beneficial structure configuration. The inter-observer agreement of AHA and modified AHA score was 46.6% and 48.3% respectively.

Table 2.14 Carotid plaque morphology.

Percentage	Group A	Group B	Group C	p value
AHA classification type				
III	0	0	1.8	
IV	71.7	60.7	59.6	
V	15.1	32.2	29.8	
VI	13.2	7.1	8.8	
Mean rank	**79.5**	**85.9**	**84.9**	**0.42**
Modified AHA classification				
Pathological intimal thickening(Rank1)	7.5	7.1	7.0	
Fibrous cap atheroma (Rank2)	**66.0**	55.4	54.4	
Thin fibrous cap atheroma (Rank3)	**15.1**	28.6	22.8	
Erosion (Rank4)	1.9	1.8	0	
Plaque rupture (Rank5)	5.7	5.4	5.3	
Mean rank	**74.3**	**83.1**	**79.2**	**0.52**

* Jonckheere-Terpstra test

2.4.10.2 Histomorphometry of carotid plaques

The plaques in the patients in group A tended to have a greater number of lipid than those in the other groups (Table 2.15), whereas the plaques in the patients in the group taking sunflower oil had a higher proportion of macrophages and lymphocytes. However no statistically significant differences were found in the three groups. Inter-observer agreement of histomorphometry was 92%.

Table 2.15 Carotid plaque histomorphometry (under 20 x power of magnification).

Percentage	Group A	Group B	Group C	p Value*
Fibrous tissue	31.7 ± 2.7	33.5 ± 3.3	36.9 ± 3.4	0.50
Recent haemorrhage	2.5 ± 0.9	1.7 ± 0.5	2.0 ± 0.6	0.76
Old haemorrhage	0.3 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0.51
Amorphous lipid	**55.4 ± 3.2**	53.5 ± 3.3	50.4 ± 3.4	0.58
Crystalline lipid	**2.4 ± 1.3**	1.2 ± 0.4	3.0 ± 1.6	0.52
Macrophage	0.74 ± 0.36	**0.86 ± 0.37**	0.35 ± 0.15	0.49
Lymphocyte	1.3 ± 0.3	**2.6 ± 0.7**	1.7 ± 0.5	0.23
Blood vessel$^{\tau}$	< 0.1	< 0.1	< 0.1	0.34
Calcification	2.4 ± 0.8	4.3 ± 1.2	2.2 ± 0.6	0.20

Data are present as mean ± SE *Anova test , $^{\tau}$ neovascularisation.

2.4.11 Immunohistochemistry of carotid plaques

The mean rank of macrophages was significantly lower in group A than in the other groups (p = 0.03), but no significant differences were found for other immunohistochemistry parameters in the three groups (Table 2.16). Inter-observer agreement of immunohistochemistry was 72.2%.

Table 2.16 Immunohistochemistry scores.

Antigen	Group	Rank Frequency(% cases)			Mean rank	P value*
		0	1	2		
T-cell	**A**	0	26.1	73.9	33.41	1.00
(CD3)	**B**	5.0	30.0	65.0	30.20	
	C	4.8	19.0	76.2	33.69	
VCAM-1	**A**	8.3	33.3	58.3	37.58	0.64
(CD 106)	**B**	36.8	26.3	36.8	27.18	
	C	17.4	30.4	52.2	34.46	
ICAM-1	**A**	9.5	14.3	76.2	32.74	0.28
(CD54)	**B**	12.5	43.8	43.8	24.63	
	C	21.1	21.1	51.9	27.08	
Macrophage	**A**	**0**	**38.1**	**61.9**	**51.10**	**0.03**
(CD68)	**B**	0	19.4	80.6	61.82	
	C	2.6	13.2	84.2	63.54	

T-cells = T-lymphocytes, VCAM-1 = vascular adhesion molecules-1, ICAM-1 = intercellular adhesion molecule-1, * Jonckheere-Terpstra test

2.5 Discussion

2.5.1 Recruitment

In this study patients took supplements for a median of 50 days before surgery. Patients in the three groups were well matched. 11% dropped out from the study (Figure 2.9). No subject was excluded because of a poor outcome, so results were not biased because of this. After checking with patients and examining their diaries, it was felt that compliance with the dietary regimens was reasonably good.

2.5.2 Characteristics of patients

In this study patients were placed randomly into one of three dietary groups. It was therefore important that there be no major differences in characteristics of patients between the groups (Table 2.5). Indeed no major differences were found between the groups in terms of age, gender distribution, BMI, intake of macro- or micronutrients, blood lipid concentrations (baseline), adipose tissue fatty composition (baseline), and proportion of patients being treated with statins (Table 2.5, 2.6, 2.8, 2.10, 2.11).

Although there were no specific gender inclusion or exclusion criteria, it turned out that the majority of patients were male, not surprising as male gender is a risk factor for atherosclerosis, as other studies have shown (Table 2.5) (Castelli 1984). Similarly hypertension and smoking history, atherosclerotic risk factors, were also found frequently in this study. Most of the patients recruited for the study had hypertension (66.7%) and 84.5% of patients smoked or used to smoke. 32.1% of these (168 patients) had plasma total cholesterol levels greater than 5.0 mmol/L (Chapter 3 for more detail).

2.5.3 Neurological symptoms and associated heart disease

There were more symptomatic patients than asymptomatic patients in this study (Table 2.7), which agrees very well with the fact that in medical practice in the UK asymptomatic carotid stenosis is not a common indication for carotid endarterectomy. This contrasts with practice in the USA, where 48% of surgical procedures are

performed for asymptomatic disease (Masuhr, Busch & Einhaupl 1998). It is also interesting that about half of the patients in this study had associated heart disease. This may explain why a major cause of death of post-operative CEA patients in some studies, including our study, has been myocardial infarction (Musser, Nicholas & Reed, III 1994). Clinicians should consider ischaemic heart disease very carefully when selecting patients for surgery.

2.5.4 Anti-platelet agents

All patients in this study were given pre-operative anti-platelet agents. The dosages of pre-operative aspirin of 93.7% of patients in this study were less than 200 mg/day. In contrast in the USA most patients take more than 500 mg/day of aspirin (Carson, Demling & Esquivel 1981). However a large trial has furnished evidence for taking smaller doses of aspirin post-operatively. In the ACE trial it was found that patients taking lower doses (81 or 325 mg/day) of aspirin post-operatively had lower risks of stroke, myocardial infarction, and death within 30 days and 3 months of endarterectomy than those taking higher doses (650 mg or 1300 mg/d) of aspirin (Taylor *et al.* 1999a). It can therefore be assumed that patients in this study benefited from aspirin after surgery if they continued the pre-operative dosage.

2.5.5 Intake of fatty acids

In this study the normal intakes of fatty acids including n-3 PUFA and n-6 PUFA as recorded in the patients' diaries did not differ between the three groups (Table 2.8). This shows that it was unlikely that the patients'normal dietary intakes interfered with the results of this study.

2.5.6 Levels of fatty acids in adipose tissue

Adipose fatty acid concentration is mainly determined by dietary intake. This is especially so for linoleic acid and α-linolenic acid, which cannot be synthesized by man. Adipose tissue composition has served as a biological marker of chronic ingestion of several dietary PUFAs (Heffernan 1963). This study found that in group A the levels

of EPA and DHA increased significantly in adipose tissue after supplements (Table 2.11), whereas in a previous study this effect was seen with higher doses of fish oil (10 g/day) and longer supplementation (12 months) (Leaf *et al.* 1995). This result indicates that the composition of adipose tissue can be changed by short-term changes in n-3 PUFA intake.

2.5.7 Levels of plasma fatty acids

It has been shown that when the high dose supplementation of fish oil (16.0-21.3 g/day of n-3 PUFA) is incorporated into the diet, plasma concentrations of EPA and DHA increase several-fold (Rapp *et al.* 1991). In our study it was found that the modest supplementation (1.7 g/day of n-3 PUFA) with fish oil caused a significant enrichment of long chain n-3 PUFA in all major fractions of LDL lipids: CE, PL, TAG. The relationship between plaque EPA content in CE fraction and the increase in plasma LDL EPA in CE fraction was highly significant, suggesting that LDL CE may be a major source of plaque EPA (Figure 2.11). Thus if the modest supplementation of fish oil used in this study affects the composition of LDL, it might also affect atherosclerotic plaques. In particular, the levels of plasma EPA and DHA in LDL increased by factors of 3 and 2 respectively after fish oil supplements, comparable to results in the GISSI-P trial (1999). The author believes that the levels of EPA and DHA in atherosclerotic plaques in coronary artery of patients in the GISSI-P study might have been elevated also like the carotid plaques in this study.

Another potential benefit of fish oil in this study was the reduction of the levels of plasma TAG (Table 2.10), a result that has been reported consistently by other studies. In the studies of Bang (Bang, Dyerberg & Nielsen 1971), for example, they found a similar effect. N-3 PUFA is believed to decrease TAG concentration by decreasing synthesis of hepatic very low-density lipoprotein-TAG (Harris *et al.* 1990). A meta-analysis of seventeen population-based prospective studies showed that an increase in plasma TAG concentration was associated with a significant increase in the risk of coronary heart disease (Hokanson & Austin 1996). A reduction of TAG in patients

taking n-3 PUFAs may partially explain the cardioprotective effect observed in some studies (Bang, Dyerberg & Nielsen 1971; Bang, Dyerberg & Sinclair 1980). As stated above, the cardioprotective effect in some studies such as the GISSI-P study (1999) was seen, even though the level of TAG was not significantly lower.

2.5.8 Composition of fatty acids in plaques

The levels of n-3 PUFA in plaques were higher in patients taking short-term n-3 PUFA supplements. One of the objectives was to see how rapidly these n-3 PUFA supplements were incorporated into plaques. This was seen after a short term of supplements. The incorporation of EPA into plaque lipids was linear with respect to time and there was no plateau effect (Figure 2.10), so the longer supplementation might have had a greater effect. This incorporation of EPA into plaque has previously shown by Rapp *et al.* (1991). In this study the daily intakes of n-3 PUFA (1.7 g/d) were about one tenth of those in the Rapp study (16.0-21.3 g/d). The results of the current study suggest that modest daily intakes of fish oil supplements suffice for incorporation into plaques. This also reflects the fact that atherosclerotic plaque is dynamic with some degree of lipid turnover and contradicts the earlier belief that atheroscleorotic disease was static.

It is important to note that there were more participants (55 patients) in this study taking supplements of n-3 PUFA than in the Rapp study (11 patients) (1991). This study considered only carotid plaques, whereas the Rapp study considered plaques in various locations, including the femoral and carotid arteries. In this way this study avoided any confounding factors, such as haemodynamic factors, which might have arisen from consideration of plaques in different locations.

2.5.9 Histomorphometry of plaques and AHA score

First it was found that the histomorphometry of plaques from the bifurcation and other sections (internal carotid and common carotid artery sections) was the same (Table 2.3). Consequently it was decided to study the histomorphometry of the bifurcation sections, which were expected to show all the changes of histological components especially the

major components of plaque. The results of histomorphometry were the same for all the groups (Table 2.15).

Using the AHA score and its modification, there was some evidence of less complicated plaques in patients in group A (Table 2.14), but this evidence was not statistically significant. Using the modified AHA criteria, plaques in group A tended to have fewer thin fibrous cap atheromas, a characteristic of unstable plaques, than those in other groups. It was suggested that plaques with thin fibrous cap atheroma are most likely to rupture (Virmani *et al.* 2000).

2.5.10 Immunohistochemistry of plaques

The number of macrophages was significantly lower in plaques from patients in group A than those in the other two groups (Table 2.16). However this result contradicts the results from histomorphometry. This may be because in histomorphometry the average proportions of the components such as macrophages of the plaques in 6-20 fields were considered, whereas in the immunohistochemistry technique all the macrophages in one section were counted. The proportion of macrophages in the histomorphometry was also small (< 1% in all treatment groups) (Table 2.15). The counting of macrophages in histomorphometry was not as accurate as it was in the immunohistometric technique, which focused specifically on stained cells (macrophages) (Figure 2.8e). In fact the purpose initially of histomorphometry in this study was to examine the major components of plaque such as lipid and fibrous tissue, which are more than 80% of the components of plaque.

There has been a lot of evidence that unstable plaques have many more macrophages than stable plaques (Felton *et al.* 1997; Plutzky 1999; Stary *et al.* 1995). For example Felton *et al.* (1997) studied 334 aortic plaques of 30 men who died from myocardial infarction and found that the ruptured plaques (unstable plaques) had many more macrophages, greater lipid area, and thinner fibrous caps than intact or stable plaques. In fact, macrophages were first recognised by van der Wal *et al.* (1994) as an important

factor involved in plaque rupture. They studied coronary artery plaques in 20 patients who died from MI and found there were many inflammatory cells, especially macrophages, at the sites of plaque rupture and superficial erosion (van der Wal *et al.* 1994). Human leukocyte antigen DR was also prevalent in these areas, reflecting active inflammation (van der Wal *et al.* 1989). They hypothesized that macrophages played a primary role in both the expansion of the lipid core and the thinning of fibrous caps (Felton *et al.* 1997). In other words, macrophages played a leading role in plaque instability. Not only did this occur in patients with coronary heart disease, but a study of carotid plaques found a similar result with unstable carotid plaques having many more inflammatory cells than stable ones (Carr *et al.* 1997; Husain *et al.* 1999; Golledge, Greenhalgh & Davies, A.H. 2000; Schumacher *et al.* 2001). Husain *et al.* (1999) found that the number of macrophages in fibrous caps of patients with symptomatic neurological deficit was significantly greater than those of asymptomatic patients. The medians of the numbers of macrophages of the former and latter were 1.28% and 0.57% respectively. As stated in Chapter 1, macrophages may cause plaques to become more unstable by four mechanisms that have been proposed: inhibition of VSMC protein synthesis (fibrous cap), apoptosis of VSMCs, production of matrix metalloproteinase, and as the main source of tissue factor for thrombosis. These mechanisms may explain the observations of fewer macrophages within plaques and fewer plaques with thin fibrous caps in group A (Table 2.14). Taken together, as in this study, fish oil supplements reduced the number of macrophages in plaques, so fish oil might improve plaque stability. If that is the case, supplements of fish oil might prevent progression of plaques and make them more stable. Perhaps this also explains the cardiovascular benefits in trials such as the the DART trial (Burr *et al.* 1989) and the GISSI-P trial (1999). It is interesting to note that the GISSI-P trial reported further recently that the reduction in risk of sudden death was statistifically significant at 4 months after randomization (relative risk 0.47, $p = 0.048$) (Marchioli *et al.* 2002). The investigators of this trial support the hypothesis of an antiarrhymic effect of fish oil for cardiac protection. However the author believes that the novel finding of the current study can explain this event as well because the effect on macrophages was seen in a

short period (median 53 days) with doses of supplements similar to those in the GISSI-P trial.

Whether the decrease in macrophage numbers within the plaque is due to fewer monocytes/macrophages entering the plaque or a greater number leaving the plaque is unclear. Three mechanisms for the reduced number of macrophages are possible.

Firstly, a lower expression of adhesion molecules in the plaque might suggest decreased entry of monocytes/macrophages. Inflammatory cells must have these molecules to pass into atherosclerotic plaques. Several studies have shown that patients taking fish oil had lower levels of adhesion molecules. Miles *et al.* (2000) studied mice given various types of fatty acid diets, low fat, coconut oil, safflower oil, and fish oil, and found that mice fed fish oil had significantly lower expressions of intercellular adhesion molecules-1 (ICAM-1) and macrophage scavenger receptor-A type I+II, which is important in recruitment of macrophages to the aortic intima, their activation, and subsequent conversion to foam cells. De Caterina *et al.* (1995) found that DHA decreased cytokine-induced expression of endothelial leukocyte adhesion molecules, secretion of inflammatory mediators, and adhesion of leukocytes to endothelial cells. In another study of 12 healthy volunteers, Hughes *et al.* (1996) found that subjects taking n-3 PUFA had fewer surface molecules on their monocytes, which is important for antigen-presenting cells. However in the current study immunohistochemistry did not show any significant differences of ICAM-1 and vascular adhesion molecules-1 (VCAM-1) between the three groups. It is important to note that previous studies used high dose of n-3 PUFA supplements and detect the level of adhesion molecule with flow cytometry. Also previous studies looked at the effect of n-3 PUFA supplements on cells such as monocytes or endothelial cells, but no studies have been conducted on the effect of fish oil on atherosclerotic plaque. These may explain the differences in results.

The second mechanism is a reduction of chemotactic factors by fish oil. Several cytokines and growth factors secreted by mononuclear cells influence atherogenesis

(Baumann *et al.* 1999; Endres *et al.* 1989). Baumann *et al.* (1999) studied growth factor production in macrophages in 28 volunteers who took one of four types of supplements: n-3 PUFA, n-6 PUFA, n-9 PUFA, or a placebo used for the control group. They found that only patients taking n-3 PUFAs had decreased production of platelet-derived growth factor-1 (PDGF-1), PDGF-β, monocyte chemoattractant protein-1 mRNA in both stimulated and unstimulated mononuclear cells.

The third mechanism is departure of macrophages from plaques. The number of monocyte/macrophages in the plaques of patients given fish oil might decrease is because of an increased rate of cell death by either apoptosis or necrosis. Avula *et al.* (1999) found that n-3 PUFA increased induced and spontaneous apotosis of immune cells in mice by increasing the generation of lipid peroxide and mediator of apotosis.

So far it is not known what the real mechanisms are that explain the decrease in macrophages in plaques of patients taking n-3 PUFA. Future studies should try to identify the real mechanisms of this decrease. For example, the relationship between incorporation of n-3 PUFA into plaques and plaque monocyte/macrophage death should be studied to determine the mechanism responsible for this.

2.5.11 Limitations

Since macrophages play a primary role in plaque instability, fish oil supplements might improve plaque stability, as suggested by this study. However there was no long-term follow-up of cardiovascular events in this study, so it cannot be confirmed from this study that fish oil improves the stability of plaques through macrophage reduction. For confirmation of this concept, another study should be carried out with long-term clinical follow-up to monitor the number of macrophages in plaques. However there is the question of how to monitor the number of macrophages in vivo. Positron emission tomography (PET) seems to be the most promising tool for monitoring inflammation in plaques (Weissberg 2000). Recently Rudd *et al.* (2002) found that Fluorodeoxyglucose PET can identify and quantify the inflammatory process of carotid plaque in patients

with advanced carotid stenosis. When PET was combined with CT scans or magnetic resonance imaging, the hot spot on the vessel wall on the PET image coincided with the part of the arterial wall thickened by atheroma. Autoradiography of these carotid plaque specimens confirmed accumulation of deoxyglucose (hot spot areas) in macrophage-rich areas. However such a study could not include patients with symptomatic severe carotid stenosis, because it is clear that CEA benefits these patients, so there would not be enough time for long-term follow-up. Perhaps patients with asymptomatic severe carotid stenosis, for whom CEA has marginal benefits, would be good candidates for another study. In addition, in the analysis of food diaries patients were encouraged to record food intake in as much detail as possible so that the components of their habitual intake could be determined accurately, but some patients in the current study recorded only the name of the food. In these cases, the ingredients of the food had to be estimated using the database of the Royal Society of Chemistry, which might have led to some inaccuracy.

2.6 Conclusions

Short-term supplements of n-3 PUFA were incorporated into carotid plaques and adipose tissue, and this incorporation of n-3 PUFA into plaques was associated with a reduced number of macrophages. As macrophages play a primary role in plaque instability, this study suggests n-3 PUFA supplements might have a role in plaque stability. This might explain the significant cardioprotective effects that fish oil has against both fatal and non-fatal MI (Burr *et al.* 1989; GISSI-P 1999; Singh *et al.* 1997).

Chapter 3

The prevalence and treatment of hypercholesterolaemia in patients with carotid artery disease

3.1 Summary

Objective: Although hyperlipidaemia is a critical problem in patients with peripheral vascular diseases, and several large trials and many guidelines have stressed the importance of detection and treatment of hyperlipidaemia, it is not known to what extent this has been applied to patients with carotid artery disease. The objective of this study was to investigate the prevalence of hypercholesterolaemia (total choletsterol concentration ≥ 5 mmol/L) and the adequacy of hyperlipidaemia management in patients awaiting carotid endarterectomy.

Design: Cross-sectional study.

Methods: A prospective study was conducted of 168 consecutive patients (the same as those in the study in Chapter 2) admitted electively for carotid endarterectomy. A medical history, medication history, and fasting venous blood sample were taken for each patient. The plasma concentrations of cholesterol were determined.

Results: 32.1% of patients awaiting carotid endarterectomies still had plasma total cholesterol levels greater than or equal to 5 mmol/L (the recommended level). 28% of patients who were aware of their hyperlipidaemia problem still had higher levels of plasma total cholesterol than the recommended value. 31.9% of patients who denied having a problem of hyperlipidaemia had plasma cholesterol concentrations greater than or equal to 5 mmol/L. 22% of these patients had never had their plasma cholesterol levels checked before.

Conclusion: Although the risk factors in vascular disease management are well understood and many guidelines have been published, hyperlipidaemia does not seem to be well managed in patients awaiting carotid endarterectomy.

3.2 Introduction

3.2.1 Hyperlipidaemia and vascular disease

Hyperlipidaemia is one of the most important risk factors in atheroscleorosis, which is the major cause of vascular diseases (Castelli 1984). It has also been reported that hypercholesterolaemia is associated with myocardial infarction (MI) (Kannel *et al.* 1971). In addition, hyperlipidaemia is a risk factor modifiable by diet or drugs, whereas other risk factors, such as sex and many genetic predisposing factors, are not modifiable. Therefore many studies have been carried to examine the effects of decreasing plasma cholesterol with lipid-lowering drugs. Many randomised controlled trials of lipid-lowering medications, both primary and secondary prevention, have shown clear benefit in reducing cardiovascular events (Shepherd *et al.* 1995; Sacks *et al.* 1996). The Cholesterol And Recurrent Event (CARE) study found that patients with myocardial infarction taking statins had lower coronary death rates than those in a control group (Sacks *et al.* 1996). A great number of protocols and policies have therefore stressed the importance of detection and control of hyperlipidaemia in patients with atherosclerosis (Joint British recommendations 1998; The Intercollegiate Working Party for Stroke 2000). In particular patients with coronary artery disease (CAD) have usually been thoroughly checked for these risk factors. Even so, one survey has found that physicians still do not fully recognise the importance of treatment of hyperlipidaemia in patients with CAD (Cohen *et al.* 1991).

Hyperlipidaemia is common in patients with peripheral vascular disease. Greenhalgh *et al.* (1971) found that 44% of patients with peripheral vascular disease had high serum lipid levels, compared to 6% of control subjects. The correct treatment of hyperlipidaemia is important not only in patients with obvious CAD, but also in patients with peripheral vascular disease because most patients with peripheral vascular disease have coronary artery disease. Hertzer *et al.* (1985) investigated the incidence of CAD in 506 patients awaiting extracranial cerebrovascular reconstruction by using coronary angiograms and found that only 13.5% of patients with no clinical suspicions of

coronary artery disease had normal coronary arteries. Patients with peripheral vascular disease also have a very high risk of death from cardiovascular disease (relative risk 5.9) (Criqui *et al.* 1992). Therefore hyperlipidaemia in all patients with vascular disease should be managed properly.

3.2.2 Hyperlipidaemia and post-operative outcomes of carotid endarterectomy

Cardiac mortality, especially MI, is a main cause of death after carotid endarterectomy (CEA) (Musser, Nicholas & Reed, III 1994). Kerdiles *et al.* (1997) studied the long-term outcomes after CEA of patients in 2 groups: 252 patients aged 75 or over and 660 patients under 75. The mean follow-up period in this study was 58 months. They found that the actuarial survivals after 10 years of patients 75 years of age or over and under 75 were 36% and 54% respectively. The most common cause of death was a coronary event (39%). There has been a lot of evidence that has to confirm that hyperlipidaemia is associated with MI (Castelli 1984). Both primary and secondary trials such as the West Of Scotland Coronary Prevention Study (WOSCOPS) (Shepherd *et al.* 1995) and the CARE study (Sacks *et al.* 1996) have shown clearly the benefit of statins in reducing the incidence of MI. However up to 2001, most of the evidence of the statin benefit was based on patients already known to have coronary disease, but there was little direct evidence of the benefit in patients without diagnosed coronary disease who had cerebrovascular disease or peripheral vascular disease. Recently the results of the landmark Heart Protection Study (HPS 2002) were reported. This randomised controlled trial compared the 5-year risk of vascular events for simvastatin and a placebo in patients with a high risk of of coronary death. Men and women aged about 40-80 years with non-fasting blood total cholesterol concentrations of at least 3.5 mmol/L were the subjects. They were considered to have a substantial 5-year risk of death from coronary heart disease because of a past history of (1) coronary disease; or (2) occlusive disease of a non-coronary artery (TIA, intermittent claudication, carotid endarterectomy or other arterial surgery); or (3) diabetes or hypertension. This study found that patients taking 40 mg/day of statins had significantly less coronary death (5.7%) than those in the control group (6.9%) ($p = 0.0005$). They also had markedly

fewer non-fatal coronary events, non- fatal strokes, and revascularisation rates than those in the control group. In a subgroup of this study, patients with cerebrovascular disease taking statins also had significantly lower risks of major vascular events (combined rate of coronary events, strokes and revascularisations) (24.7%) than those in the control group (29.8%). Taking all of this into consideration, it follows that patients undergoing carotid endarterectomy should survive longer or have less chance of major vascular events if hyperlipidaemia is managed properly. Moreover there is compelling evidence that hyperlipidaemia is a risk factor for carotid restenosis after CEA (Das *et al.* 1985; Rapp *et al.* 1987).

It is interesting to note that statins reduce the incidence of MI even in the first year of their administration in WOSCOPS study, which probably cannot be attributed to the significant reduction of preexisting atheroma or to the prevention of progression of lesions to a clinically relevant stage induced by these statins (Shepherd *et al.* 1995). The WOSCOPS Study found that a reduction of LDL was associated with a decrease in plasma and whole blood viscosity (Rumley *et al.* 1997). Perhaps this partially explains how statins reduce the incidence of systemic ischaemic events, including MI, in early stages (Rumley *et al.* 1997; Vaughan, Murphy & Buckley 1996). There is also much evidence that statins have anti-inflammatory effects. As mentioned earlier (Chapter 1), carotid plaques from patients taking statins had fewer macrophages, fewer lymphocytes and a higher collagen content than those from patients in a control group (Crisby *et al.* 2001). Following statin treatment plaque inflammation subsided markedly and there was an increase in stable plaque. In other words, statins make plaque more stable. Another piece of evidence that shows the anti-inflammatory effects of statins is the reduction of CRP following statin treatment (Ridker 1999b).

3.2.3 Recommendations

No recommendation has been made specifically for carotid disease patients, but it is recommended that for patients with major atherosclerotic disease, the plasma total cholesterol level be kept at less than 5 mmol/L. This has been proposed and supported by several societies: the British Cardiac Society, the British Hyperlipidaemia

Association, the British Hypertension Society, and the British Diabetic Association (Joint British recommendations 1998; McCormick *et al.* 2001; The Intercollegiate Working Party for Stroke 2000). In 1997, Standing Medical Advisory Committee (SMAC 1997) also suggested that the level of plasma cholesterol following lipid-lowering treatment should be less than 5 mmol/L.

3.2.4 Hypothesis

Despite much evidence about the danger of hyperlipidaemia in patients with peripheral vascular disease including carotid artery stenosis, clinicians may not be managing plasma lipid levels of patients with carotid artery stenosis appropriately.

3.2.5 Objectives

The first objective was to determine the prevalence of patients awaiting carotid endarterectomy with a plasma total cholesterol level greater than or equal to 5mmol/L. A second objective was to study the extent to which participants in this study had been managed with regard to hyperlipidaemia and plasma cholesterol.

3.3 Methods

3.3.1 Patients and design of study

168 consecutive patients were included prospectively; they were participating in the ongoing trial (Chapter 2), the objective of which was to look at the effect of n-3 PUFA supplements on the carotid plaques compared with n-6 PUFA supplements and a placebo.

3.3.2 Analysis of habitual nutrient intakes

Food diaries were analysed for habitual nutrient intakes using a modification of FOODBASE (Institute of Brain Chemistry, London, UK), which was validated for determination of nutrient intake (see section 2.3.7.1 for details).

3.3.3 Concentration of total cholesterol

Medical histories, especially the patients' awareness of hyperlipidaemia, were taken. Patients' records and the drug charts in the patient's notes were checked for record of use of any lipid-lowering medications. On the initial visit (before any nutritional intervention) fasting venous blood samples were taken and stored in vacutainer tubes containing 0.12 ml of 15% EDTA. Plasma was prepared by centrifugation at 2500 rpm (revolutions per minute) for 10 min and stored at -70° C. Concentrations of plasma total cholesterol were determined using a commercially available, enzyme-based diagnostic kit (Sigma Chemical Co., Poole, UK) (see Appendix 2 for details).

3.3.4 Analysis

Patients were classified into two groups: those with plasma cholesterol concentrations greater than or equal to 5 mmol/L and those with plasma cholesterol concentrations less than 5.0 mmol/L. Patients in these two groups were evaluated for their awareness of hyperlipidaemia, which was classified in three ways. Group 1 patients were those who were aware of an abnormality in their blood lipids and had been given treatment or advice. Group 2 patients claimed that their doctors had said that their blood tests were normal. Group 3 patients were unaware that any testing of blood lipids had ever been done. Patients in the two groups based on plasma cholesterol levels (≥ 5.0 and < 5.0 mmol/L) were also checked for lipid-lowering treatment. Analyses were done with SPSS version 10 (SPSS, Chicago, IL USA).

3.4 Results

There were 106 males and 62 females (Table 3.1) whose mean age was 69.4. Interestingly half of these patients had histories of either angina pectoralis or myocardial infarction. 10.7% of these patients had had carotid endarterectomies before. 58 patients were undergoing statin treatment (Table 3.2). These patients had been under treatment for different durations: less than 6 months (30%), 6-12 months (30%), 12-24 months (14%), 24-36 months (12%) and greater than 36 months (14%). All of these patients had

been on statins for at least 1 month except 2 patients, who had been on statins for only 2 weeks before blood samples were taken for cholesterol tests.

Table 3.1 Baseline clinical data.

Baseline clinical data	Number of patients (percent)
Mean age (yrs)	69.4
Gender: males sex	106 (63.1%)
History of hypertension	112 (67.7%)
History of diabetes	31 (18.5%)
Current smoker	21 (12.5%)
History of angina pectoralis or myocardial infarction	87 (51.8%)
History of intermittent claudication	46 (27.4%)
Previous carotid endarterectomy	18 (10.7%)
Contralateral carotid stenosis	93 (55.4%)
Daily nutrient intakes	
- **Total fat intake (% energy)**	28.1 (≤ 30% AHA recommendation)*
- **Saturated fat (% energy)**	10.8 (8 - 10% AHA recommendation)*
- **Polyunsaturated fat (% energy)**	4.4 (≤ 10% AHA recommendation)*
- **Mono-saturated fat (% energy)**	9.1 (≤ 15% AHA recommendation)*
Sodium (g/day)	2.8 (≤ 2.4 AHA recommendation)*

*American Heart Association recommendation in parenthesis (Krauss *et al* 1996)

Table 3.2 The relationship between the type of statin and dosage given

Type of statin	10 mg	20 mg	40 mg	Total
Atorvastatin	7	2	-	9
Fluvastatin	1	1	-	2
Pravastatin	4	3	1	8
Simvastatin	29	10	-	39

Data are presented as number of patients

3.4.1 Concentrations of plasma cholesterol and awareness of hyperlipidaemia

The mean of plasma total cholesterol in this current study was 4.8 mmol/L. 54 of 168 patients (32.1%) awaiting carotid endarterectomies had levels of total cholesterol greater than or equal to 5 mmol/L (Table 3.3). 23 of the 83 patients who were aware of their hyperlipidaemia (group 1) still had total cholesterol levels greater than the recommended value. It is of interest that in group 1, 39 patients had histories of ischaemic heart disease and 9 patients (23.1%) of this subgroup still had total cholesterol levels greater than the recommended value. 48 patients denied having any problem of hypercholesterolaemia (group 2); 15 patients (31.9%) in this group had levels of total cholesterol greater than the recommended value. 37 of 168 patients (22%) had never had their cholesterol levels checked before or were not aware of any previous cholesterol tests (group 3). It is interesting that 16 patients in this group (44.4%) had total cholesterol levels greater than or equal to 5.0 mmol/L.

Table 3.3 The relationship between awareness of patient in hyperlipidaemia and the plasma total cholesterol concentration (≥ 5 mmol/L versus < 5 mmol/L).

Awareness of hyperlipidaemia	Plasma chotesterol level ≥ 5 mmol/L	Plama cholesterol level < 5 mmol/L	Total
Group 1	23 (28.0)	60 (72.0)	83 (49.4)
Group 2	15 (31.9)	33 (68.1)	48 (28.6)
Group 3	16 (44.4)	21 (55.6)	37 (22.0)
Total	54	114	168

Data are present as number of patients (percentage)

3.4.2 Concentrations of plasma cholesterol and histories of lipid-lowering medications

In group 1, 58.4% of patients were on statins and 39.3% were being treated by dietary control (Table 3.4). Although 48 patients denied having any hyperlipidaemia, in fact four of these patients were still on statins, based on their medication charts. Similarly, eight out of 41 patients in group 3 were on lipid-lowering medications.

Table 3.4 The patients' true awareness of their hyperlipidaemia and lipid lowering management.

	Lipid lowering medication			Total
	On statin	On fibrate	No	
Group 1	49 (58.4)	2 (2.2)	32 (39.3)*	83
Group 2	4 (8.3)	0	44 (91.7)	48
Group 3	5 (12.2)	3 (7.3)	29 (80.5)	37

Data are presented as number of patients (percentage). * dietary control

3.4.3 Concentrations of plasma cholesterol and statin medication

In group 1 although 49 patients were on statin, 12 patients (34.8%) still had levels of total cholesterol greater than or equal to 5 mmol/L (Table 3.5). Similarly 18.7% of the patients in group 3 had total cholesterol levels greater than the same limit.

Table 3.5 The data of all patients taking statin classified into 3 types according to the patients' awareness of hyperlipidaemia compared with the plasma total cholesterol concentration.

All patients taking statin according to awareness of hyperlipidaemia	Chotesterol level ≥ 5 m.mol/L(%)	Chotesterol level < 5 m.mol/L(%)	Total
Group 1	12 (34.8)	32 (65.2)	49
Group 2	0	4 (100)	4
Group 3	1 (18.7)	4 (81.3)	5

Data are presented as number of patients (percentage).

3.5 Discussion

3.5.1 Concentrations of cholesterol and clinical practice

32.1% of patients who came for carotid endarterectomy had plasma total cholesterol levels greater than or equal to 5 mmol/L (the recommended level). Although a huge amount of evidence supports proper the treatment of hyperlipidaemia in vascular patients, it does not seem to be managed well in clinical practice (Aspry, Holcroft & Amsterdam 1995; Evans at al 1999; Harrison & Holdsworth 2001). Indeed mismanagement is evident in patients in this study, 22% of whom had never had their lipid profiles checked or were not aware of any previous plasma lipid tests (Table 3.3). Rechecking the level of cholesterol after treatment is also important. In this study 22.4% of the patients who were on statins still had plasma cholesterol levels greater than or equal to 5 mmol/L (Table 3.5). Four patients who denied having any hyperlipidaemia (group 2) were on statins (Table 3.4). It seems that some patients are not aware of this problem, which may imply that clinicians fail to stress the importance of the risk of hyperlipidaemia to patients with carotid stenosis. Some believe that once a patient has severe carotid stenosis and is waiting for carotid surgery, lipid-lowering drugs are no longer beneficial. In other words, it is already too late to treat a severely stenotic lesion. While it is true that a severely stenotic lesion is highly unlikely to regress in a short

period, in many trials statins have reduced the incidence of cardiovascular events, especially myocardial infarction, which is the main cause of death peri-operatively and long-term after CEA (Kerdiles *et al.* 1997; Musser, Nicholas & Reed, III 1994). In particular 51.8% of the patients in this study had histories of either angina or myocardial infarction (Table 3.1). To achieve a good outcome for these patients adequate hyperlipidaemia management would be beneficial.

This study found that hyperlipidaemia was not managed well in clinical practice in the UK. The main reason is probably that no one takes responsibility for the management of hyperlipidaemia. Some general practitioners treat hyperlipidaemia intensively in patients with peripheral vascular disease and some do not. Some vascular surgeons do not treat it because of the expense and ask general practitioners to handle it instead, which makes this matter even more complicated if general practitioners do not understand the importance of hyperlipidaemia management in these patients. There had been a lack of direct evidence about the benefits of lipid-lowering therapy for patients with cerebrovascular disease. Some clinicians might be concerned about the cost-effectiveness of lipid-lowering treatment, more specifically about its lack of cost-effectiveness for patients with carotid stenosis. This might come from confusion about the correct values for control of plasma cholesterol. There are several recommendations in clinical practice. For example in the USA the National Cholesterol Education Program (NCEP 1993) uses 5.2 mmol/L as the desirable blood cholesterol level. However for vascular patients who have a high risk of developing associated ischaemic heart disease the recommendation is 5.0 rather than 5.2 mmol/L (Joint British recommendations 1998). Therefore it is important to educate all clinicians that take care of vascular patients about proper management of hypercholesterolaemia.

Interestingly one of the most striking findings in the Heart Protection Study (HPS 2002) was that for high-risk patients (minimum plasma total cholesterol $\geq$ 3.5 mmol/L at entry) there appeared to be no threshold cholesterol value below which statin therepy was not beneficial, even for those with pre-treatment cholesterol levels below the

current national recommended target (Joint British recommendations 1998). As the mean of plasma total cholesterol in this current study was 4.8 mmol/L, this implies that almost all patients would benefit from statins.

In addition, serum cholesterol is an independent predictor of carotid atherosclerosis (Joensuu *et al.* 1994; Rittoo *et al.* 2001) and the level of hyperlipidaemia is a prognostic factor for recurrence of carotid artery stenosis following CEA (Das *et al.* 1985; Rapp *et al.* 1987). If patients are treated for hyperlipidaemia adequately, the patency benefit of CEA may last longer. Ultrasonic evidence has shown that lipid-lowering treatments result in regression of carotid plaques and Furberg *et al.* (1994) found that the average intimal medial thickness was significantly lower in patients taking lovastatin than in those of a control group. This might be particularly beneficial for patients with bilateral carotid stenosis who have a unilateral carotid endarterectomy and conservative treatment for the other lesion. It was found in this study that 55.4% of patients had some degree of stenosis on the contralateral side (Table 3.1). Thus proper management of hyperlipidaemia after carotid surgery on one side may prevent progression of the disease on the non-operative side.

Patients had too much saturated fatty acids and too much salt in their diet, in spite of the fact that excessive intake of saturated fatty acids can increase the level of plasma cholesterol (Table 3.1) (Calder PC 2001). Saturated fatty acids were 10.8% of the total calories of the diets of patients in this study, a little more than the 8%-10% recommended by the American Heart Association (Krauss *et al.* 1996). Patients also had more salt than the recommended upper limit of 2.4 g/day, which is based on evidence of the association between dietary sodium chloride intake and high blood pressure (Krauss *et al.* 1996). This situation might improve if health authorities put more emphasis on the importance of management of hyperlipidaemia and dietary intake.

Another risk factor that can be managed is smoking, which 21 patients (12.5%) in our study were still doing (Table 3.1). It is well known that smoking is strongly associated

with atherogenesis, and it has been reported in many papers that smoking is associated with recurrent carotid stenosis after carotid endarterectomy (Cuming *et al.* 1993). Cessation of smoking rapidly decreases the risk of ischaemic stroke and myocardial infarction (Ockene & Miller 1997; Wolf *et al.* 1988). This is another way in which long-term results for these patients may be improved.

3.5.2 Limitations

There are some limitations of this study. Firstly, all patients in this study were awaiting carotid surgery, most of them had severe stenosis, and the cases reported were from one institution only, which might not be very representative of all vascular patients, including those with mild disease and in other settings. Secondly, only the total cholesterol level was used as a measure of lipid abnormality, but low serum HDL cholesterol levels may be better measures of lipid abnormality. Lastly, in this study other atherosclerotic risk factors such as smoking and hypertension were not been considered in detail, which is part of atherosclerotic risk factor management.

3.6 Conclusion

In this study it was found that one-third of patients undergoing carotid endarterectomy had higher plasma cholesterol levels than the recommended value. Awareness of the significance of hyperlipidaemia is inadequate in clinical practice. This problem persists in spite of much evidence of the benefits of lowering lipid levels. Management of plasma lipid levels should be taken more seriously. In particular now Heart Protection Study found the clear benefit of simvastatin in patients with peripheral vascular disease including patients undergoing carotid endarterectomy. The findings in current study support the need for greater education of clinicians involved in the management of lipid levels of vascular surgery patients. In fact, hyperlipidaemia is one of many risk factors of atherosclerosis including smoking, hypertension, diabetes, and diet. To control the majority of these risk factors effectively, much expertise is needed and many services are involved, including those that lead to a cessation of smoking. A risk factor clinic with a multidisciplinary approach might therefore be beneficial.

Chapter 4

Higher levels of C-reactive protein in symptomatic patients with carotid artery disease compared with those in asymptomatic patients

4.1 Summary

Objective: Many cohort studies have shown that many inflammatory markers can predict coronary events. However few studies have investigated the role of these markers in patients with carotid artery disease. The objective of this study was to investigate the levels of inflammatory markers in symptomatic and asymptomatic patients with carotid stenosis.

Design: Cross-sectional study

Materials and Methods: A prospective study was conducted of 137 consecutive patients, admitted electively for carotid endarterectomy in the period 1998-2000. 125 patients had cerebrovascular symptoms: either stroke (neurological deficit greater than 24 hrs), transient ischaemic attack (neurological deficit less than 24 hrs), or amaurosis fugax. 12 patients were asymptomatic. A medical history and fasting venous blood sample were taken for each patient. The plasma concentrations of cholesterol and of inflammatory markers (high sensitivity C-reactive protein (hs-CRP), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and soluble endothelial leukocyte adhesion molecule-1 (sE-selectin)) were determined.

Results: The concentration of hs-CRP in the symptomatic group (3.9 mg/L) was significantly higher than in the asymptomatic group (2.1 mg/L; $p = 0.04$). These concentrations were within the normal range (< 10 mg/L). There were no differences between the two groups for sICAM-1, sVCAM-1, sE-selectin and total cholesterol concentrations.

Conclusion: Plasma hs-CRP was elevated in symptomatic patients with carotid artery disease compared to asymptomatic patients. High sensitivity C-reactive protein has been shown to be of prognostic value for a number of coronary conditions. This study suggests it may be of value in identifying patients at high risk of developing neurological deficits.

4.2 Introduction

4.2.1 Asymptomatic carotid artery stenosis

The Asymptomatic Carotid Artery Stenosis Trial (ACAS 1995) has shown the benefit of carotid endarterectomy (CEA) in preventing future strokes, however the cost-effectiveness of this procedure is limited. Over 5 years, only 1 stroke will be prevented for approximately every 17 CEAs undertaken. In particular, asymptomatic patients with diabetes mellitus and contralateral siphon stenosis have a significantly higher risk of peri-operative stroke than other patients, so the benefit of CEA in preventing strokes in this group is marginal (Young *et al.* 1996). A simple test that could predict the risk of a carotid atherosclerotic plaque leading to a stroke might be useful in identifying patients with asymptomatic carotid stenosis who have higher risks of atheroembolism and would benefit the most from surgery.

4.2.2 Inflammatory mediators and cardiovascular risk factors

C-reactive protein is an acute phase protein, a 1.15 kD peptide composed of five identical subunits (Albert & Ridker 1999). Its name is derived from its ability to react with the somatic C-polysaccharide of streptococcus pneumonia. It is synthesized by a heptocyte response to many cytokines, especially interleukin-6. Its level can increase 100-fold in response to various inflammatory stimuli. Traditionally CRP tests have been useful in monitoring many inflammatory diseases such as pelvic infection, systemic lupus erythematosus, and inflammatory bowel disease and their response to treatment. More recently high sensitivity CRP (hs-CRP) tests have become available that can detect a change even in the normal range. It has been shown that this hs-CRP test can detect low-grade inflammation in the vascular system. As is well known, inflammation is the basic pathology of atherosclerosis, and greater degrees of inflammation of atherosclerotic lesions are associated with clinical events (Davies, M.J.1996; Husain *et al.* 1999). Therefore hs-CRP has been investigated for its potential to predict vascular events.

Many studies have shown the association between C-reactive protein (CRP) and atherosclerotic lesions, especially in coronary artery disease. Firstly in healthy people, 5 cohort studies investigated the association between the levels of CRP in people without any history of coronary events and the risk of future coronary events (Albert & Ridker 1999). All studies demonstrated that hs-CRP was a predictor of cardiovascular events. In a large prospective cohort study in healthy US male physicians, Ridker *et al.* (1997) found that men in the quartile with the highest CRP values had a 3-fold increased risk of future myocardial infarction (MI) compared to those in the lowest quartile. Secondly, in patients with active coronary artery diseases (angina) in the European Concerted Group, Haverkate *et al.* (1997) found angina patients in the highest quintile of CRP had twice the risk of coronary events of those in other groups. For patients with acute coronary syndromes (MI), Marrow *et al.* (1998) investigated the predictive power of CRP in patients with non-Q wave myocardial infarction (NQMI) or unstable angina. They found that elevated CRP in patients who presented with unstable angina or NQMI correlated with an increased 14-day mortality. Also in the Toss study (1997), which followed up patients with NQMI and unstable angina, it was found that patients with elevated CRP levels were associated with an increased risk of death. CRP has been studied in patients with peripheral vascular disease. In a large prospective cohort study Ridker *et al.* (1997; 1998a) found that high concentrations of hs-CRP were associated with a 2-fold increase in risk of ischaemic stroke and a 2-fold increase prevalence of symptomatic peripheral vascular disease. Similarly Curb *et al.* (1999) studied the correlation between CRP and subsequent thromboembolic stroke. They found that when patients were divided into three groups by CRP levels, subjects in the highest tertile had a greatest risk of thromboembolism than those in the first tertile. Gronholdt *et al.* (2001) found that elevated levels of CRP were associated with increased carotid plaque volumes, which were calculated by a histological technique.

There is also some evidence that chronic low-grade infections such as those caused by Chlamydia pneumoniae are associated with atherosclosis (coronary events). Most systemic microbial infections are associated with a high level of serum C-reactive

protein (Albert & Ridker 1999). Thus one could argue that the correlation between increased CRP levels and coronary events might be the result of chronic infection rather than the result of advanced atherosclerotic activity. However in the Heathy Physician study, no relationship was found between IgG titers and Chlamydia pneumoniae and subsequent MI risk (Ridker *et al.* 1999a). There was no association between IgG and CRP either.

There is still uncertainty about the pathogenic role of CRP. It is still not known whether increased CRP is a cause or an effect of atherosclerotic activity in plaques. For instance some theories have been proposed for CRP as a cause. CRP activates complement within plaques and CRP stimulates production by monocytes of tissue factor (the coagulation responsible for occlusive thrombotic events) (Pepys & Berger 2001). On the other hand, CRP might be the result of an extensive inflammatory process in plaque or plaque instability. It was found that macrophages, which are abundant in unstable plaques, secrete interleukin-6, which stimulates the production of CRP in the liver (Albert & Ridker 1999; Morrow & Ridker 2000). Furthermore increased CRP levels are associated with elevated levels of intercellular adhesion molecules such as intercellular adhesion molecules-1 (ICAM-1) and endothelial leucocyte adhesion molecules (E-selectin) (Albert & Ridker 1999). These adhesion molecules are associated with transmigration of leucocytes into the vascular endothelial wall, a critical process in atherogenesis.

Not only have CRP levels been used to reflect the activity of atherosclerotic lesions, but other mediators have also been used as well. Many mediators are involved in atherogenesis such as ICAM-1, vascular cell adhesion molecules-1 (VCAM-1), and E-selectin. The Atherosclerosis Risk In Communities study found a correlation between baseline ICAM-1, VCAM-1 and E-selectin levels and the risk of coronary heart events and the thickness of carotid arteries (Hwang *et al.* 1997). It was found that only ICAM-1 and E-selectin could predict these outcomes. In addition hyperlipidaemia, a traditional risk factor, has been correlated with the development of coronary heart

disease (Castelli 1984). However in a head-to-head comparison study high hs-CRP concentrations had a higher correlation with stroke and MI than serum amyloid A, soluble intercellular adhesion molecule-1 (ICAM-1), interleukin-6, total cholesterol, low density lipoprotein, cholesterol, apolipoprotein A-I, and homocysteine (Ridker *et al.* 2000). It is interesting that few studies have explored the levels of hs-CRP versus other inflammatory markers in patients with symptomatic carotid stenosis and asymptomatic carotid stenosis.

4.2.3 Hypothesis

From extensive correlations between inflammatory markers and coronary events, it was hypothesized that levels of inflammatory markers in symptomatic patients with carotid stenosis, in whom the plaques were presumed to be unstable, would be elevated compared to those in asymptomatic patients.

4.2.4 Objective

The objective of this chapter is to compare the levels of plasma inflammatory markers and total cholesterol concentrations in patients with symptomatic and asymptomatic carotid stenosis.

4.3 Methods

4.3.1 Patients and study design

137 consecutive patients were prospectively included who were participating in the ongoing trial (Chapter 2), the objective of which was to look at the effect of the n-3 PUFA supplementation on carotid plaque compared with n-6 PUFA and a placebo. Since the study in this chapter began after the main trial of Chapter 2 had begun, these 137 consecutive patients were the last 137 of the 168 patients in the ongoing main trial. The indications for surgery in most cases included symptomatic severe carotid stenosis (> 70%) and asymptomatic bilateral severe carotid stenosis (> 90%). The degree of

stenosis of all patients was assessed by duplex scan. Medical histories were recorded and a physical examination carried out.

4.3.2 Cholesterol and inflammatory marker measurement

At the initial visit, fasting venous blood samples were collected in vacutainer tubes containing 0.12 ml of 15% EDTA. Plasma was prepared by centrifugation at 2500 rpm for 10 min and stored at -70°C. Plasma hs-CRP was measured by an immunoturbidimetric technique using a commercially available kit, produced by Wako Laboratories and available from Alpha Laboratories (Eastleigh, UK) (detail in Appendix 5). The technique had inter- and intra-assay coefficients of variation of 6.4% and 3.4% respectively. Plasma soluble E-selectin (sE-selectin) concentrations were measured using Quantikine ELISA kits from R & D Systems Europe (Abingdon, UK). The limit of detection was 0.1 ng/mL, and inter- and intra-assay coefficients of variation were less than 10% and 5%, respectively (detail in Appendix 6). Plasma sICAM-1 and soluble vascular cell adhesion molecule-1 (sVCAM-1) concentrations were measured using Cytoscreen ELISA kits from BioSource (Nivelles, Belgium) (detail in Appendix 6). Limits of detection were 0.04 ng/mL (sICAM-1) and 0.5 ng/mL (sVCAM-1). Inter- and intra-assay coefficients of variation were less than 5% for both assays. Plasma total cholesterol concentrations were measured using a commercially available, enzyme-based diagnostic kit (Sigma Chemical Co., Poole, UK) (detail in Appendix 2).

4.3.3 Statistical analysis

Since the data were not normally distributed, univariate analysis was performed using the Mann-Whitney U-test and Spearman's rank correlation test. Hs-CRP concentration was logarithmically (ln) transformed prior to multivariate analysis to obtain a normal distribution. Analyses were done with SPSS version 10 (SPSS, Chicago, IL USA) and in all cases a value for p less than 0.05 was considered as statistically significant.

4.4 Results

There were 137 patients: 85 males and 52 females. The median age was 73 years (range 45-85 years). 12 patients were in the asymptomatic group and 125 patients in the symptomatic group. Patients were defined as asymptomatic if they had never had any experience of neurological deficit, either a transient ischaemic attack (TIA) or stroke related to the affected vessel. Baseline clinical data and cardiovascular events were evenly matched in the two groups (Table 4.1 & 4.2). In the symptomatic group the median duration between the last reported symptom and venous blood sampling was 3 months.

Table 4.1 Clinical characteristics of the patients.

Variable	Symptomatic Group N=125	Asymptomatic Group N=12
Median Age (years) (range)	73 (45-85)	73 (56-78)
Body-Mass Index (median; kg/m^2) (range)	26 (19-43)	25 (19-31)
Gender: Males (%)	78 (62)	7 (58)
Hypertension (%)	88 (70)	8 (67)
Diabetes mellitus (%)	25 (20)	2 (17)
Hypercholesterolaemia (%) (Plasma cholesterol ≥ 5 mmol/L)	36 (28.9)	7 (54.5)
Smoking		
Former smoker (%)	81 (65)	9 (75)
Current smokers (%)	18 (14)	1 (8.3)
Non-smokers (%)	26 (21)	2 (17)
Angina pectoralis (%)	38 (30)	3 (25)
Previous MI (%)	11 (9)	2 (17)
Intermittent claudication (%)	32 (26)	4 (30)
Critical limb ischaemia (%)	0 (0)	0 (0)

N = Number of patients, MI = Myocardial infarction

Table 4.2 The degree of internal carotid artery stenosis and medication between symptomatic and asymptomatic patients.

Variable	Symptomatic Group N=125	Asymptomatic Group N=12
Ipsilateral ICA stenosis		
- Moderate (51-74%) (%)	18 (14)	0 (0)
- Severe (75-99%) (%)	108 (86)	12 (100)
Contralateral ICA stenosis		
- Moderate (51-74%) (%)	15 (12)	3 (25)
- Severe (75-99%) (%)	19 (15)	4 (33)
Lipid lowering drug (Statin, Fibrate) (%)	48 (38)	2 (17)
Aspirin (%)	125 (100)	12 (100)

Data are presented as number of patients (percentage).

The median concentration of hs-CRP for all patients was 3.5 mg/L (range 0.2-84.9 mg/L). In univariate analysis, the concentration of hs-CRP in the symptomatic group was significantly higher than it was in the asymptomatic group (Table 4.3). However there were no differences between these groups in terms of the concentrations of sICAM-1, sVCAM-1, sE-selectin, and total cholesterol. The median concentration of hs-CRP in stroke patients (4.7 mg/L, range 0.5-84.9 mg/L) was not significantly higher than it was in the transient neurological deficit group (3.5 mg/L, range 0.2-36.9 mg/L) ($p = 0.36$). No correlation was found between hs-CRP concentration and the time delay between onset of last symptoms and blood withdrawal ($r = 0.08$; $p = 0.33$). The median concentration of hs-CRP in males (3.7 mg/L, range 0.2-84.9) was not significantly different to that in females (3.5 mg/L, range 0.3-29.1) ($p = 0.81$).

Table 4.3 The comparison of concentration of inflammatory markers between symptomatic and asymptomatic patients (median-range) (Mann-Whitney U test).

Concentration of Markers (median) and (range)	Symptomatic group N= 125	Asymptomatic group N= 12	p value
hs-CRP (mg/L)	**3.9 (0.2-84.9)**	**2.1 (0.3-9.9)**	**0.04**
sICAM-1 (ng/mL)	323 (130-719)	337 (235-597)	0.66
sVCAM-1 (ng/mL)	616 (319-2 192)	582 (396-860)	0.44
sE-selectin (ng/mL)	43 (14-109)	44 (22-87)	0.44
Total cholesterol (mmol/L)	4.6 (3.0-11.0)	5.3 (3.4-7.2)	0.08

In multivariate analysis, the significant association between hs-CRP concentration and symptoms remained unaffected when adjusted for the duration from the last event (Table 4.4) and became even more pronounced when possible confounders were taken into account (Table 4.5).

Table 4.4 hs-CRP regression coefficients from multivariate regression analysis of the presence of neurological deficit on ln hs-CRP adjusted for the time period since the last event.

	B	SE	95% CI	p value
Symptomatic/ Asymptomatic	0.75	0.32	0.12-1.38	**0.02**
Duration since last event (months)	-0.03	0.01	-0.01-0.01	0.56

B: Regression coefficient, SE: standard error, CI: confidence interval.

Table 4.5 hs-CRP regression coefficients from multivariate regression analysis of the presence of neurological deficit on ln hs-CRP adjusted for reported confounding factors.

	B	SE	95% CI	p value
Symptomatic/Asymptomatic	0.83	0.33	0.18-1.50	**0.01**
Duration since last event (months)	-0.004	0.01	-0.01-0.01	0.43
History of intermittent claudication	0.01	0.29	-0.56-0.58	0.98
History of angina pectoralis or MI	0.52	1.08	-1.61-2.65	0.63
Age	-0.01	0.01	-0.03-0.02	0.63
Smoking status	-1.91	1.07	-4.03-0.21	0.08
Body-mass index	0.07	0.03	0.02-0.12	0.01
Gender	-0.16	0.19	-0.55-0.22	0.40
Hypertension history	0.28	0.21	-0.15-0.70	0.20
Hypercholesterolaemia	-0.22	0.20	-0.62-0.19	0.29
History of diabetes mellitus	0.03	0.75	-1.45-1.51	0.97

B: Regression coefficient, SE: standard error, Hypercholesterolaemia (plasma cholesterol ≥ 5mmol/L), CI: confidence interval, MI: myocardial infarction

4.5 Discussion

4.5.1 Hs-CRP and carotid disease patients

This study shows that symptomatic patients with severe carotid stenosis have elevated hs-CRP compared with asymptomatic patients. This is the first study to show the difference of the level of hs-CRP between symptomatic and asymptomatic carotid stenosis (Table 4.3). However, it cannot be concluded from this data that a high CRP level is a predictor of the progression of carotid disease because this study is cross-sectional. This issue can only be resolved by a large-scale cohort study. If a high CRP level turns out to be a predictor of unstable plaque, hs-CRP may have a benefit in identifying asymptomatic carotid stenosis patients at a higher risk of neurological deficit. To confirm this benefit, a randomised controlled trial would need to be carried

out on asymptomatic patients with high levels of hs-CRP who are being treated surgically and medically.

The upper limit of normal for hs-CRP is 10 mg/L (Tracy 1998). The median levels of hs-CRP in the carotid stenosis patients in both the symptomatic and asymptomatic groups were less than this (3.9 and 2.1 mg/L respectively) (Table 4.3). However hs-CRP did not correlate with the severity of the clinical condition (stroke, TIA) or with the duration between the onset of the last neurological symptom and sampling. This supports the findings of Canova *et al.* (1999), who showed that CRP levels in 138 patients with acute neurological deficit were not different for patients in five clinical categories: transient ischaemic attack, reversible neurological deficit, complete stroke and recovery, stroke without recovery, and cerebral haemorrhage. The concentration of hs-CRP increases in response to acute ischaemia, like most other acute phase proteins (Pepys 1996) and raised hs-CRP concentrations have been reported in many conditions, such as cigarette consumption (Das 1985). Therefore it is likely that cerebral ischaemic events and other factors (e.g. smoking) were confounding variables in the current study. However, the association between symptoms and the concentration of hs-CRP remained significant after adjusting for the duration since the most recent neurological deficit (Table 4.4). Furthermore the difference was still significant after adjusting for the possible confounding variables, including smoking status and ischaemia of the heart and the lower extremities (Table 4.5).

4.5.2 Hs-CRP and other mediators

A number of prospective studies have identified peptide mediators such as other acute-phase proteins, cytokines, and soluble adhesion molecules to be markers of cardiovascular disease (Harris *et al.* 1999; Hwang *et al.* 1997; Mocco *et al.* 2001). However, hs-CRP appears superior to other markers, a large cohort study in women showed hs-CRP to be more closely associated with cardiovascular events than other parameters (Ridker *et al.* 2000). The plasma concentration of CRP can increase several hundred fold following stimulation and is not affected by hormonal changes or

anti-inflammatory drugs (Morrow & Ridker 2000; Tracy 1998). Furthermore, in contrast to many other inflammatory markers, the assay techniques for hs-CRP are highly sensitive and can detect even minor elevation of CRP within the normal range (Morrow & Ridker 2000). On the whole the hs-CRP assay provides a simple and reliable measurement for the assessment of systemic inflammation.

Although CEA has proved to be beneficial in severe symptomatic carotid stenosis in two large trials (ECST 1991; ECST 1998; NASCET 1991), 8-10 CEAs are needed to prevent one stroke over three years. Rothwell *et al.* (1999) tried to increase the efficacy of surgical intervention by developing a model that targeted patients who had a high chance of developing further neurological events. Hs-CRP concentration after stroke has been found to be a marker of increased one-year risk in recurrent ischaemic stroke (Di Napoli, Papa & Bocola 2001). If a future cohort study shows that hs-CRP is able to predict future neurological events in patients with severe symptomatic carotid stenosis, surgical targeting might be improved.

4.5.3 Mechanism of raised hs-CRP

As mentioned earlier, the mechanism of the association between raised hs-CRP and cardiovascular symptoms is still uncertain. The raised level of this inflammatory marker may reflect an epi-phenomenon associated with atherosclerotic plaque and some experimental studies identified CRP binding in the foamy macrophages of atherosclerotic lesions (Hatanaka *et al.* 1995). It has been hypothesized that CRP might encourage lipid uptake and cytolysis of the atherosclerotic lesion, which would enlarge the necrotic area in the lesion (Hatanaka *et al.* 1995). An alternative hypothesis is that raised CRP concentrations might be related to cardiovascular events via a causal pathway such as increased thrombus formation, lipid oxidation, and cell activation and proliferation (Tracy 1998). Tohgi *et al.* (2000) found activation of the coagulation/fibrinolysis system and platelet function in patients with acute thrombotic stroke with raised CRP.

4.5.4 Limitations

CRP level is usually elevated in injury or inflammation, including ischaemia or infection. In this study blood samples were taken after some of the patients had had symptoms, so the results might have been confounded by the effect of cerebral ischaemia. To overcome this, a longitudinal study should be carried out. Although in this study blood samples were not taken when patients had any kind of obvious infection such as a common cold, this did not exclude all infections. This study also contained a potential selection bias. There was a low proportion of asymptomatic cases (12 asymptomatic patients/ 125 symptomatic patients), so the conclusions of this study are based on only 12 patients, resulting in a high probability of a type 1 error. There was a high proportion of bilateral severe asymptomatic carotid stenosis in these 12 patients, because unilateral severe carotid stenosis in asymptomatic patients is not a routine indication of surgery in the unit when this study was conducted.

4.6 Conclusion

In this study, patients with symptomatic carotid stenosis had elevated hs-CRP compared with asymptomatic patients. This elevation of hs-CRP concentration was associated with the presence of neurological events. The hs-CRP level might be useful in selecting asymptomatic carotid stenosis patients who will benefit from CEA. A large prospective cohort study would be necessary to confirm this observation before clinical application.

In the first section of this thesis, the risk factors associated with atherosclerotic plaques in the carotid artery have been investigated. Unstable plaques are associated with many vascular events such as strokes and MI. To reduce the risk of these vascular events, the potential of n-3 PUFA supplements in patients with carotid atherosclerotic plaques has been studied and the incidence and management of hyperlipidaemia in these patients has also been investigated. In addition in this chapter the potential of inflammatory markers, especially CRP, has been explored, which might help to predict the risk of neurological event in patients with carotid plaque. Surgical removal of atherosclerotic plaque around the carotid bifurcation in carotid endarterectomy has also decreased the risk of

ischaemic stroke in some patients (NASCET 1991; ECST 1991). However carotid endarterectomy may be harmful in these patients because of substantial associated morbidity and mortality. Whether the operation will benefit a patient depends on the balance between the risk of stroke and death resulting from surgery and the risk of ipsilateral ischaemic stroke without surgery. In section 2, the risk and the risk factors of stroke and death following carotid surgery have been investigated.

Section 2

Risk factors associated with the outcome of carotid endarterectomy

Chapter 5

Overview of carotid endarterectomy

5.1 Summary

Recent findings regarding carotid endarterectomy (CEA) are reviewed, starting with the history of carotid surgery back to the period of John Hunter, and the relevance of carotid artery disease in clinical practice is discussed. Clinical trials of the treatment of carotid stenosis are reviewed extensively, and finally the complete range of clinical management of carotid stenosis and complication of carotid surgery are presented.

5.2 Evolution of carotid surgery

Atherothrombotic stenosis at or around the carotid bifurcation is associated with an increased risk of ipsilateral ischaemic stroke in carotid territory. This risk has been reduced by several methods such as risk factor management and anti-platelet therapy. This risk has also been lowered for some patients by CEA.

Stroke was first described a thousand years ago by Hippocrates, but at that time he used the term apoplexy, meaning "to be stricken by an external force". He prescribed medication for it on the basis of scientific principles (Robinson & Bailes 2002). Two hundred years ago, John Hunter, who is regarded as the founder of scientific surgery, became interested in carotid disease and there are two interesting specimens in the Hunterian Museum at the Royal College of Surgeons of England (Barker 1996). One demonstrated an atherosclerotic plaque with ulceration of the carotid bifurcation, found incidentally at a post-mortem examination of a patient who had died of ruptured thoracic aneurysm. No one knows what led John Hunter to identify and dissect this specimen. Another specimen is a carotid artery aneurysm, which is described in the notes of the display as follows "An aneurysm sac of an oval form, and about three fourths of an inch in its chief diameter, from the right internal carotid artery of a lady. It is nearly full of coagulum". In 1835 Barth, a French physician, reported the results of a physical examination of one of his patients, who had intermittent claudication caused by a thrombosis of the terminal aorta. The report specifically mentioned "no murmurs were heard over the carotid arteries in the neck" (Barker 1996). Why should Barth have noted

that particular item in a physical examination at that time? It may be that he knew that the disease of claudication and carotid disease were the same pathology. Not long after that, evidence suggests that some physicians had started to suspect that the neck vessels were the cause of cerebrovascular insufficiency. In 1856 William Savory described a young patient who had episodes of giddiness, indistinct vision in the left eye, and a carotid bruit on physical examination (Barker 1996). On post-mortem examination of this patient he found diffused atherosclerotic lesions of the innominate artery and both left and right carotid arteries. He suspected some relationship between a deficiency of carotid blood flow and neurological deficit in the ipsilateral hemisphere. In 1905 Chiari proposed that a thrombus in the carotid sinus could cause stroke. He based this on 400 autopsies of patients, in which four out of seven with carotid thrombi had had a history of cerebral emboli while they were alive (Fisher 1951). In 1915 Ramsay Hunt described the syndrome of internal carotid occlusion. He emphasized the importance of obstructive lesions of the internal carotid artery in the causation of cerebral infarction (Eastcott, Pickering & Rob 1954). However at that time the work of both Chiari and Hunt had not been taken seriously by other clinicians (Fisher 1951) and most of the evidence for the relationship between carotid disease and stroke was based on post-mortem material.

Following the development of X-rays in animals and cadavers by Wilhelm Röentgen, there was a great deal of exploration of the use of cerebral angiograms, which had the potential of allowing study of cerebral artery disease in living humans. Egas Moniz of Lisbon consolidated this fragmentary work and established on a firm basis the specialty of angiographic radiology, which for many years focused only on intracranial vessels (Barker 1996). In 1937 Moniz and colleagues found based on angiograms that hemiplegic patients had carotid occlusions more frequently than non-hemiplegic patients (Fisher 1951). As a result of these clinicians focused much attention on the correlation between brain infarction and stenosis. In 1948 Fisher (1951) proposed the same idea based on evidence from a large number of post-mortem examinations. He studied several patients suffering from embolic ischaemic phenomena but could not find

thrombi in any of the usual sources of systemic emboli such as the left heart, ascending aorta, or pulmonary vein. He established the cervical carotid bifurcation as a common source of emboli in patients with embolic strokes of the middle cerebral artery.

Many investigators have examined the nature of the association between stroke and extracranial artery stenosis found by Fisher. In 1962 Hollenhorst (1961) reported the presence of bright orange, yellow, or copper plaques at various bifurcations of the retinal arterioles that may have resulted from embolic crystals of cholesterol from any proximal point, including ulcers in carotid plaques. In 1963 Ormand and co-workers recognised the importance of ulceration in plaques, especially in those with a thrombus in the ulcer crater (Julian *et al.* 1963). This suggested that these ulcers were a source of intracranial emboli of both thrombotic and atheromatous material.

As the evidence of the correlation between atheromatous plaque and stroke was becoming clear, surgeons commenced exploring the role of carotid surgery in patients with stroke. Many surgical procedures have been advocated. Resection of a diseased segment was reported by Chao in 1938, cervical sympathectomy by Johnson and Walker in 1951, internal carotid artery and external carotid artery bypass by Carrea in 1951, thromboendarterectomy by Strully in 1953, and resection of the diseased segment and anastomosis of the internal carotid artery to the common carotid artery by Eastcott (1954) (Barker 1996; Vanmaele & Pickut 1991). On 7 August 1953, De Bakey (1975) carried out the first successful CEA, although he did not report it until 19 years later. His patient had presented with symptoms of circulatory insufficiency in the left carotid artery, including intermittent episodes of weakness of the right arm, hesitancy in speaking, and difficulty in writing clearly. De Bakey performed this CEA based on the good outcomes that he had had with endarterectomy for segmental atherosclerotic lesions causing circulatory insufficiency in other arteries, particularly those in the legs. He believed that the same procedure would restore normal circulation to the patient's brain. After this operation the patient returned to work as a school bus driver and showed definite improvement in speech. He had no further manifestations of transient

cerebral attacks. He died 19 years later from coronary artery disease. After 1960, CEA emerged as the most frequently used technique when dealing with carotid atheroma (Movius, Zuber & Gaspar 1966).

At the beginning CEA was used for all types of carotid stenosis, including patients with carotid artery occlusion. There appeared to be little rationale for patient selection based on the nature of the carotid disease. CEAs were also performed on patients with poor prognoses, including those who had just suffered a severe disabling stroke. As a result the outcomes were not good. It was soon recognised that CEA did not have great benefit when the carotid artery was completely occluded. However they were of value in an earlier stage, the stenotic period (Rob 1961). In addition, with time Thompson *et al.* (1962) proposed that the outcomes of CEA in acute stroke were poor. Javid (1966) suggested using this operation to prevent strokes in particularly good candidates, such as those with transient ischaemic attack (TIA), based on his own experience. Then in 1991 the benefits of CEA were established firmly on the basis of the interim results of two big studies, the European Carotid Surgery Trial (ECST 1991) and the North American Symptomatic Carotid Endarterectomy Trial (NASCET 1991). However there are still some controversies about this operation, such as the role of this operation in asymptomatic cases, anaesthetic technique, cerebral protection, temporary intraluminal shunting, and patch angioplasty.

5.3. Epidemiology, morbidity and mortality

Stroke is a great problem in the western world. It is the third most common cause of death (Wolfe 2000). In 1997, there were 57,747 deaths in England and Wales from stroke. In addition, treatment of patients with stroke accounted for at least 4-6% of the NHS budget in the UK. 20% of acute beds and 25% of long-term beds were used for stroke patients (Wolfe 2000), causing the UK government to focus on it as a major health issue (National service framework 2001).

Stroke is defined as an acute loss of focal or occasionally global cerebral function with symptoms exceeding 24 hours or leading to death, which after due investigation has a vascular origin (Naylor 1999). A transient ischaemic attack (TIA) is defined as a focal neurological deficit of sudden onset that resolves completely within 24 hours. TIA appears mainly in two ways: one is as a hemispherical symptom (cerebral TIA) and the other is as an eye symptom, amaurosis fugax.

From the Harvard Cooperative Stroke Registry (Mohr *et al.* 1978), the incidence of stroke caused by infarction and haemorrhage was 84% and 16% respectively. Of 694 patients in this registry, 364 (53%) were diagnosed as having thrombosis, 215 (31%) as having cerebral embolism, 70 (10%) as having intracerebral haematoma, and 45 (6%) as having subarachnoid haemorrhage from aneurysm or arteriovenous malformations. Of the 364 patients diagnosed as having thrombosis, 233 (34% of all 694 patients) had thrombosis believed to involve a large artery. Of these patients with large vessel thromboses, 102 (14.7% of 694 patients) were found by angiography to have carotid stenosis or occlusion. Carotid stenosis is therefore a main cause of strokes due to thrombosis.

5.4 Clinical features

Extracranial carotid stenosis can present into two ways: symptomatic and asymptomatic.

5.4.1 Symptomatic carotid stenosis

The typical symptoms of carotid disease are as follows (Toole, Dibert & Harpold 1996):

A. Focal hemisensory/hemimotor deficits, on contralateral side.
B. Visual defects: unilateral blindness, ipsilateral blindness (amaurosis fugax).
C. Speech and/or language defect: dysarthria or dysphasia.

5.4.2 Asymptomatic carotid stenosis

It is not uncommon for the disease to be asymptomatic. About 10% of people older than 70 years of age have asymptomatic carotid stenosis, with a degree of stenosis greater than 50% (Naylor 1999). This problem is usually detected incidentally, for example from auscultation of the neck or following carotid artery scanning of the contralateral symptomatic artery.

5.5 Investigation

The duplex scan has largely replaced carotid angiography as the first line of investigation. This has been supported by the results of a randomised controlled trial, in which no difference in neurological complication was found between pre-operative duplex scan alone or pre-operative duplex scan together combined with angiography (Deriu *et al.* 2000). Duplex scan combines real-time (B-mode) imaging with Doppler waveform analysis. Now colour duplex scan (Figure 5.1) has greater accuracy with a sensitivity of up to 90%. However, since duplex scan is dependent on flow and may be less accurate in the presence of severe contralateral internal carotid artery (ICA) disease because of altered flow dynamics. Several groups have modified the criteria for duplex in patients with severe contralateral ICA disease, and this modified criteria has correlated very well with angiograms (AbuRahma *et al.* 1995). Pre-operative angiograms (Figure 5.2) are still used routinely in some centres (Long *et al.* 2001). However the most worrying problem of angiography is the incidence of stroke following a selective carotid angiogram, which is 0.5%. In symptomatic patients with carotid stenosis greater than 50%, there is an associated incidence of stroke of 2% following an angiogram (Naylor 1999). In practice angiograms tend to be used in some limited situations, such as when trickle flow is suspected, or there are technical difficulties with duplex.

Figure 5.1 Color duplex scan of carotid artery.

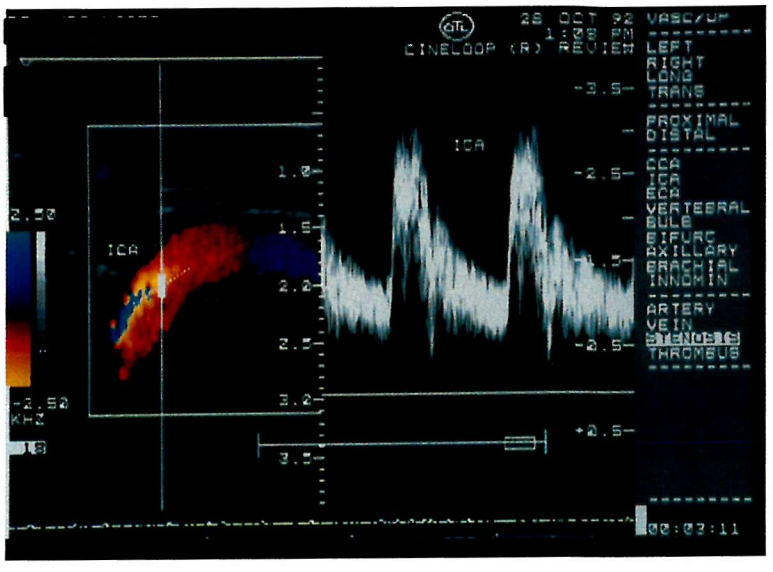
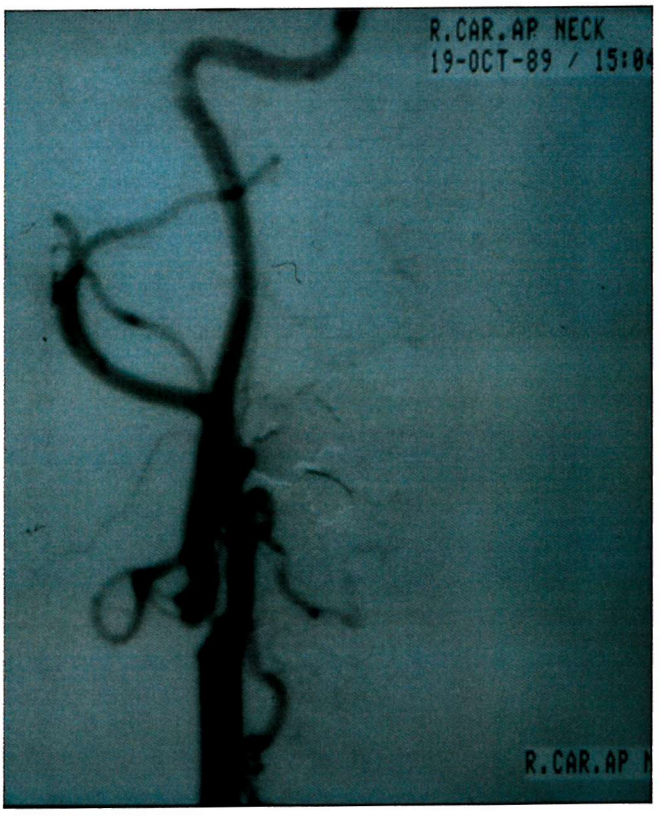


Figure 5.2 An example of a carotid angiogram showing the severe stenosis of internal carotid artery.



It is important to note that there is a discrepancy in the degree of stenosis measured in the angiograms of NASCET (1991) and ECST (1991). In the NASCET study, the principal neuroradiologist measured the diameter of the internal carotid artery at the point of greatest stenosis and at the normal part of the artery beyond the carotid bulb. The percentage of stenosis was then calculated by using the ratio of these two measurements. In ECST, a neurologist at the trial centre measured the diameter at the point of the greatest stenosis and the estimated carotid bulb. The ratio of these two measurements was then used to calculate the percent of stenosis. However it is possible to convert the measurements made by one method to those of the other method using a simple equation (ECST% stenosis = 0.6 NASCET% stenosis + 40%) (Rothwell *et al.* 1994).

Magnetic resonance angiography has developed enormously in the last ten years. The main advantage of magnetic resonance angiography over duplex scan is the ability to image the arch vessels, the carotid siphon, and intracranial circulation. However there may be problems of interpretation because of a state of low flow above a critical inflow stenosis and the tortuosity of the distal ICA (Humphrey 2000a). Further refinements are needed.

The role of CT brain imaging in extracranial carotid artery disease remains controversial. Although NASCET and ECST showed a clear benefit of CEA overall, CEA in some patients with intracranial lesions such as intracranial aneurysms have excessive risk. However the incidence of intracranial aneurysm associated with significant extracranial occlusive lesions is around 2% (Donaldson 2000). The majority of these aneurysms are small and the risk depends on the size. The usual indication for surgery is a size of 7-10 mm. On the basis of this, routine pre-operative brain scanning does not seem to be cost-effective (Donaldson 2000). In practice, it tends to be used in some circumstances: for patients in the acute phase of stroke to exclude haemorrhage, for any patient under consideration for urgent or emergency CEA, or for patients with atypical symptoms or examination findings (Thomas & Donaldson 2000).

The pre-operative cardiac investigation is of paramount importance, since myocardial infarction (MI) remains the principal cause of mortality following CEA, in particular for patients with pre-existing cardiac disease. The pendulum between extensive pre-operative cardiac evaluation and no pre-operative cardiac evaluation has swung freely over the past decade, usually ending at the midpoint. In the early 1980s Hertzer *et al.* (1985) at the Cleveland Clinic studied the true incidence of coronary artery disease in patients undergoing CEA. They performed coronary angiography pre-operatively on 506 patients, sixty percent of whom had a clinical history of coronary artery disease (CAD). In the patients who had a history of CAD, 35.6% were found to have severe correctable coronary artery disease and 9.8% were found to have severe inoperable coronary artery disease. 16% of patients without any history of CAD were found to have severe correctable CAD and 9.8% were found to have severe inoperable CAD. Only 13.5% of patients with no clinical suspicions of CAD had normal coronary arteries. The pendulum began to swing in the other direction in the early 1990s as a result of cost considerations. It might be sensible and sufficient to have an initial pre-operative cardiac evaluation of a history, physical examination, and an electrocardiogram for patients with no unstable angina, no angina at rest, no severe arrhythmias, and no limitations of daily life functions (William *et al.* 1999). On the other hand, patients with these significant cardiac problems should undergo further cardiac testing. Several types of cardiac testing are available including exercise stress testing, dipyridamole myocardial scintigraphy, echocardiography with or without stress, and Holter monitoring for ischaemia. The medical management and risk factors are optimised for patients who are found to be low-risk based on these non-invasive tests, and they proceed to surgery (Belkin & Sebatian 2000). Patients who are classified as high risk based on these non-invasive tests are evaluated for indications of coronary revascularisation. If this does not show any indications of coronary reconstruction such as class III-IV angina or left ventricular ischaemic dysfunction, patients may be considered for carotid surgery with the precautions as mentioned above. If patients have indications of coronary revascularisation, they undergo an aggressive program of

medical optimisation and risk factor modification. It is appropriate to consider cancellation of surgery for patients with asymptomatic carotid stenosis in this subgroup. Those who have indications of coronary revascularisation undergo coronary arteriography. If the lesion is correctable, patients will be referred for coronary revascularisation such as coronary artery bypass grafting (CABG) or angioplasty. The scheduling of CEA as prior, simultaneous or post-CABG depends on the presentation and degree of disease that each patient has and remain very controversial.

5.6 Indications for surgery

In this era of evidence-based medicine, prospective randomised controlled trials are considered as the gold standard (level 1 evidence), which tends to have a great effect on clinical practice. There are two types of trials in this area: symptomatic and asymptomatic trials.

5.6.1 Symptomatic carotid stenosis trials

There have been three clinical trials comparing CEA and the best medical treatment in symptomatic carotid stenosis: the European Carotid Surgery Trial (ECST) (1991), the North American Symptomatic Carotid Endarterectomy Trial (NASCET) (1991), and the Veterans Administration Symptomatic Stenosis Trial (VASST) (Mayberg *et al.* 1991). The primary outcomes of these three trials were reported in 1991. The inclusion criteria of these trials were very similar: patients with a recent history of TIA, amaurosix fugax, or minor stroke in the distribution of the internal carotid artery. The ECST and NASCET studies recruited patients from populations in a multi-centre collaboration, whereas VASST included only men at Veterans Administrative Centres in the USA. All trials used angiography, although there were variations in inclusion criteria: 0% to 99% stenosis in the ECST and 30% to 99% in the NASCET studies and 50% to 99% stenosis in the VASST. Exclusion criteria were similar in all three trials. Patients were excluded who would not have been good surgical candidates because of their overall medical conditions. The primary end point for all three trials was defined as cerebral infarct

ipsilateral to the stenosed carotid artery. The VASST trial also included crescendo TIA distributed appropriately as a primary end point.

In the ECST trial (1991; 1998), patients were stratified into three groups: those with mild (< 30%), moderate (30-69%), and severe (70-99%) carotid stenosis. This study was terminated with an interim analysis of 2200 patients with a mean follow-up interval of 2.7 years for patients with symptomatic mild and severe carotid stenosis. Only patients with severe carotid stenosis (778) had outcomes favoring carotid surgery. In the severe carotid stenosis (70-99%) group, the incidence of any peri-operative stroke was 7.5% and there was an additional 2.8% risk of post-operative stroke at 3 years after surgery compared with 16.8% after medical management (the control group), a six fold reduction ($p < 0.0001$) (ECST 1991). The ECST final report (1998) stated that in general on weighing the risk of peri-operative complications against the long-term risk of stroke without surgery, CEA was worthwhile when the degree of stenosis was greater than 80%.

In the NASCET study, patients were stratified into three groups: those with mild (< 50%), moderate (50-69%), and severe (70-99%) carotid stenosis (Barnett *et al.* 1998; NASCET 1991). This study was terminated early because carotid surgery had reduced the risk overwhelmingly in severe carotid stenosis (659 patients) compared with medical treatment (NASCET 1991). From life table analysis, the estimate of the risk of ipsilateral stroke in two years in the medical group was 26%, whereas the same figure in the surgical group was 9% ($p < 0.001$). Therefore the absolute reduction of risk was 17% at two years. The number of patients that had to be treated to prevent one stroke was about six (NASCET 1991). The final report of the NASCET study stated that for patients with moderate stenosis (50-69%; 2226 patients), surgery was marginally beneficial, but 15 CEAs were able to prevent one stroke in five years (Barnett *et al.* 1998). There was no surgical benefit for patients in the mild stenosis group (Barnett *et al.* 1998).

The VASST study (Mayberg *et al.* 1991) was terminated prematurely because of strong evidence of the benefit of CEA in the ECST and NASCET studies. The results of this study are based on only 193 men who had symptomatic carotid stenosis greater than 50%. Two thirds of these patients had severe carotid stenosis (> 70%). The mean follow-up period was 11.9 months. For patients in the severe carotid stenosis group, the risk of stroke and crescendo TIA in the surgical group (7.9%) was much less than that of the medical group (25.6%). The absolute risk reduction was 17.7% ($p = 0.004$). Unfortunately in the moderate stenosis group it was difficult to interpret the results because there were only 58 men in this group.

In summary, the ECST and NASCET trials provided solid evidence that compared to medical treatment CEA reduced the risk of subsequent stroke for patients with symptomatic high-grade stenosis (> 70% by the NASCET angiographic method). The benefit of this surgery for patients with a moderate degree of stenosis (50-69% by the NASCET method) was marginal. No benefit was found for patients with mild stenosis (< 50% by the NASCET method) for surgery compared with the best medical treatment. In spite of these results, several issues must be considered. For example, a major determinant for a successful outcome was low surgical risk associated with the procedure. This implies that an operation should be done by experienced surgeons or those training under their supervision. Also the results must be applied carefully to general practice. The patients in the trials may not have been the same as the normal population of patients. For example the NASCET study excluded any patients older than 80 and anyone with severe medical conditions.

5.6.2 Asymptomatic carotid stenosis trials

There have been four major published randomised studies of asymptomatic trials: the Carotid Artery Stenosis Asymptomatic Narrowing Operation Versus Aspirin (CASANOVA 1991), the Mayo Asymptomatic Carotid Endarterectomy (MACE 1992), the Veterans Administration Asymptomatic Stenosis Trial (VAAST) (Hobson *et al.* 1993) and the Asymptomatic Carotid Atherosclerosis Study (ACAS 1995). In addition

there is an ongoing trial: the Asymptomatic Carotid Surgery Trial (ACST), a large European trial that is still recruiting and has now randomised in excess of 2000 patients (Halliday, Thomas & Mansfield 1994). It is expected that the results of this study will be published after 2002.

These trials recruited patients who had never had any symptoms of ipsilateral cerebral ischaemia caused by carotid stenosis, although patients with contralateral symptoms were accepted for ACAS and VAAST. The cut-offs for carotid stenosis were similar: 60% for ACAS and 50% for the others. All patients in VAAST were investigated by angiograms, whereas the ACAS used angiography only for patients in the surgical group. The CASANOVA and MACE trials required only non-invasive assessment. ACAS screened participating surgeons for peri-operative morbidity and mortality rates of less than 3%. Patients were excluded if they were poor surgical candidates because of general medical criteria (e.g. renal failure or diabetes), had a condition that might have affected stroke outcome, namely neurological deficit (e.g. seizure), or had a cardiac condition (e.g. atrial fibrillation). All of the asymptomatic trials used the best medical management, including risk factor management and aspirin for both surgical and medical groups, except the patients in the MACE surgical group, who did not have any aspirin.

The CASANOVA (1991) study did not find any difference in the stroke and fatal stroke rates between the surgical group (10.7% of 206 patients) and the medical group (11.3% of 160 patients). However this study had serious flaws in the design, including the exclusion of patients with carotid stenosis greater than 90% and a high crossover rate of patients from the medical group to the surgical group. The CASANOVA study has failed to have a significant impact on clinical care because of these shortcomings.

The MACE (1992) trial was terminated prematurely after 30 months of recruitment (158 patients) because there was an increase in frequency of myocardial infarcts and TIAs in the surgical group. These patients did not receive aspirin. Because there were

too few primary outcome events in this study, it was difficult to draw any conclusions. However the authors did suggest that the use of aspirin was appropriate throughout the peri-operative and post-operative period.

The VAACET study found a significant difference in the combined incidence of ipsilateral events (TIA, amaurosix fugax, and stroke) between the surgical group (8.0% of 221 patients) and the medical group (20.6% of 233 patients) ($p < 0.001$) (Hobson *et al.* 1993). However it did not show any significant difference in the incidence of stroke between these two groups. No difference between groups was found in mortality, perhaps because the sample size was too small. For these reasons, this study has not been accepted for clinical decision-making.

The results of ACAS (1995) indicated that CEA might prevent stroke in selected patients with asymptomatic carotid stenosis. The trial projected the aggregated risk over five years for ipsilateral stroke and peri-operative stroke or death to be 5.1% for the surgical group (mean follow-up interval 2.7 years, 825 participants) compared with 11% for patients managed medically (834 participants). This resulted in an aggregate risk reduction of 53% ($p < 0.001$). In other words, around 17 operations were required to prevent one stroke over five years.

In summary, on the basis of the ACAS studies, CEA can marginally reduce the risk of stroke in selected patients with asymptomatic carotid stenosis of greater than 50% to 60%. It is interesting to note in this study that patients, surgeons, and institutions were specifically selected for low surgical risk. Therefore any centre that would like to apply these results in practice must be sure that it has done everything possible to maximise good surgical outcomes, as most centres in the trials did. In particular the morbidity and mortality rates of surgeons must be less than 3%. It is interesting to note that in the ACAS study, the surgical benefit for women was not apparent.

5.7 Treatment

5.7.1 Non-operative treatment

Disease of the internal carotid artery is mostly atherosclerotic in origin. It should be kept in mind that the cause of death in patients with carotid atherosclerosis is most frequently MI and not stroke (Caplan & Silver 2000). Modification of atherosclerotic risk factors is therefore vitally important not only for stroke prevention but also for prevention of premature cardiac death. Also, regardless of the need for surgery, every patient with carotid stenosis requires both anti-platelet and risk factor management.

5.7.1.1 Anti-thrombotic therapy

It is generally accepted that aspirin is the first choice for anti-platelet treatment for patients with carotid stenosis. Aspirin can reduce subsequent stroke and MI by about 20% (Humphrey 2000b). Following the MACE (1992) trial, it has been recommended that CEA patients should take aspirin throughout the operative period; otherwise patients have an increased risk of MI. Furthermore alternative anti-platelet drugs such as clopidogrel or a combination of aspirin and dipyridamole are available. Fibrinogen may also play a primary role in pathogenesis of a carotid-related atherothrombotic neurological event, and omega-3 fish oils, pentoxophylline, atromid, and ticlopidine have some fibrinogen-lowering effect (Caplan & Silver 2000; Haglund *et al.* 1991). However the clinical relevance of their effect on fibrinogen is unknown. Anticoagulants such as warfarin have a role only in atrial fibrillation and at present there is no evidence to support the routine use of warfarin for TIA or minor stroke (Humphrey 2000b). On the whole, aspirin remains the drug of first choice.

5.7.1.2 Risk factor management

The Framingham study found that many risk factors such as hyperlipidaemia, hypertension, cigarette smoking, and elevated blood sugar are associated with coronary heart disease. These risk factors should be addressed seriously in order to improve long-term outcomes in patients with carotid stenosis (Castelli 1984). For instance cessation of

smoking also decreases the risk of ischaemic stroke and MI rapidly (Ockene & Miller 1997). As the Heart Protection Study has shown the clear benefit of simvastatin in patients with cerebrovascular disease (HPS 2002), hyperlipidaemia should be treated properly. Furthermore some risk factors are associated with the peri-operative outcomes of CEA. High blood pressure is the most important risk factor to be corrected. Rothwell *et al.* (1999) have reported the risk factors associated with peri-operative stroke and death following CEA using the ECST database. If patients have pre-operative systolic blood pressures greater than 180 mmHg, they have a high probability of peri-operative stroke and death (Hazard ratio 2.21, 95% CI 1.29-3.79).

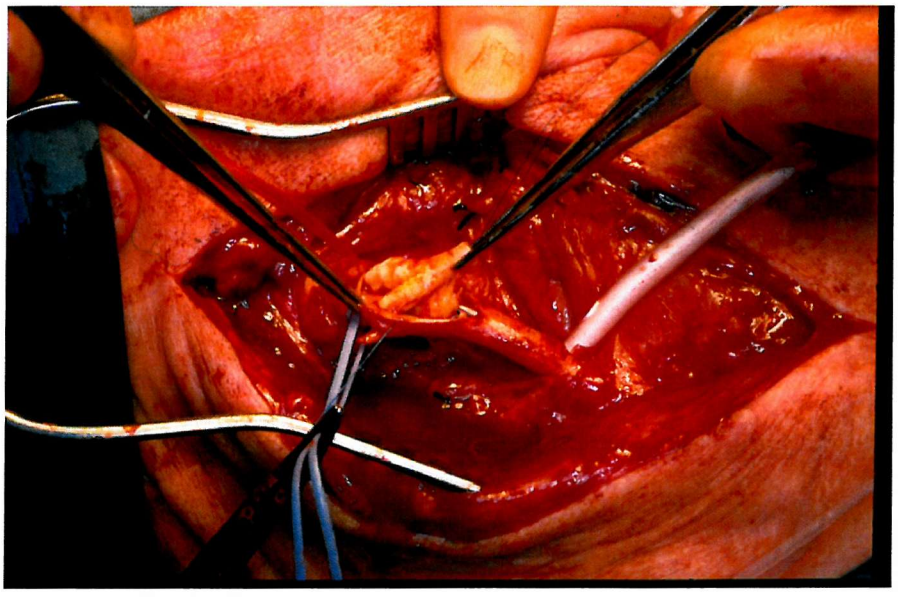
5.7.2 Carotid endarterectomy

The ECST and NASCET trials demonstrated that the benefit of CEA in patients with severe symptomatic carotid stenosis is clear. However carotid surgery only benefits fit patients operated on by surgeons with good track records. One has to take the physical status of a patient and the surgeon's operating record into account before advising patients to have a CEA. Careful discussion with patients is very important, especially about the benefit of surgery as secondary prevention and the risk of peri-operative stroke and death (Shearman 1993).

Two techniques have been described for CEA. In a standard endarterectomy, the most popular technique, carotid plaque is removed by a longitudinal arteriotomy (Figure 5.3). An eversion endarterectomy is performed through an oblique transection of the ICA from the common carotid artery, endarterectomy by eversion of the ICA, and reimplantation of the ICA on the common carotid artery. There is a lot of controversy about the technique of CEA. Thus surgeons tend to operate following their own preferences and many aspects still need to be studied on a prospective randomised basis. This controversy is discussed in more detail in the next section. However, certain aspects of general technique should be considered (Gough, Tan & Maritati 1999).

- Careful positioning of patients on the operating table. Cerebral blood flow may be compromised if patients undergo excessive rotation or extension of the neck.
- Minimal manipulation of the carotid artery during dissection to avoid embolisation.
- Good knowledge of anatomy, careful technique, especially during deployment of self-retaining retractors, and circumspect use of diathermy can minimise cranial nerve injury.
- The use of magnifying loupes for endarterectomy for the removal of small fragments of residue and for vessel repair.
- The use of sharp bent-on-flat scissors to cut the proximal part of plaque and appropriate use of proximal and distal tacking sutures.
- Careful flushing of debris or air with heparinised saline and continuous back bleeding of the internal carotid artery during final closure of the arteriotomy, followed by initial reperfusion of the external carotid artery.

Figure 5.3 Carotid plaque removal during carotid endarterectomy.



5.7.2.1 Controversies about carotid endarterectomy

As in most operations, surgeons continue to try to improve the outcomes of CEA to achieve the best results. In 1970 the overall morbidity and mortality of CEA in symptomatic carotid stenosis was 13%, whereas this figure is recently about 5% (Rothwell, Slattery & Warlow 1996a) (more detail in Chapter 6). However the debate continues about how to improve the results of this procedure, particularly its cost-effectiveness.

5.7.2.1.1 Anaesthetic technique

There is a controversy about the use of a local anaesthetic (LA) versus a general anaesthetic (GA) (see Chapter 9 for detail). A GA also provides some protection for the brain, as many volatile anesthetic agents such as isoflurane can reduce neuronal activity and the cerebral requirements for oxygen (McCleary, Maritati & Gough 2001). A fast-acting barbiturate has been used in some centres because it can protect against cerebral ischaemia because of its reduction of oxygen metabolism and oxygen demand by reduction of electricity activity. Nevertheless, loss of electrical activity cannot offer protection against severe ischaemia. In fact barbiturates provide other mechanisms of cerebral protection such as redistribution of regional cerebral blood flow, reduction of intracranial pressure, and prevention of oedema and inhibition of calcium influx, but in these situations, patients require unacceptably high doses of these drugs(McCleary, Maritati & Gough 2001). Only one prospective study has showed a clinical benefit of using small doses of barbiturates in clinical practice (Nussmeier, Arlund & Slogoff 1986). Clearly patients under a GA do not have anxiety during a surgical procedure, which eliminates the effect anxiety has on cardiovascular function. On the other hand, some surgeons believe that with LAs there is greater sensitivity in monitoring neurological function (awake testing), reducing the need for intraluminal shunts and their embolic complications. In addition, there is evidence that physiological protective mechanisms may be preserved when LAs are used (McCleary *et al.* 1996). In the Cochrane systematic review of non-randomised studies it has been suggested that the

use of LAs reduced the odds ratio of stroke or death, MI, and pulmonary complications during the peri-operative period and also reduced days in hospital (Tangkanakul, Counsell & Warlow 1997). LA may also reduce costs (Syrek *et al.* 1999). However this topic still needs to be explored in large well-designed randomised trials and is an ongoing project at the moment in the UK (GALA trial) (McCleary, Maritati & Gough 2001).

5.7.2.1.2 Eversion carotid endarterectomy

Eversion carotid endarterectomy (eCEA) involves the disconnection of the common carotid artery and the ICA and eversion endarterectomy of the ICA followed by endarterectomy of the carotid bifurcation and the external carotid artery. Compared to the conventional method, the eversion technique avoids longitudinal arteriotomy of the ICA, and there is no need for patching. The eversion technique also provides optimal correction of an elongated ICA. However with this technique it is difficult to see the distal end of plaque. Raitel and Kasprzak performed angioscopy on the ICA after eCEA and found that in 20% of cases the ICA had to be revised because of intimal flaps (London 2000). In addition, a shunt could not be inserted until the endarterectomy was completed, so the operation had to be rushed.

In randomised controlled trials (1353 participants) in Italy between conventional CEA and eCEA, no differences were found between these two techniques in short term results for peri-operative stroke and death, incidence of early carotid occlusion, and other peri-operative complications including TIA, minor stroke, MI and neck haematoma (Cao *et al.* 1998). Unfortunately this study did not answer the question of how durable eCEA is and what the rate of restenosis is.

5.7.2.1 3 Patch angioplasty

A CEA can be closed directly or closed using a patch (Figure 5.4). The use of patches remains controversial. On the positive side, they result in a far lower incidence of peri-operative stroke (AbuRahma *et al.* 1996a; Archie, Jr. 1986). A patch may also reduce early and late restenosis compared with primary closure (AbuRahma *et al.* 1996a; AbuRahma *et al.* 1999). However on the negative side, operating times and clamping times are longer. It is interesting that there is even a debate about patching material. Two randomised controlled trials found that there was no significant difference between vein or synthetic patching in both short term and long-term outcomes (Gonzalez-Fajardo, Perez & Mateo 1994; Katz & Kohl 1996). On the whole, the issue of patching or primary closure remains controversial. Although there have been many small-randomised controlled trials, a large randomised controlled trial is needed for a solid conclusion.

5.7.2.1.4 Temporary intraluminal shunting

This issue remains debatable (Figure 5.5). On the whole, surgeons use one of three protocols: they always shunt, they shunt selectively, or they never shunt (see more detail in section 5.7.3.1). In the Cochrane Library there are only two clinical trials involving 590 patients in which routine shunting has been compared with no shunting (Counsell *et al.* 1998). A seemingly promising but statistically non-significant 40% reduction has been found in both death and strokes within 30 days of surgery in favour of routine shunting. However, because the number of participants was too small to draw any conclusions, a study on a larger scale is required to answer this question. There have been no good randomised trials of selective shunting to compare intra-operative monitoring methods (Counsell *et al.* 1998). There are several shunts in use, which may also confound any analysis. In clinical practice there are two common types of shunts: Javid shunts and Pruitt-Inahara shunts. The Javid shunt is 32 cm in length and tapers from 17 FG proximally to 10 FG distally (Beard 2000). External clamps hold the

Figure 5.4 This picture depicts a patch angioplasty with vein.

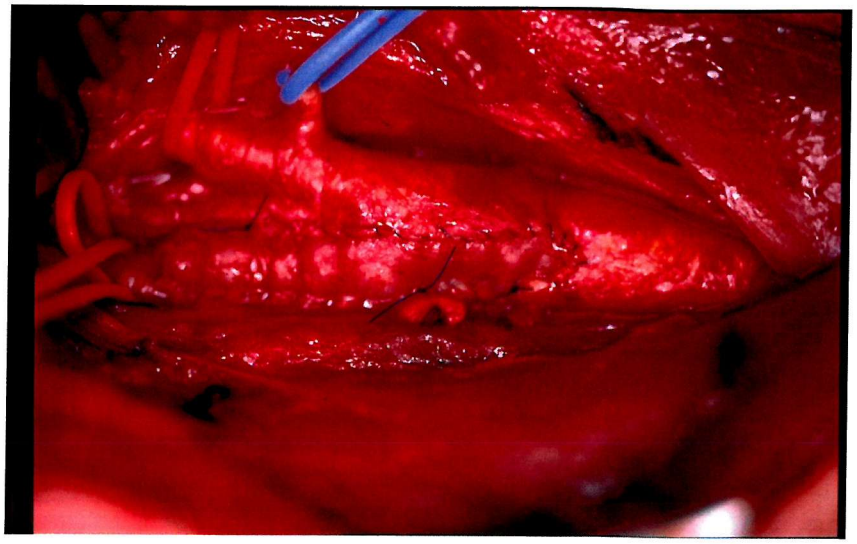
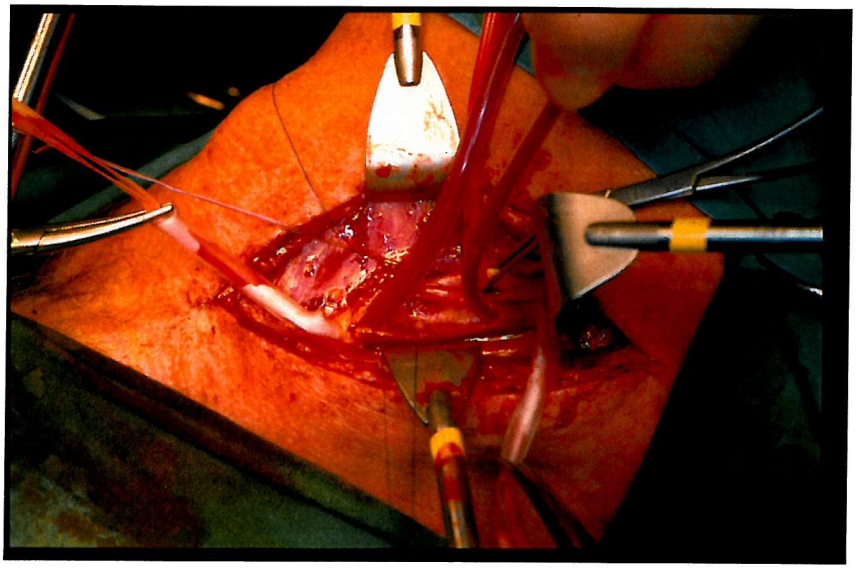


Figure 5.5 This picture shows intraluminal shunting (Javid) during carotid endarterectomy.



fusiform swellings at each end of the shunt in position. The Pruitt-Inahara shunt is as long as the Javid shunt but has an internal lumen of 10 FG and uses inflatable balloons at each end to hold it in position. The blood flow through the Javid shunt has been shown to be much greater than that through the Pruitt-Inahara shunt, but the Pruitt-Inahara is more flexible than the Javid. The balloons of the Pruitt-Inahara cause less trauma to blood vessel walls than the Javid. There has been a randomised controlled trial at Sheffield which showed that the middle cerebral artery velocity in the Pruitt-Inahara group was lower than that in the Javid group, but the number of emboli on declamping was greater for the Javid group (Counsell *et al.* 1998). Patients in the Javid group also had a higher incidence of post-operative disabling stroke than those in the Pruitt-Inahara group, although this did not reach a significant level. In conclusion, the authors seem to prefer the Pruitt-Inahara shunt, which causes less vessel wall injury and subsequently may result in fewer embolic strokes, too (Beard 2000).

5.7.2.1.5. Angioplasty and stents versus open carotid Endarterectomy

Since angioplasty was first performed in 1992 this has been a very controversial topic because of the high risk of embolisation. There have been many reports with different success rates and complications. In a non-randomised study comparing 377 patients who underwent carotid angioplasty (n = 268) and CEA under LA (n = 109) at the same institution during the same period it was found that there were a 9.7% stroke or death rate with angioplasty and a 0.9% rate with CEA (p = 0.0015) (Jordan, Jr. *et al.* 1998). It was concluded that carotid angioplasty was not an acceptable alternative to CEA under LA. This was also supported by a systematic review of cases in the period 1990-1999, which found that the risk of stroke was significantly higher with angioplasty than CEA (Golledge *et al.* 2000). However in 2001 in a randomised controlled trial (CAVATAS 2001) comparing CEA (253 patients) and an endovascular method (251 patients; 74% had balloon angioplasty alone and 26% had stenting) it was reported that there were no significant differences in incidences of peri-operative disabling stroke or death (major outcomes) between these two treatments in three years: that for CEA was 6.4% vs 5.9% for endovascular treatment. The endovascular method avoided minor complications,

namely cranial nerve injuries and haematomas. The rates of complications for endovascular treatment and CEA were 0% vs 8.7% respectively ($p < 0.0001$) for cranial nerve injury and 1.2% vs 6.7% for haematomas (groin and neck) ($p < 0.0015$). However for recurrent severe carotid stenosis in one year, CEA had a significantly lower rate than endovascular treatment: 4% vs 14% respectively ($p < 0.001$) (CAVATAS 2001).

5.7.2.2 Intra-operative monitoring for detecting cerebral ischaemia

5.7.2.2.1 Cerebral ischaemia during carotid artery clamping

Normally when the carotid artery is clamped, there is a reflex rise in blood pressure in order to preserve cerebral circulation, the so-called cerebrovascular reflex (cerebral autoregulation) (McCleary, Maritati & Gough 2001). This physiological response has been attributed to many mechanisms such as those of the baroreceptors in the carotid body or those of the reticular activating system in the medulla oblongata (McCleary, Maritati & Gough 2001). However this reflex may not compensate enough during carotid artery clamping in some patients. The global reduction in cerebral flow during cross clamping of the carotid artery can cause stroke. From their experience, Riles *et al* (1994) found that this explained 15% of all peri-operative strokes. Global ischaemia may be avoided by insertion of an intraluminal shunt. Some surgeons use shunts routinely because they believe that they provide an optimal blood flow to the brain. On the other hand, some studies have reported personal series in which CEA without shunts have been safe and the blood supply to the brain during clamping has presumably been sufficient to avoid stroke (Ott *et al.* 1980). Furthermore, shunts can cause complications during insertion such as dissection, thrombus formation, subsequent embolisation, and even embolisation from air or atherosclerotic plaque. Kinking of the shunt or impact of the shunt against the vessel wall can cause global ischaemia.

Currently most surgeons do the operation using at least one method to detect cerebral ischaemia (indication for the insertion of an intraluminal shunt), so-called cerebral protection monitoring, such as awake testing or measurement of carotid stump pressure,

middle cerebral artery blood flow velocity using transcranial Doppler (TCD), or regional cerebral oxygen saturation using reflected near infrared light spectroscopy.

5.7.2.2.2 Awake testing

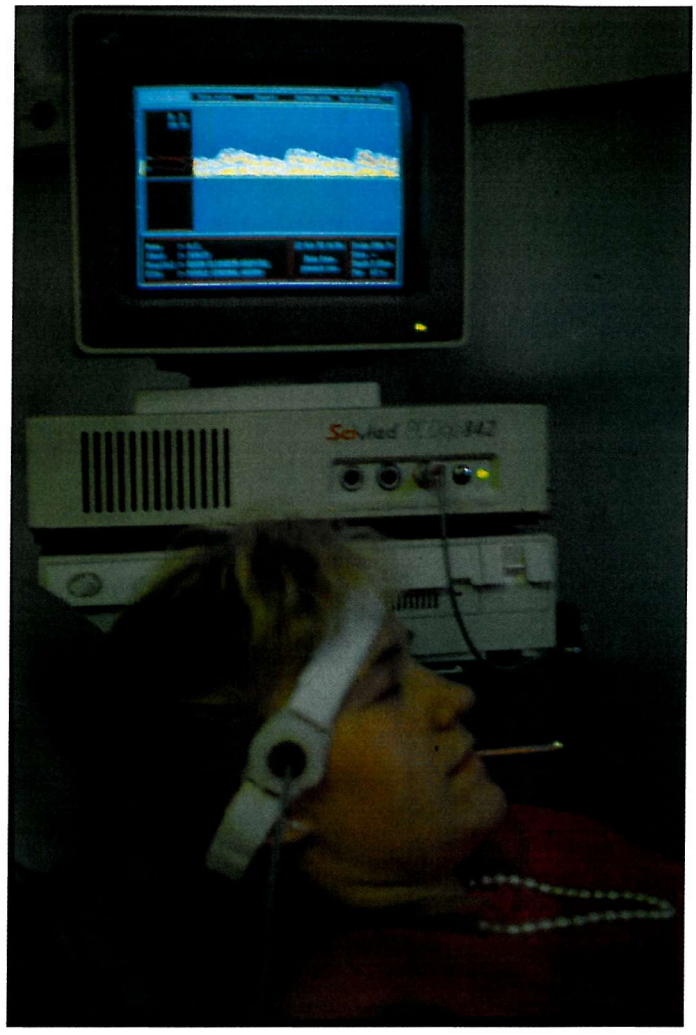
During a CEA when the patient is under regional and local infiltration of local anaesthetic cerebral function can be assessed by asking the patient to speak or to squeeze a fluid-filled bag connected to a pressure transducer (Fearn & McCollum 1998). If the patient has any problems during the test such as focal weakness, the surgeon can insert a shunt. However, in an awake patient all cerebral activity cannot be monitored, especially higher cortical function, which may be affected by silent emboli or ischaemia (Beard 2000; Sise *et al.* 1989).

5.7.2.2.3 Transcranial Doppler

TCD can measure directly the velocity, flow pattern and embolisation in the middle cerebral artery through the window in the thin temporal bone (Figure 5.6). However, one out of ten people do not have this window. The change in velocity of blood flow in the middle cerebral artery determines the collateral circulation and the need for a shunt. One author has suggested that a middle carotid artery velocity of less than 30 cm/s or a clamp/preclamp ratio of less than 0.6 indicates the need for a shunt (Fearn & McCollum 1998). TCD also detects changes in cerebral blood flow more reliably than electroencephalography or somatosensory evoked potentials (Arnold *et al.* 1997). Therefore TCD is also a useful method for monitoring the function of an intraluminal shunt. Recently this technology has been improved so that emboli can be detected, although their size cannot be measured. Generally, signals showing emboli have a duration of less than 100 msec and energy levels at least 3 dB above the background Doppler blood velocity spectrum (Fearn & McCollum 1998). They appear at random during the cardiac cycle and produce audible chirps, whistles, or clicks. There is an association between signals of emboli during carotid dissection or shunt manipulation and cerebral complications as well as new ischaemic lesions detected by magnetic resonance imaging (Ackerstaff *et al.* 1995; Ackerstaff *et al.* 2000). TCD has also been

able to identify patients with hyperperfusion syndrome (Ghali *et al.* 1997; Magee, Davies, A.H. & Horrocks 1994), which is diagnosed if at 15 sec after releasing a cross clamp middle carotid artery velocity exceed twice the pre-clamp levels (Ghali *et al.* 1997).

Figure 5.6 Transcranial Doppler.



5.8 Complications of carotid endarterectomy

5.8.1 Death

Generally the peri-operative mortality is 1.31% for asymptomatic stenosis and 1.81% for symptomatic stenosis (Rothwell PM, Slattery J & Warlow CP 1996b). Two main causes of mortality are stroke and MI. In 1945 CEA in Georgia in 1993, the 30-day mortality was 1.9% (37 patients): there were stroke-related deaths in 0.7% of patients and MI-related deaths in 0.5 (Karp *et al.* 1998). Any policy that can prevent stroke or MI must therefore be encouraged.

5.8.2 Neurological deficit

The neurological deficit following CEA ranges from TIA to disabling stroke and fatal stroke. Stroke is the most worring problem. The risk of fatal stroke in symptomatic and asymptomatic patients is 0.47% and 0.91%, respectively (Rothwell PM, Slattery J & Warlow CP 1996b). Fatal stroke may be caused by a massive cerebral infarction or haemorrhage. There are many mechanisms of peri-operative stroke. Riles *et al.* (1994) reported the mechanisms of peri-operative stroke using their database (2365 CEAs; 66 strokes): ischaemia during carotid artery clamping (15%), post-operative thrombosis and embolism (38%), intracerebral haemorrhage (18%), stroke from other mechanisms associated with the surgery (12%), and stroke unrelated to the reconstructed artery (12%). They also found that patients with hypertension and pre-operative stroke had a high risk of stroke. 65% of the 66 strokes were attributed to errors in technique. Patient selection and surgical technique are therefore the most important factors in the risk of stroke (Riles *et al.* 1994).

Many studies have identified the risk factors that are associated with stroke and death. These risk factors may help clinicians to estimate operative risks for individual patients, but the risk factors reported vary enormously. Even the results of the two landmark studies ECST and NASCET were different, although the methodologies were quite similar. In ECST cerebral presentation (cerebral transient ischaemic attack or stroke),

female sex, hypertension, and peripheral vascular disease were the risk factors for stroke and death (Rothwell, Slattery & Warlow 1997), whereas in the NASCET study, hemispherical symptoms, left-sided procedure, contralateral occlusion, ipsilateral ischaemic lesions on CT scan, and irregular or ulcerated ipsilateral plaque were the major risk factors (Ferguson *et al.* 1999). This issue might be settled by a study with a larger database. Several methods of quality control were proposed to decrease errors in technique. For instance, TCD was used during surgery to monitor shunt performance and detect release of emboli during dissection. After CEA complete angiography, duplex scan, and angioscopy have been proposed as methods of examining the adequacy of surgical repair. These methods of quality control may identify the 6-12% of patients with lesions requiring surgical revision (Gough, Tan & Maritati 1999). However there have been no randomised controlled trials to confirm the benefit of revision, and there is no consensus either about which lesions warrant revision.

Interestingly some studies have shown that more than half of these neurological deficits occur a few days after successful operations (Whitney *et al.* 1980). It has been proposed that these events might be caused by the aggregation of platelets and fibrin at the endarterectomy site with subsequent cerebral embolisation or spontaneous thrombosis of the ICA. The administration of an anti-platelet agent such as aspirin in the post-operative period can be of value. This has been confirmed by a large randomised controlled trial (Taylor *et al.* 1999a).

Another subcategory of neurological deficit following CEA is hyperperfusion syndrome. This syndrome is caused by a great increase in blood flow to the brain after CEA. In fact, it is poorly defined and understood, because most patients undergoing CEA have a transient increase in cerebral blood flow because of dysfunction of cerebral autoregulation (Magee *et al.* 1992). However only 2% of patients overall will progress to this syndrome, which comprises seizure, irritability and confusion (Naylor 2000). High-risk patients are those with severe bilateral carotid artery disease, poor collateral

circulation in the circle of Willis, hypertension, and impaired cerebrovascular autoregulation (Naylor 2000).

5.8.3 Cardiac morbidity

The incidence of peri-operative cardiac morbidity and mortality ranges from 0.7 to 7.1% (Gunel & Awad 2000). MI is a major cause of peri-operative death (Karp *et al.* 1998; Musser, Nicholas & Reed, III 1994). Histories of stable angina, premature ventricular contraction, intra-operative hypotension, and end-stage renal disease were identified as risk factors for 30-day MI after CEA (Musser, Nicholas & Reed, III 1994). In fact, post-operative MIs were also found in patients without a history of heart disease (Riles, Kopelman & Imparato 1979).

In the long term, 30-60% of late deaths after CEA have been attributed to coronary causes (Belkin & Sebatian 2000). The five and ten year survivals of patients undergoing CEA have been 72-78% and 36-64% respectively (Belkin & Sebatian 2000). Obviously coronary artery disease influences the long-term survival of patients after CEA. It is vital for vascular surgeons to focus on atherosclerosis as a systemic disease. An effective strategy for management of the patients and their risk factors is necessary in order to maximize quality of life and survival. It seems pointless to perform CEA if patients succumb to some other, possibly avoidable, vascular event such as MI in the next few years.

5.8.4 Hypertension and hypotension

CEA may affect the baroreceptors, which are located in the adventitia of the internal carotid artery at the carotid bifurcation. These baroreceptors send impulses to the carotid sinus nerve (of Hering). High blood pressure normally stimulates a reflex arc to the medulla oblongata mediated by the sinus nerve. The body compensates then by bradycardia and hypotension.

Fluctuation of blood pressure is not an unusual problem in patients with atherosclerotic disease. The incidences of post-operative hypertension and hypotension are 9% and 12% respectively (Wong, Findlay & Suarez-Almazor 1997a). Post-operative hypertension is also associated with peri-operative risk of stroke and death (Rothwell, Slattery & Warlow 1997). Risk factors for post-operative hypertension include angiographic intracranial carotid stenosis greater than 50%, cardiac arrhythmia, pre-operative systolic pressure greater than 160 mmHg, neurological instability, and renal insufficiency (Wong, Findlay & Suarez-Almazor 1997a).

5.8.5 Cranial nerve injury

The incidence of cranial nerve injury following CEA ranges from less than 5% to more than 50% (Schroeder & Levi 2000) and the cranial nerves at risk include VII, IX, X and XII. This incidence is especially high following re-operation. AbuRahma *et al.* (2000) evaluated cranial nerve function by clinical examination and direct laryngoscope of 89 consecutive patients undergoing repeat CEAs and found that the risk of injury was 21%. The incidences of hypoglossal and vagus nerve injury were 9% and 12% respectively. 88% of these injuries were transient. A detailed knowledge of anatomy and good surgical technique are important to minimize cranial nerve injury.

5.8.6 Patch infection

Infection is an extremely uncommon complication of carotid surgery. Bell (2000) reported from his institute series that the incidence of this complication was 0.06%. Although this complication is rare, it is associated with a high risk of stroke and death. The key factor in management is prevention, which requires obsessive attention to aseptic technique and early recognition and proper management of wound complications (Gunel & Awad 2000).

5.8.7 Wound haematomas

Post-operative wound haematomas are not an uncommon problem. Kunkel *et al.* (1984) found that 80% of them were caused by capillary oozing and 20% by arteriotomy

wounds. They also found that anti-platelet drugs and post-operative hypertension were associated with the development of wound haematomas (Kunkel *et al.* 1984). Bleeding because of poor surgical technique is also a common problem. The common facial vein and other smaller vessels should be ligated securely with fine sutures to prevent delayed bleeding in the event that transient venous hypertension or straining occurs at the time the endotracheal tube is removed following GA. Fortunately most haematomas can be successfully treated conservatively (Hertzer 1996). However a large cervical haematoma can compromise the airway by shifting the trachea across the midline and can also compress the carotid bifurcation. Large haematomas might necessitate a secondary procedure, consisting of evacuation of the haematoma, haemostasis, irrigation and reclosure over a temporary vacuum drain.

5.8.8 Restenosis

The incidence of recurrent stenosis following CEA ranges from 1.2% to 35% (Gagne *et al.* 1993; Norrving, Nilsson & Olsson 1982). The most common cause of restenosis in the first two years is intimal hyperplasia, whereas late recurrent carotid stenosis is due to atherosclerosis. There is no randomised trial evidence that indicates when carotid surgery should be done again. The risks of stroke and death following revision are 3.9 and 1.0 respectively (Lattimer & Burnand 1997).

5.9 Conclusion

The major cause of strokes is ischaemia, and thromboembolism from the internal carotid artery is a relatively common cause of these ischaemic strokes. CEA clearly benefits patients with symptomatic severe carotid stenosis, compared with medical treatment only. However there are many controversies about CEA, especially about the selection of patients and the use of different operative/anaesthetic techniques during surgery. Many studies have shown differing results for the risk factors that determine the outcomes of CEA. In the next chapters, these issues will be examined.

Chapter 6

Time trends in the published risks of stroke and death due to carotid endarterectomy for symptomatic stenosis: a systematic review

6.1 Summary

Objective: Randomised trials published in the early 1990s demonstrated that carotid endarterectomy (CEA) is highly beneficial for patients with severe symptomatic stenosis, but were less conclusive for patients with moderate symptomatic disease. Surgery in the latter group is often justified on the premise that operative risks and complications have fallen over the last decade. To identify any changes in outcome of CEA, a review of all published literature reporting outcome for CEA in the last 7 years was undertaken.

Design: Systematic review and meta-analysis of reports of the operative risk of CEA.

Methods: A systematic review was done of all studies published between 1994 and 2000 reporting the risks of stroke and death because of CEA. These data were combined with previously identified studies published between 1960 and 1994 to study trends of outcomes over the last 40 years.

Results: 46 studies were identified that reported stroke and death rates specifically for symptomatic patients. The overall operative mortality was 1.3% (95% Confidence interval [CI] 1.1-1.4) and the risk of stroke and death was 4.6% (95% CI 3.9-5.2). Over the last 15 years the risk of death and stroke and death, has remained constant for symptomatic patients. There has been an increase in the mean age of patients operated ($p < 0.01$). As noted previously, the peri-operative risk of combined stroke and death when reported by independent neurologists was the same as that of the major randomised trials but higher than studies reported by surgeons alone 6.0% (95% CI 3.8-8.2) and 6.8% (95% CI 6.3-7.0) vs 3.8% (95% CI 3.1-4.6) respectively. However, reported death rates alone were identical for both the surgical case series and the major prospective trials but higher in those reported by a neurologist.

Conclusions: There has been no reduction in published risks of stroke and death for symptomatic patients for the last 15 years. There is still a difference in stroke and death rates reported by the operating surgeon and by independent neurologist assessors.

6.2 Introduction

6.2.1 Risk of stroke and death following carotid endarterectomy

Benefit from CEA relies upon the risk of stroke and death from surgery being significantly less than that for patients treated with best medical management alone. Large, prospective trials have shown that surgery is beneficial for symptomatic patients with at least 70% stenosis (NASCET method) (Barnett *et al.* 1998; ECST 1998). They have also suggested a marginal benefit for symptomatic patients with moderate stenosis (50-69%) (NASCET method) (Barnett *et al.* 1998), but this relies upon a low operative morbidity and mortality rate, which may be difficult to achieve in real life practice. Many surgeons currently justify operating upon the more borderline patients on the basis that the outcomes reported in the NASCET and ECST trials are now out of date, and that peri-operative stroke and death rates have fallen. This belief appears to be supported by stroke and death rates of 2% quoted for series published in the recent literature (Shah *et al.* 1998), which contrast with the 3 times higher rates of 6-7% seen in the major trials 10 years ago. However, since the large prospective studies were first reported in 1991 (ECST 1991; NASCET 1991), there have also been significant changes in the medical management of carotid disease. Introduction of aggressive lipid lowering and blood pressure management regimes may have reduced the risks for patients treated non-surgically (Perindopril trialists 2001). Recently the Heart Protection Study has also shown that high-risk patients taking 40 mg per day of simvastatin had significantly a lower 5-year risk of ischaemic stroke (2.8%) than those in the placebo group (4.0%) (HPS 2002). Consequently, it is important to assess whether there has truly been a reduction in surgical risk, since if there has not been, the appropriateness of surgery in the patients with moderate carotid stenosis may fall into question once more.

Assessment of "true" complication rates of surgery from the published literature is very difficult. It has been noted previously that series reported by independent neurologists have significantly higher combined stroke and death rates than those published by the surgeon performing the series (Rothwell, Slattery & Warlow 1996a). Although it is difficult to prove which are the "true" results, there is considerable similarity between

the results of neurologist led studies and those of the major prospective randomised trials (NASCET, ACE, ECST (1998)) (Barnett *et al.* 1998; Taylor *et al.* 1999a). These trials were large, well-organised, and involved prospective follow up and are therefore likely to be the most reliable estimates of outcome of surgery as it was performed at that time. If these trials represented the true results 10 years ago, then if both the degree of surgeon neurologist bias and the rates reported in the surgical literature remain constant it is likely that the risks reported in the major trials in the 1990 are still valid today.

6.2.2 Hypothesis and Objective

In order to identify any changes in outcome of CEA over time, a review of all published literature reporting outcome for CEA in the last 7 years was undertaken and then combined with data obtained from 2 previous reviews covering the years 1960 to 1994 (Rothwell, Slattery & Warlow 1996a; Rothwell, Slattery & Warlow 1996b). It was thus possible to observe the variation in surgical stroke and death rates with time as well as any trends towards changes in baseline characteristics of the patients operated upon. It was also possible to examine if the previously noted differences between who reported and followed-up patients and the published stroke and death rates were still present.

6.3 Method

6.3.1 Criteria for considering studies for review

6.3.1.1 Type of studies

All randomised trials and non-randomised studies were considered that reported the risk or risk factors of stroke or death or combined stroke and death following CEA. Mortality and the risk of stroke and/or death had to be defined or possible to calculate per operation. In other words, these studies had to report the number of operations that had complications and the number of total operations rather than giving only the percentage of complications. Only studies in English were included.

6.3.1.2 Type of participants

Studies that included any type of patient undergoing unilateral or staged bilateral CEA were considered eligible, whether symptomatic or asymptomatic carotid disease was indicated or not. Symptomatic patients were defined as those who had suffered a transient ischaemic attack in the carotid distribution or a stroke ipsilateral to a stenosis. Excluded were any studies that had participants with other arterial reconstructions such as a CEA combined with coronary artery bypass grafting (CABG) or upper extremity arterial reconstruction. Excluded also were any papers reporting the risk from simultaneous bilateral CEA.

6.3.1.3 Type of intervention

Only studies of CEA were used either the conventional or eversion technique. Studies involving resection of the carotid artery and bypass grafting were excluded.

6.3.1.4 Measures of outcome

The measures of outcome were the number of combined strokes and deaths, deaths alone, or strokes alone that occurred within 30 days of CEA or a similar time period such as four weeks, one month, or 31 days.

6.3.2 Search strategy for studies

A. The National Library of Medicine's Medline database and EMBASE were systematically searched for the period 1994-2000 using the terms "carotid endarterectomy" and "carotid surgery" with the restriction that articles had to be original and about humans.

B. The following six key journals for CEA were searched manually for the period 1994-2000:

American Journal of Surgery

Annals of Vascular Surgery

Cardiovascular Surgery

European Journal of Vascular and Endovascular Surgery

Journal of Vascular Surgery

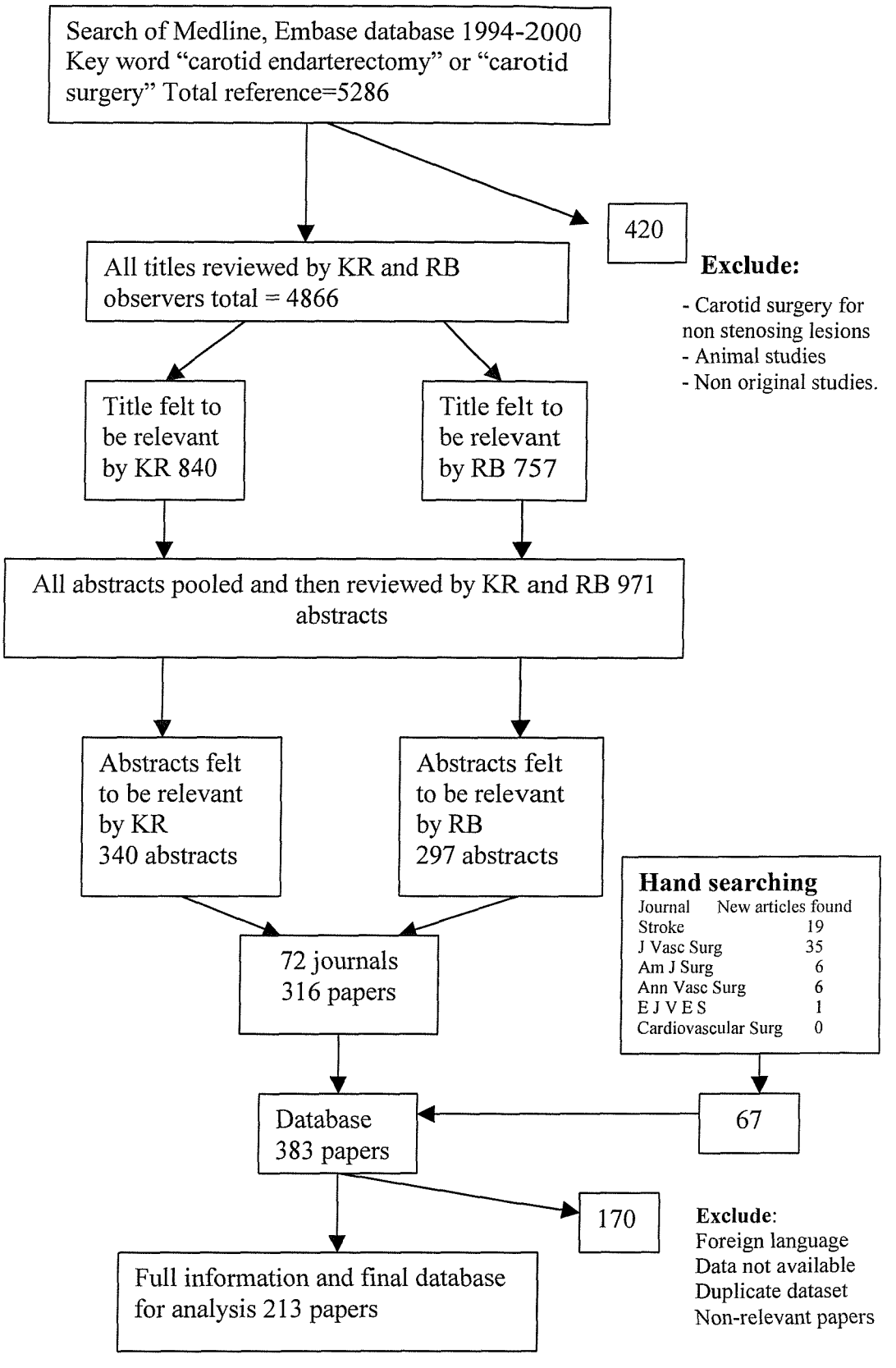
Stroke

6.3.3 Method of Review

The lists of all papers located electronically were also checked by the author and Mr. R. Bond (RB), who then screened the resulting list of 4866 references individually for any papers that might contain relevant information. These were then pooled and the process repeated using the abstracts as a guide to relevance (Figure 6.1).

After manual searching and pooling of the selected abstracts, a total of 383 articles remained from which data could be extracted. Each reader reviewed every article and recorded information about rates of surgical complication rates, reported associations between operative and pre-operative risk factors, and outcomes and also assessed each trial for its methodological correctness. The data were incorporated into a specifically designed database Microsoft Access © 1992-1999 Microsoft Corporation. Several papers reporting the same cohort were identified, in which case all papers but the most comprehensive paper were excluded. Any conflicting results between the two reviewers were identified and re-assessed in an attempt to reach a consensus. If necessary, disagreements were referred to a third reviewer Dr. P.M. Rothwell (PMR). After exclusion of duplicates or articles with inadequate data, a final database of 213 articles was available for analysis. The quality of these 213 papers (see list in Appendix 7) was assessed and is reported in Chapter 8. References of all the relevant studies identified above were reviewed and another three papers were included (Crawley *et al.* 2000; Ferguson *et al* 1999; Yates *et al.* 1997). Although the preliminary results for a small subgroup of surgical patients randomly assigned in the CAVATAS trial (Crawley *et al.* 2000) were available, it was felt that it was better to wait for the complete results of this trial, which were published later (CAVATAS 2001).

Figure 6.1. Diagram of strategy used during literature searching.



As the presenting symptoms strongly influence outcomes of carotid surgery (Rothwell, Slattery & Warlow 1996b) (Rothwell, Slattery & Warlow 1997), articles were further restricted to the 46 papers that reported stroke and death rates for symptomatic patients separately from those for asymptomatic patients. To identify trends, results of this review were combined with those of two similar reviews by a colleague (PMR) who studied similar articles in the period 1960 to 1994 (Rothwell, Slattery & Warlow 1996a) (Rothwell, Slattery & Warlow 1996b).

6.3.4 Statistical analysis

Meta-analyses were performed to calculate the overall death and combined stroke and death rates, for all the identified studies reporting outcomes for symptomatic patients. Analysis was stratified according to whether an independent assessor was involved in prospective patient follow-up, and only the operating surgeons involved.

The degree of inter-observer agreement for numerical results and methodology scoring was assessed using simple proportions. The meta-analysis values of the absolute risks of death, and stroke/death and their confidence intervals were calculated using the Mantel-Haenszel method. Heterogeneity was assessed by using the Chi-squared test to see whether the risk observed in each study differed from the overall estimate (absolute risk). Analyses were done with STATA version 6 (Stata Corporation, Texas, USA) and in all cases a value of $p < 0.05$ was taken to indicate a statistically significant difference.

6.4 Results

6.4.1 Search

383 studies fulfilled the criteria for inclusion in the review, but many reported similar or identical datasets. Therefore, wherever possible, only the most comprehensive paper was included. A final pool of 213 papers was produced, containing adequate detail to allow the stroke rate, stroke and death rate, or death rate alone to be calculated.

An overall agreement in data extraction by the two observers of 91.4% was obtained (Table 6.1). 46 papers reported the results for symptomatic and asymptomatic patients separately.

Table 6.1. Rate of agreement between observers (KR, RB) before combined decisions were made.

	Agreement	Disagreement	Percentage agreement
Total number of operations	152	14	91.6
Death rate	160	6	96.4
Stroke and death rate	143	23	86.2

6.4.2 Meta-analysis

The death rates and stroke/death rates for the meta-analysis in symptomatic patients undergoing CEA between 1994 and 2000 were 1.3% (95% CI 1.1-1.4) and 4.6% (95% CI 3.9-5.2) respectively, (Figure 6.2a-b). The stroke and death rates in symptomatic patients were 6.0% (95% CI 3.8-8.2) when the study either had a neurologist involved in prospective follow up, compared with 3.8% (95% CI 3.1-4.6) when no neurologist was involved (Figure 6.2b). However, death rates were the same (1.2%) in both the published literature (non neurologist follow-up) and the major randomised controlled trials (RCT) (NASCET, ECST, CAVATAS, ACE) but higher in neurologist reported series (1.9%, 95% CI 1.2-2.6) (Figure 6.2a).

Figure 6.2a Meta-analysis of published death rates following CEA in symptomatic patients, for the period 1994 –2000, stratified according to involvement of a neurologist or independent assessor. Risk for each study represented by a square, the area of which is proportional to statistical power of estimate. Line represents 95% CI of risk. Diamond represents overall pooled estimate. (p(het) = p value for heterogeneity).

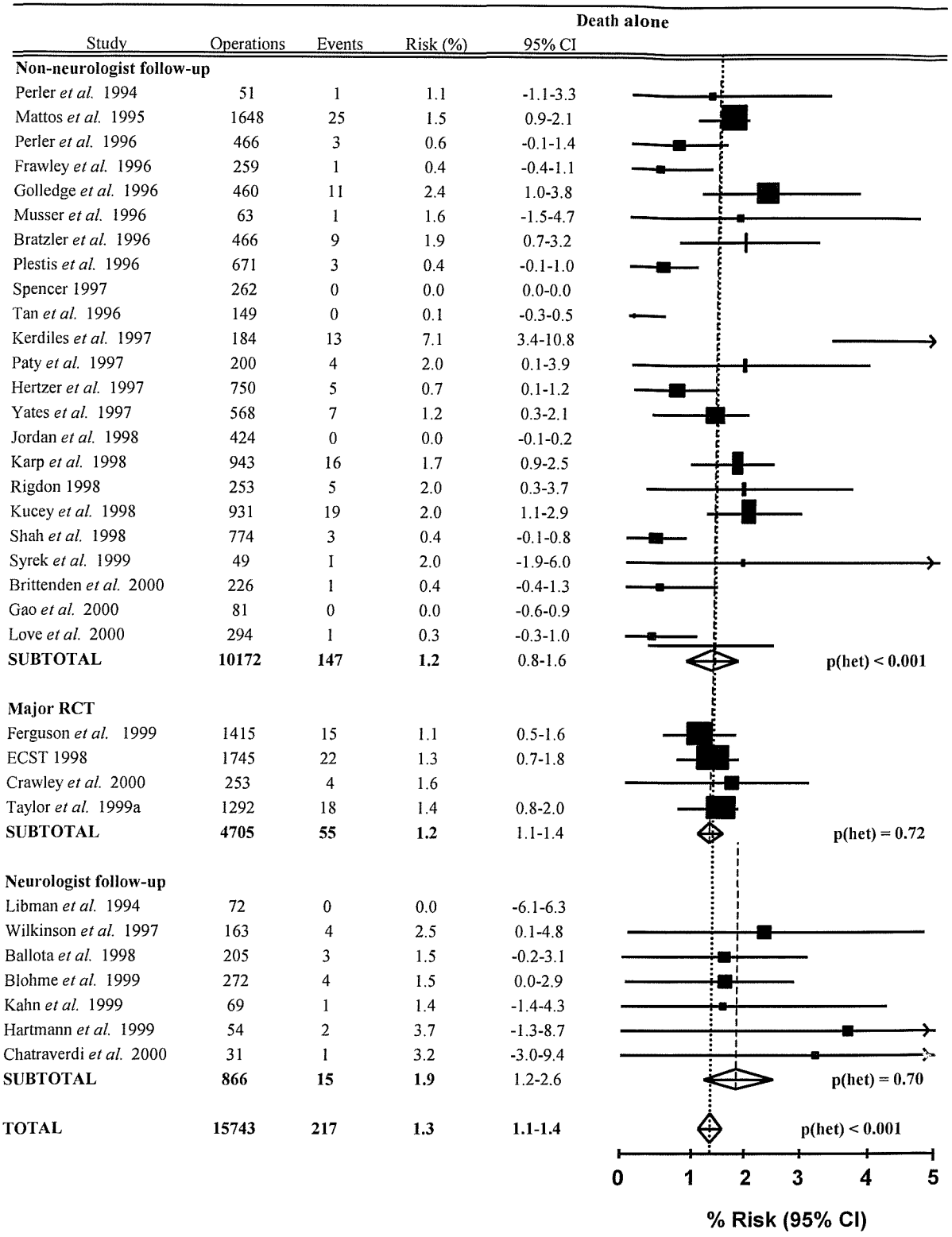
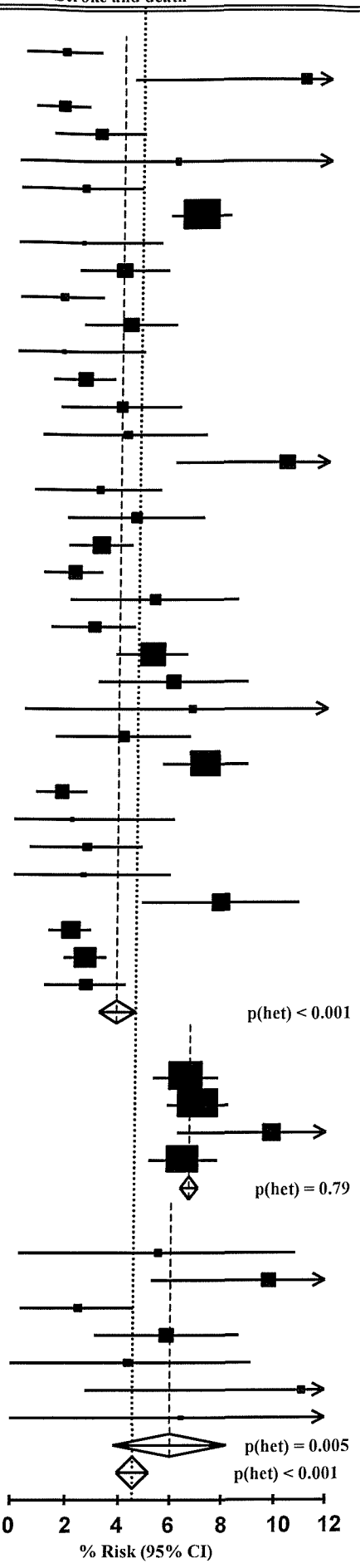


Figure 6.2b Meta-analysis of published stroke and death rates following CEA in symptomatic patients, for the period 1994–2000, stratified according to involvement of a neurologist.

Study	Operation	Event	Risk(%)	95% CI
Non-neurologist follow-up				
Boontje *et al.* 1994	320	5	1.6	0.2-2.9
Naik *et al.* 1994	82	9	11.0	4.2-17.7
Poulias *et al.* 1994	600	9	1.5	0.5-2.5
Musser *et al.* 1994	345	10	2.9	1.1-4.7
Perler *et al.* 1994	51	3	5.9	-0.6-12.3
Urbinati *et al.* 1994	172	4	2.3	0.1-4.6
Mattos *et al.* 1995	1648	113	6.9	5.6-8.1
Perler *et al.* 1995	89	2	2.2	-0.8-5.3
Perler *et al.* 1996	466	18	3.9	2.1-5.6
Frawley *et al.* 1996	259	4	1.5	0.0-3.0
Golledge *et al.* 1996	460	19	4.1	2.3-5.9
Musser *et al.* 1996	63	1	1.6	-1.5-4.7
Plestis *et al.* 1996	671	16	2.4	1.2-3.5
Spencer 1997	262	10	3.8	1.5-6.1
Tan *et al.* 1996	149	6	4.0	0.9-7.2
Kerdiles *et al.* 1997	184	19	10.3	5.9-14.7
Paty *et al.* 1997	200	6	3.0	0.6-5.4
Rigdon *et al.* 1997	226	10	4.4	1.7-7.1
Hertzer *et al.* 1997	750	23	3.1	1.8-4.3
Plestis *et al.* 1997	633	13	2.1	0.9-3.2
Wong *et al.* 1997	174	9	5.2	1.9-8.5
Jordan *et al.* 1998	424	12	2.8	1.3-4.4
Karp *et al.* 1998	943	48	5.1	3.7-6.5
Rigdon 1998	253	15	5.9	3.0-8.8
Hoffmann *et al.* 1998	60	4	6.7	0.4-13.0
Mayo *et al.* 1998	224	9	4.0	1.4-6.6
Kucey *et al.* 1998	931	67	7.2	5.5-8.9
Shah *et al.* 1998	774	13	1.7	0.8-2.6
Syrek *et al.* 1999	49	1	2.0	-1.9-6.0
Brittenden *et al.* 2000	226	6	2.7	0.6-4.8
Gao *et al.* 2000	81	2	2.5	-0.9-5.8
Love *et al.* 2000	294	23	7.8	4.8-10.9
Cl-Darling *et al.* 2000	1230	25	2.0	1.2-2.8
Kresowick *et al.* 2000	1392	36	2.6	1.8-3.4
Naylor *et al.* 2000	421	11	2.6	1.1-4.1
SUBTOTAL	**15106**	**581**	**3.8**	3.1-4.6
Major RCT				
Ferguson *et al*.1999	1415	92	6.5	5.2-7.8
ECST 1998	1745	122	7.0	5.8-8.2
Crawley *et al.* 2000	253	25	9.9	6.1-12.5
Taylor *et al.* 1999a	1292	83	6.4	5.1-7.8
SUBTOTAL	**4705**	**297**	**6.8**	6.3-7.0
Neurologist follow- up				
Libman *et al*. 1994	72	4	5.6	0.3-10.8
Wilkinson *et al.* 1997	163	16	9.8	5.2-14.4
Ballota *et al.* 1998	205	5	2.4	0.3-4.6
Blohme *et al. 1* 999	272	16	5.9	3.1-8.7
Kahn *et al.* 1999	69	3	4.3	-0.5-9.2
Hartmann *et al.* 1999	54	6	11.1	2.7-19.5
Chatraverdi *et al.* 2000	31	2	6.5	-2.2-15.1
SUBTOTAL	**866**	**52**	**6.0**	3.8-8.2
TOTAL	**20677**	**930**	**4.6**	**3.9-5.2**



6.4.3 Trends in baseline characteristics

Although there was initially a significant reduction in reported stroke and death rates and death rates in symptomatic patients leading up to 1980, there has been no statistically significant change for the last 15 years (Figure 6.3a). Reported outcomes in studies where a neurologist (independent assessor) was involved are consistently higher than when they are not, and for the last 15 years the difference between them has remained constant at approximately 1.5:1 (Figure 6.3b). There have also been significant increases in average age of patients operated ($p < 0.01$ for trend, Figure 6.4a), but no significant change in the proportion of male and female patients (Figure 6.4b).

Figure 6.3a Time trends in stroke and death rates and death rates (S/D rate) following CEA in symptomatic patients, for the years pre-1980 to 2000.

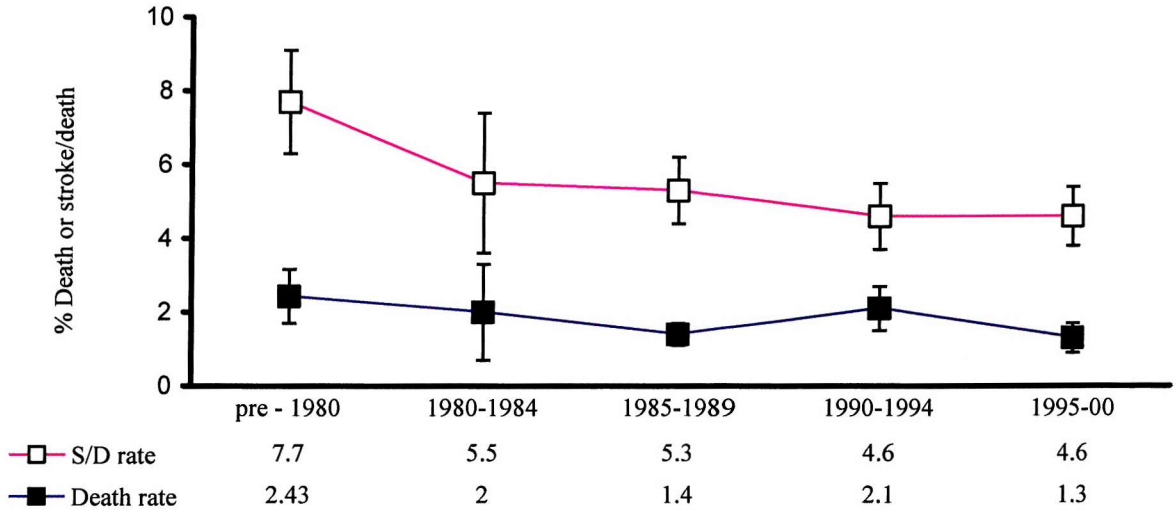


Figure 6.3b Time trends in stroke and death rates following CEA in symptomatic patients stratified according to involvement of a neurologist or independent assessor, for the years pre-1980 to 2000.

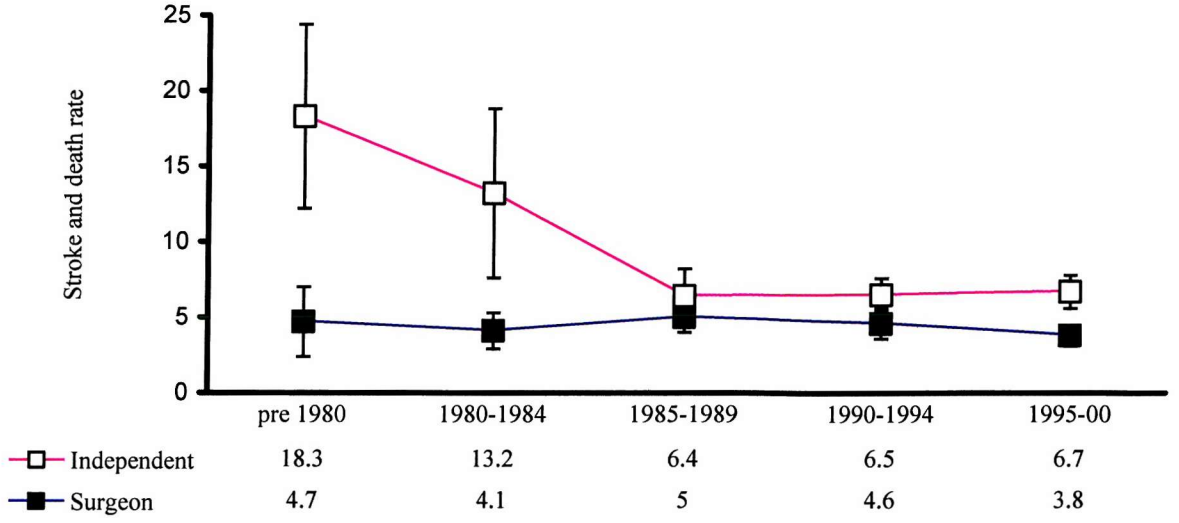


Figure 6.4a Variation over time of the average age of patients undergoing carotid endarterectomy, for the years pre-1980 to 2000.

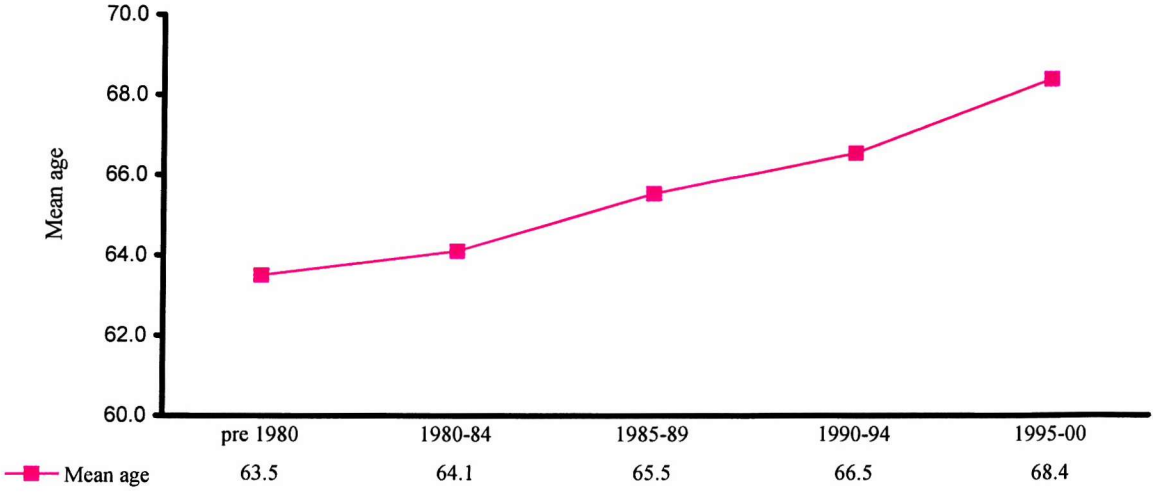
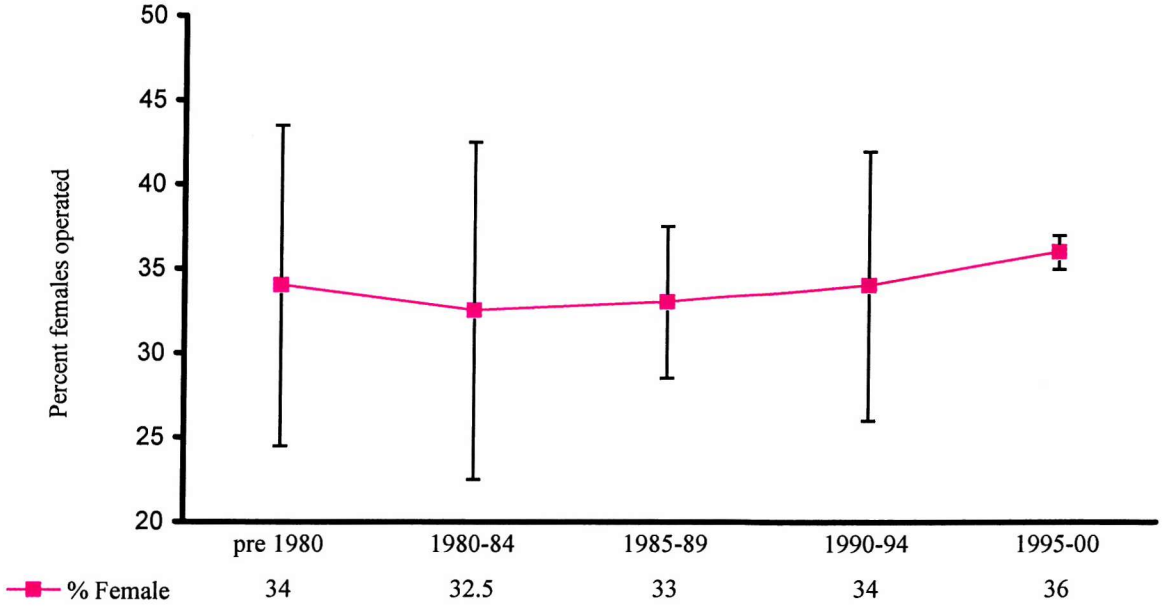


Figure 6.4b Variation over time of the percentage females undergoing carotid endarterectomy, for the years pre-1980 to 2000.



6.5 Discussion

6.5.1 Changes in the risk of complication following carotid endarterectomy with time

Large, prospective trials have shown that CEA is beneficial for symptomatic patients with at least 70% carotid stenosis. However, there is still much controversy regarding the benefit of surgery for patients with lesser degrees of stenosis (Barnett *et al.* 1998; ECST 1998). Many surgeons feel that the overall outcome of surgery is improving over time due to progression along a learning curve, the introduction of new quality control techniques and better patient selection (Lennard *et al.* 1999). It, therefore, seems justifiable to operate upon symptomatic patients with moderate stenosis. However, although it may be possible to show that specific units have good or improving results, it is less certain as to whether this is occurring in the community at large. Further, since medical management of carotid artery disease is also likely to be improving, if the

assumption about improving surgical results is incorrect, surgery may, paradoxically, becoming less appropriate for such patients. On the other hand, although medical treatment has shown promising results in reducing vascular events for high-risk patients such as patients with carotid stenosis, it also has adverse effects. For instance, statin treatment can lead to hepatoxicity and myopathy, but in the large RCT study, in which 40 mg doses of simvastatin were used, there was no significant difference in the incidence of such complications between the treatment group and the control group (HPS 2002). Similarly patients with vascular disease taking an angiotensin-converting enzyme inhibitor can have adverse effects such as cough (7.3%), hypotension or dizziness (1.9%) and angioedema (0.4%) (Yusuf *et al.* 2000).

Interpreting the results published in the literature is difficult. There is considerable disagreement as regards the "true" expected surgical mortality and stroke/death rates. Rothwell *et al.* (1996a) have noted that the published outcome of surgery when assessed, or reported, by neurologists is much more than that of single author surgeon. However, whether this is due to under-reporting by surgeons or over-reporting by neurologists is uncertain (Rothwell, Slattery & Warlow 1996a). It is human nature to publish ones own results only if they are better than, or at least equal to, the expected (published) norm. Surgeons may also be more likely to miss or discount "minor" neurological symptoms following surgery that neurologists would pick up and consider significant. Conversely, it can be argued that neurologists are only likely to audit a surgeon's results if they consider a problem to be present within their own surgical department. However, the overall meta-analysed stroke and death rates from papers reported by neurologists are similar to, and homogeneous with, those of the ECST, NASCET and ACE. Since these high quality trials are generally accepted as being reliable estimates of the results of surgery as it was practiced at the time it does appear that surgical series are under reporting combined stroke and death rates. However, unlike stroke and death rates, the mortality rates published in the surgical literature are identical to those of the major trials, whereas the rates reported by studies involving neurologists are higher. This may indicate that neurologist run studies (outside of

randomised trials) may indeed be identifying higher risk patient cohorts (Chaturvedi S 2001; Chaturvedi, Aggarwal & Murugappan 2000).

As both the overall published stroke/death rates following CEA, and the degree of bias between surgeons and neurologist have remained static, if the 6-7% risk of stroke and death for symptomatic patients reported by the ECST and NASCET were valid 10 years ago then they are likely to remain so today.

6.5.2 Changes in patient baseline characteristic with time

Several baseline characteristics have been shown to be associated with increased stroke and death rates following CEA. It is important, therefore, to try and take into account any trends in these characteristics when interpreting the changes in surgical outcome with time. There has been a steady rise in the average age of patients operated upon for carotid artery occlusive disease over the last 30 years (Figure 6.4a), and this change may hint at relaxation in attitudes towards other factors that would have previously identified a high risk group (Fisher *et al.* 1989; Plecha *et al.* 1985). The ratio of males to females operated upon has remained almost constant (Figure 6.4b) at about 65% since the inception of the procedure, and this is likely to reflect the underlying prevalence of the disease in the community.

6.6 Conclusion

The risks of death alone and combined stroke and death for symptomatic patients undergoing CEA have neither increased or decreased significantly over the last 15 years. There has also been an increase in the average age of patients and this may indicate that more high-risk patients are being accepted for surgery in general. Overall, the results support the continued policy of CEA for carefully selected symptomatic patients. There is still a significant discrepancy between the outcomes reported by neurologists and surgeons that have been constant for the last 15 years. The 6-7% risk of stroke and death for symptomatic patients reported by the ECST and NASCET were valid 10 years ago then they are likely to remain so today.

Chapter 7

A systematic review of the risks of carotid endarterectomy in relation to the clinical indication and the timing of surgery

7.1 Summary

Objective: Individual studies of the risk of stroke and death due to carotid endarterectomy (CEA) in relation to the clinical indication and the timing of surgery have been small and have produced conflicting results. Reliable data are necessary so that surgery can be targeted more effectively, patients can be properly informed of the risks, the operative risks of individual surgeons or institutions can be corrected for case-mix, and the mechanisms of operative stroke can be better understood.

Design: Systematic review and meta-analysis of reports of the operative risk of CEA.

Methods: A systematic review was done of all studies published between 1994 and 2000 that reported the risk of stroke and death due to CEA stratified by the indication for surgery and timing of surgery. The author combined these data with previously identified studies published between 1980 and 1994. Pooled estimates of the effect of the indication on operative risk were obtained by Mantel-Haenszel method.

Results: The risk of stroke and death ranged from 2.8% (95% CI 2.2-3.4, 18 studies) for CEA for ocular ischaemic events only to 19.2% (95% CI 10.7-27.8, 12 studies) for urgent surgery for ongoing cerebral symptoms. Operative risk was greater for symptomatic stenosis than for asymptomatic stenosis (Odd ratio [OR] = 1.7, 95% CI 1.6-1.9, $p < 0.00001$, 57 studies), but there was no difference in risk between CEA for asymptomatic stenosis and CEA for ocular ischaemic events (OR = 1.2, 95% CI 0.8-1.8, $p = 0.57$, 13 studies). Among symptomatic patients, there was no difference in risk between CEA for stroke and cerebral TIA (OR = 1.1, 95% CI 0.9 - 1.3, $p = 0.22$, 18 studies), but higher risks were found for cerebral TIA than for ocular events (OR = 2.2, 95% CI 1.6 - 3.0, $p < 0.001$, 17 studies) and for CEA for re-stenosis than primary surgery (OR = 1.6, 95% CI 1.2 - 3.2, $p = 0.17$, 5 studies). Urgent CEA for stroke in evolution or crescendo TIA was considerably more risky than surgery for stable symptoms (OR = 4.5, 95% CI 3.0 - 6.8, $p < 0.00001$, 12 studies), but there was no difference between early (< 2 - 6 weeks) and late (> 2 - 6 weeks) surgery in stable patients (OR = 1.1, 95% CI 0.7 - 1.6, $p = 0.9$, 11 studies).

Conclusions: The risk of stroke and death due to CEA is highly dependent on the clinical indication, and analyses of surgical risk should be stratified accordingly. Categorisation of stenosis as "symptomatic" or "asymptomatic" is an oversimplification, and is of limited use in predicting operative risk.

7.2 Introduction

7.2.1 Risk factors and presenting symptomS

Large randomised controlled trials have shown that carotid endarterectomy (CEA) is beneficial for recently symptomatic severe carotid stenosis (Barnett *et al.* 1998; ECST 1998). However, the benefit of surgery is highly dependent on the operative risk. The risk of stroke and death due to CEA is dependent on a number of patient characteristics, particularly the presence and nature of recent cerebrovascular events (Bond *et al.* 2002; Rothwell, Slattery & Warlow 1997). There is little doubt that asymptomatic patients have a lower operative risk than patients with symptomatic stenosis (Rothwell PM, Slattery J & Warlow CP 1996b), but there is uncertainty about the relative risks of surgery in patients presenting with ocular TIA versus cerebral TIA, cerebral TIA versus stroke, and patients with stable symptoms versus those with stroke-in-evolution or crescendo TIA. There are also no reliable data on the risks of re-operation for re-stenosis versus primary surgery. The American Heart Association (AHA) guidelines on CEA give target operative risks for TIA, stroke and for asymptomatic stenosis, but do not subdivide the indications further (Beebe *et al.* 1989).

Reliable data on the risks of CEA by indication are necessary so that surgery can be targeted more effectively, patients can be properly informed of the risks, the operative risks of individual surgeons or institutions can be corrected for case-mix, and that we can better understand the mechanisms of operative stroke. However, the risk of stroke and death due to CEA is relatively low and very large sample sizes (several thousand) are required in order to determine differences reliably and precisely. Meta-analysis allows the results of smaller studies to be combined in a way that achieves this. A systematic review ensures that all available data are included and minimises any

selection bias. The consistency of any findings can then be tested across studies and the causes of heterogeneity determined.

In addition to the type of event, it was postulated that the length of time between the presenting event and operation might be related to patient outcome. A small number of patients may present with diagnoses such as stroke in evolution (SIE) or crescendo TIA (CTIA) and may be considered as candidates for urgent or emergency surgery (Mentzer, Jr. *et al.* 1981). Also until recently it was considered that performing CEA early (less than 6 weeks) after established stroke was associated with a high risk of peri-operative stroke and death (Giordano *et al.* 1985; Rob 1969). This was thought to be due to the occurrence of a number of intracerebral vascular changes that render the brain more susceptible to infarction soon after stroke (Giordano *et al.* 1985). However, more recently the majority of reports have refuted these findings and noted that there is a significant risk that stroke may occur in the six-week period between presentation and CEA (Gasecki & Eliasziw 1998). Another group that has been previously identified as having a greater risk is patients undergoing surgical revision for recurrent carotid stenosis (Hertzer *et al.* 1997). However the numbers of such patients in each subgroup in any centre tend to be small. It is useful, therefore, to group available studies and use meta-analysis techniques to allow meaningful conclusions to be drawn from associations between presenting events and surgical stroke and death rates.

7.2.2 Hypothesis and objectives

To assess whether presenting symptoms and the length of time between the presenting event and operation have any effect on the surgical outcome, the author and a colleague (RB) undertook a major review of all published literature-reporting outcome for CEA in the last 7 years. These data were subsequently combined with data obtained from two similar reviews by the PMR, for the years 1960 to 1995 (Rothwell, Slattery & Warlow 1996a; Rothwell, Slattery & Warlow 1997). However, assessing true complication rates of surgery from the published literature is difficult due to differences in individual surgeons techniques and results, publication bias and inaccuracies of follow-up. Other factors, such

as who reports outcome and where it is reported, have also been shown to affect the published outcome (Rothwell & Warlow 1995). Despite this, if the degree of inaccuracy and bias are constant, it should be possible to make conclusions about relative risks between patients undergoing surgery within the same institution.

7.3 Methods

7.3.1 Review method

A systematic review of all the literature reporting peri-operative stroke and death rates following CEA between 1994 and 2000, was performed by the author and RB. The methods have been stated in Chapter 6 and resulted in a database of 213 articles. References of all relevant studies identified in this database were reviewed and another five papers were included (Gasecki *et al.* 1994; Mattos *et al.* 1995; Parrino *et al.* 2000; Tretter, Jr. *et al.* 1999; Zbornikova, Lassvik & Alm 1998). This database was combined with one formed during a previous review by PMR covering the period 1980 to 1994 (Rothwell, Slattery & Warlow 1996a; Rothwell, Slattery & Warlow 1997). From the pooled database, studies were further analysed if their authors had reported the combined stroke/death rates for patients undergoing CEA and stratified the results by presenting events and the timing of surgery. In papers which did not supply combined stroke and death figures but reported the stroke and death figures separately, it was assumed that the combined number of strokes and deaths were the total of the stroke and death figures.

7.3.2 Statistical analysis

Meta-analyses were performed to calculate both the absolute stroke/death rates for patients presenting with each type of event and the relative risks between the different types of presentation and the timing of surgery. Overall odds ratios and absolute risks of death, and stroke and/or death were calculated using the Mantel-Haenszel method. Tests for heterogeneity, the Chi-squared test, were used to assess whether the outcomes observed in each study differed from the overall estimate (overall odds ratios and absolute risks). Analyses were done with STATA version 6 (Stata Corporation, Texas,

USA) and in all cases a value of $p < 0.05$ was taken to indicate a statistically significant difference.

7.4 Results

We identified 213 studies (see Chapter 6) reporting the risk of stroke and death following CEA published during the period 1994-2000 inclusive. Of these, 49 reported the outcome of surgery but gave no information about the indication, 118 reported the proportion of symptomatic versus asymptomatic patients operated but did not report the operative risk separately. The remaining 43 studies reported results separately for symptomatic and/or asymptomatic patients, and 34 of these studies also stratified their results according to at least two different modes of presentation in symptomatic patients. A further 55 studies were included from our previous review covering the time period 1980-1994.

7.4.1 Absolute risks of surgery

Table 7.1 shows the results of the meta-analyses of the absolute risks of stroke and death due to CEA by indication, and the number of studies and operations on which the estimates were based. The absolute risk of stroke and death ranged from 2.8% (95% CI 2.2-3.4, 18 studies) for CEA for ocular events only to 19.2% (95% CI 10.7-27.8, 12 studies) for urgent surgery for ongoing cerebral symptoms.

7.4.2 Comparisons of risk by indication within studies

The meta-analyses of absolute risks across studies (Table 7.1) were highly heterogeneous i.e. there were significant differences between studies in the operative risks for the same indications. It is therefore more appropriate to determine differences in operative risk by indication within studies and then use meta-analysis to combine the within-study odds ratios. These analyses are summarised in Table 7.2.

Table 7.1. Absolute risks of stroke and death for patients suffering each presenting event.

Presenting event	Number of studies	Number of operations	Absolute risks (%)	95%CI	Heterogeneity
Symptomatic	**95**	**36482**	**5.1**	**4.6-5.6**	**p < 0.001**
Urgent	12	208	19.2	10.7-27.8	p < 0.001
Established stroke*	49	7634	7.1	6.1-8.1	p < 0.001
Cerebral TIA	26	8138	5.5	4.7-6.3	p < 0.001
Ocular ischaemic event	18	1784	2.8	2.2-3.4	p < 0.001
Non-hemispheric	24	1751	4.2	3.2-5.2	p < 0.001
Asymptomatic	**60**	**14399**	**2.8**	**2.4-3.2**	**p < 0.001**
Redo surgery	**12**	**914**	**4.4**	**2.4-6.4**	**p < 0.001**

*Excludes stroke in evolution

Table 7.2. Summary of odds ratios for risk of stroke/death in patients with various presentations.

	Urgent	Stroke	Cerebral TIA	Non-hemispheric	Asymptomatic	Ocular event
Urgent	1	4.0 (2.7-5.7)[a]	5.0 (3.1-8.3)[a]	7.3 (4.2-12.7)[a]	10.5 (6.6-16.8)[a]	10.5 (5.6-19.4)[a]
Stroke	***	1	1.1 (0.9-1.3)[b]	2.1 (1.6-2.7)[a]	2.2 (1.9-2.5)[a]	2.7 (2.0-3.6)[a]
Cerebral TIA	***	***	1	1.1 (0.8-1.7)[b]	1.5 (1.3-1.9)[a]	2.2 (1.6-3.0)[a]
Non-hemispheric	***	***	***	1	1.1 (0.8-1.5)[b]	1.9 (1.1-3.2)[c]
Asymptomatic	***	***	***	***	1	1.2 (0.8-1.8)[b]
Ocular ischaemic event	***	***	***	***	***	1

Data in brackets =95% CI

Significance = [a]$p < 0.005$, [b]$p > 0.1$, [c]$p = 0.06$

Operative risk was greater for symptomatic stenosis than for asymptomatic stenosis (OR = 1.7, 95% CI 1.6-1.9, $p < 0.00001$, 57 studies, p value for heterogeneity = 0.95). In the 13 studies where the comparison was possible (Figure 7.1), surgery for asymptomatic stenosis had a similar operative risk to surgery for ocular ischaemic events only (OR = 1.2, 95% CI 0.8 – 1.8, p = 0.57), and there was no heterogeneity between studies (p = 0.81). There was a highly statistically significant excess operative risk associated with surgery for cerebral TIA versus surgery for ocular events only (OR = 2.2, 95% CI 1.6 – 3.0, $p < 0.001$) (Figure 7.2). This trend was present in all 17 studies from which data were available and there was absolutely no heterogeneity between studies (p = 0.99). Figure 7.3 shows no difference in the relative odds of stroke and death due to CEA for in patients with stroke versus CEA in patients with cerebral TIA in 18 studies (OR = 1.1, 95% CI 0.9-1.3, p = 0.22), and no heterogeneity between studies.

The highest operative risks were reported in studies of urgent surgery for "stroke-in-evolution", "crescendo TIA" and cases that were simply termed "urgent" (Table 2, Figure 7.4). Twelve studies reported data on these groups, and although the number of cases in each individual study was small, the results were consistent with a trend towards a higher operative risk in the urgent surgery in all 12 studies. The combined relative odd of operative stroke and death for surgery for these urgent indications versus non-urgent surgery (routine CEA) across the 12 studies was 4.5 (95% CI 3.0-6.8, $p < 0.0001$). There was no difference in the odds between patients with recent stroke who had surgery less than 2-6 weeks after an established stroke compared with those who had surgery after this period (Figure 7.5).

Only five studies had figures for the risk of surgical revision for re-stenosis versus primary surgery (Figure 7.6). There was no significant difference in the odds (OR = 1.6, 95% CI 1.2–3.2, p = 0.17).

Figure 7.1 The odds of combined stroke and death risk following CEA for asymptomatic patients versus patients presenting with ocular ischaemic event (amaurosis fugax and retinal artery occlusion).

Odd ratio for each study represented by a square, the area of which is proportional to statistical power of estimate. Line represents 95% CI of odd ratio. Diamond represents overall pooled estimate.

Study	Aymptomatic Events / Operations	Ocular Events / Operations	OR	95% CI
Fode *et al*. 1981	14 / 396	14 / 461	1.2	0.6-2.5
Bernstein *et al.* 1982	12 / 92	2 / 57	4.1	0.9- >10
Rosenthal *et al.* 1983	1 / 50	1 / 58	1.2	0.1- >10
Friedman *et al.* 1988	3 / 126	5 / 173	0.8	0.2-3.5
Hafner *et al.* 1988	1 / 283	0 / 166	6.5	0- >10
Treiman *et al.* 1992	0 / 18	0 / 15	1.0	0- >10
Vigo *et al.* 1992	0 / 8	0 / 6	1.0	0- >10
Perler *et al.* 1994	0 / 12	0 / 3	1.0	0- >10
Perler *et al.* 1996	0 / 61	1 / 28	1.0	0- >10
Spencer *et al.* 1996	5 / 238	1 / 78	1.7	0.2- >10
Kucey *et al*. 1998	14 / 350	8 / 193	1.0	0.4-2.3
Tretter *et al.* 1999	1 / 29	3 / 62	0.7	0.1-7.1
Syrek *et al.* 1999	1 / 60	1 / 15	0.2	0-3.5
TOTAL	**53 / 1723**	**37 / 1315**	1.2	0.8-1.8
Significance	p =	0.572		
Heterogeneity	p =	0.812		

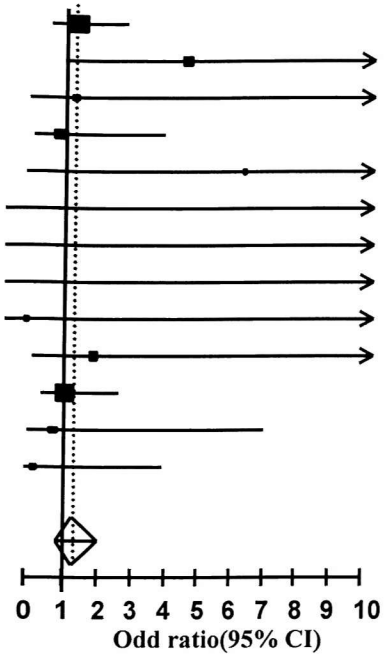


Figure 7.2 The odds of combined stroke and death risk following CEA for patients presenting with cerebral TIA versus patients with presenting with ocular ischaemic event.

Study	Cerebral TIA Events / Operations	Ocular Events / Operations	OR	95% CI
Fode *et al.* 1981	72 / 1283	14 / 461	1.9	1.1-3.4
Bernstein *et al.* 1982	13 / 123	2 / 57	3.3	0.7- >10
Rosenthal *et al.* 1983	15 / 399	1 / 58	2.2	0.3- >10
Friedman *et al.* 1988	11 / 248	5 / 173	1.6	0.5-4.6
Vigo *et al.* 1992	1 / 27	0 / 6	2.5	0- >10
Treiman *et al*. 1992	2 / 46	0 / 15	7.1	0- >10
Perler *et al.* 1994	2 / 25	0 / 3	2.7	0- >10
Golledge *et al.* 1996	7 / 199	1 / 84	3.0	0.4- >10
Perler *et al.* 1996	3 / 46	1 / 28	1.9	0.2- >10
Spencer *et al.* 1996	8 / 139	1 / 78	4.7	0.6- >10
ECST 1998	57 / 825	6 / 247	3.0	1.3-7
Zbornikova et al. 1998	2 / 10	2 / 29	3.4	0.4- >10
Barnett et al. 1998	45 / 703	6 / 167	1.8	0.8-4.4
Kucey et al. 1998	25 / 374	8 / 193	1.7	0.7-3.7
Tretter et al. 1999	11 / 146	3 / 62	1.6	0.4-6.0
Syrek et al. 1999	1 / 11	0 / 15	16.6	0- >10
Blohme et al. 1999	5 / 76	0 / 81	58.2	0.1- >10
TOTAL	**281 / 4680**	**51 / 1757**	2.2	1.6-3.0
Significance	p =	< 0.001		
Heterogeneity	p =	0.994		

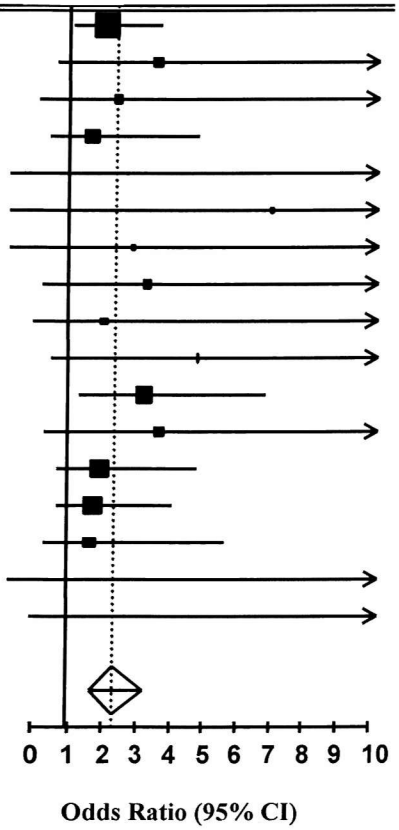


Figure 7.3 The odds of combined stroke and death risk following CEA for patients presenting with established stroke versus patients with cerebral TIA (ie excluding amurosis fugax).

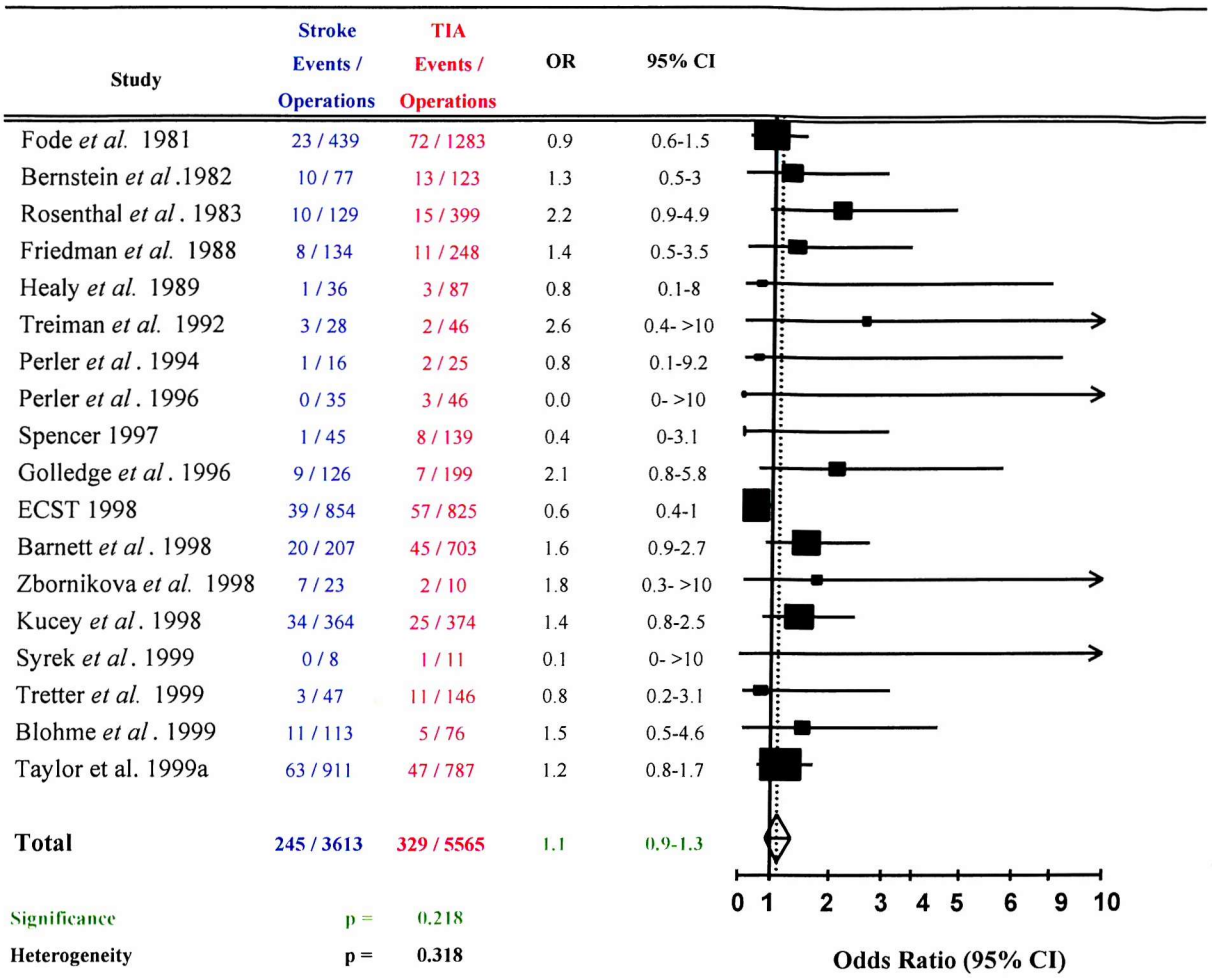


Figure 7.4 The odds of combined stroke and death risk following CEA for patients presenting urgently versus those undergoing routine operations.

Stroke in evolution (SIE) versus Routine operation.

p (sig) = p value for significance

Study	SIE Events / Operations	Routine CEA Events / Operations	Odds Ratio	95% CI
Rosenthal *et al.* 1983	1 / 12	10 / 129	1.1	0.1-9.3
Fode *et al*. 1986	6 / 38	27 / 439	2.9	1.1-7.4
Friedman *et al.* 1988	4 / 7	8 / 134	21.0	4.0- >10
Brook *et al*. 1990	2 / 4	32 / 259	7.1	1.0- >10
Mcrory *et al*. 1993	1 / 4	4 / 49	3.8	0.3- >10
Goldstein *et al*. 1994	1 / 5	11 / 183	3.9	0.4- >10
Golledge *et al*. 1996	3 / 25	17/409	3.1	0.9->10
Tretter *et al*. 1999	1 / 5	3 / 47	3.7	0.3- >10
SUBTOTAL	**19 / 100**	**112/1649**	3.7	2.1-6.3

p(sig) < 0.00001

p(het) = 0.51

Urgent CEA versus Routine CEA

For the urgent CEA papers indicated only that these patients needed immediate or emergency CEA but there were not enough details for further classification.

Study	Urgent CEA Events / Operations	RoutineCEA Events / Operations	Odds Ratio	95% CI
Schneider *et al*. 1993	1 / 43	1 / 237	5.6	0.1- >10
Mattos *et al*. 1995	5 / 9	47 / 423	10.0	2.6- >10
Rigdon *et al*. 1998	2 / 3	16 / 253	29.6	2.6->10
SUBTOTAL	**8 / 55**	**64 / 913**	10.9	3.7->10

p(sig) < 0.00001

p(het) = 0.65

Crescendo TIA (CTIA) versus Routine CEA

Study	CTIA Events / Operations	RoutineCEA Events / Operations	Odds Ratio	95% CI
Kirschner *et al*. 1989	7 / 27	13 / 119	2.9	1.0-8.0
Golledge *et al*. 1996	7 / 26	17/409	8.5	3.2->10
SUBTOTAL	**14 / 53**	**30/528**	**4.5**	**2.2-9.4**

p(sig) = 0.001

p(het) = 0.13

TOTAL	41/208	206/3090	4.5	3.0-6.8

Significance of total 12 papers p < 0.00001

Heterogeneity p = 0.42

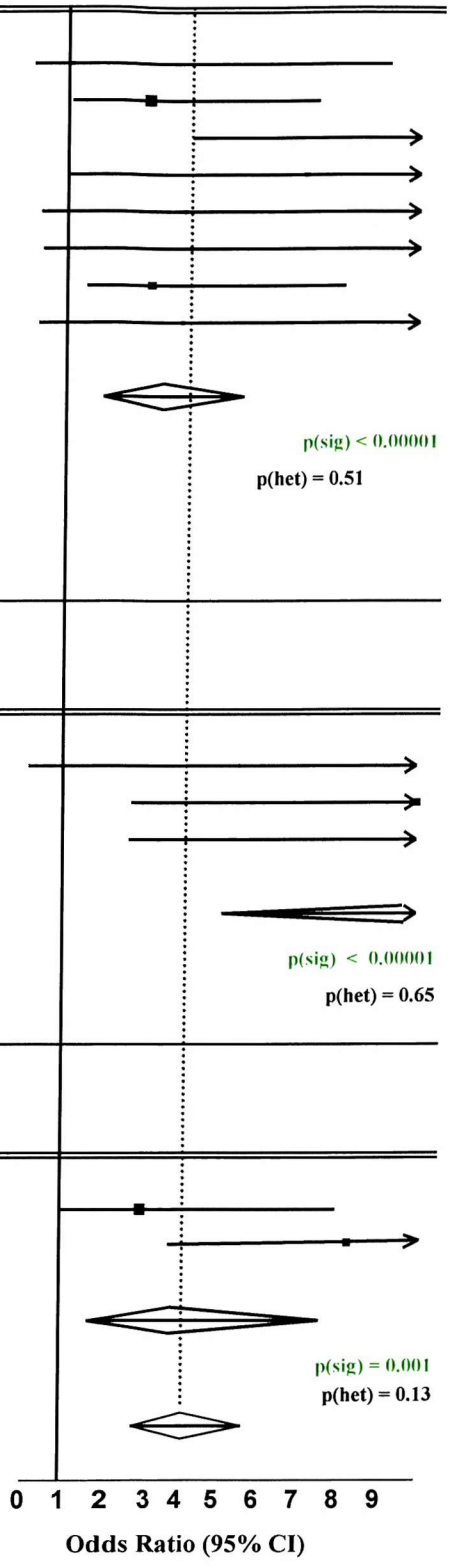
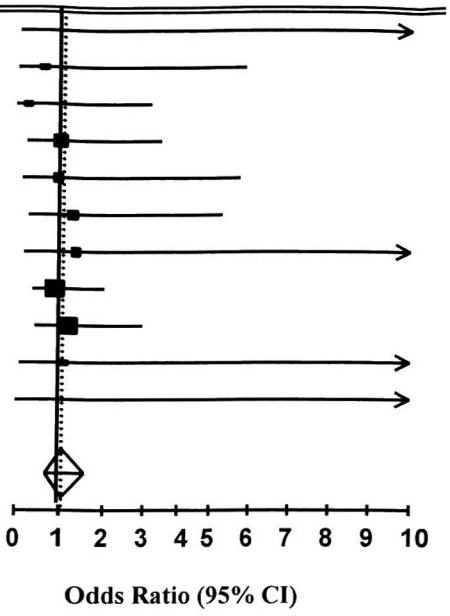


Figure 7.5 The odds of combined stroke and death risk following early CEA (< 2-6 weeks) versus late CEA (> 2-6 weeks).

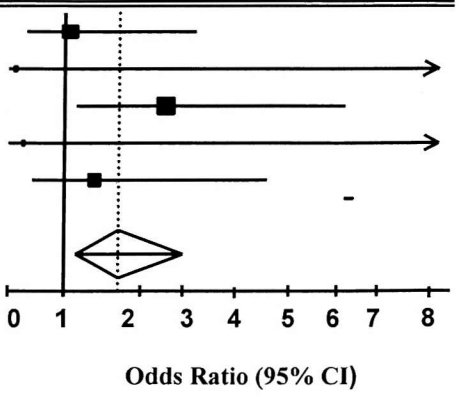
Study	Early CEA Events / Operations	Late CEA Events / Operations	Odds Ratio	95% CI
Giordano *et al.* 1985	5 / 27	0 / 22[d]	51.0	0.1- >10
Rosenthal *et al.* 1988	1 / 29	4 / 75[a]	0.6	0.1-5.9
Piotrowski *et al*. 1990	1 / 82	2 / 47[c]	0.3	0-3.1
Goldstein *et al.* 1994	4 / 68	7 / 115[b]	1.0	0.3-3.4
Gasecki *et al*. 1994	2 / 42	3 / 58[c]	0.9	0.1-5.7
Eckstein *et al*. 1998	2 / 56	53 / 1853[c]	1.3	0.3-5.3
Hoffmann *et al*. 1998	2 / 26	2 / 34[c]	1.3	0.2- >10
ECST 1998	7 / 122	45 / 692[c]	0.9	0.4-2
Barnett *et al.* 1998	8 / 71	13 / 132[c]	1.2	0.5-3
Khan *et al*. 1999	2 / 49	1 / 26[c]	1.1	0.1- >10
Parrino *et al*. 2000	0 / 20	0 / 25[c]	1.3	0- >10
TOTAL	**34 / 592**	**130 / 3079**	1.1	0.7-1.6
Significance	p =	0.856		
Heterogeneity	**p =**	**0.822**		



Time period = [a]2 weeks, [b]3 weeks, [c]4 weeks, [d]5 weeks, [e]6 weeks

Figure 7.6 The odds of combined stroke and death risk following recurrent CEA (revision CEA) versus primary CEA.

Study	Revision Events / Operations	Primary Events / Operations	OR	95% CI
Coyle *et al.* 1995	3 / 69	40 / 1003	1.1	0.3-3.6
Plestis *et al.* 1996	0 / 33	23 / 973	0.1	0- >10
Hertzer *et al*. 1997	7 / 136	34 / 1788	2.8	1.2-6.4
Hill *et al*. 1999	0 / 40	3 / 350	0.3	0- >10
Maxwell *et al*. 2000	3 / 63	64 / 2018	1.5	0.5-5
Total	13 / 341	164 / 6132	1.6	1.2-3.2
Significance	p =	0.167		
Heterogeneity	**p =**	**0.386**		



7.5 Discussion

Although it is now well recognised that the risk of stroke and death due to CEA is dependent on the indication, and the AHA recommendations specify operative risks by indication (Beebe *et al.* 1989; Moore *et al.* 1995), only a quarter of studies published between 1994 and 2000 stratified their results even according to whether the patients were symptomatic or asymptomatic. In the majority of these publications, the operative risk of CEA was the primary topic of the research, and the lack of reporting of the risk according to the indication for surgery seriously limits the usefulness of the report.

Ad Hoc Committees of the AHA Stroke Council have recommended that the combined risk of stroke and death due to CEA should be no more than 3% for asymptomatic patients, 5% for patients with TIA, 7% for patients with previous strokes and 10% for patients with recurrent stenosis (Beebe *et al.* 1989). The combined estimates of the absolute risk of stroke and death in our review correspond reasonably well to these recommendations (Table 7.1). However, for each of the indications that we studied there was statistically significant heterogeneity between studies, such that interpretation of the overall absolute risks is not straightforward. As has been shown previously (Rothwell & Warlow 1995; Rothwell, Slattery & Warlow 1996a), published absolute risks of CEA differ depending on whether the study was prospective or retrospective, and whether post-operative assessment was performed by a surgeon or neurologist. There are also likely to be other biases, such as publication bias, the extent of which will vary from one study to another. Unlike the absolute risks of surgery, the within-study relative odds of stroke and death due to CEA for one indication versus another were generally highly consistent, and it was therefore appropriate to perform meta-analyses of these within-study comparisons in order to derive precise estimates of the effects of the indication for surgery on the operative risk. These analyses have produced several original and clinically useful observations.

7.5.1 Operative risk in patients with ocular ischaemic events only

The AHA guidelines on CEA do not differentiate between surgery for cerebral TIA and surgery for ocular TIA. However, our analysis shows that patients with only ocular ischaemic events have a consistently lower surgical risk (Table 7.1). This lower risk of stroke in patients with ocular ischaemic events than those with cerebral events has also been shown in patients on medical treatment only (Hankey, Slattery & Warlow 1991). In addition Golledge *et al.* (1996) studied the association between presentation and long-term survival (36 months after surgery) and stroke rate. They found that the prognosis of patients with amaurosis fugax was best for all types of symptomatic patients, with survival significantly better than it was for patients with cerebral TIA ($p < 0.01$). Similarly amaurosis fugax was associated with a lower risk of stroke than it was for patients with cerebral TIA ($p < 0.05$).

It is also important to note that the operative risk in patients with ocular ischaemic events was non-significantly higher than that in patients with asymptomatic stenosis (Figure 7.1). This shows that, in terms of operative risk, the strict distinction between surgery for symptomatic stenosis and surgery for asymptomatic stenosis is false. In practical terms, it is clear that future studies reporting the operative risk of CEA should consider patients with ocular ischaemia and cerebral ischaemia separately.

7.5.2 Operative risk in patients with TIA versus stroke

The AHA guidelines give recommended maximum operative risks of 5% for TIA and 7% for stroke. However, they do not differentiate between ocular TIA and cerebral TIA (Figure 7.2). Our analysis shows that when surgery for ocular TIA is considered separately, there is no difference in the operative risk of CEA between cerebral TIA and stroke (Figure 7.3). There is therefore a case for revision of current guidelines.

7.5.3 Operative risk in relation to the timing of surgery

The issue of risk of CEA in relation to the timing of surgery can be separated into two main parts. Firstly, what is the risk of surgery in the acute phase i.e. in patients with stroke-in-evolution and crescendo TIA? Secondly, does the risk of surgery differ between the sub-acute phase (first six weeks) and the non-acute phase (> 6 weeks) in those patients who are neurologically stable? In relation to the first question, although the definitions of stroke-in-evolution and crescendo TIA are somewhat subjective, they are widely recognised clinical syndromes. They have a relatively poor prognosis on medical treatment alone, and some surgeons feel that urgent CEA is indicated in those patients with severe stenosis (Carter 1961; Mentzer, Jr. *et al.* 1981).

Our meta-analysis suggests that the operative risk of stroke and death in patients operated for stroke-in-evolution and crescendo TIA was in the region of 20%, and that the relative odds of a surgical stroke or death were three times greater than in patients with stable disease (Table 7.1). The number of studies was relatively small, and the definitions of urgent surgery varied, but the finding of a very high risk in this situation was consistent (Figure 7.4). This risk must be balanced against the likely outcome if surgery had not been performed, but in the absence of randomised controlled trials of CEA for this indication, the available data on operative risk do not support the routine use of CEA in the acute phase.

The second issue of whether the risk of surgery differs between the sub-acute phase (first six weeks) and the non-acute phase (> 6 weeks) in those patients who are neurologically stable is more clear-cut. Although it has long been considered that CEA early after established stroke has a high operative risk (Giordano *et al.* 1985; Rob 1969), the brain perhaps being more susceptible to infarction if exposed to a further ischaemic insult at this stage (Giordano *et al.* 1985), there was no evidence in our analysis of any increased risk due to surgery in the sub-acute phase (Figure 7.5). Moreover, any increased operative risk would have to be balanced against the significant risk of stroke

on medical treatment alone if surgery is delayed. The 30-day risks of stroke in patients randomised to medical treatment in NASCET were 4.9% (Gasecki & Eliasziw 1998).

In summary, therefore, the operative risk of CEA in the acute phase of ongoing or progressing cerebral ischaemia is probably too high to be justified in the majority of circumstances, but the fears of a similarly high risk in the sub-acute phase in patients with a stable neurological syndrome appear to be unfounded.

7.5.4 Operative risk of surgery for re-stenosis

The reported incidence of recurrent carotid stenosis after CEA varies from 1.2% to 35%, but this is dependent upon the definition of re-stenosis and the length and methods of follow up (Gagne *et al.* 1993; Norrving, Nilsson & Olsson 1982). A recent systematic review suggested that the incidence of re-stenosis (degree of stenosis > 50%) is about 10% in the first year after surgery, but then falls to a stable level of 1% per year by the third year (Frericks *et al.* 1998). It is uncertain how many patients with recurrent stenosis go on to develop symptoms, but several studies have suggested that re-stenosis has a more benign course than primary disease (Frericks *et al.* 1998). This is consistent with the observation that early re-stenosis is associated with smooth myointimal hyperplasia, rather than unstable atherelosclerotic plaque.

In the review of the current study it was found that surgical revision was not associated with a higher risk of stroke and death than primary surgery was. There were insufficient data to stratify the analysis according to the symptom status of either the primary or revision patients. The decision to perform surgery on patients with symptomatic or asymptomatic should be performed in the knowledge that there is an increased risk of local complications, such as cranial nerve injury and wound haematoma (Mansour *et al.* 1997). Carotid angioplasty with stenting may be an alternative treatment with a low risk of local complications during intervention (Vitek *et al.* 2001).

7.5.5 Limitation

The studies included in this study were of varying methodological quality (details in Chapter 8). Some were retrospective and only a minority of the remainder had independent assessment of outcomes by a neurologist. This made the assessment of absolute stroke and death rates of surgery from the published literature extremely difficult. Moreover the small number of events in some comparisons complicated matters further because the odds ratios are very sensitive-a few events more or less may cause dramatic changes--and have large confidence intervals and low statistical power. Obviously more data is required. Furthermore since some papers did not supply the combined number of strokes and deaths, it was assumed that the number of strokes and deaths was equal to the total of the separate numbers of strokes and deaths. This may have resulted in an overestimate of the true figure. In spite of this, if the degree of overestimation was constant, it should be possible to draw conclusions about relative risks for patients undergoing surgery at the same institution.

7.6 Conclusions

Although established guidelines on the use of CEA clearly state that the risk of the procedure is dependent on the clinical indication (Beebe *et al.* 1989; Moore *et al.* 1995), the majority of published reports of the risks of CEA do not stratify their results by indication. Our analyses show that the risk of stroke and death due to CEA is highly dependent on the clinical indication and the timing of surgery, and reports of surgical risk should be stratified accordingly. It is also clear that categorisation of patients as "symptomatic" or "asymptomatic" is an oversimplification, and is of limited use in predicting operative risk. There is considerable difference of risk between the different "symptomatic" groups, and patients with only ocular ischaemic events are closer in risk to patients with asymptomatic stenosis. In relation to the timing of surgery, the operative risk of CEA in the acute phase of ongoing cerebral ischaemia is probably too high to be justified in routine clinical practice, but surgery in the sub-acute phase in patients with a stable neurological syndrome is not associated with a higher operative risk than later surgery.

Chapter 8

Quality of methodology and reporting in studies of surgical risk: lessons from published reports of carotid endarterectomy

8.1 Summary

Objective: Recently there has been a great deal of concern about the quality of articles in the surgical literature. The objective of this study was to evaluate the quality of the design and reporting of clinical research in surgical papers about the risks of carotid endarterectomy (CEA).

Design: Systematic review and meta-analysis of reports of the operative risk of CEA published in English.

Methods: All English-language publications on the risk of stroke and death due to CEA were identified by electronic and manual searching. 12 criteria were used to assess their quality, and were related to the reported operative risk.

Results: 213 eligible publications were studied. Many flaws in research design and reporting were identified. For example, the definitions of stroke and stroke severity were reported in only 27.5% and 20.6% of the papers respectively, and adequate definitions of any risk factors were given in only 15.8% of reports. Studies that were prospective and studies that had independent assessment of outcome reported higher risks of stroke and death. Several criteria for the quality of reporting were also independently associated with the reported operative risk of stroke and death, including specification of inclusion/exclusion criteria, reporting of baseline patient data, definitions of stroke and peri-operative period, definitions of any risk factors that were reported, and description of surgical techniques.

Conclusions: Low quality research and inadequate reporting of research are very common in the literature of CEA. This could be improved by attention to simple quality criteria. Among published reports of the operative risk of CEA, those papers that did not satisfy the quality criteria reported the lowest operative risks.

8.2 Introduction

8.2.1 Reporting and the risk of stroke and death

Recently there has been a great deal of concern about the quality of published surgical papers. Magos *et al.* (2000) found that only 6.8% of the papers in four leading non-specialist medical journals (The Lancet, BMJ, N Engl J of Med, and JAMA) were surgical papers They felt that surgeons' papers deserved the same representation in such journals as those of physicians. One editor has attributed this meagre representation to the poor quality of surgical papers (Smith 2000) and another editor has compared surgical papers to "comic opera" (Horton 1996).

In this era of evidence-based medicine, high-quality randomised controlled trials (RCTs) are considered to be the most credible type of clinical study. In a survey of nine general surgery journals in 1996, 175 papers reporting the results of original research were identified (Horton 1996). Only 7% of these were randomised controlled trials; 46% were case series and 31% were animal experiments. Some surgeons have argued that in surgical research, studies comparing two procedures tend to be difficult and underpowered because of very small differences in outcomes (Baum 2000; Dimick, Diener-West & Lipsett 2001). In addition, there is a large variation in surgical expertise and a lack of funding for surgical trials (Baum 2000; Majeed *et al.* 1996; McLeod 1999). Some investigators have claimed that surgeons are not well trained in clinical research (McLeod & Solomon 1995) and others feel that academic surgeons find it far easier to be promoted by engaging in laboratory research than by conducting arduous RCTs (Cohen 1995).

What is the evidence that the quality of surgical papers is poor? Most studies of the quality of articles in the surgical literature have concentrated on RCTs (Dimick, Diener-West & Lipsett 2001; Moss *et al.* 2001; Solomon *et al.* 1994). Few studies have reviewed the quality of surgical case series, although this is by far the most common form of clinical research performed by surgeons. This is surprising because surgical

case-series, particularly those that report operative risks, have a major effect on surgical practice. For example, published reports of operative risks are frequently used to determine standards for "best practice" when developing clinical guidelines. It was therefore decided to study the quality of papers reporting the risk of stroke and death because of CEA, the majority of such papers being case-series.

CEA is one of the most common operations in arterial surgery. Since the publication of two landmark randomised controlled trials (ECST 1991; NASCET 1991) many hundreds of papers have been published about this operation and its major complications of stroke and death. However the reported risk of stroke and death after CEA varies enormously. It was found in one study that the risk of disabling stroke and death in symptomatic patients ranged from 1.03 to 35% (Rothwell, Slattery & Warlow 1996a). It was also found that this figure was highest in studies in which patients were assessed by a neurologist after surgery and lowest in studies with a single surgical author (Rothwell, Slattery & Warlow 1996a). It was also observed that in reports from one medical centre the risks reported by surgeons and neurologists were 0% and 11.4% respectively, a great difference, although they were based on the same data and periods (Berguer 2001; Chaturvedi, Aggarwal & Murugappan 2000). Although it was suggested that this difference may have been due to differences in inclusion criteria (Chaturvedi 2001), there is increasing evidence that the quality of research design and reporting are associated with risks of stroke and death following CEA that are reported.

8.2.2 Hypothesis and objectives

It has been suggested that the quality of articles in the surgical literature is poor. A systematic review was therefore done to determine the quality of the design of studies and the completeness of reporting in papers about the risk of stroke and death following CEA over the past seven years. It was also attempted to assess whether the criteria of the quality of papers were related to the outcomes reported. In other words, not only might criteria be indicators of a good paper, but they might also be related to a high risk of peri-operative stroke and death.

8.3 Methods

The author and RB performed independent systematic reviews of all reports of the risk of stroke and death following CEA published between 1994 and 2000 inclusive. The methods have been described in Chapter 6 and resulted in a database of 213 articles.

8.3.1 Methodological criteria

The quality of research design and the completeness of reporting were assessed using the 12 criteria in table 8.1. To date, there has been no formal scoring system for quality of CEA papers, or surgical case-series. All 12 criteria are modifications of criteria in papers that have assessed the quality of papers in the surgical literature (Evans & Pollock 1985; Solomon & McLeod 1993). The definition of hypertension was used as a surrogate measure of the adequacy of reporting of risk factor data – in those papers where hypertension was mentioned. For each criterion, each paper was evaluated as having adequate or inadequate information. Those papers with inadequate information, where it was felt that the criterion was not appropriate to the main topic, or the methods were not included. Each paper was reviewed by two independent readers, who followed a standard checklist and evaluated the paper with respect to each of the 12 criteria. A pilot study of 20 papers was done by the two readers before the quality of all 213 papers was examined. At the end of study, all data were combined and any disagreements were resolved by a joint review of a paper and discussion until a consensus was reached.

For each criterion, the papers in which the criterion was considered to be relevant were categorised as having adequate or inadequate information. The proportion of papers with adequate information was calculated. The denominators were combined only between the number of papers with adequate and inadequate information. These denominators excluded papers in which the two independent researchers agreed that it was not reasonable to expect the criterion to be satisfied. For example, if hypertension was not mentioned in the paper, the definition of hypertension was clearly unnecessary. Papers that failed a particular criterion were those where I and RB agreed that the

criterion was relevant to the methods or reporting of the research and where the criterion was not satisfied. For example, if the authors reported the risk of stroke following CEA, but did not give the definition of stroke that was used (symptoms > 24 hours *versus* symptoms > 7 days etc), or the length of the post-operative period on which the risk estimate was based (hospital discharge *versus* 7 days *versus* 30 days etc).

8.3.2 Statistical analysis

Following the assessment of quality by the two independent researchers, the agreement above and beyond what could have been expected by chance was calculated using kappa value. The Mantel-Haenszel method was used to determine the pooled risks of stroke and death across different studies. Tests for heterogeneity, the Chi-squared test, were used to assess whether the risks observed in each study differed from the pooled risks.

The association between each quality criterion and the reported risk of stroke and death was first calculated for the papers that reported risks for patients operated for symptomatic stenosis. It was necessary to consider symptomatic stenosis and asymptomatic stenosis separately because the operative risk of stroke and death is significantly higher in patients with symptomatic stenosis (Rothwell PM, Slattery J & Warlow CP 1996b). The number of papers reporting outcome separately for patients with asymptomatic stenosis was too small to allow meaningful analysis. For papers reporting the outcome of surgery for symptomatic carotid stenosis, pooled estimates of the operative risk of stroke and death were calculated according to whether each of the criteria for methodology and reporting were satisfied. In addition, the percentage of studies fulfilling criteria according 2 different types of assessors (independent versus non-independent assessors) was determined and any difference of these percentages was calculated using the Chi-squared test.

Although relatively few papers reported the operative risk in symptomatic versus asymptomatic patients separately, many papers reported the proportion of symptomatic

versus asymptomatic patients included in the series. It was therefore possible to determine the overall association between the quality of reporting and the operative risk of stroke and death in this larger group of studies by multivariate analysis, correcting for the proportion of symptomatic patients. A multivariate regression analysis was performed, with weighting of studies according to the total number of patients included, and adjusting for major confounding factors (Rothwell, Slattery & Warlow 1996a; Rothwell, Slattery & Warlow 1997), namely the proportion of symptomatic cases, whether studies were prospective, the proportion of women in the case series, and the mean age of patients in the series. Analyses were done with STATA version 6 (Stata Corporation, Texas, USA) and in all cases a value of $p < 0.05$ was taken to indicate a statistically significant difference.

8.4 Results

8.4.1 Results of searching and extraction of data

The quality of 213 papers was assessed, 16 of which were randomised controlled trials. Only 67.8% of papers indicated whether or not the series of patients reported were consecutive. A total of 31.5% of papers included a consecutive series of patients. 88.7% of the papers were published in surgical journals; the remainder were published in non-surgical or non-specialist journals. Only 4 papers (1.9%) were published in one of the four leading non-specialist medical journals (The Lancet, BMJ, N Engl J of Med, and JAMA). The authors were mainly from the USA (59.2%) and Europe (33.2%). No studies by either reader were selected, so there was no conflict of interest.

The kappa coefficients for agreement between the independent observers in application of the quality criteria ranged from 0.68 to 1.00 (Table 8.1). These values represent *good agreement* (kappa = 0.61 – 0.80) and *very good agreement* (kappa > 0.8) (Altman 1993).

8.4.2 Quality of papers

Of these 213 papers, 22.6% were prospective studies and 11.7% had independent assessors (Table 8.1). There were very low rates of adequate reporting of the definition of stroke (27.5%), the definition of stroke severity (20.6%) and the definition of hypertension (15.8%) (Table 8.1). Of 213 papers, 66.7% defined the peri-operative period, 87% of which used the 30 days following surgery (or similar time period).

8.4.3 Criteria of quality and the risk of stroke and death.

The papers that were prospective studies and papers that had independent assessment of outcome reported higher operative risks of stroke and death than those that did not (Table 8.2). In relation to the quality of reporting, all of the quality criteria were associated with a higher reported operative risk, apart from the adequate reporting of surgical detail. The trend towards a higher risk of stroke and death in papers that satisfied the quality criteria was statistically significant for all the criteria apart from the reporting of whether or not the series was consecutive and the adequate reporting of surgical details (Table 8.2).

Table 8.1 The criteria used to assess the quality of methodology and reporting of 213 studies of the risks of carotid endarterectomy. The number of studies fulfilling each criterion, and the inter-observer agreement for the assessment of each criterion are given.

Methodology	Fulfilling criteria (%)	% Agreement	Kappa value	95% CI	p value
Were data collected prospectively?	48/213 (22.6)	94	0.83	0.64 - 1.00	< 0.0001
Was post-operative assessment performed by an independent physician or neurologist?	25/213 (11.7)	94	0.70	0.38 - 1.00	< 0.0001
Reporting					
Are the inclusion and/or exclusion criteria specified?	175/213 (82.2)	85	**0.68**	0.48 - 0.88	< 0.0001
Are the indications for surgery specified?	195/213 (91.6)	100	**1.00**	Not estimable	< 0.0001
Is it clear whether the patients are consecutive or non-consecutive?	**139/205 (67.8)**	86	0.70	0.52 - 0.89	< 0.0001
Are basic demographic data reported?	159/213 (74.7)	90	0.77	0.59 - 0.96	< 0.0001
Are risk factors defined: is a definition given for hypertension?	**23/146 (15.8)**	94	0.90	0.79 - 1.00	< 0.0001
Is the definition of stroke reported?	**58/211 (27.5)**	96	0.89	0.75 - 1.00	< 0.0001
Are data on the severity of stroke reported?	**42/204 (20.6)**	98	0.95	0.85 - 1.00	< 0.0001
Is the period of assessment of operative risk defined?	**142/213 (66.7)**	87	0.72	0.53 - 0.91	<0.0001
Are adequate data reported on surgical technique?	155/207 (74.9)	85	0.69	0.50 - 0.88	<0.0001
Are any statistical methods described?	145/166 (87.3)	96	0.90	0.76 - 1.00	<0.0001

CI = confidence interval

Table 8.2 The operative risk of stroke and death in 46 studies of carotid endarterectomy for symptomatic stenosis in relation to the quality of study methodology and reporting.

Methodology	Risk (95% CI)*	p value
Were data collected prospectively?		
- Yes	**6.3 (5.2 - 7.3)****	0.003
- No	4.2 (3.4 - 5.0)	
Was post-operative assessment performed by neurologist?		
- Yes	**6.7 (5.6 - 7.7)****	< 0.00001
- No	3.8 (3.1 - 4.6)**	

Reporting	Adequate	Inadequate	p value
	S/D rate*(95% CI)	S/D rate*(95% CI)	
Are the inclusion and/or exclusion criteria specified?	5.1 (4.4 - 5.8)	2.2 (1.3 - 3.2)**	< 0.00001
Are the consecutive patients specified?	4.8 (4.0 - 5.6)**	3.3 (1.9 - 4.6)**	0.070
Are basic baseline demographic data reported?	6.8 (5.5 - 8.1)	4.6 (3.9 - 5.3)**	0.004
Is a definition given for hypertension?	6.7 (5.2 - 8.2)	4.3 (3.4 - 5.3)**	0.007
Is the definition of stroke reported?	5.5 (4.7 - 6.3)**	3.7 (2.7 - 4.8)	0.006
Are data on the severity of stroke reported?	6.2 (5.5 - 6.9)	3.0 (2.3 - 3.8)	< 0.00001
Is the period of assessment of operative risk defined?	5.3 (4.5 - 6.0)**	2.9 (1.6 - 4.1)	0.002
Are adequate data reported on surgical technique?	4.3 (3.4 - 5.2)	5.3 (4.1 - 6.5)**	0.191
Are any statistical methods described?	5.3 (4.6 - 6.1)**	2.5 (1.4 - 3.6)	< 0.00001

*Mantel-Haenszel method, **p value for heterogeneity < 0.001, S/D = stroke and death rate, CI = confidence interval

Papers that had independent assessment of outcome (usually by neurologists) tended to have a higher quality of reporting than papers with other methods of assessment (Table 8.3). Similar differences were found between prospective and retrospective studies (data not shown), but there was a strong association between independent assessment of outcome and prospective methodology. All papers with independent assessors had adequate details of the indications for surgery, the baseline patient data, and the statistical methods used. The differences in quality between papers reporting studies with independent assessors and those with other types of assessment were statistically significant for description of baseline patient data, and for the definition of stroke (Table 8.3). The only criterion that favoured studies with non-independent assessment was the reporting of adequate detail of the surgical techniques used.

Studies with independent assessors were excluded from the multivariate analysis of the association between the quality of reporting and the risk of stroke and death. This was done because this relatively small group of studies were not typical of the majority of studies in that they had high risks of stroke and death, and generally had a high quality of reporting. It was therefore useful to determine whether quality of reporting was still associated with an increased operative risk in the majority of studies that did not have independent assessment. When each criterion of reporting was associated with the stroke and death rate together with adjustment for the other possible confounding factors detailed in the methods section (univariable regression analysis), five quality criteria were significantly associated with high risk of stroke and death (Table 8.4). When all criteria were included together in a multivariate analysis, six out of ten reporting criteria were independently associated with a higher risk of stroke and death (Table 8.5).

Table 8.3 The number of studies fulfilling 10 criteria for 2 different types of assessors.

Criterion	Number of studies fulfilling criteria (%)			
	Independent assessors N=25	**Non-independent assessors** N=188	**Risk ratio (95% CI)**	**p value***
Are the inclusion and/or exclusion criteria specified?	22/25 (88.0)	153/188 (81.4)	1.08 (0.92 - 1.27)	0.580
Are the indications for surgery specified?	**25/25 (100.0)**	170/188 (90.4)	1.11 (1.06 - 1.16)	0.140
Is it clear whether the patients are consecutive or non-consecutive?	21/25 (84.0)	118/180 (65.6)	1.28 (1.05 - 1.57)	0.060
Are basic baseline demographic data reported?	**25/25 (100)**	134/188 (71.3)	1.40 (1.28 - 1.54)	**0.002**
Is a definition given for hypertension?	4/24 (16.7)	19/122 (15.6)	1.07 (0.40 - 2.87)	1.000
Is the definition of stroke reported?	12/25 (48.0)	46/186 (24.7)	1.94 (1.20 - 3.13)	**0.010**
Are data on the severity of stroke reported?	9/24 (37.5)	33/180 (18.3)	2.05 (1.12 - 3.73)	0.060
Is the period of assessment of operative risk defined?	20/25 (80.0)	122/188 (64.9)	1.23 (0.99 - 1.54)	0.130
Are adequate data reported on surgical technique?	14/23 (60.9)	141/184 (76.6)	0.79 (0.57 - 1.11)	0.100
Are any statistical methods described?	**21/21 (100)**	124/145 (85.5)	1.17 (1.09 - 1.25)	0.080

* p value calculated by Chi-squared test, CI = confidence interval, N= number of studies.

Table 8.4 Univariable regression analysis of effect of study methodology on reported operative risk of stroke and/or death in studies without independent assessors adjusted for possible confounding factors (proportion of symptomatic cases, females, prospective studies and mean age)

Study characteristic	Parameter estimate	SE	Mean square	p value
Are the inclusion and/or exclusion criteria specified?	0.36	0.35	1353.06	0.300
Are the indications for surgery specified?	1.02	1.25	851.92	0.410
Is it clear whether the patients are consecutive or non-consecutive?	0.42	0.31	2296.95	0.180
Are basic baseline demographic data reported?	0.95	0.34	9284.77	**0.006**
Is a definition given for hypertension?	2.05	0.42	16786.49	**< 0.0001**
Is the definition of stroke reported?	1.20	0.25	26156.23	**< 0.0001**
Are data on the severity of stroke reported?	0.53	0.27	2412.45	0.150
Is the period of assessment of operative risk defined?	0.96	0.29	13041.18	**0.001**
Are adequate data reported on surgical technique?	-1.07	0.28	21348.41	**< 0.0001**
Are any statistical methods described?	0.49	0.55	587.63	0.630

SE: standard error

Table 8.5 Multivariate regression analysis of effect of study methodology on reported operative risk of stroke and/or death in studies without independent assessors adjusted for possible confounding factors (proportion of symptomatic cases, females, prospective studies, and mean age).

Study characteristic	Parameter estimate	SE	Mean square	p value
Are the inclusion and/or exclusion criteria specified?	0.86	0.30	6155.80	**0.005**
Are the indications for surgery specified?	2.04	0.99	31.83	0.839
Are the consecutive patients specified?	0.26	0.26	761.13	0.322
Are basic baseline demographic data reported?	0.06	0.31	3250.55	**0.042**
Is a definition given for hypertension?	1.59	0.38	6970.09	**< 0.001**
Is the definition of stroke reported?	0.55	0.27	3136.03	**0.046**
Are data on the severity of stroke reported?	0.05	0.29	138.45	0.836
Is the period of assessment of operative risk defined?	0.64	0.25	4959.35	**0.012**
Are adequate data reported on surgical technique?	-0.74	0.28	7885.91	**< 0.0001**
Are any statistical methods described?	0.03	0.46	98.87	0.881

SE: standard error

8.5 Discussion

8.5.1 Agreement on individual criteria

Agreement on individual quality criteria was good to very good (Altman 1993), despite the subjective element of the decision as to whether the reporting was "adequate". In keeping with previous reviews of surgical research (Horton 1996), only a small proportion of studies (16 of 213) were randomised controlled trials (Solomon & McLeod 1993). The vast majority of papers were reports of surgical case series, and might be prone to biases, particularly when the study data were not collected prospectively specifically for research purposes (Davey Smith 1992; Solomon & McLeod 1993). In this respect, it is also disappointing that in only one third of the papers was it clear that the study was definitely confined to a consecutive series of patients. Non-consecutive studies may be biased because of exclusion of cases with a poor outcome.

8.5.2 Fulfillment of criteria

Although many hundreds papers on the risks of CEA have been published, the majority of them unfortunately have methodological shortcomings. Relatively few studies were prospective. It has been shown previously that prospective studies report higher risks of stroke and death than retrospective studies (Rothwell, Slattery & Warlow 1996a). Retrospective case-note reviews might miss minor strokes that were not documented. As a rule of thumb, the ratio of post-carotid endarterectomy stroke to death should be about six to one (Barnett 2001). Reports of a lower ratio should raise the possibility that minor strokes have been missed.

Only 11.7% of papers had independent assessment of outcome. It has been shown previously that studies with independent assessors (neurologists) report higher risks of stroke and death due to CEA than those with other types of assessment. The type of assessor should be reported in all papers. However since a large proportion of papers are retrospective studies (77.4%), it might be impossible to indicate this because of a lack of any systematic protocol for assessment of patients.

The definitions of important terms were often omitted or inadequate in these papers. The definitions of stroke and stroke severity were only given in 27.5% and 20.6% of articles respectively. These should have been stated to allow proper interpretation of the data, and comparison between studies. When it was defined, most papers used stroke to mean any neurological deficit persisting for more than 24 hours, but some studies used the term reversible ischaemic neurological deficit (RIND) for neurological deficit longer than one day, but not permanent (Buchbinder *et al.* 1994). The number of these strokes would be smaller. In addition, many papers used the term disabling or major stroke, but few papers gave a definition. This is important for reporting of outcomes because the number of disabling strokes or major strokes will be smaller than the total number of strokes. Consequently the figures for stroke and death at such centres would be lower than those at other centres, but would not represent the true risks of surgery.

The risk of stroke and death due to CEA is dependent on a number of risk factors (Rothwell, Slattery & Warlow 1997). For example, hypertension, defined as pre-operative systolic blood pressure greater than 180 mmHg, has been shown to be an important risk factor of stroke and death following CEA (Rothwell, Slattery & Warlow 1997). The prevalence of hypertension is therefore commonly given in reports of the risks of CEA. However, since the definition of hypertension varies considerably between different clinical studies (Kunz *et al.* 1997), it is important that the term is properly defined. In the current study, only 15.8% of papers had adequate definitions of hypertension.

One third of the papers did not define the term 'peri-operative period'. In those studies that did define the peri-operative period, several different definitions were used, namely 7, 10, 28, 30 post-operative days or time to hospital discharge. The reported risk is likely to be higher in studies using 30 days than those using shorter follow-up periods, and the duration of follow-up should be defined in all studies.

8.5.3 The quality of papers and risk

The current study has demonstrated that many criteria of quality are significantly associated with a higher risk of stroke and death – usually in an inverse fashion. Studies that were well designed and reported had higher risks than those that were not. This is analogous to the finding in reviews of randomised controlled trials that the benefits of treatment are exaggerated by about 30% in trials that do not state the method of concealment of randomisation assignment (Schulz *et al.* 1995). The risk of complications is an important parameter for assessment of the performance of surgeons, but it is clear from this study that the quality of research methods and reporting should be taken into account before judging the performances of surgeons, at least in published reports.

This study has confirmed the findings of a previous study that papers with independent assessors have higher risks of stroke and death than those without (Rothwell, Slattery & Warlow 1996a). In addition, the current study has further demonstrated that papers that have independent assessors are more likely to be adequately reported (Table 8.3). After excluding these studies, almost all of the remaining papers were non-randomised studies or case series, the most common types of papers in the surgical literature. In multivariate analysis of these studies, six quality criteria were associated independently with the risk of stroke and death (Table 8.5).

On the whole, the quality of study design and reporting of most papers was poor, which is consistent with the results of the assessment of quality of RCT surgical papers (Dimick, Diener-West & Lipsett 2001; Moss *et al.* 2001; Solomon *et al.* 1994). The weakest points of most papers were the definitions of terminology and the details of follow-up. Similar findings have been reported in a review of papers on the results of excision of the rectum for cancer (Dent *et al.* 2001). It could be argued that the quality criteria will depend on the objective of a study. The main objective of some papers may not have been to report the risk of stroke and death. However once papers give figures

for stroke and death, readers should have some information that will allow them to assess the reliability of such figures.

In randomised trials, there are many guidelines for reporting (Begg *et al.* 1996), but there are no guidelines for reporting of case series. Guidelines for writing up case series should be formulated, similar to the Consolidated Standards of Reporting Trials (CONSORT) statement for randomised controlled trials (Begg *et al.* 1996). Surgeons may also have to collaborate with epidemiologists to improve research design and reporting of case series. Adherence to the criteria of the current study should help to standardise the reporting of risk of stroke and death following CEA and to assure that differences in stroke and death rate are real, rather than the result of differences in definitions, methodology, and follow-up.

8.5.4 Limitations

In this study only papers in English were considered. Since the quality of studies has not been found to be related to the language in which those studies have been reported (Moher *et al.* 1996), the conclusions here based on English language publications only are unlikely to have any substantial sampling bias. In the results of the meta-analyses, some quality criteria across studies (Table 7.1) were highly heterogeneous, i.e. there were significant differences between studies in the operative risks for the same criteria, so that interpretation of the overall absolute risks was not straightforward.

In this discussion, I have assumed that inadequate reporting of research probably reflects inadequate methodology. However, it is possible that some studies are well conducted but badly reported, whereas others might be biased but well reported. Whichever is correct, it can be concluded that most poor quality methodology and reporting is associated with a low operative risk in published studies of the risks of CEA.

8.6 Conclusions

In general the quality of articles about CEA in the surgical literature was poor in many respects, especially the definitions of important terms and the method of follow-up. Many criteria of quality were associated with a high risk of stroke and death after CEA. One must be cautious in evaluating performances of surgeons based only on risks reported in papers. Greater attention needs to be paid to research design and reporting methods.

Chapter 9

A systematic review of randomised and non-randomised comparisons of local versus general anaesthetic for carotid endarterectomy

9.1 Summary

Objectives: Although it is clear that patients with symptomatic severe carotid stenosis need carotid endarterectomies (CEAs), to date the optimal type of anaesthetic for this operation is still controversial. The objective of this review was to assess the effect of performing carotid endarterectomy under local anaesthetic (LA) versus general anaesthetic (GA).

Design: A systematic review and meta-analysis study

Method: Two reviewers searched the Cochrane Stroke Group trials register, Medline (1966 to 2001) and Embase (1980 to 2001). The author hand-searched 13 relevant journals up to September 2001. Reference lists of articles were searched. We also advertised our search in Vascular News (a European newspaper for vascular specialists) in August 2001. Any randomised trial or non-randomised study comparing carotid endarterectomy under LA vs GA was included. The author selected studies for inclusion and RB independently checked the decisions. Two reviewers assessed trial quality and independently extracted the data.

Result: Seven randomised trials involving 554 operations, and 41 non-randomised studies involving 25622 operations were included. The methodological quality of the non-randomised trials was questionable. In 41 non-randomised studies, only 11 were clearly prospective and in only 29 was it clear that the data were from a consecutive series of patients Among the randomised studies, there were significant reductions in the risk of local haemorrhage (odd ratio [OR] = 0.31, 95% confidence interval [CI] 0.12 - 0.79, p = 0.01) within 30 days of the operation with LA. However, although there were trends towards reduced risks of death (OR = 0.23, 95% CI 0.04 - 1.33, p = 0.10) and stroke and death (OR = 0.63, CI 0.25 - 1.62, p = 0.30) with LA, these were not statistically significant. The non-randomised studies showed significant reductions with LA in the odds of death (OR = 0.67, 95% CI 0.46 - 0.97, p = 0.04), stroke (OR = 0.56, 95% CI 0.44 - 0.70, p < 0.00001), stroke or death (OR = 0.61, 95% CI 0.48 - 0.77, p = 0.00003), myocardial infarction (OR = 0.55, 95% CI 0.39 - 0.80, p = 0.001), and pulmonary complications (OR = 0.31, 95% CI 0.15 - 0.63, p = 0.001) within 30 days of

the operation. There was no significant heterogeneity between studies for any of these comparisons.

Conclusions: There is a large amount of non-randomised evidence to suggest that LA is associated with lower operative risks of death, stroke, stroke/death, myocardial infarction and pulmonary complications than GA. However, although there are some encouraging trends in the few small randomised trials, there are insufficient randomised data to allow any firm conclusions to be drawn. The ongoing GALA Trial is likely to provide reliable data within the next few years.

9.2 Introduction

CEA has been shown in large, well conducted randomised controlled trials to reduce the risk of stroke in patients with recently symptomatic severe (> 70%) carotid artery stenosis (Barnett *et al.* 1998; ECST 1991) and to a lesser extent in patients with moderate (50-69%) symptomatic stenosis (Barnett *et al.* 1998) and severe asymptomatic stenosis (ACAS 1995). In all of these trials there was a clinically significant operative risk of CEA. In patients with recently symptomatic carotid stenosis the risk of stroke or death within 30 days of the surgery is between 5-10% (Rothwell, Slattery & Warlow 1996a). If the risk of peri-operative stroke could be reduced, the benefits from CEA would be increased.

9.2.1 Anaesthetic and intraluminal shunting

Many operative strokes occur during the procedure and may be due to reduced blood flow during carotid artery clamping (Riles *et al* 1994). If the onset of such strokes could be recognised early, it may be possible to minimise the damage by placing a shunt across the clamped artery, thereby increasing blood flow. In patients operated on under GA, the neurological deficit can only be identified after recovery from the anaesthetic. To minimise the operative risk of stroke, several different approaches to shunting have been adopted when the procedure is performed under GA: placement of a shunt in all

patients (Binder *et al.* 1999); placement of a shunt in some patients thought to be at high risk of an operative stroke (Corson *et al.* 1987; Forssell *et al.* 1989; Gabelman *et al.* 1983; Ghali *et al.* 1997; Hartsell *et al.* 1999; Love & Hollyoak 2000); or avoiding a shunt altogether (Ott *et al.* 1980). Since only a small minority of patients do not tolerate arterial clamping without a shunt, and shunting may cause arterial dissection and embolic stroke, selective shunting is currently popular among surgeons. However, there is little consensus about how best to identify those patients who require a shunt. Several methods have been used including pre-operative assessment (e.g. a history of recent stroke or occlusion of the contralateral artery), and a variety of methods designed to indirectly or directly monitor cerebral blood flow during surgery (e.g. electroencephalographic monitoring, somatosensory evoked potential monitoring, transcranial Doppler monitoring, measurement of the internal carotid artery back pressure (Counsell *et al.* 1998). However, none of these methods are particularly sensitive or specific for detecting intraoperative stroke (McCleary, Maritati & Gough 2001).

9.2.2 Anesthesia and cerebral protection

Performing CEA in awake patients under LA has the advantage of accurate assessment of the clinical state of the patient during surgery and during the early post-operative period (McCleary, Maritati & Gough 2001). Any neurological change, either during test clamping or during surgery itself, can be detected early and therefore allow more appropriate use of selective shunting. Furthermore LA may preserve cerebral autoregulation reflex (post-clamping hypertension). Using near infrared spectroscopy and continuous jugular venous oximetry to monitor cerebral oxygenation, McCleary *et al.* (1996) found that cerebral oxygenation was preserved throughout CEA done under LA via cerebral autoregulation. However this reflex does not exist in CEA done under GA. The mechanism of these changes in blood pressure occurs remain obscure. It is believed that some volatile anaesthetic agents like isoflurane might block or dampen some cerebral perfusion receptors (McCleary *et al.* 1996). In addition, the cardiac and pulmonary morbidity of GA may be avoided (Becquemin *et al.* 1991; Corson *et al.*

1987). Operation under LA may also be associated with a shorter hospital stay, and consequent economic savings (Godin *et al.* 1989; McCarthy *et al.* 2001b).

CEA under LA may, however, be associated with certain problems. The operation may be more hurried and technically more difficult, and this may increase the risk of a poor result from surgery. Patients may also undergo undue stress and pain during the operation, which may increase the risk of myocardial ischaemia. It is also possible that there may be certain advantages to operating under GA. Some gases improve cerebral circulation, decrease the brain's oxygen requirement, and have neuroprotective effects. For instance, some volatile anaesthetic agents such as isoflurane, desflurane or sevoflurane reduce neuronal activity and cerebral oxygen consumption (McCleary, Maritati & Gough 2001). Similarly propofol produces a reduction in cerebral oxygen consumption and inhibits liberation of free fatty acids. Also it may also be easier to train junior surgeons during an unhurried procedure. In the early 1990s, most surgeons in the United Kingdom favoured the use of GA (Murie, John & Morris 1994), but there is now increasing enthusiasm for LA.

9.2.3 Hypothesis

There has been much recent controversy about whether LA or GA in CEA is the most efficacious. The only way to reliably assess the relative risks and benefits of operation under LA vs GA is by direct comparison in randomised controlled trials (RCTs). We therefore undertook this review of all such RCTs. In the absence of definitive data from RCTs, we also reviewed the non-randomised comparisons.

In fact, Tanganakul *et al.* (1997) reported such a study in the Cochrane systematic review in 1996. Since then many studies, both randomised and non-randomised in nature, have been reported. The author think this issue could benefit from being revisited again.

9.2.4 Objectives

1. To determine whether CEA under LA reduces the risk of peri-operative stroke and death compared to operation under GA.

2. To determine whether CEA under LA reduces the risk of other complications of surgery e.g. myocardial infarction (MI), pulmonary complications, wound haematoma, and cranial nerve palsy.

9.3 Methods

9.3.1 Criteria for considering studies for this review

9.3.1.1 Types of studies

All randomised trials and non-randomised studies that compared LA vs GA for CEA and that measured clinically relevant outcomes were eligible. Non-randomised studies were however analysed separately because they tend to overestimate treatment effects (Chalmers *et al.* 1983).

9.3.1.2 Types of participants

Trials, which included any type of patient undergoing unilateral or bilateral CEA, were considered eligible, whether the initial indication was symptomatic or asymptomatic carotid disease.

9.3.1.3 Types of interventions

We sought to identify all trials comparing CEA under GA of any sort with CEA under LA of any sort including both epidural and skin/deep infiltration.

9.3.1.4 Types of outcome measures

The intended measures of outcome were:

(a) Deaths from all causes within 30 days of the operation. The author tried to classify each death as stroke-related, related to other vascular disease (cardiac disease, pulmonary embolism, haemorrhage or other vascular disease) or non-vascular.

(b) Any stroke (fatal or non-fatal, contralateral or ipsilateral or brainstem, haemorrhage or infarction) within 30 days of the operation, and during long term follow up.

(c) Stroke ipsilateral to the operated artery within 30 days of operation and during long-term follow-up.

(d) Any MI (fatal or non-fatal) within 30 days of the operation.

(e) The number of arteries (operations) that were shunted during the operation.

(f) Other significant complications related to surgery e.g. local haemorrhage from the artery or neck wound, pulmonary complications including pneumonia, pulmonary embolism, atelectasis, prolonged intubation and pulmonary oedema, and cranial nerve palsies.

(g) The duration of total hospital stay and intensive care unit stay.

(h) The numbers of patients with hypotension or hypertension during or after operation.

(i) The overall satisfaction and preference of patients with each type of procedure. The author hoped this would indirectly assess outcomes such as pain and anxiety during and after the procedure.

(j) The overall satisfaction and preference of surgeons.

(k) The feasibility of operation under LA. This was assessed by calculating the percentage of patients allocated to have the operation under LA who were subsequently operated under GA.

9.3.2 Search strategy for identification of studies

Relevant trials were identified in the Stroke Group's Specialised Register of Controlled Trials (Cochrane Library). The following methods were also used:

1) The National Library of Medicine's MEDLINE database was systematically searched from 1966 – September 2001 using the terms "carotid endarterectomy" and "carotid surgery".

2) EMBASE was searched from 1980 – September 2001 using the terms "carotid endarterectomy" and "carotid surgery".

3) The following 13 journals were hand-searched up to September 2001:

Annals of Surgery (1981 – 2001)
Annals of Vascular Surgery (1996-2001)
British Journal of Surgery (1985-2001)
Cardiovascular surgery (1995-2001)
European Journal of Vascular and Endovascular Surgery (1988 – 2001)
International Journal of Angiology (1995-2001)
Journal of Cardiovascular Surgery (1995-2001)
Journal of Vascular Surgery (1996-2001)
Neurology (1996-2001)
Neurosurgery (1996-2001)
Surgical Neurology (1996-2001)

Stroke (1996-2001)
World Journal of Surgery (1978 – 2001)

4) References list of all relevant studies identified above were reviewed.

5) An advertisement was placed in Vascular News (a European newspaper for vascular specialists) in August 2001, issue 11, page 14.

9.3.3 Methods of the review

The author selected which trials met inclusion criteria and another (RB) independently reviewed these decisions. All disagreements were discussed, and if necessary, were referred to a third reviewer (PMR). For the randomised trials, details of the method of randomisation, the blinding of outcome assessments, losses to follow-up, crossovers and exclusions after randomisation were extracted from the publications. For non-randomised trials, the method of allocation to general anaesthetic or local anaesthetic was recorded along with whether the series was prospective or retrospective, and whether consecutive or selected patients were included. For all studies, patient characteristics (age, sex, vascular risk factors, indication for surgery) and details of the operation (type of cerebral monitoring, use of carotid patching, use of shunts, use of peri-operative anti-platelet therapy) were compared between the treatment groups in each trial. The outcome events were then extracted independently by two reviewers (KR, RB) using a standardised form, and cross-checked. Discrepancies were resolved by discussion or referral to PMR.

The number of operations was extracted and used for calculation. However some studies included patients who had bilateral operations but only recorded the number of patients in each group. However, since the frequency of bilateral endarterectomy was generally low, we had to assume the number of patients equalled the number of operations in such papers.

9.3.4 Statistical analysis

Proportional risk reductions were calculated based on a weighted estimate of the odds ratio using the Peto method (APT 1994). However the Peto method may be inaccurate in studies with large treatment effects and a small number of outcomes in each group (Greenland & Salvan 1990). The author therefore also calculated Mantel Haenszel (MH) summary odd ratios. In cases where the results differed because of such limitations, we have quoted MH summary odd ratios. The absolute risk reductions were calculated from the crude risks of each outcome in all trials combined (APT 1994). Tests for heterogeneity, the Chi-squared tests, were used to assess whether the outcome observed in each study differed from the overall estimate of treatment effect. Analyses were done with STATA version 6 (Stata Corporation, Texas, USA) and in all cases a value of $p < 0.05$ was taken to indicate a statistically significant difference.

9.4 Result

9.4.1 Description of studies

There were 41 non-randomised studies (list in Appendix 8) including about 25622 operations (the exact number of patients is unknown since some reports only gave the number of operations). Nine studies reported only the number of patients and did not apparently perform any bilateral operations. For these studies, the author assumed that the number of patients and the number of operations were the same. There were seven RCTs (list in Appendix 8; including 554 patients) that compared LA and GA for CEA. All RCTs reported the number of operations. All reports were in English except two RCT which were translated from French (Pluskwa *et al.* 1989) and German (Binder *et al.* 1999). Four non-randomised studies were published in languages other than English: two in Italian (Bartoloni *et al.* 1991; Rignano *et al.* 1999) and two in Spanish (Mertens *et al.* 2000; Reina *et al.* 1998). Papavasiliou's report (2000) was excluded because it was identical to Harbaugh's study (Harbaugh, R.E. 2000) and another three reports (Adelman *et al.* 1995; Riles *et al.* 1994; Rockman *et al.* 1998) were excluded because they were from the same database that was used by Imparato (1998) and

Rockman (1996). One ongoing randomised controlled trial (GALA trial) has been identified (McCleary, Maritati & Gough 2001).

9.4.1.1 Randomised studies

Six studies used a cervical block and one study (Pluskwa *et al.* 1989) used an epidural block to provide LA. All of them used standard medication in the GA group. Five trials reported the indication for shunting (Binder *et al.* 1999; Forssell *et al.* 1989; Kasprzak *et al.* 2002; McCarthy *et al.* 2001a; Sbarigia *et al.* 1999). Binder *et al.* (1999) used intraluminal shunting in all patients in both treatment groups. Four trials defined the post-operative period of follow-up: 30 post-operative days (Kasprzak *et al.* 2002; Sbarigia *et al.* 1999); 2 post-operative days (Binder *et al.* 1999); time to hospital discharge (Forssell *et al.* 1989). In the other trials, the period of follow up was unclear and the author assumed that it was to the time of hospital discharge.

9.4.1.2 Non-randomised studies

41 papers (75.6%) reported operative risk in relation to LA vs GA. Most of the studies used only a cervical block in the LA group. In one study, the surgeons used either cervical block or cervical epidural block (Becquemin *et al.* 1991) and another three studies used local infiltration of skin (Imparato *et al.* 1998; Palmer 1989; Schwartz *et al.* 1988). The indications for shunting in LA and GA were reported in 28 and 22 studies respectively. In LA patients, 27 of 28 studies used a shunt if there were neurological symptoms during test clamping in LA group. In the GA group, there were many indications for shunting, including electroencephalography changes (7 studies), stump pressure (10 studies: <50 mmHg in 5 studies; <40 mmHg in 4 studies; and <25 mmHg in 1 study), severe contralateral stenosis (9 studies), recent ipsilateral stroke (5 studies) and routine in all patients (8 studies). Nineteen studies followed-up patients until 30 days, 11 studies followed-up patients until hospital discharge, and one study only followed-up patients until 24 hours post-surgery. In eight studies, the duration of follow-up was unclear.

In both the randomised and non-randomised studies some important outcomes were not assessed. It was usually not possible to determine whether the strokes were ipsilateral to the operated artery or not (although most strokes will have been ipsilateral). The cause of operative death was generally not reported, nor was the severity of operative stroke (e.g. disabling vs non-disabling etc). Patient and surgeon satisfaction were not formally assessed.

9.4.2 Methodological quality of included studies

9.4.2.1. Randomised studies

Two out of seven RCT studies were published only as abstracts (Kasprzak *et al.* 1999; McCarthy *et al.* 2001a). For one of these (Kasprzak *et al.* 2002), additional unpublised data were obtained from the authors. This trial is due to be published in full during 2002-2003. The reporting of methodological quality was poor. In terms of randomisation, two studies used sequential numbers (Forssell *et al.* 1989; Sbarigia *et al.* 1999), and one study used block randomisation (Binder *et al.* 1999). Regarding concealment, one study used sealed envelopes but it was not stated whether these were numbered or opaque (Forssell *et al.* 1989), whilst three trials used computer concealment (Binder *et al.* 1999; Kasprzak *et al.* 2002; Sbarigia *et al.* 1999). In the other three trials, the method of concealment of allocation was unknown. In most trials, the blinding of outcome assessment was unclear, but only one trial had a neurologist (blinded to treatment allocation) as the assessor (Sbarigia *et al.* 1999). In two studies (Kasprzak *et al.* 2002; Sbarigia *et al.* 1999), some patients who were randomised to have their operation under LA actually had it done under GA. These patients were excluded from the analysis in all two trial reports, and so intention-to-treat analyses were not possible. In one trial (Forssell *et al.* 1989), 11 (11%) patients underwent staged bilateral endarterectomies and were randomised twice. Some of these patients may have had one operation under GA and the other under LA.

None of the trials reported major differences in baseline prognostic factors between the two groups of patients, although only limited data were provided in some reports. Only three trials commented on the use of patching. Two used selective patching (Kasprzak *et al.* 2002; Sbarigia *et al.* 1999), and the other used patching in all patients (Binder *et al.* 1999). None of the trials reported on the use of peri-operative anti-platelet therapy in the two groups.

9.4.2.2. Non-randomised studies

Of the 41 non-randomised studies, two were published only as abstracts. Unpublished data were obtained from three large randomised trials that had been designed to study other issues relating to CEA but in which the type of anaesthetic had been recorded (Barnett *et al.* 1998; ECST 1998; Taylor *et al.* 1999a). Most (28/41) studies were reported after 1991. Only 11 studies were prospective, and only 29 stated that they included consecutive patients. Patients were divided between GA and LA by a variety of methods, including by surgeon or patient preference (8 studies), by hospital of admission (1 study), and by year of the operation (7 studies), whilst fifteen studies did not report how treatment was determined. Nine studies reported the outcome only according to the number of patients, and so the number of operation in each group was unclear.

Twenty-six studies reported a comparison of major vascular risk factors at baseline. In 15 studies, no significant differences between the LA and GA groups were found. Five studies had more asymptomatic patients in the LA group (Allen *et al.* 1994; Bartoloni *et al.* 1991; Becquemin *et al.* 1991; McCarthy *et al.* 2001b; Schwartz *et al.* 1988), and one study had more asymptomatic patients in the GA group (Gabelman *et al.* 1983). In one study (Rockman *et al.* 1996), the proportion of transient ischaemic attacks was higher in the GA group than the LA group.

Patients who undergo combined CEA and coronary artery bypass grafting (CABG) require a GA. These patients may be at increased risk of serious complications

following surgery, and inclusion may lead to bias against GA. In nine studies, patients who underwent a combined procedure were excluded from the GA group. In another study, the results of patients having a combined procedure were reported separately (Shah *et al.* 1994) and these were excluded from this review. In the remaining 32 studies, it was unclear whether combined procedures were included in the GA group. Nine studies reported the use of carotid artery patching. In four of these, patching was more frequent in the GA group (Allen *et al.* 1994; Fiorani *et al.* 1997; Shah *et al.* 1994; Stone, Jr. *et al.* 2000). Only one study (Gabelman *et al.* 1983) reported the use of anti-platelet agents in the peri-operative period, and these agents were used more frequently in the LA group (50% vs 26%).

9.4.3 Outcomes

Data from 41 non-randomised trials (25622 operations) and seven randomised trials (554 patients) were included in this review. Only outcomes within 30 days of operation were assessed.

9.4.3.1 Randomised studies (Table 9.1)

9.4.3.1.1 Death from all causes

There were 5 deaths: 3 coronary artery diseases, 1 stroke and 1 pneumonia. All of these occurred in the GA groups. This difference between the LA and GA groups was statistically significant (OR = 0.13, 95% CI 0.02 - 0.76, p = 0.02) (Figure 9.1a) with the Peto method, but it was not statistically significant with the MH method (OR = 0.23, 95% CI 0.04 - 1.33, p = 0.10) (Figure 9.1b).

Table 9.1 The outcome between the local and general anaesthesia in randomised controlled trial section within about 30 days of surgery

	Events / operations		Peto method			Mantel Haenszel method		
	LA	GA	Odds ratio (95% CI)	p value	p value for heterogeneity	Odds ratio (95% CI)	p value	p value for heterogeneity
Death	0/246	5/241	0.13 (0.02 - 0.76)	0.02	1.00	0.23 (0.04 - 1.33)	0.10	0.87
Any stroke	6/246	6/241	1.01 (0.32 - 3.18)	1.00	0.24	1.00 (0.35 - 2.91)	1.00	0.43
Stroke and death	7/280	11/274	0.63 (0.25 - 1.62)	0.30	0.33	0.66 (0.26 - 1.63)	0.36	0.56
MI	3/246	3/241	0.96 (0.19 - 4.85)	1.00	0.40	0.96 (0.19 - 4.85)	0.96	0.40
Local haemorrhage	4/223	14/221	0.31 (0.12 - 0.79)	0.01	0.64	0.29 (0.10 - 0.85)	0.02	0.67
Cranial nerve injury	4/167	2/166	1.98 (0.39 - 9.97)	0.40	0.21	1.84 (0.39 - 8.72)	0.44	0.33
Arteries shunted	56/223	60/221	0.66 (0.40 - 1.14)	0.14	< 0.00001	0.70 (0.42 - 1.15)	0.16	< 0.0001

LA = local anaesthesia, GA = general anaesthesia, CI = confidence interval, MI = myocardial infarction

Figure 9.1a Meta-analysis of death rates following carotid endarterectomy comparing LA versus GA calculated with Peto method. Odd ratio for each study represented by a square. Line represents 95% CI of odd ratio. Diamond represents overall pooled estimate.

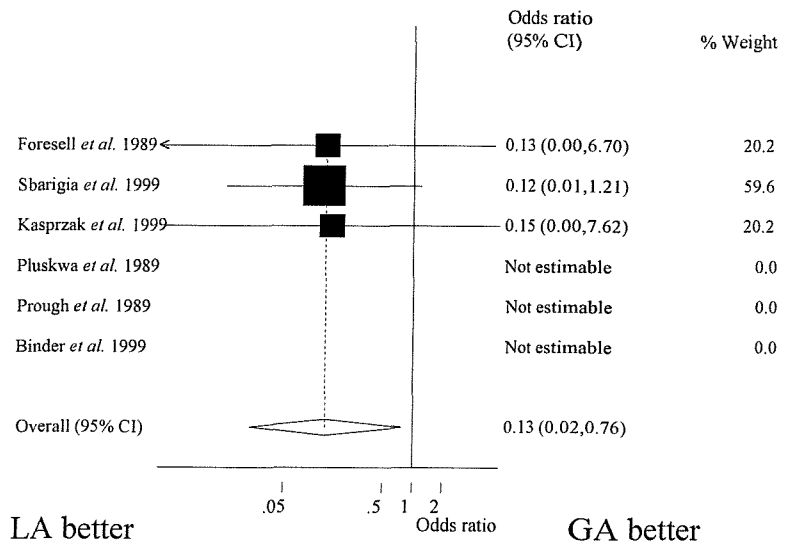
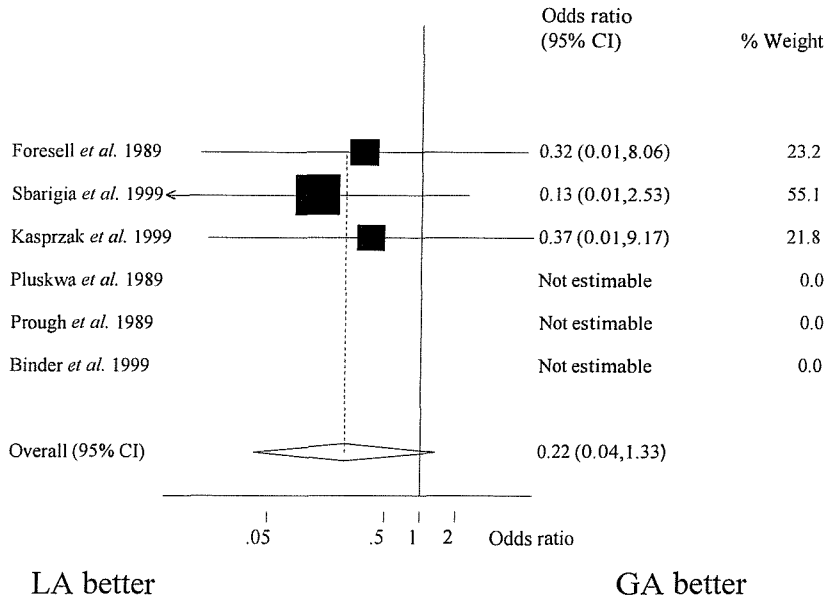


Figure 9.1b Meta-analysis of death rates following carotid endarterectomy comparing LA versus GA calculated with Mantel Haenszel method.



9.4.3.1.2 Any stroke

There were 12 recorded strokes of any type within 30 days (overall risk = 2.46%). There was no significant difference in risk between the LA and GA groups (OR = 1.01, 95% CI 0.32 - 3.18).

9.4.3.1.3 Any stroke or death

The combined risk of stroke or death in LA group (7/280, 2.5%) was lower than in the GA group (11/274, 4.0%) but this was not statistically significant (OR = 0.63, 95% CI 0.25 - 1.62).

9.4.3.1.4 Myocardial infarction

Only six patients suffered an MI within 30 days of surgery and there was no significant difference in risk between LA and GA.

9.4.3.1.5 Other operative complications

9.4.3.1.5.1 Wound haematoma (local haemorrhage)

Four studies reported the incidence of wound haematoma. There were significantly fewer haematomas in the LA group than in the GA (1.8% vs 6.3%; OR = 0.31, p = 0.014 by the Peto method; OR = 0.29, p = 0.024 by the MH method). This was based on eighteen haematomas overall. There was no indication of the severity of these bleeds.

9.4.3.1.5.2 Cranial nerve injury

3 trials reported the incidence of cranial nerve palsies. The overall risk of cranial nerve injury was 2.4% under LA and 1.2% under GA (OR = 1.98, 95% CI 0.39 - 9.97).

9.4.3.1.5.3 Other complications

No trial reported pulmonary complications

9.4.3.1.6 Blood pressure

Six trials recorded the blood pressure during and after the operation. However, neither the number of patients with significant hypotension or hypertension, nor the mean arterial pressures during and after the operation were always given. The definitions of hyper- and hypotension also varied between trials. The author has therefore simply described the individual results rather than perform any meta-analysis.

Four trials reported blood pressure after induction of anaesthesia (Forssell *et al.* 1989; McCarthy *et al.* 2001b; Pluskwa *et al.* 1989; Prough *et al.* 1989). In each case, blood pressure dropped in the GA group, and in one trial (Forssell *et al.* 1989) more patients in the GA group had significant hypotension during or after the operation (25% vs 7%). However, this was not confirmed in another trial (Pluskwa *et al.* 1989). Three trials reported that blood pressure tended to increase during clamping of the carotid artery in the LA group (Forssell *et al.* 1989; Pluskwa *et al.* 1989; Prough *et al.* 1989), but this was not found in another trial (McCarthy *et al.* 2001b). In two trials, there were more patients with significant hypertension during surgery in the LA group (Forssell: 0% vs 36%; Pluskwa 20% vs 80%). Two trials suggested that hypotension was more common in the post-operative period in those who had LA (Pluskwa *et al.* 1989; Prough *et al.* 1989), and one trial reported patients under GA were more likely to have post-operative (day 1) hypertension in the GA group (Kasprzak *et al.* 2002).

9.4.3.1.7 Shunting

Four trials reported the number of arteries that were shunted during surgery. The use of LA was associated with fewer shunts (OR = 0.66, 95% CI 0.40 – 1.14), but there was a significant heterogeneity between studies ($p < 0.00001$).

9.4.3.1.8 Hospital stay

The duration of hospital stay was reported one trial. The mean time in hospital after surgery was 7.9 days in the LA group and 9.2 days in the GA group (Binder *et al.* 1999).

9.4.3.1.9 Patient satisfaction

Patient satisfaction was not formally assessed. However, in one trial (Forssell *et al.* 1989), three patients who had LA required a further CEA subsequently, and none refused repeat randomisation (LA). In this same trial, it was reported that one patient in the LA group became extremely agitated during the procedure. Another trial evaluated patients' satisfaction by questionnaires (Binder *et al.* 1999). Both types of anaesthesia were equally acceptable to patients, but full details of the questionnaires and results were not reported. All patients said that they would prefer the same type of anaesthetic again if they need a second operation, except one LA patient who stated that he/she would prefer a GA.

9.4.3.1.10 Surgeon satisfaction

The satisfaction or preference of the surgeon was not assessed in any trial.

9.4.3.1.11 Feasibility of performing operation under local anaesthetic

One trial recorded the number of patients randomised to have the operation under LA, but who had it under GA (Forssell *et al.* 1989). Eight patients (14%) crossover from LA to GA whilst none switched from GA to LA. The most common reasons for crossover were that the patient changed his or her consent or that the patient had unstable cardiac disease. In another trial, 6 patients who were randomised to LA were converted to GA due to severe agitation, but these patients were excluded from the analysis (Kasprzak *et al.* 2002).

9.4.3.2 Non-randomised studies (Table 9.2)

9.4.3.2.1 Death from all causes

Thirty-five studies reported peri-operative mortality. There was a trend towards a reduced mortality in the LA group (LA: 79/8202, 1.0% vs GA: 116/9498, 1.2%) and this was statistically significant, (OR = 0.67, 95% CI 0.46 - 0.97, p = 0.04).

9.4.3.2.2 Any stroke

Thirty-one studies reported the risk of peri-operative stroke separately from death. There were fewer peri-operative strokes in the LA group (166/8474 - 1.4% vs 398/8355 - 4.8%) and this was statistically significant (OR = 0.56, 95% CI 0.44 - 0.70, p < 0.00001). Some studies may only have reported ipsilateral strokes but this was rarely defined. There was very little data on the severity of the stroke.

9.4.3.2.3 Any stroke or death

These data were available from 26 studies. Significantly fewer patients in the LA group suffered a stroke or died (99/4122, 2.4%) compared to the GA group (505/9425, 5.4%) (OR = 0.61, 95% CI 0.48 - 0.77, p = 0.00003).

9.4.3.2.4 Myocardial infarction

Performance of the CEA under LA was associated with a significantly lower odd of MI within 30 days of surgery (1.1% vs 3.3%, OR = 0.55, 95% CI 0.39 - 0.80, p = 0.001). It was unclear how many of these MIs were fatal.

9.4.3.2.5 Other operative complications

9.4.3.2.5.1 Wound haematoma (local haemorrhage)

Thirteen studies reported the number of patients with rupture of the artery or wound haematoma. There was no significant difference between the two groups (45/2425, 2.0% LA versus 152/5001, 3.0% GA; OR = 0.74, 95% CI 0.51 - 1.07). The severity of the haemorrhage was not reported.

Table 9.2 The outcome between the local and general anaesthesia in non-randomised controlled trial section within about 30 days of surgery

	Events / operations		Odds ratio (95% CI)	p value	Heterogeneity
	LA	GA			
Death	79/8202	116/9498	0.67 (0.46 - 0.97)	0.040	0.46
Any stroke	166/8474	398/8355	0.56 (0.44 - 0.70)	< 0.00001	0.41
Stroke and death	99/4122	505/9425	0.61 (0.48 - 0.77)	0.00003	0.46
Myocardial infarction	84/7572	239/7201	0.55 (0.39 - 0.80)	0.001	0.40
Local haemorrhage	49/2425	152/5001	0.74 (0.51 - 1.07)	0.110	0.69
Cranial nerve injury	51/2198	228/4675	0.92 (0.64 - 1.33)	0.670	0.32
Pulmonary complication	6/1635	27/1292	0.31 (0.15 - 0.63)	0.001	0.20
Arteries shunted	1022/7697	4037/8153	0.11 (0.10 - 0.12)	< 0.00001	< 0.00001

LA = local anaesthesia, GA = general anaesthesia, CI = confidence interval

9.4.3.2.5.2 Cranial nerve palsies

Only eleven studies assessed this outcome. There was no significant difference in the rate of cranial nerve palsy between the LA and GA groups (LA - 51/2198, 2.3% vs GA - 228/4675, 5.0%; OR = 0.92, 95% CI 0.64 - 1.33). The majority of cranial nerve injuries are likely to have recovered spontaneously (Hartsell *et al.* 1999; Stone, Jr. *et al.* 2000).

9.4.3.2.5.3 Pulmonary complications

Only seven studies reported pulmonary complications. The definition of pulmonary complication varied between different studies and included pneumonia in five studies, pulmonary emboli in two studies, pulmonary oedema in one study and prolonged intubation in one study. These complications were rare, but were significantly less frequent with LA (0.4% vs 2.1%, OR = 0.31, 95% CI 0.15 - 0.63, p = 0.001).

9.4.3.2.6 Blood pressure

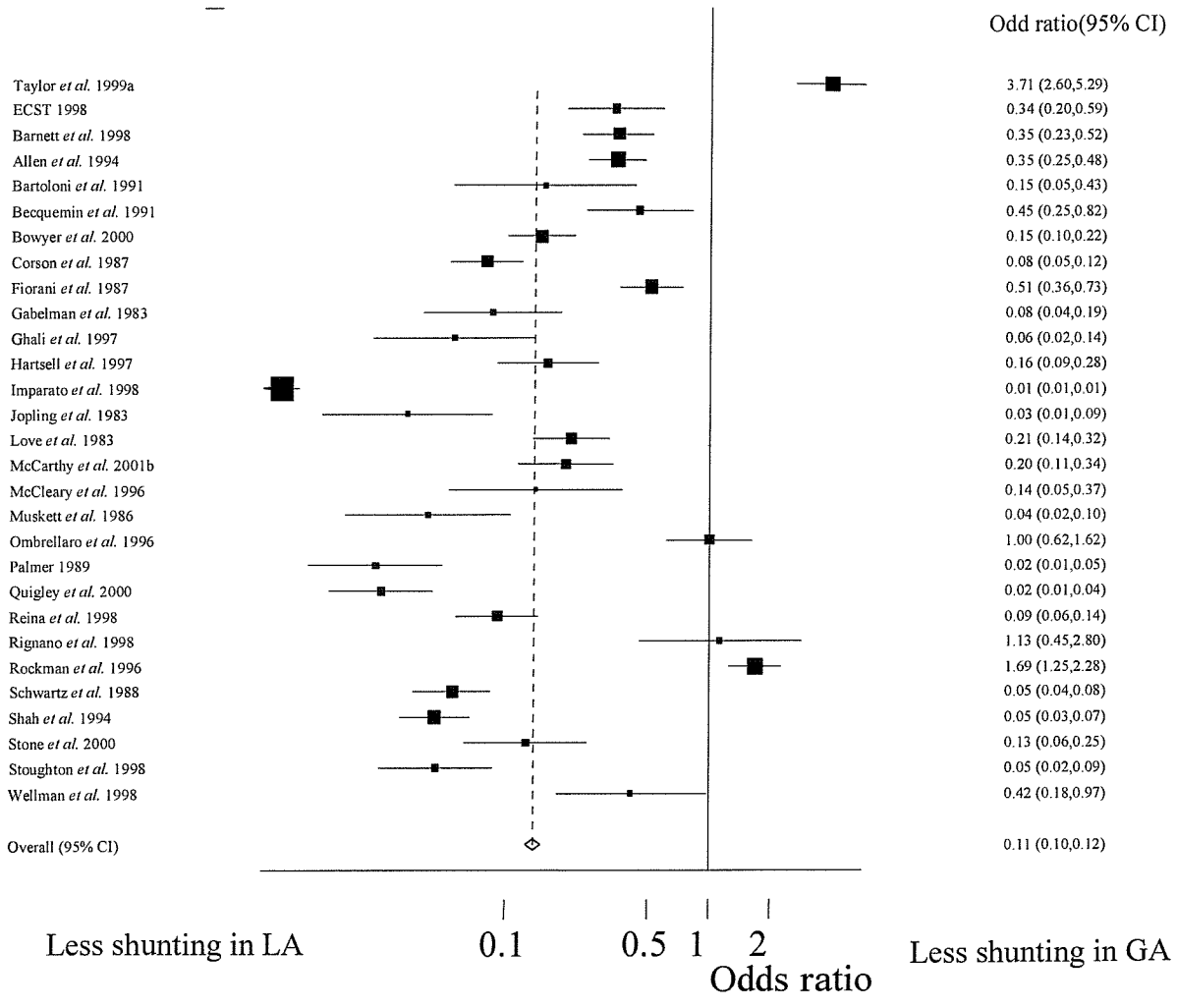
Seventeen studies reported some data on blood pressure during and after the operation. However, we were unable to assess the number of patients with hypertension or hypotension because of differences between studies in definitions and because the number of patients with each type of outcome was not usually reported. Rather, the number of episodes of hyper- or hypotension was reported or the mean blood pressure.

Intraoperative hypertension was found more commonly in the LA group in three studies (Gabelman *et al.* 1983; McCarthy *et al.* 2001b; Stullken *et al.* 1984) and in the GA group in one study (Corson *et al.* 1987). Post-operative hypertension was found more commonly in the GA group in six studies (Allen *et al.* 1994; Corson *et al.* 1987; Godin *et al.* 1989; Jopling *et al.* 1983; Kraiss *et al.* 1995; McCarthy *et al.* 2001b) and in the LA group in one study (Gabelman *et al.* 1983). However, post-operative hypotension was more common in the GA group in three studies (Allen *et al.* 1994; Corson *et al.* 1987; Jopling *et al.* 1983), and in the LA group in one study (Gabelman *et al.* 1983). In the two other studies, patients in the LA group required more administration of fluid to maintain blood pressure at a systolic value greater than 120mmHg (Scuderi *et al.* 1989; Stullken *et al.* 1984), but another study reported that patients in the GA group requiring more administration of fluid to maintain blood pressure (Muskett, McGreevy & Miller 1986).

9.4.3.2.7 Shunting

Many fewer shunts were inserted in those who had the operation under LA compared to GA (13.3% vs 49.5%, OR = 0.11, 95% CI 0.10 - 0.12) (Figure 9.2). However, there was enormous heterogeneity between studies ($p < 0.00001$), making the overall result difficult to interpret reliably. There was also enormous variation in the use of shunting in the GA group, depending on the selection criteria for shunt insertion in these patients, and to a lesser extent in the LA group. The percentage of arteries shunted in the GA group ranged from 9% to 100%, and in the LA group from 1% to 43%.

Figure 9.2 Meta-analysis of shunt rates following carotid endarterectomy between local versus general anaesthesia in non-randomised controlled trial section.



9.4.3.2.8 Hospital stay

Twelve of the non-randomised studies gave some data about the total duration of hospital stay and four provided the duration spent in the intensive care unit. Meta-analysis could not be performed because the data were not normally distributed and I was unable to transform the data because I did not have access to the original individual patient data. The four studies, which measured intensive care stay, showed that those who had the operation done under LA required less time in intensive care (Bowyer (2000) - mean 24 vs 30 hrs; Godin (1989)- 0.1 vs 1.2 days; Corson (1987)- 0.8 vs 1.7 days; and Stoughton (1998)- 1.1 vs 1.2 days).

Eleven studies reported the total hospital stay. All of these reported shorter stays for the LA groups, on average shorter by 1.5 days. Mean lengths of stay (days) for LA vs GA groups were: Allen (1994) 3.0 vs 4.0, Bowyer (2000) 3.5 vs 4.5, Gabelman (1983) 7.1 vs 9.2, Godin (1989) 4.1 vs 6.1, Harbaugh (Harbaugh, R.E.2000) 1.3 vs 3.5, Kraiss (1995) 1.3 vs 3.1, McCarthy (2001b) 2 vs 3, Muskett (1986) 3.2 vs 5.6, Palmer (1989) 5 vs 5.8, Schwartz (1988) 3.1 vs 5.1, Stoughton (1998) 1.3 vs 1.9 days (although as previously stated, means were inappropriate measures). McCarthy *et al.* (2001b) reported that the cost saving per CEA performed under LA compared with GA was £225.

9.4.3.2.9 Patient satisfaction

Patient satisfaction was only formally assessed in one study (Quigley, Ryan & Morgan 2000). No significant difference was found between the two types of anaesthesia in terms of discomfort in peri-operative period, perception of the attentiveness of staff, satisfaction with the hospital stay, time to complete recovery and recollection of events in the operating room. Two studies reported the use of LA in those patients who went on to have a second CEA. In one study, 8% (31/368) of patients had bilateral-staged CEA, and no patient who previously had the operation under LA elected to have contralateral surgery under GA (Corson *et al.* 1987). In another study, only two of the

116 patients who had bilateral carotid endarterectomies changed from LA to GA for the second operation because of patient preference (Shah *et al.* 1994).

9.4.3.2.10 Surgeon satisfaction

This was not assessed in any of the studies.

9.4.3.2.11 Feasibility of performing the operation under LA

As expected, crossovers were poorly reported in the non-randomised studies. Only sixteen studies reported the number of patients in whom operation under LA was not possible. In those studies, between 0% and 8.6% of patients in the LA group had to have the operation under GA.

In addition to the overall analyses of the non-randomised data, the unpublished data from the large randomised trials (NASCET, ECST, and ACE) was analysed separately from the remaining published data. On the whole, the summary odd ratios were less than 1 in both groups (Table 9.3).

Table 9.3 The outcome between the local and general anaesthesia in non-randomised controlled trials within about 30 days of surgery between large trial data (NASCET, ECST, ACE) and the remaining non-randomised trials.

	ECST, NASCET, ACE			Other non-RCT studies		
	Odds ratio (95% CI)	**p value**	**p value for heterogeneity**	**Odds ratio (95% CI)**	**p value**	**p value for heterogeneity**
Death	0.62 (0.21 - 1.84)	0.390	0.62	0.68 (0.46 - 1.01)	0.060	0.35
Any stroke	0.80 (0.48 - 1.32)	0.380	0.12	0.51 (0.39 - 0.66)	< 0.0001	0.61
Stroke and death	0.82 (0.51 - 1.32)	0.410	0.22	0.56 (0.43 - 0.73)	< 0.0001	0.58
MI	0.78 (0.37 - 1.65)	0.510	0.66	0.50 (0.33 - 0.76)	0.001	0.32
Local haemorrhage	0.81 (0.32 - 2.03)	0.650	0.67	0.73 (0.49 - 1.08)	0.120	0.55
Cranial nerve injury	0.93 (0.47 - 1.81)	0.820	0.10	0.92 (0.60 - 1.43)	0.710	0.37
Pulmonary complication	N/A	N/A	N/A	0.31 (0.15 - 0.63)	0.001	0.20

CI = confidence interval, MI = myocardial infarction, N/A = not available data

9.5 Discussion

The main finding of this review is that there are too few randomised studies to allow the balance of the risks and benefits of LA vs GA for CEA to be determined reliably. More randomised trials are required. However, some conclusions can be drawn from the data that are available. There was no evidence, for example, that surgery under LA increased the risk of operative stroke, death or other complications. Indeed, both the randomised and non-randomised studies suggested that LA may have some benefits.

CEA under LA was associated with a lower risk of post-operative death and also a lower risk of local haemorrhage than surgery under GA (Table 9.1), with relative odds reductions of 87% and 69% respectively. The non-randomised studies suggested that surgery under LA was associated with a 39% reduction in the relative odds of stroke and death and a 45% reduction in the relative odds of MI. There were no increases in the risk of other operative complications with LA.

The reduction in operative mortality due to surgery under LA in the meta-analysis of randomised trials is encouraging (Figure 9.1a), but was based on very small numbers of patients and events. In addition, this reduction was not statistically significant with MH method (Table 9.1) (Figure 9.1b). Greenland *et al.* suggested that the Peto method should avoid in situations where the treatment effect is large (OR = 0.13) and that the result from MH method was more appropriate in such cases (Greenland & Salvan 1990).

The modest reduction that was reported in the much larger group of patients included in the non-randomised studies might be more interesting (Table 9.2), but non-randomised studies are highly susceptible to bias (Chalmers *et al.* 1983). It is possible that the type of patients who had LA may have differed from those who had GA, such that they were at a different risk of a poor outcome even before they had the procedure. As with other operative procedures, it is possible that high-risk patients were more likely to be offered surgery under GA. Some studies definitely included high-risk

patients in the GA group, such as those with a CEA combined with a coronary bypass grafting, and this may also have distorted the results. This difficulty in determining this size and extent of any bias in the non-randomised studies highlights the need for more randomised data. In some studies there appeared to be more patients in the LA group who had asymptomatic carotid disease. The LA groups in these studies may therefore have been at lower risk of poor outcome (Rothwell, Slattery & Warlow 1996b). There may also have been differences in the ways that the patients in the LA and GA groups were treated. Few studies reported data on other treatments systematically, but in at least two non-randomised studies there were differences in the use of patching (Agrifoglio *et al.* 1987) and anti-platelet therapy (Gabelman *et al.* 1983).

The definition of some of the complications, such as wound haematoma, varied between studies. Some studies reported only haematoma requiring reoperation, whereas others reported all haematomas. Nevertheless, the apparent consistency of the findings between the randomised and non-randomised comparisons, and the lack of significant heterogeneity within comparisons, is reassuring. In other words, there was a significantly lower rate of wound haematoma in patients operated under LA in the randomised studies, and a trend in the same direction in the non-randomised studies, and no significant heterogeneity among either.

There are other potential biases in the non-randomised studies. Most of the studies were retrospective and some included non-consecutive cases. It is therefore likely that some cases were missed or excluded, and this may have introduced bias. Outcome assessment was not blind and the studies were not analysed on an intention-to-treat basis. Publication bias may have affected the results of the non-randomised studies. It is possible that studies showing a poor outcome with LA may have been less likely to be submitted for publication than studies showing a good outcome (Dickersin & Min 1993).

As stated above, there are many methodological problems associated with non-randomised studies. In the current study, therefore, further analysis of non-randomised data was done in which the studies were divided into two groups: unpublished data from the large trials (NASCET, ECST, and ACE (Taylor *et al.* 1999)) and the remaining data. Although still non-randomised, the data from large trials were less effected by some of the potential biases. For example, the trials did not allow patients to undergo combined carotid and cardiac surgery. They were also of a higher quality in general than the published studies, with independent assessment of outcome and complete follow-up. However, most importantly, they were not published analyses and the results could not therefore be due to publication bias. In general, the directions of the summary odds ratios from large trials agreed with those from the remaining published (non-randomised) studies.

There were also some potential methodological problems in the randomised trials. The method of concealment of allocation was inadequately reported in four trials, and the duration of follow up was limited. It was also unclear from most trials whether the outcome had been assessed blind to treatment allocation. Only one study stated clearly that they had neurologists as blinded assessors – which is generally regarded as the optimal trial design (Rothwell, Slattery & Warlow 1996a). Two of the trials also excluded some of the randomised patients from the analysis – particularly those patients who crossed over from LA to GA. If patients excluded differed systematically from those patients who remained in the analysis, this may bias the results. Furthermore, as in the non-randomised studies, some important outcome measures were not reported. There was no data on how many of the strokes were disabling, the patient's or surgeon's satisfaction or preference, or the duration of intensive care and overall hospital stay.

The effect of LA on the rate of carotid artery shunting during surgery varied between studies in both the randomised and non-randomised studies (Figure 9.2). Most studies used a selective shunting protocol, but these were based on many different methods of

detecting cerebral ischaemia (such as awake testing, trancranial Doppler (TCD), electroencephalography, stump pressure and somatosensory evoked potential) and different indications of shunting (even in the same method of detecting cerebral ischaemia).

9.6 Conclusions

Although this review could not provide a reliable answer as to whether CEA is best performed under LA or GA, there are some data from both randomised and non-randomised studies that suggest that LA may be superior. These results should encourage investigators, such as the GALA trialists, who are performing randomised trials.

Chapter 10

General discussion: implications for clinical practice and further research

10.1 Summary

Results of the study and their applications to clinical practice and further research are discussed.

10.2 Risk factors associated with atherosclerotic lesions

Atherosclerosis, the most common underlying fatal disease in the UK (Marmot & Mann 1996), is the major pathology underlying cerebrovascular diseases such as stroke. This has prompted a great deal of research in the field, as a result of which the role of plaque instability has become much better understood. In contrast with earlier concepts, atherosclerotic plaques are modifiable and dynamic (Weissberg 2000). Consequently clinicians now attempt to improve plaque stability in several ways by using new lipid-lowering drugs, antioxidants, and n-3 polyunsaturated fatty acids (n-3 PUFA). Many guidelines have also been published about the control of hyperlipidaemia, a known risk factor. At the same time, many novel risk factors indicating plaque instability have emerged.

Rapp *et al.* (1991) found that the level of n-3 PUFA in plaque was significantly higher in patients taking n-3 PUFA (16 g of n-3 PUFA/ day) than in those of a control group. In the current study it was found that after a modest intake of n-3 PUFA supplements (1.7 g of n-3 PUFA/ day) for a short period (median 53 days), the level of n-3 PUFA in patients' plaques was significantly higher than in those of a control group. This demonstrates that n-3 PUFA is incorporated into even advanced and complicated plaques. Not only is n-3 PUFA incorporated into plaque, but it also reduces the number of macrophages in plaque. An increased number of macrophages in atherosclerotic plaques is a risk factor for plaque instability, as are an increased expression of tissue factor, a reduced number of smooth muscle cells, and a large lipid core (Davies, M.J. 1996). In fact, n-3 PUFA has other effects on atherosclerotic plaques that may have an effect on plaque stability. This has been shown by the results of a study on rhesus monkeys, which were given fish oil supplements for 12 months. Investigators found that the monkeys in the fish oil group had less cholesterol composition, smaller

atherosclerotic lesion and fewer macrophages in their plaques than those in the control group (Davies, H.R.1987). Whether fish oil supplements improve carotid plaque stability or not can only be settled by a randomised controlled trial with long-term follow-up such as a randomised controlled trial in patients with asymptomatic carotid stenosis. Since these patients have no clear benefit from carotid endarterectomy (CEA) (ACAS 1995), there is an opportunity for a long-term follow-up of vascular events. Not only such a study can assess the stability of plaque with the risk of clinical event, but also it can be done with transcranial Doppler. Transcranial Doppler can quantify the extent of cerebral microembolisation, which is associated with carotid plaque ulcer or haemorrhage if it is extensive (Gaunt *et al.* 1996). In addition, further investigation is needed of the effect of n-3 PUFA supplements on other factors that may affect plaque stability such as thickness of fibrous caps, size of lipid cores, and the number of vascular smooth muscle cells. It is unclear what the mechanism is by which n-3 PUFA reduced the number of macrophages in carotid plaques in the current study. Future studies should examine the relationship between the incorporation of n-3 fatty acid into plaques, expression of inflammatory mediators in plaques, and the death of monocytes/macrophages in plaques in order to determine the mechanism(s) responsible for the reduction of plaque macrophages. It is also unknown whether the results seen in this study occur in other atherosclerotic lesions such as those in coronary arteries; this warrants further investigation. Furthermore, although the benefits of fish oil supplements have been demonstrated in several large randomised controlled trials (Burr *et al.* 1989; GISSI-P 1999; Singh *et al.* 1997), these trials are secondary prevention studies of patients with a history of myocardial infarction. There has been little direct evidence about the role of the n-3 PUFA in primary prevention and its effect specifically on cerebrovascular diseases. Further studies should explore this.

An understanding of the events associated with non-lipid-lowering effects of statins in atherogenesis leads one to contemplate the development of drugs affecting specific events in atherogenesis that may act synergistically with statins (Weissberg 2000). Possible targets for these drugs include endothelial nitric oxide, adhesion molecules, the

matrix mettalloproteinases, and inflammatory cells and their cytokines. This current study found that n-3 PUFA can decrease the inflammatory response of atherosclerotic lesions by decreasing the number of macrophages. Currently n-3 PUFA supplements are recommended in some lipid-lowering guidelines. For example, they are recommended in managing hyperlipidaemia, particularly for patients with coronary artery disease whose diets lack fish, and assist in managing young men with hypertriglyceridaemia and severe dyslipidaemia (Brown 2001). In addition, the scientific advisory committee of the American Heart Association (AHA) has found that although there are many effective kinds of intervention for cardiovascular disease, the burden of cardiovascular disease in the United States is still excessive. They are seeking better ways to decrease the incidence of cardiovascular disease. Recently they have been impressed with the marked cardioprotective effects of the Mediterranean style diet (Robertson & Smaha 2001), for which α-linolenic acid (18:3 n-3 PUFA) was provided as a supplement. This study showed that patients in the intervention group had a 50% to 70% reduction of cardiac end points. However further studies are needed before features of this Mediterannean style diet can be integrated into current AHA guidelines.

It is well known that hyperlipidaemia is associated with cardiovascular events (Castelli 1984). Myocardial infarction (MI) is also the most common cause of death in patients with carotid stenosis (Caplan & Silver 2000). Several lipid-lowering drug trials, both primary and secondary prevention trials, have showed that lipid-lowering drugs reduce the incidence of cardiovascular events (Sacks *et al.* 1996; Shepherd *et al.* 1995). Statins are also beneficial in reducing inflammation, inhibiting formation of thrombi, and increasing proliferation of smooth muscle cells (Weissberg 1999). Although many guidelines such as the recommendation from the Standing Medical Advisory Committee (SMAC 1997) and the joint British recommendations (1998) on the prevention of coronary heart disease in clinical practice (British Cardiac Society, British Hyperlipidaemia Association, British Hypertension Society, British Diabetic Association) have been published, it has been shown in the current study that in practice these guidelines are not being followed by clinicians as well as they should be. The

Department of Health should pay more attention to this problem and ensure not only that guidelines reach general practitioners, but also that general practitioners adhere to them in practice. In fact some might argue that during the 1990s there was no clear evidence at that time supporting the use of statins in patients with peripheral vascular disease. However recently the Heart Protection Study has showed clearly that statins benefit high-risk patients (including patients with peripheral vascular disease and patients requiring carotid endarterectomy (CEA)) with either high or even normal plasma cholesterol concentration (HPS 2002). This trial found that deaths from all causes in a 5-year follow-up were reduced from 14.7% in patients taking a placebo to 12.9% in those taking simvastatin, mainly because of an 18% relative reduction in coronary death rate. Patients taking simvastatin had a 12% reduction in total mortality, a 17% reduction in vascular mortality, a 24% reduction in coronary events, a 27% reduction in all strokes, and a 16% reduction in non-coronary revascularisations (Collins, Peto, & Armitage 2002; HPS 2002). So evidence does exist that supports early management of hyperlipidaemia in patients with peripheral vascular disease, and the problem of inadequate treatment should lessen. The current study also showed that perception by patients of the problem of hyperlipidaemia was quite poor and that clinicians should emphasise the relevance of this problem to every patient with peripheral vascular disease.

High levels of CRP have been shown to be associated with coronary events in many cohort studies, but there is little evidence of the association between this marker and cerebrovascular events. Some authors have even proposed that CRP has no relevance in cerebrovascular disease (Canova, Courtin & Reinhart 1999). In the current study it was found that levels of hs-CRP in patients with symptomatic carotid stenosis were significantly higher than in patients with asymptomatic carotid stenosis. Perhaps this could be used as a basis for a test to select for operations only asymptomatic carotid stenosis patients at a high risk of neurological events, as in coronary studies. Of course the results of the current study cannot be applied for general use because of many limitations. To establish the association between hs-CRP concentration and the

probability of the development of symptoms in initially asymptomatic patients, hs-CRP concentrations must be studied in long-term cohorts such as in asymptomatic carotid stenosis patients. If the level of hs-CRP is associated with the incidence of cerebrovascular events, a randomised controlled trial should be carried out on asymptomatic carotid stenosis patients with high levels of hs-CRP to compare surgical and medical treatments. In addition, the mechanism responsible for increased levels of CRP in patients with cardiovascular events is still unknown. Perhaps polymorphisms of the gene that controls CRP production could provide some clues. Ridker *et al.* (1999b) also found that statins reduced the incidence of coronary events as well as the level of CRP. It might be possible to use the level of hs-CRP as a surrogate to indicate the response to many novel medical treatments for atherosclerosis, such as lipid-lowering drugs or gene therapy.

10.3 Risk factors associated with the outcome of carotid endarterectomy

For patients with symptomatic severe carotid stenosis, in NASCET (1991) and ECST (1991) the clear benefits of CEA over medical treatment were shown in terms of secondary prevention of future stroke. In NASCET, the 9% risk of ipsilateral stroke, including post-operative stroke, in the two-year period after operation was much lower than the 26% risk of stroke in patients on medical treatment alone. In spite of this, some surgeons proposed that the risk of stroke and death following CEA is currently much lower than the result of the landmark studies (ECST and NASCET), so it is appropriate to conduct CEA in patients with marginal benefit such as those with symptomatic moderate stenosis (50-69% NASCET method). Based on our study, this risk has not been changed over 15 years, so there is a lack of evidence to support this assumption.

An ad hoc Committee of AHA council has recommended that the combined stroke and death rate following CEA should be no more than 5% for patients suffering TIA including amaurosis fugax (Beebe *et al.* 1989; Moore *et al.* 1995). This current study found that the absolute risk of cerebral TIA was 5.5%, but the risk of ocular ischaemic event was 2.8%, which is close to the risk of asymptomatic patients. When comparing

relative odds ratios, it was found that the odds for patients with cerebral TIA were not different to those in stroke patients. Similarly the odds for patients with ocular ischaemic events were not different to those in asymptomatic patients. Perhaps it is more appropriate to separate these two types of patients into two different categories (cerebral TIA, amaurosis fugax) in the future. Most trials in CEA have been conducted with patients classified as symptomatic or asymptomatic. Patients with amaurosis fugax have been classified with other symptomatic patients, including those with established stroke. In further studies in carotid surgery, patients should be reported with details of how they present. It is not sufficient to simply note that the patient is symptomatic or asymptomatic.

As discussed above, CEA itself can cause stroke or fatal stroke and even death. Many hundreds of papers have reported the risk of stroke and death following CEA, but these figures vary greatly between centres. Rothwell found that the risk reported in papers where the authors, surgeons, were assessors was much lower than that in papers with independent assessors (Rothwell, Slattery & Warlow 1996a). This has been confirmed by the results of the current study. This seemingly implies that the audit of carotid surgery should be carried out by independent assessors (neurologists). The author agrees that this is ideal. It is difficult and expensive, however as it requires experts (neurologists or stroke physicians) and these experts need to be interested in this small part of the stroke field.

This current study found that the figure of the risk also depended on the quality of reporting and the research design. Prospective studies had higher risks than retrospective studies. Similarly the papers that covered many topics adequately such as inclusion/exclusion criteria, specification of baseline data, definitions of risk factors, definitions of stroke and peri-operative period, and description of surgical techniques were independently associated with higher risks of stroke and death following CEA than those that did not. Risk is supposed to be indicative of performance; surgeons with high incidences of complications are believed to be poor surgeons. The recent events in

Bristol are an example. Public opinion turned against the two pediatric cardiac surgeons because of the high operative mortality rates they had with children with congenital heart diseases. It is generally believed that any surgeon who has a high morbidity and mortality rate should not be allowed to continue to practice. In the meta-analysis of the current study, it was found that there was great variation in the risk of stroke and death after CEA and that this risk was associated with the person reporting the study and research design. One has to bear in mind that in comparing performances between surgeons or institutions, one should not forget that the quality of reporting and research design must be taken into account as well as clinical risk factors. In practice, it seems unfair to dismiss a surgeon based only on a figure for risk in one paper without considering who reported it and the design of the study. A comparison of the performance of surgeons should be made using the same criteria. For instance patients should have the same level of risk factors of peri-operative stroke and death such as age, sex, hypertension history, and presentation together with an independent assessor. Although this is the ideal, it is difficult to do because in real life patients come with different risk factors. A way to get round this is to have a large database such as a national database, and use statistical methods to adjust for this difference. However this needs the complete detail of patients' risk factors rather than a database such as the retrospective study, which had a large proportion of missing data.

It is of interest that the quality of research methodology and reporting of papers about the risk of stroke and death following CEA was poor. Of course this result is only a type of surgical papers; it may not reflect the quality of all surgical research. However a large proportion of case series in this study are similar to those in other surgical papers (Horton 1996). There are several obstacles in conducting randomised controlled trials for surgical research such as time-consuming operations in theaters or difficulties in blind intervention. The majority of surgical papers may therefore have to draw on case series. In fact case series is very useful, because many guidelines, such as best clinical practice and guidelines, have been derived from this type of research. Also the outcome of this research might be closer to the real world patients. Some randomised controlled

trials tend to restrict patient selection in some way. For instance NASCET study included only fit patients without significant heart disease (such as unstable angina) or older than 80 years old and only surgeons with a good track record (stroke and death rate following CEA < 6%) were selected to participate in the trial. However it would be better to have the case series, conducted using proper methodology. Another problem is that no guidelines are available for writing up case series. This issue is just as important as research methodology, because a well-conducted but badly reported study may be overlooked.

10.4 Anaesthetic technique during carotid endarterectomy

Although it is clear that patients with symptomatic severe carotid stenosis need CEAs, to date the optimal type of anaesthetic (local anaesthetic (LA) versus general anaesthetic (GA)) for this operation is still controversial. In 1995 Tangkanakul *et al.* did the first systematic review of general anaesthesia versus local anaesthesia in CEA by examining three RCTs (143 operations). They did not find any significantly difference. In the current review, although there were 7 RCTs (554 operations), no definite conclusions could be reached because the samples were too small. More RCTs are needed. In spite of this the data from non-randomised trials in both the Tangkanakul and the current review suggest that LA is preferable for several reasons, namely the lower incidences of stroke, stroke and death, and myocardial infarction associated with it. Hopefully the ongoing GALA trial, the biggest trial ever, will shed more light on this issue. In the GALA trial the calculation of the sample size is based on the non-randomised section of the Tangkanakul study. For a 50% reduction in the relative odds of peri-operative stroke and death, the GALA trial must have about 2000 patients for a 90% chance of detecting such a reduction with a 5% significance level. In fact, this is quite a cause for worry. If 2000 patients do participate in the GALA trial, there may not be a 90% chance of detecting a reduction, because in the current review LA was associated with a 39% reduction of odds of post-operative stroke and death. As stated above, this figure was derived from meta-analysis of data associated with several methodological flaws. In fact when a meta-analysis was done using data from big trials (NASCET, ECST (1998), and

ACE) (Barnett *et al.* 1998; Taylor *et al.* 1999a), which is more credible and less biased than other non-randomised studies, it was found that LA was associated with only about a 18% reduction in the relative odds of stroke and death. This result might be useful for GALA in recalculating the sample size. In fact this unpublished result of my study has been used by the GALA steering committee to apply for the further funding at the moment.

Given that the overwhelming majority of CEAs are presently performed under GA, much stronger evidence will be required from well-designed randomised trials before it can be recommended that a LA be used for patients undergoing this operation. Such a recommendation would have major implications for the training of neurological and vascular surgeons. If LA is proven to be better than GA, this should be taken into account before interpreting the results of any trials in carotid surgery. For instance, it has also been found as in the CAVATAS trial (2001) that percutaneous angioplasty has as good an outcome as CEA. Perhaps it would be appropriate to compare percutaneous transluminal angioplasty and CEA under LA in future. However, even if LA is shown to be safer than GA, it is unlikely that all operations could be performed under LA. In some patients the operation may be more difficult technically under LA, eg in those with short, fat necks. Some patients, perhaps 10%, (Forssell *et al.* 1989) will refuse to have the operation under LA, and some surgeons may also be unhappy to perform the operation under LA especially if they use vein patch.

10.5 Conclusion

In conclusion, now that the importance of plaque instability is better understood than before, it is clear that composition of plaque is a major determinant of the risk of an ischaemic event, especially a coronary event. New knowledge about cellular and biochemical mechanisms involved in the progress of disease should be used to stabilize atherosclerotic lesions. N-3 PUFA is a candidate for this. At the moment, there is firm evidence about management of risk factors for patients with atherosclerosis. One must ensure that this evidence is disseminated to and used by clinical practitioners. In papers

that have been published over the last 15 years there is a lack of evidence about the reduction of stroke and death following CEA, so one cannot assume that the performance of surgeons has improved on the whole. Presentations of patients strongly influence the incidence of stroke/death, so reporting should always include this. Research design and the quality of reporting are also associated with the risk of stroke/death. Furthermore it is important to monitor and compare the performance of surgeons fairly. Several controversial issues about CEA remain, however, such as the optimal type of anesthetic. More randomised controlled trials are needed.

Appendices

Appendix 1 Example of a one-day food diary (data taken from a patient's diary).

Day 1 Date 19/9/00

Time	Description of food or drink	Manufacturer or Brand	Weight of serving (grams)	Weight of left-overs (grams)
7.30	• Breakfast			
	2 slices bread		75	
	Margerine		20	
	Marmalade (60% sugar)		15	
	Instant coffee		4	
	Milk		70	
	Kiwi Fruit		78	16
12.00	• Packed lunch			
	Bread wholemeal		120	
	Margerine		20	
	Thin cut ham		35	
	Homemade pickle		50	
	Jam-filled doughnut (purchased)		47	
1.00	Single glass red wine (pub)	Housewine	108	
	Handful peanuts		32	
4.00	Homemade beer		250	
	Homemade beer		250	
6.00	• Dinner			
	Cauliflower cheese		244	
	Marrow & tomato-hot		130	
	Mushrooms-hot		25	
	2 slices wholemeal bread		75	
	Apple crumble		212	
	Icecream		111	
6.30	Coffee instant		3	
	Milk		25	
8.00	Homemade wine		150	

Appendix 2 Plasma lipid assay

Blood samples were taken before intervention at the time that patients agreed to participate in this study and after intervention but before CEA; these samples were collected in vacutainer tubes using EDTA (15%; 0.12ml) as an anticoagulant. Plasma was isolated by low-speed centrifugation (2500 rpm for 10 min).

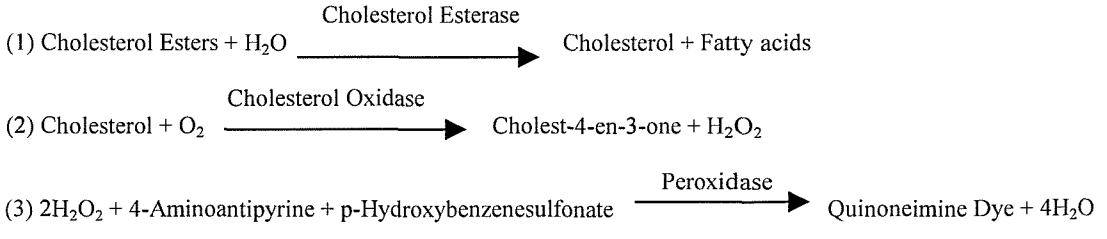
Appendix 2.1 Measurement of total cholesterol

The cholesterol concentrations of plasma were measured using an enzymatic assay (Sigma Diagnostics, Procedure No.352).

In this assay cholesterol esters are first hydrolysed by cholesterol esterase to cholesterol. The cholesterol produced by hydrolysis is oxidised by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide produced is then coupled with the chromagens 4-aminoantipyrine and p-hydroxybenzenesulfonate in the presence of peroxidase to yield a quinoneimine dye (reaction 3), which has an absorbance maximum at 500 nm. The intensity of the colour produced is directly proportional to the total cholesterol concentration in the sample.

A. Principle:

Enzymatic reactions involved in this procedure are as follows:



B. Procedure

1. Cholesterol reagent was prepared according to instructions.
2. The spectrophotometer wavelength was set to 500 nm, the absorbance reading to zero with water as a reference.
3. A series of cuvettes was set up for the blank, standard, and samples.
4. The reagent was warmed to assay temperature.
5. 1 ml of reagent was pipetted into each cuvette.
6. 10 ml of deionised water (blank), a cholesterol standard, and the sample were added to the appropriate cuvettes, which were mixed by gentle inversion.
7. The cuvettes were incubated for 10 min at room temperature.
8. The absorbances were read at 500 nm.
9. The cholesterol concentration in the sample was calculated as follows.

$$\text{Cholesterol (mg/dL)} = \frac{\text{Absorbances}_{\text{TEST}} - \text{Absorbances}_{\text{BLANK}}}{\text{Absorbances}_{\text{STANDARD}} - \text{Absorbances}_{\text{BLANK}}} \times \text{Standard concentration (mg/dL)}$$

To convert to mmol/L multiply by 0.0259.

C. Calibration: The procedure was calibrated using a cholesterol standard, also from Sigma.

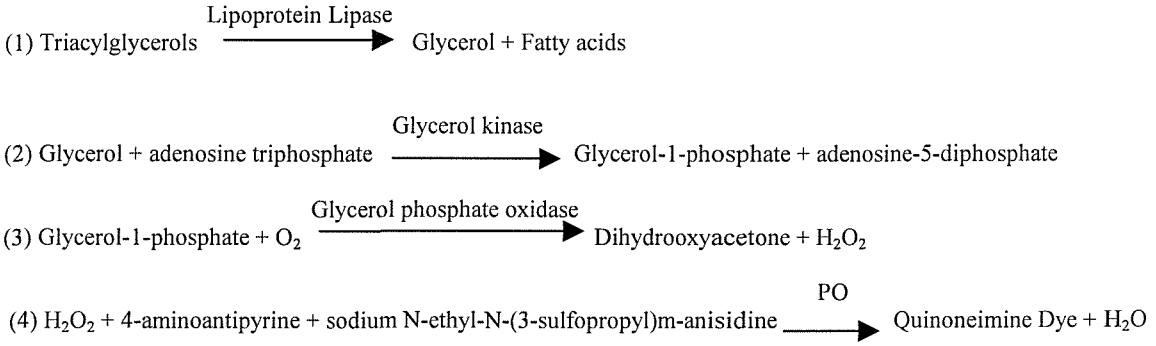
D. Precision: The same sample was assayed ten times on the same day to test intra-assay variation and was assayed once on each of ten consecutive days to test inter-assay variation. The intra- and inter- assay coefficients of variation were 1.9% and 5.0% respectively.

Appendix 2.2 Concentration of triacylglycerols

The concentrations of triacylglycerols in plasma were measured using an enzyme assay kit (Sigma Diagnostics, Procedure No. 337).

A. Principle:

The enzymatic reactions in the assay procedure are as follows:



Triacylglycerols are first hydrolysed by lipoprotein lipase to glycerol and three free fatty acids (reaction 1). Glycerol is then phosphorylated by adenosine triphosphate forming glycerol-1-phosphate and adenosine-5-diphosphate in a reaction catalysed by glycerol kinase (reaction 2). Glycerol-1-phosphate is then oxidised by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide (H_2O_2) (reaction 3). A quinoneimine dye is produced by the peroxidase (PO)-catalysed coupling of 4-aminoantipyrine and sodium N-ethyl-N-(3-sulfopropyl)m-anisidine with H_2O_2 (reaction 4), which has an absorbance maximum at 540 nm. The increase in absorbance at 540 nm is directly proportional to the concentration of triacylglycerols in the sample.

B. Procedure:

1. Triglyceride Reagent A and Reagent B were prepared according to the instructions.
2. The spectrophotometer was set to 540 nm and the absorbance reading to zero with water as a reference.
3. The reagents were warmed to room temperature.
4. A series of cuvettes for the blank, the standard, and the sample was set up.
5. Reagent A (0.8 ml) was pipetted into each cuvette.

6. Deionised water, the standard, and the sample (10 ml) were added to the cuvettes, which were then mixed by gentle inversion.
7. Cuvettes were incubated for 5 min at room temperature.
8. The initial absorbances of the blank, the standard, and the sample cuvette were read at 540 nm with water as a reference.
9. Then 0.2 ml of reagent B were added to each cuvette, and the contents were mixed and incubated for a further 15 min at room temperature.
10. The final absorbances of the blank, the standard, and the sample cuvettes were read.
11. The concentration of triglycerides in the sample was calculated as:

$$\frac{\text{Final absorbances}_{\text{SAMPLE}} - (\text{Initial absorbances}_{\text{SAMPLE}} \times \text{Dilution factor})}{\text{Final absorbances}_{\text{STANDARD}} - (\text{Initial absorbances}_{\text{BLANK}} \times \text{Dilution factor})} \times \text{Concentration of Standard}$$

Where F (dilution factor) = 0.81/1.01 = 0.80
To convert to mmol/L divide by 92.

C. Calibration: Sigma Diagnostics glycerol standard was used for calibration of the assay.

D. Precision: The same sample was assayed ten times on the same day to test intra-assay variation and was assayed once on each of ten consecutive days to test inter-assay variation. The inter- and intra-assay coefficients of variation were 5.0% and 2.5% respectively.

Appendix 3 The assay of fatty acid composition

Appendix 3.1 Composition of adipose tissue fatty acids

Total lipids were extracted from adipose tissue using a solution of chloroform, methanol, and water (2:1:0.8 vol/vol/vol). Each lipid extract was then separated into cholesteryl ester (CE), triacylglycerol (TAG), and phospholipid (PL) fractions by thin-layer chromatography (detail in Appendix 3.2.2). A homogenate of the sample and solvent was closed under nitrogen and then vortexed vigorously for 1 min and chloroform and KCl (0.88%) were added. Again the sample was closed under nitrogen and vortexed and the lower phase was then collected after centrifugation (10 min, 2500 rpm). The procedure was repeated, adding more chloroform and KCl.

Fatty acid methyl esters were formed by reaction of the total lipid extract with hot methanolic boron trifluoride for one hour at 80°C. These fatty acid methyl esters were then purified using a solution of hexane and butylated hydroxytoluene and a solution of saturated aqueous sodium chloride. The methyl esters were then separated by gas chromatography in a Hewlett-Packard 6890 gas chromatograph (Hewlett-Packard Ltd., Bracknell, Bucks, U.K.) fitted with a 25 m x 0.32 mm SGE BPX70 capillary column with a film thickness of 0.25 mm. Helium at 1 ml/min was used as the carrier gas, and the split/splitless injector was set to the split mode with a split ratio of 20:1. Injector and detector temperatures were 280 °C and 275 °C respectively, and the column oven temperature was held at 170 °C for 12 min and then programmed to increase by 5 °C/min until it reached 200 °C. The detector was a flame ionising detector. Fatty acid methyl esters were identified by comparison with standards run previously. These samples were analysed by Dr. J. M. C. Garry and Dr F. Thies of the Institute of Human Nutrition, University of Southampton.

Appendix 3.2 Composition of LDL fatty acids

Appendix 3.2.1 LDL preparation

Plasma was adjusted to a density of 1.24 g/ml by the addition of 381.6 mg of solid potassium bromide per ml of plasma. A two-step density gradient was created by a layer

of 1.7 ml of density-adjusted plasma below a layer of 3.3 ml of phosphate-buffered saline (d = 1.006 g/ml) in centrifuge tubes. These tubes were then sealed and centrifuged in an ultracentrifuge (TLA-100.4 rotor) at 100,000 rpm for 2 h at 15 °C to separate VLDL, LDL and HDL fractions. Each lipoprotein fraction was then removed. Firstly the VLDL fraction was removed from the top of the tube using a needle and syringe, after which the top of the tube was cut off and the remaining VLDL, LDL, and HDL fractions were removed separately using Pasteur pipettes. VLDL and HDL fractions were frozen (-70°C) for use in other studies. Aliquots of the LDL fractions were frozen (-70°C) for later analysis.

Appendix 3.2.2 Fatty acid composition assay in LDL

Total lipids were extracted from LDL using a solution of chloroform, methanol, and water as described above for adipose tissue (Appendix 3.1). Each lipid extract was then separated into CE, TAG, and PL fractions by thin-layer chromatography using a solution of hexane, ether, and acetic acid (90:30:1 vol/vol/vol) in the elution phase. Fatty acid methyl esters from each lipid fraction were formed by reaction with hot methanolic boron trifluoride. These fatty acid methyl esters were then purified using solution of hexane and butylated hydroxytoluene and a saturated aqueous solution of sodium chloride. The composition of fatty acids of each fraction was then determined by Dr F. Thies, as described above (Appendix 3.1).

Appendix 3.3 Composition of plaque fatty acids

Total lipids were extracted from the plaque using the solution of chloroform and methanol described by Rapp *et al* (1991). Plaque lipids were separated into cholesterol ester (CE), triacylglycerol (TAG) and phospholipid (PL) fractions by thin layer chromatography as described above for LDLs (Appendix 3.2.2). Fatty acid methyl esters were formed by reaction with hot methanolic boron trifluoride. The fatty acid methyl esters of each fraction were then purified using solution of hexane and butylated hydroxytoluene and a saturated aqueous solution of sodium chloride. The fatty acid

composition of each fraction was then determined by Dr. F. Thies, as described above (Appendix 3.1).

Precision: The same sample was assayed ten times on the same day to test intra-assay variation and was assayed once on each of ten consecutive days to test inter-assay variation. The inter- and intra-assay coefficients of variation in n-3 PUFA were less than 5.0%. The inter- and intra-assay coefficients of variation of other fatty acid components ranged from 3% to 15%.

Appendix 4 Immunohistochemistry technique of carotid plaques

In this procedure, cellular or tissue components (antigens) are localised in situ by the use of an antibody-antigen reaction. Steptavidin horseradish peroxidase was used as a system. Sections of plaque where the specific primary antibody had been omitted served as negative controls. Normal human tonsil tissue was used as a positive control for antibody staining; antibodies were titrated to determine the optimal concentration and conditions for use. The procedure consisted of the following steps:

1. 4-6 mm cryostat sections of the frozen plaques (section 2.3.7.4.1) were mounted on organosilan-coated microscope slides.
2. The slides were then air-dried overnight, either stained the following day or wrapped in Parafilm foil face-to-face, and stored in a closed box at –70 °C until needed.
3. If the slides had been frozen they were brought up to room temperature before being unwrapped.
4. Then the slides were fixed in acetone for 10 min at 4 °C and air-dried briefly for 2 min.
5. The tissue sections were then encircled with an Immedge pen (Vector Laboratories, Cat. No. H-4000).
6. Endogenous peroxidase activity was blocked using 50 mg of Na-azide and 500 ml of hydrogen peroxide (30%) in 50ml tris-buffered saline (TBS) for 20 min at room temperature. The slides were then rinsed three times with TBS.
7. The sections were covered with 10% normal goat serum and incubated for 15 min at room temperature.
8. The 10% normal goat serum was blotted off and the sections were incubated with the primary antibodies and diluted in TBS + 1% bovine serum albumin for 60 min at room temperature; see Table 2.4 for antibodies and dilutions used.
9. Sections were washed in TBS, secondary antibodies were applied, and sections were incubated for 30 min at room temperature. Secondary antibodies were diluted in TBS with 10% human antibody serum; see Table 2.4 for antibodies and dilutions used.

10. Sections were washed in TBS. Strepavidin/ horseradish peroxidase reagent (DAKO, P397; diluted 1:400 in TBS + 1% bovine serum albumin) was applied to the slides, and they were incubated for 30 min at room temperature.
11. Sections were again washed in TBS, and enzyme peroxidase enzyme activity was detected using 3,3-amino-9-ethyl carbazole. Graham's method was used.(Graham & Karnovsky 1965) 25 mg of 3,3-amino-9-ethyl carbazole (Sigma A5754) were added to 25 ml of N, N demethylformamide. 2.5 ml of this stock solution were mixed with 45 ml of H_20 and 2.5 ml of acetate buffer (1.0 M, pH 4.9) and the resulting mixture was filtered immediately. 20 ml of 30% H_2O_2 were added just before use and then sections were stained with the mixture for 10 min at room temperature.
12. Sections were fixed with 4% buffered formalin for 5 min, washed with running tap water for 5 min, and counterstained with Harris haematoxylin for 15 sec.
13. Sections were then differentiated in an acid solution (0.1% HCl) for 15 sec and washed again in running tap water for 5 min.
14. Coverslips were then placed on the sections using an aqueous mounting medium such as glycerin-gelatin (DAKO).

Values of 0, 1, or 2 were assigned to indicate the number of monocytes, lymphocytes, ICAM-1 or VCAM-1 per slide. Staining was graded 0 (0-10 stained cells (points) per section), 1 (moderate staining: 11-50 stained cells (points) per section) or 2 (heavy staining: > 50 stained cells (points) per section) (Figure 2.8a-d).

Appendix 5 Immunoturbidity assay of high-sensitivity CRP

A. Principles of method

CRP in the sample combines specifically with anti-human CRP antibodies in the buffer-antibody reagent mixture to form an aggregate. This insoluble aggregate causes an increase in turbidity, which can be measured optically, the degree of turbidity being proportional to the amount of CRP in the sample. The measurement of turbidity was done by Dr. David Rowe, Chemical Pathology, Southampton General Hospital, using the Wako kit (code No. 419-22007).

B. Collection of samples

Fasting venous blood samples were collected in vacutainer tubes containing 0.12 ml of 15% EDTA. Plasma was prepared by centrifugation at 2500 rpm for 10 minutes and stored at -70°C.

C. Storage and preparation of reagents

The reagents needed are CRP-HS buffer (Wako product No. 419-22015) and CRP-HS antibody (Wako product No. 419-22025). The control reagents are Dade Behring N/T rheumatology control SL/1 (product No. OQDB 13 and product No. OQDC 13).

D. Procedure

At room temperature, 14 mL of plasma sample were mixed with 330mL of buffer and left at room temperature for 5 minutes. Then 50mL of antibody were added and left for 5 minutes. The concentration of CRP was then determined by using an automated analyzer with a wavelength of 340 nm.

E. Precision

The same sample was assayed twenty times on the same day to test intra-assay variation and was assayed twice on each of ten consecutive days to test inter-assay variation.

Appendix 6 ELISA assay

Appendix 6.1 The assay of soluble E selectin (sE-selectin)

A. Principles of the ELISA assay for sE-selectin

To measure sE-selectin in plasma samples, Quantikine ELISA kits from R & D Systems Europe (Abingdon, UK) were used. This involved a reaction between sE-selectin and specifically directed antibodies; the presence of sE-selectin in the sample or standard reacted with two antibodies present in the assay. For this to occur, antibodies (monoclonal) were coated on the wall of the microtiter wells, while another antibody (polyclonal) was conjugated to the enzyme horseradish peroxidase. Any sE-selectin present in the sample formed a bridge between the two antibodies.

Unbound material was removed by aspiration and washing. The amount of conjugate bound to the well (and thus sE-selectin in the sample) was proportional to a colored product, which was the result of a reaction between substrate and conjugated horseradish peroxidase. The enzyme reaction yields a blue product that turn yellow when the stop solution is added. The colored product was quantified photometrically.

To determine the concentration of sE-selectin from the corresponding optical density a standard curve was required. This involved assaying the eight standards of known sE-selectin concentrations, quantifying the colored product photometrically, and plotting a standard curve of optical density to give concentration in the plasma sample.

B. Sample collection and storage

Fasting venous blood samples were collected in vacutainer tubes containing 0.12 ml of 15% EDTA. Plasma was prepared by centrifugation at 2500 rpm for 10 min and stored at -70°C.

C. Preparation of reagents

While plasma samples were defrosting at room temperature, the standards and wash buffer concentrate were prepared 25 ml of ionized water was added to buffer concentrate until reaching a final volume of 625 ml. The standard was added with 5 ml of Calibrator Diluent RD5-26 before use.

D. Assay procedure

First all 137 plasma samples were diluted 1:50 with sample diluent. These were archived by using 10 ml of sample and 490 ml of sample diluent. The control was then reconstituted with 1 ml of distilled water and left to stand at room temperature for 10 minutes, after which it was then gently mixed, dissolving all contents and avoiding any agitation or frothing.

Then 50 ml of Calibrator Diluent RD5-21 was added to each well, following which 50 ml of diluted sample, standard, and diluted control were added to each well of the microtitre plate. The diluted control and standard were added in duplicate. The plate was covered with a plate sealer and left to incubate for 2 hours at room temperature.

In order to remember the layout of the plate, the positions of the samples, standard and control were recorded. After incubation, the contents were then decanted from each well and washed by adding 400ml of wash buffer per well. This procedure was repeated 4 times for a total of 5 washes. After the last wash, the inverted plate was tapped firmly on a clean paper towel. Then the plate was added with 100 ml of sE-selectin conjugate each well and left to incubate for 30 minutes at room temperature. Immediately thereafter, 100 ml of substrate were added to each well. The plate was then covered with a new plate sealer and left to incubate at room temperature for 30 minutes.

Stop solution (100ml) was added to each well in the same order as the substrate. The optical density of each well was determined within 30 minutes, using a microtiter plate reader set at 450 nm with a correction wavelength of 540 nm.

E. Calculation of results

The mean absorbance values were calculated for each set of duplicate standards. A standard curve was created by plotting the mean absorbance for each standard concentration on the y-axis against sE-selectin concentration on the x-axis. A best-fit curve was drawn through the points.

The concentration for each sample was determined by calculating the concentration of sE-selectin corresponding to the mean absorbance from the standard curve. The concentration of samples and control were obtained by multiplying by the dilution factor, 50.

F. Precision

The same sample was assayed twenty times on the same day to test intra-assay variation and was assayed twice on each of ten consecutive days to test inter-assay variation.

Appendix 6.2 The assay of sICAM-1 and sVCAM-1

Principles of the ELISA Assay for plasma soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) concentrations were also measured by the same ELISA principle in section Appendix 6.1 (A) using Cytoscreen ELISA kits from BioSource (Nivelles, Belgium) (Catalog number KHS5402 and KHT0602/KHT0601 respectively).

Appendix 7 Reference list for Chapter 6-8

Reference list of 213 papers

AbuRahma *et al.* 1996b

AbuRahma, Robinson & Short 1996

Ackerstaff *et al.* 2000

Akbari *et al.* 1997

Allen *et al.* 1994

Ammar 1996

Andrikopoulos *et al.* 1996

Appleberg *et al.* 1995

Archie, Jr. 2000

Aune, Amundsen & Trippestad 1997

Ballotta *et al.* 1997

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Bhattacharya *et al.* 1999

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Branchereau *et al.* 1998

Brittenden & Bradbury 2000

Calaitges *et al.* 1996

Campron, Cartier & Fontaine 1998

Cantelmo *et al.* 1999

Cao *et al.* 1996

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Cao *et al.* 1999

Cebul *et al.* 1998

Chaturvedi, Aggarwal & Murugappan 2000

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Economopoulos, Gentile & Berman 1999

Fabiani *et al.* 1994

AbuRahma *et al.* 1999

Ackerstaff *et al.* 1995

Ahari *et al.* 1999

Akbari *et al.* 2000

Allen *et al.* 1997

Anderson *et al.* 1999

Anthony & Johansen 1994

Archie 1999

Ascer *et al.* 1996

Back *et al.* 1997

Ballotta, Da Giau & Renon 1999

Ballotta *et al.* 1999b

Ballotta, Da Giau & Guerra 1998

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Berman *et al.* 1994

Blohme *et al.* 1999

Bowyer *et al.* 2000

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Bratzler *et al.* 1996

Buchbinder *et al.* 1994

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De Letter *et al.* 1994

Deriu *et al.* 1994

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Eckstein *et al.* 1998

ECST 1991

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Frawley *et al.* 1996
Friedman & Tortolani 1995
Gasecki *et al.* 1995
Gibbs, Guzzetta & Furmanski 1995
Goldman *et al.* 1999
Golledge *et al.* 1997
Graham, Salasidis & Ferneini 1995
Hallett, Jr. *et al.* 1998
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Hertzer *et al.* 1997
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Hoffmann, Robbs & Abdool-Carrim 1998
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Jackson *et al.* 1996
Jordan, Jr. *et al.* 1998
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Kaufman *et al.* 1996
Koskas *et al.* 1995
Kresowik *et al.* 2000
Ladowski *et al.* 1997
Lawrence *et al.* 1998
Levy *et al.* 1997
Lingenfelter, Fuller & Sullivan 1995
Locati *et al.* 2000
Lohr *et al.* 1995
Luna & Adye 1995
Martin *et al.* 1997
Mauney *et al.* 1995
Mayo *et al.* 1998
McConnell *et al.* 1996
McKinsey *et al.* 1996
Merlo *et al.* 1998
Mingoli *et al.* 1997
Morasch *et al.* 1996
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Frawley, Hicks & Woodforth 2000
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Habozit, Derosier & Gaillard 1997
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Hobart *et al.* 2000
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Jones *et al.* 1996
Kahn *et al.* 1999
Karp *et al.* 1998
Katz & Kohl 1996
Kerdiles *et al.* 1997
Kraiss *et al.* 1995
Kucey *et al.* 1998
Lawhorne, Jr., Brooks & Cunningham 1997
Levison *et al.* 1999
Libman *et al.* 1994
Little & Meyer 1997
Loftus *et al.* 1998
Love & Hollyoak 2000
Marcinczyk *et al.* 1997
Mattos *et al.* 1995
Maxwell, Maxwell & Brinker 2000
McCarthy *et al.* 1996
McGrath, Wagner & Shabot 1996
Melissano *et al.* 1997
Meyer & Windschitl 1998
Morasch *et al.* 1995
Mueller *et al.* 1996
Musser *et al.* 1996
Myers *et al.* 1994
Naylor *et al.* 2000

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Ombrellaro *et al.* 1996
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Passerini 1996
Paty *et al.* 1999
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Wilkinson *et al.* 1997
Wong, Findlay & Suarez-Almazor 1997a

Appendix 8 Reference list for Chapter 9

Reference list of all papers used for the systematic review of randomised and non-randomised comparisons of local versus general anaesthetic for carotid endarterectomy

Appendix 8.1 Randomised papers

Binder *et al.* 1999

Kasprzak *et al.* 1999; Kasprzak *et al.* 2002

Pluskwa *et al.* 1989

Sbarigia *et al.* 1999

Forssell *et al.* 1989

McCarthy *et al.* 2001a

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Appendix 8.2 Non-randomised papers

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Bowyer *et al.* 2000

Corson *et al.* 1987

Fiorani *et al.* 1997

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