A Study of Risk Factors for Peripheral Arterial Disease

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Declaration

I declare that the work presented within this thesis was composed by myself. Those who contributed to the literature reviews in their specialist areas of rheology, blood pressure, and genetics in joint papers are named in the acknowledgements. All other literature reviews were conducted by myself as were all statistical analyses.

Abstract

This thesis set out to consider risk factors, in isolation and in combination, for symptomatic and asymptomatic Peripheral Arterial Disease (PAD), which unlike Coronary Heart Disease, has not been undertaken in a sample from the general population aged 55 - 74 years.

PAD shares a common aetiology with coronary heart disease and so it would be expected that these two conditions would have similar risk factors. Study of the risk factors for peripheral arterial disease would therefore shed light on important factors for atherosclerosis in general and hence is relevant to millions in the Western world suffering from atherosclerosis. The main difference in relative importance of the risk factors appears to be that smoking is a more important risk factor for peripheral arterial disease than coronary heart disease.

The risk factors were considered in a hierarchy with health related behaviours or lifestyle factors such as diet and physical activity preceding the intermediate factors such as blood viscosity and fibrinogen. Each set of risk factors was considered separately, followed by consideration of the two clusters of lifestyle and intermediary factors. Finally, all factors were analysed simultaneously. Social class was strongly related to many of the lifestyle and intermediary factors.

The results showed that the risk factors in relationship to the Ankle Brachial Pressure Index (ABPI), an indicator of the extent of asymptomatic and symptomatic PAD, differed for men and women and for smokers and non-smokers. In women smoking was the main risk factor, while blood viscosity and fibrinogen although univariately related to the ABPI, were not independent risk factors.

In men who had never smoked, nonHDL cholesterol and diabetes were the important risk factors. In men who had ever smoked there were synergistic effects of

smoking on the relationships between the ABPI and fibrinogen, blood viscosity, leisure activity, and energy adjusted vitamin C. Other independent risk factors for men who had ever smoked were diabetes, social class, nonHDL and HDL cholesterol. This suggests that in considering arterial disease, analyses should be carried out separately for men and women and perhaps for smokers and non-smokers.

These results indicated that some health education programmes could be aimed at specific groups, such as female smokers. On the other hand dietary programmes to reduce cholesterol level could be aimed at the general population.

Finally, the calculation of power and sample size for epidemiological studies showed that the simple two group comparison calculations generally used underestimated the sample size required when adjusting for numerous confounders and when subgroup analyses are likely, as in a cross-sectional study.

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Abbreviations

ABPI	Ankle Brachial Pressure Index
CHD	Coronary Heart Disease
CI	Confidence interval
CV	Canonical Variable
DNA	Deoxyribonucleic acid
FFQ	Food Frequency Questionnaire
GLM	Generalised Linear Models
HDL	High density lipoprotein
IC	Intermitttent Claudication
IHD	Ischaemic Heart Disease
MI	myocardial infarction
MCC	Multiple Correlation Coefficient
MVN	Multivariate Normal
ND	Nutrient density
OR	Odds Ratio
PAD	Peripheral Arterial Disease
PCA	Principal Components Analysis
PV	Phenotypic Variability
RFLP	Restriction Fragment Length Polymorphism
SD	Standard Deviation
se	standard error
SHHS	Scottish Heart Health Study
WHO	World Health Organistion
WI	Weighed Intake

CHAPTER 1

INTRODUCTION

1.1 Introduction

Research into arterial disease has tended to concentrate on coronary heart disease and stroke because of their high morbidity and mortality. Arterial disease of the lower limbs has largely been ignored in epidemiological studies, even though it is an important source of morbidity in the general population especially among those over 55 years (Fowkes 1988c). In addition, study of risk factors for peripheral arterial disease could shed light on coronary artery disease as these conditions share a common aetiology.

1.2 Aims and Objectives

The main aim of this thesis is to assess risk factors in isolation and in combination for symptomatic and asymptomatic Peripheral Arterial Disease in the general population of Edinburgh aged 55 - 74 years. The term risk factor can have a number of interpretations. This thesis uses the term to mean an attribute or exposure that is associated with an increased probability of peripheral arterial disease as outcome. This implies that a risk factor is not necessarily causal, but may be a risk marker (Abrahamson 1988).

1.3 Plan of the Thesis

In order to meet this aim the analysis of peripheral arterial disease was assessed using data from the Edinburgh Artery Study (Fowkes et al 1991). The Case-Control study used a subset of the population from the main cross-sectional study and the background to both these studies is described in chapter 2.

For the purposes of this analysis risk factors can be approximately grouped into

two clusters, that is, lifestyle and personality factors, and intermediary factors. Under the umbrella of the term intermediary were included rheological and lipid risk factors. Lifestyle or health behaviour factors included diet, leisure activity, smoking and alcohol. Lifestyle factors influence rheological factors and plasma lipid levels which in turn are risk factors for arterial disease. In this sense, lifestyle factors precede or influence the lipids and rheological factors (intermediary), which may be considered to be more directly related to peripheral arterial disease. On this basis, the lifestyle factors in relation to disease are assessed in the next three chapters, dietary factors in chapter 3, followed by the relationship of leisure activity when aged 35-45 to current disease status in chapter 4, and finally, the role of personality, especially measures of hostility in chapter 5.

The next step is concerned with the effect of rheological factors and these are analysed in chapter 6. There is evidence that lifestyle factors such as exercise are related to the resistance to flow of blood or viscosity (Ernst 1985) which in turn may be related to arterial disease status. As blood pressure is also a risk factor for arterial disease (MacMahon et al 1990) as well as directly and indirectly part of the outcome measures it is difficult to assess the role of blood pressure in relation to peripheral arterial disease. Chapter 7 considers the relationship between rheological variables and systolic and diastolic blood pressure and thereby relates blood pressure indirectly to peripheral arterial disease through the rheological factors.

Plasma fibrinogen is an important risk factor for atherosclerosis and it is not clear whether smoking or genetic factors are the cause of raised levels in the general population. The role of fibrinogen haplotype in determining the level of serum fibrinogen is considered in chapter 8. In addition, fibrinogen haplotype as a risk factor independently of serum fibrinogen level is examined. This analysis used data from the

case-control study which is a subset of the Edinburgh Artery Study and therefore the sample size was very much smaller.

Chapter 9 brings together the simultaneous consideration of all risk factors to assess the relative importance of risk factors and their independent relationship to peripheral arterial disease. Since social class may be related to atherosclerosis it is introduced in this chapter and the relationship between social class and other risk factors is also examined.

Having examined the role of risk factors in relation to peripheral arterial disease chapter 10 turns to the problem of estimating power and sample size in epidemiological studies with specific examples from the Edinburgh Artery study to illustrate the procedures discussed.

Finally, chapter 11 discusses the results from previous chapters focusing on the independence of risk factors for peripheral arterial disease and presents some implications of these results.

CHAPTER 2

BACKGROUND TO THE EDINBURGH ARTERY STUDY

2.1 CROSS-SECTIONAL STUDY

2.1.1 Study Population

The population for the cross-sectional study in 1988 consisted of the men and women of Edinburgh aged 55 to 74 years. Ten general practices with catchment populations spread geographically and socioeconomically throughout the city were chosen and an age stratified random sample was selected from the age-sex registers of these practices. The sample size of approximately 1500 was estimated on the basis of the number required to conduct a subsequent follow-up study. In order to produce at least 1500 participants, 272 subjects were selected from each practice; 34 males and 34 females from each five year age band. General practitioners reviewed lists of their patients selected for the study and excluded those who had severe mental illness or terminal disease, those who had moved from the practice or those who had died. These exclusions were replaced by other randomly sampled patients.

The study was publicised in the local media and letters of invitation signed jointly by the study director and a partner in each general practice were sent to the subjects inviting them to attend a university clinic for a medical examination. An examination at home was available for those who had difficulty in attending the clinic. Letters returned by the post office were replaced with invitations to other randomly-sampled patients. An appointment, map, and details of the examination were sent to those who agreed to take part in the study. Nonresponders were sent a second letter of invitation. Respondents who did not attend were offered another appointment, usually by telephone.

Approximately 20% of subjects in each practice who did not respond or attend were randomly selected for follow-up. Each was sent a letter enclosing a short questionnaire. Subjects not returning the questionnaire were telephoned or visited at home on up to three occasions at different times of the day and evening.

Out of the 2720 subjects aged 55-74 years selected from the age-sex registers, 19% were subsequently replaced; 13% had been excluded by general practitioners and 6% had invitation letters returned by the Post Office. Invitations were finally sent to 2709 subjects of whom 1592 attended for examination, which gave a crude response rate of 59%. The follow-up of the random sample of nonresponders showed that 19% had moved and 3% were dead or in hospital. Extrapolation of these results to the whole sample suggested that the response rate of those receiving an invitation was 65%.

The responders were reasonably typical of the target population. The crude response rate did not differ substantially by age or sex, although there was some under-representation of women aged 70-74 years and males aged 55-59 years who comprised 21.3% and 22.5% (instead of 25%) of women and men respectively (Table 2.1). The social class distribution of responders (Table 2.2) was similar to that of Edinburgh adult residents in the 1981 census except that the responders contained fewer social classes IV and V (13% compared to 19%). The crude response rate varied between the ten general practices from 47% to 71% with the lower response rates occurring in practices serving deprived areas, thus also suggesting a slight under-representation of lower social classes.

Table 2.1 Age-Sex Distribution in the Edinburgh Artery Study

	Age Group				Total
	55-59	60-64	65-69	70-74	
Females	216 (27.6%)	201 (25.7%)	199 (25.4%)	167 (21.3%)	783 (49.2%)
Males	182 (22.5%)	205 (25.3%)	228 (28.2%)	194 (24.0%)	809 (50.8%)
Total	398 (25.0%)	406 (25.5%)	427 (26.8%)	361 (22.7%)	1592 (100%)

Table 2.2 Social Class(OPCS 1980) distribution in the Edinburgh Artery Study

Social Class	Edinburgh*		EAS	
	n	Percentage	n	Percentage
I Professional	3056	9.9%	168	10.6%
II Intermediate	7962	25.9%	505	31.7%
III Non-manual/ clerical	4873	15.9%	254	16.0%
III Skilled manual	8881	28.9%	452	28.4%
IV Semi-skilled manual	4028	13.1%	148	9.3%
V, VI Unskilled manual, unknown	1890	6.0%	65	4.1%

* 1981 Census

2.1.2 The Questionnaire

The questionnaire was self-administered and contained mostly validated questions enquiring about personal characteristics, social class (OPCS 1980), intermittent claudication and angina by the WHO questionnaire (Rose 1962), medical history including diabetes, smoking, physical activity, personality (Bedford and Foulds 1978, Bortner 1969), diet, and alcohol consumption (Duffy 1985). The complete questionnaire is in appendix 1, while detailed description of the personality questionnaires are left to later chapters.

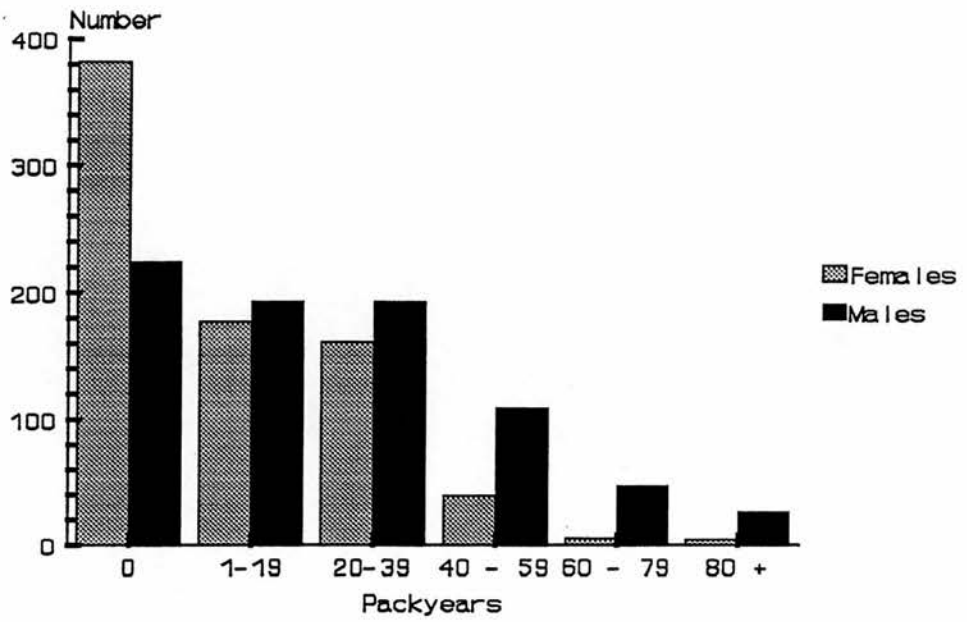
Unemployment or change of occupation can often occur in middle-age or later, because of ill-health and this may bias social class comparisons if based on current occupation. Consequently social class was based on either current occupation or if retired or unemployed, the respondents longest held occupation. The classification (OPCS 1980) was I - Professional, II - Intermediate, III Non-manual/clerical, III - Skilled, manual, IV - Semi-skilled, manual, V - Unskilled, manual, VI - Unknown, unclassified. Married women were classified according to their husband's occupation. Social deprivation was also calculated based on small area statistics (Carstairs and Morris 1991).

Information on Ischaemic Heart Disease was obtained using doctor recall questions (appendix 1) concerning angina, heart attack and stroke and also from the WHO questionnaire. Doctor recall questions gave a lower level of angina than the WHO definition, while doctor recall of heart attack gave a slightly higher prevalence than the WHO questionnaire (Table 2.3)

Table 2.3 Prevalence of Ischaemic Heart Disease in the Edinburgh Artery Study

	n (%)	
	Yes	No
Doctor recall:		
Heart Attack	134 (8.4%)	1458 (91.6%)
Angina	166 (10.4%)	1426 (89.6%)
WHO Questionnaire		
Heart Attack	99 (6.2%)	1491 (93.7%)
Angina	256 (16.1%)	1336 (83.9%)

Smoking was measured by current, former and never smoked categories, while a measure of cumulative lifetime smoking (packyears) was calculated as the average number of packs of cigarettes multiplied by the number of years as a smoker. As the packyears included a large number of individuals who had never smoked (Fig 2.1a) a square root transformed variable was used in all the analyses. Similarly, the number of alcohol units was also positively skewed (fig 2.1b) and a square root transformation was also used in all analyses.



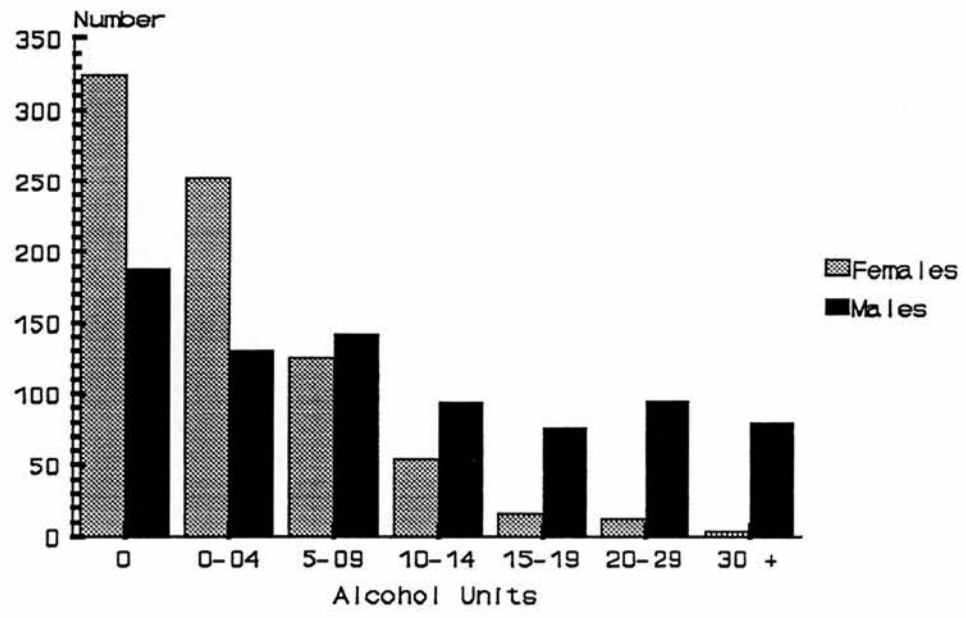


Figure 2.1 Distribution of a) Packyears and b) Alcohol in the Edinburgh Artery Study

2.1.3 Clinical Examination

The clinical examinations were held each weekday morning from August 1987 to September 1988. Ten subjects were invited to each session and were asked to fast from 11 p.m. the previous evening (if not diabetic) and to refrain from smoking for two hours prior to the examination. Each subject had two sets of clinical procedures performed by one of two teams each comprising a nurse and technician. The self-administered questionnaire was also completed at the clinical examination and then checked by a member of the survey team.

Peripheral arterial disease was assessed in the clinical examination by measurement of the Ankle Brachial Pressure Index (ABPI) and by a stress test; the reactive hyperaemia test. Variability studies of the ABPI suggested that biological variability was greater than inter- or intra-observer variability (Fowkes et al 1988a). Reproducibility of the reactive hyperaemia measurements was also assessed (Fowkes et al 1988 b). Prior to the main study, a pilot study of all clinical and laboratory procedures was carried out on 50 volunteers from the general public. The quality of the clinical measurements were checked before and during the study by repeat measurements taken intermittently by the study co-ordinator.

At the beginning of the clinical examination, 20 ml of venous blood was taken initially for subsequent estimation of biochemical, haemostatic and rheological factors. From this the levels of serum total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, blood viscosity, plasma viscosity, haematocrit, and leucocyte elastase were determined. NonHDL cholesterol was calculated as the total cholesterol minus HDL cholesterol. Standing height was measured once to the nearest 5mm without shoes using a free-standing metal ruler on a heavy base. Weight, without shoes and outer clothing, was measured once to the nearest 100g on a digital Soehnle

scale. A 12-lead ECG and rhythm strip were taken using a Hewlett Packard 'Pagewriter' electrocardiograph. ECGs were later coded independently by two trained staff using the 'Minnesota code' (Prineas et al 1982). A third member of staff checked the two results and, in cases of disparity , coded a third time. If the third code did not agree with either of the first two, the ECG was read by a consultant cardiologist and a final code agreed following discussion between the coders.

Next, after ten minutes rest in the supine position, systolic and diastolic (Phase V) blood pressures were taken in the right arm using a Hawksley random zero sphygmomanometer. The femoral, posterior tibial and dorsalis pedis arteries were palpated in both legs. Ankle systolic blood pressures in the right and left legs were taken using the random zero sphygmomanometer and a Sonicaid Doppler probe. Blood flow was detected where possible in the posterior tibial artery. The ankle brachial pressure index (ABPI) was defined as the minimum ratio of ankle pressure to brachial pressure of the left and right limbs. The distribution of the ABPI in males and females was reasonably symmetric with a slight negative skewness (Figure 2.2).

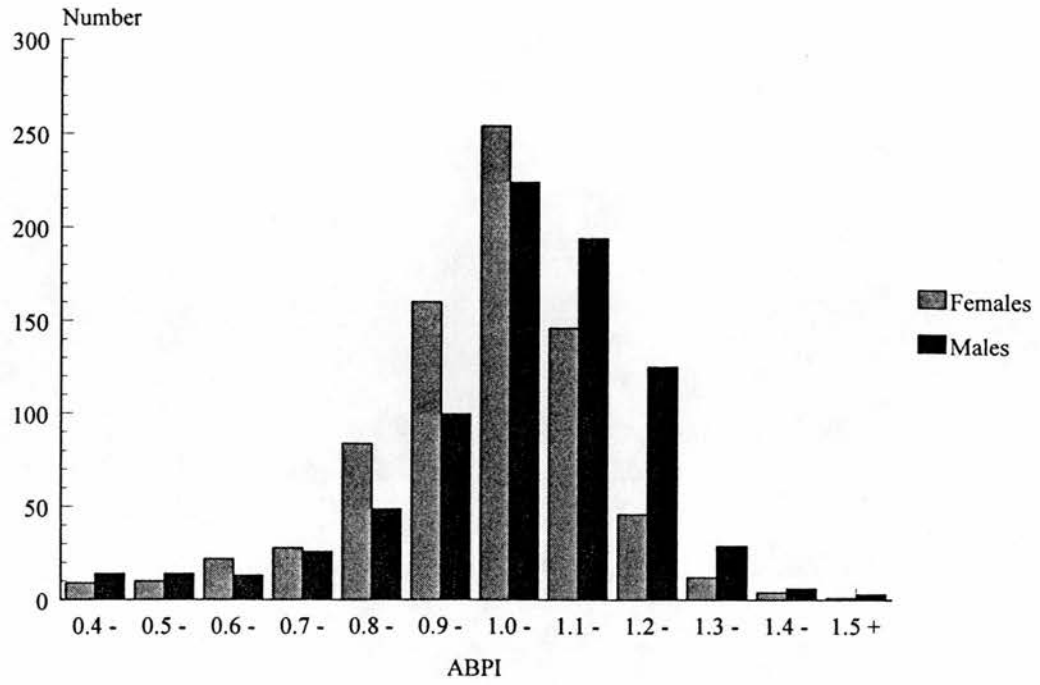


Figure 2.2 Distribution of the ABPI in the Edinburgh Artery Study

In the reactive hyperaemia test which followed, ankle systolic pressure was measured in the right and left legs 15 seconds after the release of a cuff occluding arterial flow just above the knee for four minutes at about 50 mm Hg above systolic pressure. The timing was standardised using an electronic timer. The maximium percentage reduction of pressure on reactive hyperaemia was calculated.

Using the WHO intermittent claudication questionnaire, the ABPI and the reactive hyperaemia test, the following categories of severity of peripheral arterial disease were constructed:

1) Intermittent Claudication - positive on WHO questionnaire

2) Major disease - ABPI < 0.7 **or** maximum percentage reduction on reactive hyperaemia > 35% **or** ABPI < 0.9 plus maximum percentage reduction on reactive hyperaemia > 20%

3) Minor disease - ABPI < 0.9 **or** maximum percentage reduction on reactive hyperaemia > 20%

4) Normal

5) Not classified - one or more of the tests missing (mainly due to incomplete reactive hyperaemia tests.)

This classification gave 4.6% with intermittent claudication and 6.7% with major peripheral arterial disease (Fig 2.3)

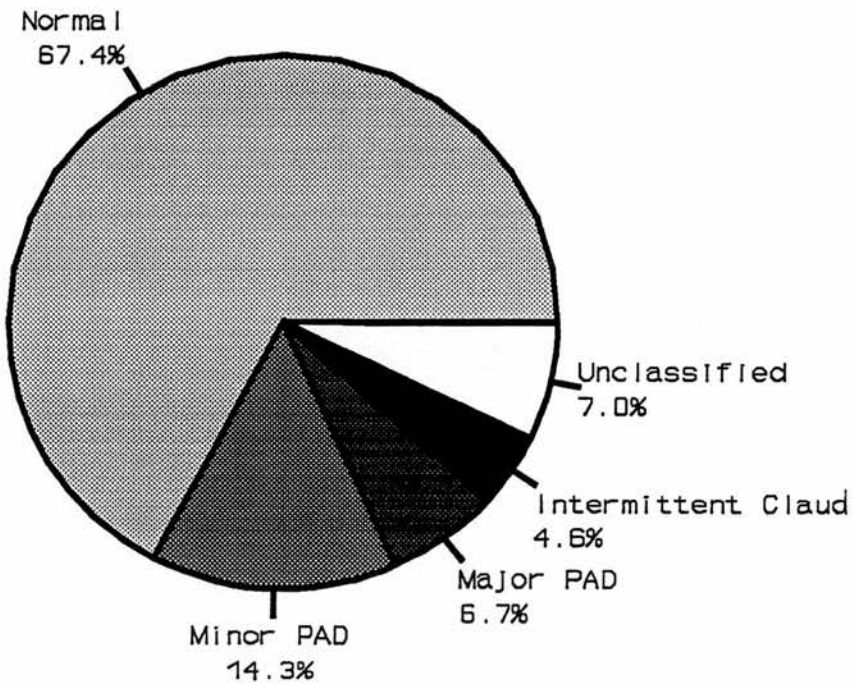


Figure 2.3 Distribution of Peripheral Arterial Disease in the Edinburgh Artery Study

As might be expected there is some overlap between ischaemic heart disease and peripheral arterial disease; 30.2% of those with intermittent claudication or major peripheral arterial disease also had angina or myocardial infarction (table 2.4) as indicated by WHO questionnaire

Table 2.4 Overlap of IHD and PAD in the Edinburgh Artery Study

	WHO MI or Angina		Total
	Yes	No	
Intermittent claudication or Major PAD	54 (30.2%)	125 (69.8%)	179
None	243 (17.2%)	1170 (82.8%)	1413
	297	1295	1592

Statistical analyses were carried out using SPSS-X (1986), or BMDP (Dixon et al 1990) statistical packages, while all plots were produced using the statistical packages S (Becker et al 1988) or Harvard Graphics (1991). Details of particular statistical methods are described in the relevant chapters.

Before the analysis of the first lifestyle risk factors in the cross-sectional study, namely, dietary factors, in the following chapter, a brief description of the case-control study follows.

2.2 CASE-CONTROL STUDY

The primary aim of the case-control study was to examine the role of haemostatic factors in thrombogenesis and atherogenesis. This has been reported elsewhere (Smith et al 1992) and will not be discussed in this thesis. It was not yet known to what extent alterations in plasma levels of fibrinogen were due to genetic or lifestyle factors and so fibrinogen haplotype was measured in the case-control study in order to address this question. In addition, the role of fibrinogen haplotype independently of plasma fibrinogen level, will be explored as possible risk factors for peripheral arterial disease (chapter 8).

2.2.1 Cases

Cases of peripheral arterial disease included all subjects from the Edinburgh Artery Study who fulfilled the following criteria:

1) Ankle Brachial Pressure Index ≤ 0.7 or;

2) A drop in ankle pressure following occlusion of $\geq 35\%$ or;

3) Ankle Brachial Pressure Index ≤ 0.9 plus Intermittent claudication according to WHO questionnaire. Specificity was 98% (Criqui et al 1985) or;

4) A drop in ankle pressure following occlusion of $\geq 20\%$ plus Intermittent Claudication.

Subjects excluded were those who had rest pain, ulcer, gangrene or previous arterial surgery. The cut-off point of 0.9 for the ABPI was based on hospital studies and was over 95% specific (Carter 1968).

2.2.2 Controls

For each case an age and sex frequency matched control was selected within a five year age band. Controls had:

1) no history of coronary artery disease, cerebrovascular disease or arterial surgery;

2) no angina pectoris or intermittent claudication according to WHO questionnaire;

3) no evidence of ischaemia or previous infarction on ECG (Minnesota coding).

Confirmation that the subject was a case or control was made by repeat administration of the WHO questionnaire on angina and intermittent claudication and measurement of ankle and brachial systolic pressures using Hawksley random zero sphygmomanometer and Doppler probe.

Using information on the variability of plasma fibrinogen obtained in one of the pilot studies, it was estimated by another statistician that in a study of 120 cases and 120 controls, after allowing for smoking in the two groups, analysis of variance would have 80% power at the 5% level of significance to detect a difference in fibrinogen of 0.22 g/l. In fact, 153 cases and 153 controls were selected for the study.

2.2.3 Statistical Methods

Odds ratios were estimated using logistic regression and since the cases were frequency matched by age and sex to the controls the appropriate procedure was to stratify by age and sex in all analyses (Schlesselman 1982). All multiple logistic regressions were carried out using the statistical package BMDP (Dixon et al 1990).

CHAPTER 3

DIET

3.1 Introduction

The first lifestyle factor or health related behaviour considered was diet. As the underlying mechanism of peripheral arterial disease and coronary heart disease is atherosclerosis, similarities in dietary risk factors for these two conditions would be expected. However, most research has concentrated on the relationship between diet and coronary heart disease.

Epidemiological evidence suggests that high levels of consumption of fish oil (Shekelle et al 1985a), dietary fibre (Morris et al 1977) and polyunsaturated fats reduce the risk of coronary heart disease, while smoking is associated with a higher incidence of coronary heart disease (Reid et al 1976). Lower levels of serum total cholesterol have been shown to significantly lower the risk of coronary heart disease (Kannel et al 1971, MRFIT 1982, Frick et al 1987) as well as lower total mortality (Anderson et al 1987, Hjermann et al 1986). Recently it has been suggested that there may be a link between cholesterol lowering trials and increased risk of death from accidents/suicides (Muldoon et al 1990), so that cholesterol lowering in the general population still remains controversial.

In contrast to coronary heart disease the relationship between diet and peripheral arterial disease has not been studied extensively. In a hospital based case-control study in Greece (Katsouyanni et al 1991) dietary intake of saturated fatty acids, proteins and cholesterol were associated with increased risk of peripheral arterial disease, while high intake of polyunsaturated fatty acids and crude fibre reduced risk. They concluded that substitution of olive oil for saturated fats and/or the consumption

of high levels of vegetables and fruit and other fibre containing foods may help to explain the low occurrence of atherosclerotic disease in Mediterranean countries. In dietary intervention studies on patients with peripheral arterial disease clinical improvement has been demonstrated in patients on low fat, high-fibre diets (Hutchinson et al 1983, Brown et al 1984, Heller et al 1989). Fish oil (eicosapentaenoic acid) supplementation produced a reduction in blood viscosity (Woodcock et al 1984).

There has been considerable interest in the past in the role of vitamin E and atherosclerosis. Vitamin E has been shown to be an effective inhibitor of platelet adhesion. Intermittent claudication has been treated with α-tocopherol (Vitamin E) supplementation (Livingstone and Jones 1958, Haegar 1974, Pinsky 1980, Williams et al 1971) as has angina (Gillilan et al 1977) and thrombo-embolic disease (Kanofsky 1981).

The limited research on diet and peripheral arterial disease has been conducted only on hospital patients. In The Edinburgh Artery Study one aim was to determine the relationships between diet and a wide spectrum of peripheral arterial disease in a representative sample from the general population of men and women aged 55-74 years surveyed in the Edinburgh Artery Study.

3.2 Dietary Survey Methodology

The influence of nutrition on the genesis of chronic disease has become an important area of research, but work is hindered by the difficulty of measuring people's habitual diets. Diet assessment methods currently available include weighed food records, food consumption with estimated weight of food, the daily (24 hour) recall method, seven day recall, the diet history method, and the food frequency questionnaire (FFQ). Each method may have random or systematic errors and each has advantages and disadvantages in use (Block 1982). For example , the diet history method measures the usual pattern of intake but requires a long interview with a trained nutritionist.

The literature on dietary survey methodology is scattered and few textbooks consider methods in detail. However, two comprehensive reviews have been published (Bingham 1987, Cameron and Van Staverne 1988) which cover description of diet assessment methods available and their accuracy. Biological markers of food consumption and their potential validatory use are discussed by Bingham (1987) and by Block (1982) who also make recommendations on the selection of suitable methods for clinical and epidemiological research.

Diet assessment methods used for CHD epidemiological research have ranged from weighed records of food intake (Keys 1970) to food frequency questionnaires (Tunstall-Pedoe et al 1989). Weighed food records are considered to give reasonably accurate measures of the actual nutrient intake. They usually cover a seven day period which is not necessarily the most appropriate way to assess the habitual dietary intake of all nutrients of all individuals within a population. However, this is usually sufficient to assess an individual's current energy intake and percentage energy derived from protein, fat and carbohydrate with an acceptable degree of precision and

to place individuals correctly at the very extremes of the distribution for more variable nutrients (Thomson et al 1988). A single 24-hour record or recall should never be used to assess dietary status or to test association between diet and other risk factors such as serum lipids (Marr 1971, Bingham 1987), as a single day's intake may not be representative of an individual's usual diet.

Among the short-cut methods available for dietary assessment is the use of a questionnaire to assess the frequency of consumption of foods. Wiehl and Reed (1960) suggested that a questionnaire should be used in epidemiological studies of cardiovascular disease and a number were subsequently developed (Hankin et al 1975, Jain et al 1982, Yarnell et al 1983). Quantitative estimation of their accuracy is not possible in many cases, because food frequency methods and questionnaires are designed to assess the intake of a specific nutrient or food, rather than intake of all foods and despite their routine use in epidemiology, attempts to validate them are sparse. Yarnell et al (1983) compared the results of a food frequency questionnaire with a seven-day weighed record in a Welsh population and found correlation coefficients for dietary intake ranged from 0.27 to 0.41, and 0.75 for alcohol. Although correlation coefficients do not measure agreement it is clear that consistency between FFQ and weighed intake (WI) is very good for alcohol and moderate for other nutrients. The same questionnaire with some modifications for the alcohol intake was used in the Scottish Heart Health Study (SHHS) of coronary heart disease risk factors (Tunstall-Pedoe et al 1989). Bolton-Smith and Milne (1990) validated the FFQ used in the SHHS and found correlations between 0.18 for sugar to 0.46 for fat, and 0.64 for alcohol. Whenever the percentage energy from nutrients or nutrient density was calculated the correlation increased to 0.33 for protein to 0.71 for fat, and 0.68 for alcohol, indicating the necessity of adjusting for total energy intake (Willett et al

1986). The better agreement of the FFQ used in the SHHS compared to Wales (Yarnell et al 1983) may have been due to the age differences, the longer time interval of weighed diet assessment (14 days compared to 7 days), and the higher percentage of non-manual workers (84%) in the Scottish sample. A more recent validation by Hankin et al (1991) found good agreement between a quantitative diet history method and detailed food records using intraclass correlation coefficient and weighted Kappa statistics as measures of agreement. These few comparisons of the two methods suggest reasonable agreement , and this is especially the case for alcohol intake. The WI method is time-consuming and requires training to be administered properly, but is considered to be the 'gold standard' for assessing dietary intake of nutrients. An advantage of the FFQ is the ease of administration in large epidemiological surveys where WI is not feasible for such large numbers. Many important epidemiological questions can still be usefully addressed by FFQ's, even if they do not yield completely accurate measures of nutrient intake, as required by nutritionists.

3.3 METHODS

In the Edinburgh Artery Study, a version of a food frequency questionnaire employed in a previous study (Tunstall-Pedoe et al 1989) was used to obtain nutrient information. This was completed by all who attended the clinic, and a nurse was available to go through the questionnaire if there were any problems of interpretation. Calculation of energy and nutrient intakes for individuals was estimated by multiplying the nutrient content of a typical portion size of the specified food item by the frequency of consumption and summing over all food items. Where participants failed to complete all questions, nutrients were still calculated providing a response had been given to those foods which are major sources of a specific nutrient. Consequently, some nutrients may have been slightly underestimated.

3.3.1 Statistical Methods

The results of the food frequency questionnaire in terms of nutrients have been expressed as medians along with the upper and lower quartiles since many of the nutrients had positively skewed distributions. The nutrients were expressed as percentages of total energy intake or as energy adjusted nutrients using a procedure described later. The Mann-Whitney test was used to compare differences in absolute nutrient intakes between males and females. In addition, the relationships between the frequency of consumption of individual foods and peripheral arterial disease were explored by testing for linear trends across the frequency categories: rarely/monthly, 1-3 times/week and 4-7 times/week.

Multiple linear regression was used to assess the independence of associations between dietary factors and the minimum ankle brachial pressure index of the two limbs.

3.3.2 Total Energy Intake

The interpretation of total energy intake, nutrients and their relationship to each other and to disease in epidemiological studies is often difficult. Total energy intake is related to body size, physical activity, metabolic efficiency and net energy balance. Body size is easily obtained in terms of weight and height, while the other components are more difficult to measure.

Willett et al (1986) suggests that it is important in epidemiological analyses to use measures of nutrient intake which are independent of total energy intake, especially whenever energy is related to disease. In the Edinburgh Artery Study total energy intake was weakly correlated with disease status ($r = 0.04$) as measured by the ankle brachial pressure index (ABPI). Nevertheless, Willett et al (1986) suggests nutrient measures independent of total energy intake are preferable. Most nutrients are positively correlated with total energy intake with weaker correlations for vitamin C and β-carotene. In order to produce uncorrelated nutrient measures, one method is to express them as a percentage of total energy intake for macronutrients; and for micronutrients, simply divide the absolute value by total energy intake in kilocalories. In either case the resulting measure is known as nutrient density (ND). However, for those nutrients only weakly correlated with total energy intake and disease, the result of calculating ND is a measure which is more correlated with energy intake and correlated in the opposite direction with respect to disease. Another potential drawback is that this procedure may lead to a measure which has greater variance than the original measure.

Alternatively, 'energy-adjusted ' nutrient intakes (Willett et al 1986) were obtained from the residuals of the regression of each of the nutrients with total energy intake as predictor, applying the usual assumptions of regression. The residuals were added

to the expected nutrient intake for the mean total energy intake of the population to give more meaningful measures of nutrient intake. This procedure gave relatively uncorrelated measures of nutrient intake which were used in the multiple linear regression models with ABPI as outcome.

Further models were fitted to assess the independence of dietary factors from other factors known to be associated with the ABPI such as age, sex, height and cigarette smoking. Smoking history was defined in two ways. Firstly, consumption was estimated in terms of packyears which is a measure of the amount smoked and the number of years as a smoker. A square root transformation of packyears was used in the models to give a more symmetrical distribution for the analyses. Secondly, when interactions with smoking were found, the population was divided into those who had never smoked and those who were ex-smokers or current smokers (ever smokers).

3.4 RESULTS

The absolute values of nutrient intakes and as a percentage of energy for males and females in the population of the Edinburgh Artery Study are shown in Table 3.1. Most levels of nutrient consumption were higher in males with the exception of vegetable fibre and vitamin C which were significantly higher in females. In addition, females had significantly greater intake of protein and polyunsaturated fat as a percentage of total energy compared to males. Table 3.2 shows the trend in mean ABPI in females across three categories of frequency of food consumption: rarely/monthly, 1-3 times per week, and 4-7 times per week. There were significant reductions in mean ABPI in females with increasing consumption of low fibre breakfast cereal, meat pies and pasties and pork/bacon/ham, and significant trends in mean ABPI with increasing frequency of consumption of onions and biscuits/sweets. Among males (table 3.3) there were significant linear reductions in mean ABPI with increasing consumption of white bread and meat pies and pasties, while significant increases in mean ABPI were associated with increasing consumption of wholemeal bread, high fibre breakfast and pears. Increasing lard consumption in males and females was associated with lower levels of mean ABPI but this did not reach statistical significance. Caution should be applied in interpreting these results because of the problem of multiple testing, although the number of significant tests is greater than would be expected by chance alone. Tests of association and for linear trend were carried out. As the test for trend may be more powerful, these are presented and the significant linear trends (tables 3.2 and 3.3) provide stronger evidence of cause and effect than the more general tests for association.

Table 3.1 Medians and upper and lower quartiles of absolute nutrient intake and as a percentage of energy in males and females aged 55-74 years in the Edinburgh Artery Study

	FEMALES(n=783)			MALES(n = 809)			¶
		Quartiles			Quartiles		
	Median	Lower	Upper	Median	Lower	Upper	
Total Energy (kcal)	1774	1492	2090	2040	1731	2449	***
(%) of Total Energy (excluding alcohol) from:							
Total fat	38.1	34.4	42.0	38.0	33.6	44.6	
Carbohydrate	44.2	40.3	47.8	45.2	41.5	49.5	***
Protein	17.4	15.9	19.1	16.8	15.5	18.3	***
Saturated fat	16.5	14.0	19.5	16.2	13.9	18.8	
Polyunsaturated fat	4.9	3.7	6.7	4.5	3.5	6.4	**
P:S ratio	0.31	0.20	0.46	0.30	0.19	0.44	
Alcohol (g)	1.2	0.0	6.2	9.5	1.2	21.9	***
Cereal fibre (g)	8.9	6.2	12.8	9.7	6.9	14.0	***
Vegetable fibre (g)	12.2	9.5	15.0	10.5	7.8	13.7	***
Vitamin C (mg)	63.5	46.3	85.4	48.3	35.0	67.5	***
Cholesterol (mg)	309.4	238.2	396.4	328.2	258.8	433.6	***
Retinol (μg)	646.7	404.6	956.9	657.9	427.3	985.3	
B-carotene (mg)	3.3	1.8	5.8	3.3	1.8	4.8	
α-tocopherol (mg)	8.1	6.1	12.2	8.2	6.1	12.7	
Linoleic acid (g)	7.2	5.1	11.7	7.7	5.4	11.9	*

¶ * $p<0.05$; ** $p<0.01$; *** $p<0.001$; Mann-Whitney test, Males v. Females

Table 3.2 Age adjusted mean(se) ABPI by frequency of individual food consumption in Females in the Edinburgh Artery Study

	Age adjusted mean (se) ABPI			
Frequency of food consumption	Monthly/ Rarely	1-3 per week	4-7 per week	Linear Trend (p - value)
White bread	1.00 (0.01)	1.01 (0.02)	1.01 (0.01)	0.91
Wholemeal bread	1.01 (0.01)	1.01 (0.02)	1.01 (0.01)	0.93
Low fibre breakfast cereal e.g.porridge, Rice Crispies	1.02 (0.01)	0.98 (0.02)	0.98 (0.02)	0.04
High fibre breakfast cereal e.g. Bran Flakes, Puffed Wheat	1.01 (0.01)	1.01 (0.03)	1.01 (0.02)	0.75
Pork, bacon, ham	1.03 (0.01)	1.00 (0.01)	0.94 (0.03)	0.002
Meat pies and pasties	1.01 (0.01)	0.98 (0.01)	0.84 (0.24)	0.03
Kippers, herrings, tuna, mackerel	1.02 (0.01)	1.00 (0.01)	0.94 (0.06)	0.06
Carrots	0.99 (0.02)	1.02 (0.01)	0.99 (0.01)	0.71
Onions (raw, cooked)	1.00 (0.01)	1.00 (0.01)	1.03 (0.01)	0.04
Sweets, biscuits, jellies, etc.	0.99 (0.01)	1.01 (0.01)	1.04 (0.01)	0.002
Lard, dripping, solid veg. oil	1.01 (0.01)	1.00 (0.01)	0.97 (0.04)	0.09
Apples	0.99 (0.01)	0.99 (0.01)	1.03 (0.01)	0.11
Pears	1.01 (0.01)	1.01 (0.01)	1.02 (0.03)	0.46

Table 3.3 Age adjusted mean(se) ABPI by frequency of individual food consumption in Males in the Edinburgh Artery Study

	Age adjusted mean (se) ABPI			
Frequency of food consumption	Monthly/ Rarely	1-3 per week	4-7 per week	Linear Trend (p-value)
White bread	1.08 (0.01)	1.05 (0.02)	1.04 (0.01)	0.0008
Wholemeal bread	1.05 (0.01)	1.08 (0.02)	1.07 (0.01)	0.03
Low fibre breakfast cereal e.g.porridge, Rice Crispies	1.06 (0.01)	1.04 (0.02)	1.06 (0.02)	0.97
High fibre breakfast cereal e.g. Bran Flakes, Puffed Wheat	1.05 (0.01)	1.08 (0.03)	1.10 (0.02)	0.02
Pork, bacon, ham	1.05 (0.01)	1.05 (0.01)	1.06 (0.03)	0.80
Meat pies and pasties	1.07 (0.01)	1.04 (0.01)	1.05 (0.07)	0.03
Kippers, herrings, tuna, mackerel	1.05 (0.01)	1.04 (0.01)	0.99 (0.09)	0.29
Carrots	1.03 (0.02)	1.05 (0.01)	1.07 (0.01)	0.07
Onions (raw, cooked)	1.04 (0.01)	1.06 (0.01)	1.08 (0.02)	0.08
Sweets, biscuits, jellies, etc.	1.05 (0.01)	1.06 (0.01)	1.07 (0.01)	0.22
Lard, dripping, solid veg. oil	1.07 (0.01)	1.04 (0.01)	1.04 (0.03)	0.06
Apples	1.05 (0.01)	1.05 (0.01)	1.08 (0.01)	0.14
Pears	1.05 (0.01)	1.08 (0.01)	1.13 (0.03)	0.004

In the multiple linear regression model with ABPI as outcome, there were significant relationships between ABPI and age ($p < 0.0001$), height ($p = 0.03$) and smoking (ever vs. never, $p < 0.0001$). Sex ($p = 0.22$) and total energy intake ($p = 0.76$) were also added to the model and this formed the core model to which energy adjusted nutrients were added simultaneously (Table 3.4). The number of diabetics in the study (known or positive in glucose tolerance test) was small (5.7%) and when a dummy variable representing diabetes was added to the model, this was not statistically significant and did not greatly alter the regression coefficients of the nutrients.

The energy adjusted intake of alcohol had the strongest correlation with the ABPI and a significant interaction with sex was found in the regression analysis at the low 10% level. Alcohol was positively associated with the ABPI in males ($p = 0.01$) when added to the core model, while there was no significant association in females. Similarly, cereal fibre intake had a significant interaction with sex ($p = 0.05$) and was significantly related to the ABPI in males ($p = 0.002$), but not in females. A highly significant interaction ($p = 0.006$) was found between vitamin C and smoking (ever vs. never). In those who were ex-smokers or current smokers vitamin C was significantly related to the ABPI ($p=0.01$) but this association was not significant in lifelong non-smokers (Table 3.4). There were no other significant sex by nutrient or smoking by nutrient interactions. Finally, the antioxidant α - tocopherol (Vitamin E) was significantly associated ($p = 0.03$) with an increase in the ABPI, after adjustment for all variables in the core model. Vegetable fibre and β-carotene were univariately related to high levels of the ABPI ($p < 0.1$), although not reaching statistical significance. In the multiple regression analysis these along with the other non-significant nutrients were not independently related to the ABPI.

Table 3.4 Multiple linear regression of age, sex, height, smoking and energy adjusted nutrients on the ABPI in the Edinburgh Artery Study

	Change in ABPI X 100 (se)	p-value
Sex (1 = Female, 2 = Male)	-6.23 (5.09)	0.22
Age (+ 10 years)	-5.77 (0.81)	<0.001
Height (+ 10 cm)	1.44 (0.67)	0.03
Cigarette smoking (ever vs never)	-14.11 (2.57)	<0.001
Total Energy Intake (+1000 kcal)	9.71 (32.1)	0.76
Energy Adjusted Nutrients (+ 1 unit)		
Vitamin C : Never Smoked	-0.02 (0.03)	0.39
Ever Smoked	0.06 (0.02)	0.01
Cereal Fibre (ln) : Males	4.91 (1.57)	0.002
Females	0.91 (1.49)	0.54
Alcohol (ln) : Males	1.41 (0.58)	0.01
Females	0.17 (0.62)	0.78
Retinol (ln)	-0.77 (0.96)	0.42
α- tocopherol (ln)	4.73 (2.11)	0.03
β- carotene (ln)	0.43 (0.66)	0.52
Carbohydrate (ln)	-0.01 (0.02)	0.74
Vegetable fibre	0.01 (0.17)	0.95
Protein (ln)	-2.56 (3.87)	0.51
Saturated fat (ln)	1.75 (3.05)	0.57
Polyunsaturated fat (ln)	5.17 (8.62)	0.55
Cholesterol	-0.002(0.006)	0.69
Linoleic acid (ln)	-8.92 (7.74)	0.25

ln - natural logarithm

3.5 DISCUSSION

3.5.1 Bias in regression coefficients due to measurement error

The FFQ method of assessing nutrient intake is prone to error, both within subject random error and systematic error (more likely underestimation). The consequence of error in one measure is to bias the regression coefficient for that particular measure towards the null value, that is, zero and hence it is less likely to be significant. The problem is greater when a number of confounders are present and correlated with the variable of interest. In this case the bias can be in either direction and so inflate or reduce the size of the regression coefficient. Thus measurement error can contribute to either giving falsely 'independent' associations or falsely non-significant results (Phillips and Davey Smith 1991).

A number of methods have been proposed to deal with this problem. One method based on logistic regression (Rosner et al 1989, Rosner et al 1990) requires a validation study that will provide an independent, unbiased estimate of each subject's true nutrient intake value. This does not have to include all the subjects, a random sample is sufficient. In a dietary study, for example, a random sample of subjects would have nutrient intake assessed by the 'gold standard' method, weighed record as well as by FFQ. Based on the regression coefficients λ relating the true measure to the surrogate measure in the subsample, the true logistic regression coefficients β^* are estimated by $\beta' \lambda^{-1}$ where β' is the regression coefficient obtained from the logistic regression on the surrogate measure for the whole sample. This method also corrects the confidence interval, allowing for a component of variability due to estimation of the measurement error from the validation study (Rosner et al

1989). The method assumes multivariate normality for the error terms in the regression of the surrogate measures on the true measures and is only suitable for continuous exposures. It also assumes that the probability of disease is small. Rosner et al (1992) extended this method further for measures such as cholesterol which does not have a gold standard measure by using a reproducibility substudy to estimate corrected regression coefficients for logistic regression.

Phillips and Davey Smith (1991) used a simulation method to estimate the correct regression coefficients β^* and found good agreement with the method of Rosner et al (1989). The advantage of this method is that it requires a replicate study rather than a validation study but its disadvantage is that it can only be used when a limited number of exposures are being considered.

Thus, one way of overcoming the problem of bias in the regression coefficients in dietary studies would be to have a validation study for a random sample of the full study in which a 'gold standard' method such as the weighed record method is also used to assess nutrient intake. However, one advantage of the procedure of calculating energy adjusted nutrient measures (Willett et al 1986) was to produce relatively uncorrelated variables and so the problem of confounding between the nutrients is minimised. With regard to other non-dietary confounders, the general rule that the relationship between an exposure and outcome cannot be due to confounding by a factor more weakly associated with the outcome (Schlesselman 1978), it is clear that smoking, being highly correlated with the ABPI, could explain the significance of dietary associations. Unfortunately, the Edinburgh Artery study does not have information on the measurement error of the nutrient intakes or smoking, so that these methods cannot be applied to the analysis.

In any case, these methods only demonstrate biases and do not completely

'correct' for them. Hence the problem needs to be addressed at the design stage of a study either by a replication study or a validation study which uses a 'gold standard' measure. Another suggestion made by Phillips and Davey Smith (1991) was to study populations in which the confounding is not present, but this may be difficult in practice, for example, when studying peripheral arterial disease in a population where smoking is not related to the ABPI.

3.5.2 Dietary Risk Factors

The FFQ is a common tool in epidemiological studies of diet and disease. One drawback of this method of estimating nutrient intake is the lack of validation studies. The energy adjusted alcohol intake showed relatively high Spearman Rank correlations with HDL cholesterol ($r = 0.28$ males; $r = 0.18$ females) and this suggested that reasonably valid estimates of alcohol intake were obtained (Jackson et al 1991). As discussed above, another disadvantage of the FFQ method is in terms of measurement imprecision both in the dietary measures and confounders, and systematic error in estimating the nutrients, that is, underestimation. These problems can be alleviated to some extent and a validation substudy in which nutrients are estimated using a 'gold standard' is recommended for future work in this area (Phillips and Davey Smith 1991). Despite these methodological drawbacks significant associations of dietary factors with peripheral arterial disease in terms of the ABPI were detected in the Edinburgh Artery Study.

The total energy intake and nutrient intakes (Table 3.1) in males were slightly lower than might be expected from a previous survey in Edinburgh (Thomson et al 1985), although time trends in consumption indicate some consistency with this finding (MAFF 1989). The exception to the general decrease is a slight increase in

cereal fibre in the Edinburgh Artery Study. However, comparisons are difficult since the Edinburgh Artery Study population is older.

When the frequencies of consumption of individual foods were considered, low ABPI was significantly associated with high frequency of consumption of low fibre containing foods (white bread in males, low fibre breakfast cereals in females) and high frequency of consumption of meat and meat products in both males and females. The significant tests for trend indicate a dose-response relationship and provide stronger evidence of cause and effect than the more general tests of association. The results are consistent with the general consensus concerning a "healthy" diet, that is, high fibre intake and a low intake of saturated fats.

The differences found in the frequency of consumption of individual foods, especially those containing fibre, according to the ABPI and the calculated nutrient intakes were also reflected in the regression analyses. In a multiple linear regression model high cereal fibre intake in males was significantly associated with high levels of the ABPI, and this relationship was independent of smoking. This finding was consistent with the recent case-control study (Katsouyanni et al 1991) in Greece, in which it was found that a significant relationship existed between intake of crude fibre, which presumably included cereal fibre, and peripheral arterial disease independently of smoking. In addition, in the Edinburgh Artery Study alcohol intake in males was associated with an increase in the ABPI. Alcohol intake in males was significantly related to the ABPI in males and not in females. This was consistent with a case-control study of New Zealand men and women which showed a lower risk of myocardial infarction in moderate drinkers compared to total abstainers (Jackson et al 1991). There is also some evidence that high wine consumption may be beneficial for coronary heart disease (Renaud and de Longeril, 1992). In the Edinburgh Artery

Study current drinking habit will be confounded with disease status and this may explain the positive association.

Dietary cholesterol was not significantly associated with the ABPI and this may be due to inadequacies of the FFQ to measure cholesterol or that serum cholesterol is the more important risk factor for peripheral arterial disease.

Food supplements contribute to the intake of nutrients, especially vitamin intake. Although this was not measured in the Edinburgh Artery Study, a British survey (Gregory et al 1990) suggests that the prevalence of food supplementation is low; 16% in the 50-64 age range compared to 51% over one year or a daily use of 21%-40% in the 55-74 age range in the USA (Subar and Block, 1990). Food supplementation is lower in males than females and the British survey (Gregory et al 1990) consisted of mainly English and Welsh respondents, and vitamin supplementation was possibly even lower in Scotland. Thus, our measures of vitamin intake will be underestimates, but it is unlikely that this could explain the significant findings with vitamin C and α-tocopherol. Nevertheless, food supplementation is growing in importance as a source of vitamins and so should be included in any future studies using a food frequency questionnaire.

It is interesting that α - tocopherol (Vitamin E) had a significantly positive effect on the ABPI ($p=0.03$), independently of smoking. This finding is consistent with population studies where low vitamin E was correlated with ischaemic heart disease (Gey et al 1989) and angina (Riemersma et al 1991). In fact, Vitamin E has been used to treat intermittent claudication (Haegar 1974, Livingstone and Jones 1958, Williams et al 1971, Pinsky 1980) and angina (Gillilan et al 1977). It is thought that the mechanism of prevention of thrombo-embolic disease (Kanofsky 1981) by α-tocopherol is through inhibition of platelet adhesion (Jondak et al 1989). The

association of α - tocopherol and the ABPI occurred despite the fact that food frequency questionnaire derived measures of vitamin E are not as accurate as blood measures. The other main dietary antioxidant, Vitamin C, which is also thought to be related to atherosclerosis, showed some positive relationship to the ABPI, but this was only significant for those who had ever smoked. Non-smokers already had significantly higher levels of the ABPI and vitamin C than smokers, and so high intake of Vitamin C would have little effect in non-smokers. By contrast, high dietary vitamin C intake may partly compensate for the reduction of vitamin C through smoking, although never reaching the levels in non-smokers. Hence smoking remained one of the strongest risk factors for peripheral arterial disease.

CHAPTER 4

PHYSICAL ACTIVITY

4.1 Introduction

4.1.1 Physical Activity and Ischaemic Heart Disease

One of the first studies to show a relationship between physical activity and ischaemic heart disease found greater mortality among London bus drivers compared to the more active bus conductors, and also among post office supervisors compared to postmen (Morris et al 1953). Since these early studies were carried out, increasing mechanisation and automation has resulted in leisure activity becoming a major component of total physical activity. Morris et al (1973) showed that mortality from ischaemic heart disease was significantly reduced in London civil servants who had taken part in physical activity. Many studies have shown an increased risk of coronary heart disease associated with a lack of physical activity in leisure time (Kannel et al 1979a, Morris et al 1973, Paffenbarger et al 1978, Morris et al 1980). More recently Zimmet et al (1991) showed that physical activity in Mauritians improved their cardiovascular risk factor profiles, in that HDL cholesterol was significantly higher, while fasting triglyceride levels, uric acid, 2-hour plasma glucose concentration were significantly lower among more active subjects. In addition, lifelong physical activity has been shown to reduce the risk of stroke (Shinton and Sagar 1993).

4.1.2 Physical Activity and Peripheral Arterial Disease

Few studies have concentrated on peripheral arterial disease and it has generally only been mentioned in studies where the main concern was ischaemic heart disease. In the Framingham Study (Kannel et al 1979a) an association was found between low levels of physical activity and the development of intermittent claudication, which became non-significant on adjustment for age. Levels of physical activity decline with increasing age. Hence the Edinburgh Artery Study represents the first examination of physical activity as a risk factor for peripheral arterial disease in the general population.

4.2 Methods

4.2.1 Physical activity vs Physical Fitness

The distinction between physical fitness and physical activity should be made. Physical fitness is a biological marker of the ability to perform physical activity and so is the outcome of participation in activity and not the process. Most epidemiological surveys have been concerned with measuring physical activity, although it has been suggested that physical fitness is a more powerful predictor of disease (Sobolski et al 1987). In a study of coronary heart disease risk factors Lochen et al (1992) found that physical fitness was more closely related to other risk factors but also found physical fitness to be significantly correlated with physical activity. They suggested that fitness and activity may be two different dimensions with largely related but independent information. It is also more difficult to measure physical fitness with suitable precision in population surveys compared to activity. In any case it is physical activity which promotes fitness and studies have shown activity to be protective against coronary heart disease (Morris et al 1990).

4.2.2 Measurement of Physical Activity

The usual method of measuring physical activity in large population studies is through recall questionnaires. These are simple to administer in epidemiological studies but the grading is crude and can result in over and under-estimation. Among the other methods which show promise is telemetry which measures the heart rate response minute by minute (Armstrong et al 1990), although this presents problems for analysis, with large numbers of correlated observations. There have also been attempts to measure activity with a single question (Shectman et al 1991) but this has not been sufficiently validated. Recall questionnaires have been shown to be reliable

(Blair et al 1991). A recent study (Jacobs et al 1993) showed good correlation between the Minnesota leisure time physical activity questionnaire and nine other questionnaires with treadmill estimation of oxygen uptake and body composition, both of which are gold standard measures of physical activity. They found poor agreement between the questionnaire and occupational activity.

4.2.3 Statistical Methods

The Edinburgh Artery Study used an adapted version of the questionnaire employed in the Welsh Heart Health Study (1985 a), which had been validated against measures of cardiovascular fitness (Welsh Heart Health Study 1985 b). This consisted of three broad classifications of activity into light, moderate and strenuous (Appendix 1). Respondents were asked to assess the number of twenty minute periods of activity in the summer and winter in the last year and when aged 35-45 years old. This last category is important to obtain some measure, albeit crude, of past physical activity which can be related to present disease status. The summer and winter responses were then combined and classified into a single measure of leisure activity aged 35-45 with the following categories; 0 = no leisure activity; 1 = maximum of light activity; 2 = maximum of moderate activity; 3 = maximum of strenuous activity. Three dummy variables were created representing each of these categories relative to no activity for the purposes of multiple regression analyses. The measure of leisure activity at the time of the survey was correlated ($r = 0.56$) with leisure activity when aged 35-45 but present activity was not used in the analysis because of the methodological difficulties of interpretation in a cross-sectional survey. Present activity would be reduced because of symptomatic disease. Occupational activity was assessed by two questions for present activity and for activity aged 35-45. There was no significant association

between occupational activity and the ABPI and so this was also not explored further.

The analysis concentrates on the ABPI as a continuous outcome which gave greater power to detect associations. Spearman rank correlations of the ABPI (Chapter 2) which is an indicator of peripheral arterial disease with leisure activity were calculated and the mean ABPI levels across leisure activity categories was tested for linear trend. The independent relation between leisure activity and peripheral arterial disease was assessed using multiple linear regression with the ABPI as the dependent variable, adjusting for age, sex, BMI, cumulative lifetime smoking as measured by the packyears and alcohol, lifestyle variables which have been shown to be related to the ABPI (Fowkes et al 1991). Square root transformed variables for smoking and alcohol were used in the regression analyses because of positive skewness. Tests for interaction between other risk factors and leisure activity were also carried out. It has been shown that social class is related to leisure activity at age 36 (Kuh and Cooper 1992) and so the effect of social class was also assessed in the multiple regression model. A more thorough analysis of the relationship between lifestyle factors and social class is discussed in chapter 9.

4.3 Results

A greater proportion of men (65.7%) indulged in moderate or strenuous leisure activity when aged 35-45 years (table 4.1), compared to women (39.7%).

Table 4.1 Percentages (n) of men and women taking part in leisure activity when aged 35-45 years in the Edinburgh Artery Study

	Leisure Activity aged 35-45 years			
	None	At most Light	At most moderate	At most Strenuous
Females	11.9% (93)	48.5% (379)	36.6% (286)	3.1% (24)
Males	8.0% (65)	26.3% (213)	48.0% (388)	17.7% (143)

The Spearman Rank Correlations show a positive relationship between leisure activity aged 35-45 and the ABPI, alcohol intake and being male (Table 4.2). High social class is associated with greater leisure activity. Leisure activity in females is associated with low BMI and high alcohol intake while in males leisure activity is associated with low smoking. A leisure activity by sex interaction is suggested by the differing correlations with the ABPI in males compared to females.

Table 4.2 Spearman Rank Correlations of leisure activity aged 35-45 years with the ABPI and risk factors for Peripheral Arterial Disease in males and females

	Leisure Activity (0 = none, 1=light,2 = moderate,3=strenuous)		
	Females (n = 782)	Males (n = 809)	Total (n = 1591)
ABPI	0.02	0.13***	0.13***
Sex(1=F,2=M)	-	-	0.28***
BMI kg/ m^2	-0.15***	-0.006	-0.06*
Packyears(square root)	-0.004	-0.11**	0.02
Alcohol (square root)	0.15***	0.05	0.20***
Social Class#	-0.32***	-0.23***	-0.27***

1= I, 2 = II, 3 = III Non-manual, 4 = III Manual, 5 = IV, 6 = V, VI

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

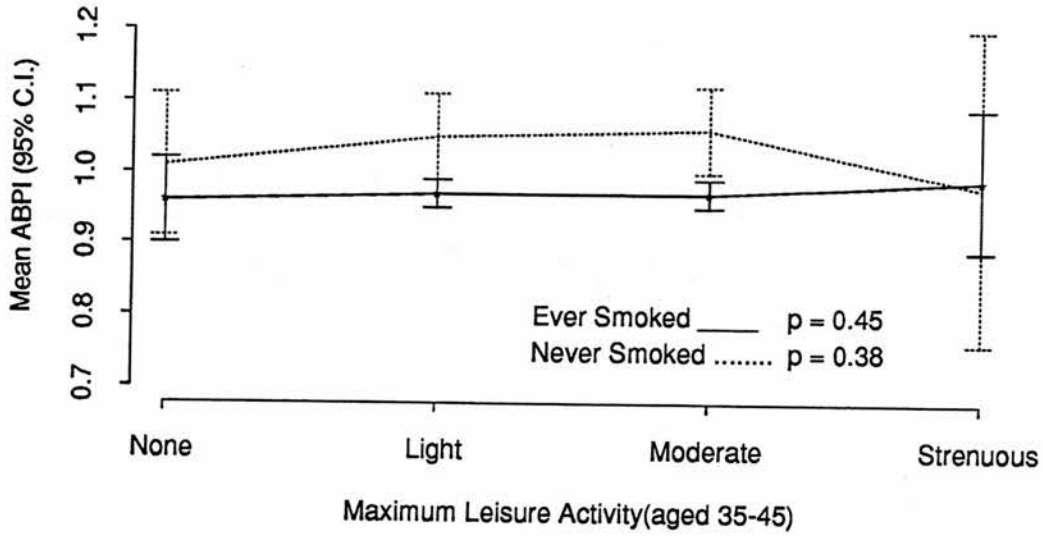
This is reinforced by the significant positive linear trend (p = 0.0004) in the mean ABPI with increasing leisure activity in males but not in females (table 4.3).

Table 4.3 Mean (se) ABPI by degree of leisure activity aged 35-45 years in males and females.

Leisure activity	Mean (se) ABPI	
	Females	Males
None	0.99 (0.02)	0.98 (0.02)
At most light	1.01 (0.01)	1.04 (0.01)
At most moderate	1.01 (0.01)	1.06 (0.01)
At most strenuous	0.99 (0.03)	1.09 (0.02)
Test for trend	p = 0.41	p = 0.0004

Table 4.2 showed a significant relationship between smoking and leisure activity in males and figure 4.1 suggests that the strongest linear relationship between the ABPI and leisure activity is amongst males who ever smoked. Note also that the mean levels of the ABPI are higher in the never smoked in both males and females, so that although the greatest increase in the mean ABPI with leisure activity is in the ever smoked these mean levels never reach the mean ABPI levels found in the never smoked, irrespective of the degree of leisure activity.

(a) Females



(b) Males

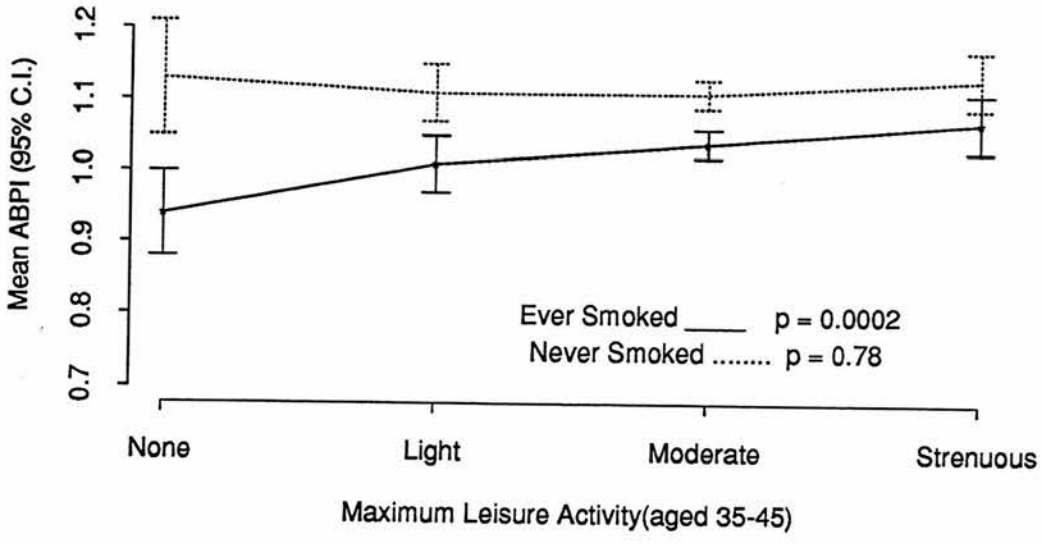


Figure 4.1 Trends in mean(± 2 se) ABPI by degree of leisure activity aged 35 - 45 in (a) females (b) males

In the multiple regression analysis with the ABPI as the dependent variable a significant leisure activity by sex by smoking (ever vs never) interaction was found ($p = 0.02$). Consequently twelve dummy variables were created for each of the leisure categories relative to no activity separately for males and females in the ever and never smoked categories. When this model was fitted adjusting for age, BMI, sex, smoking (ever vs never) and alcohol, increasing leisure activity was significantly associated with higher ABPI only in males who ever smoked (table 4.4). A dose response is indicated by the increasing size of the regression coefficients with increasing degree of activity.

Table 4.4 Results of multiple regression of leisure activity aged 35-45 on the ABPI

Maximum Leisure activity relative to none	Change in the mean ABPI (se)*			
	Females		Males	
	Ever smoked	Never smoked	Ever smoked	Never smoked
Light	0.04 (0.03)	0.01 (0.03)	0.06* (0.03)	0.05 (0.04)
Moderate	0.03 (0.03)	0.01 (0.03)	0.08*** (0.03)	0.03 (0.04)
Strenuous	0.07 (0.05)	-0.07 (0.06)	0.10*** (0.03)	0.05 (0.04)

* Adjusted for age, sex, smoking, BMI and alcohol.

There is some indication of a similar positive effect of activity in females, especially strenuous in the ever smoked, but this does not reach statistical significance perhaps because of the low numbers of women who indulged in moderate or strenuous activity aged 35-45, compared to males and hence the larger standard error. On adding social class to this model it just failed to reach statistical significance ($p = 0.06$) and the regression coefficients for leisure activity did not change.

4.4 Discussion

Although the questionnaire was relatively crude for measuring leisure activity it did show relationships between the degree of leisure activity aged 35-45 and arterial disease, as indicated by the ABPI. This was consistent with multiple regression analyses of ischaemic heart disease in which it has been shown that physical activity was an independent risk factor, mainly in men (Kannel et al 1979a). A meta-analysis of 27 cohort studies showed a reduced summary risk of coronary heart disease for those who were physically active compared to those who were more sedentary (Berlin and Colditz 1990).

Relatively few women indulged in moderate and strenuous activity when aged 35-45 (39.7%) compared to men (65.7 %), which was consistent with other findings (Kuh and Cooper 1992), although a possible contributing factor is that the Edinburgh Artery Study scale emphasised competitive sport especially in the strenuous category, which may be more relevant to males than to females. In the list of items for moderate or strenuous activity, exercise to music, housework and childminding were not listed, activities which a high proportion of females carry out compared to males (Kuh and Cooper 1992). In addition horse riding and sailing, for example, in the light category could be described as moderate or strenuous depending on their intensity and duration. The other main problem is that of recall bias but it is difficult to assess how important this could be in the present study. A recent study suggested that the validity of recall of previous activity over a ten year period was satisfactory, and independent of recall interval and age (Blair et al 1991).

Despite these drawbacks there is a highly significant linear increase in the mean ABPI with leisure activity in males ($p = 0.0004$). The multiple regression analysis showed that this increase was most marked amongst those who had ever

smoked. It seems that exercise aged 35-45 goes some way to ameliorate the drawbacks of smoking in terms of the ABPI, but cannot compensate completely. For those who never smoked there is a weak positive relationship between leisure activity and the ABPI but the increase does not reach statistical significance. Clearly, smoking remains one of the strongest risk factors for peripheral arterial disease and the risk is increased in the absence of adequate exercise, aged 35-45 years.

CHAPTER 5

PERSONALITY FACTORS

5.1 Introduction

Since Friedman and Rosenman (1974) identified an excess of what later became known as Type A behaviours in their patients controversy has raged over the possible role of personality in the pathogenesis of atherosclerosis. They found that certain types of individuals seemed to be overrepresented in their patients. These Type A individuals (as opposed to Type B) tended to have a competitive craving for achievement and recognition, a tendency towards hostility and aggression, and a sense of extreme time urgency and impatience. They tended to act and speak quickly, interrupt more often in conversation, show impatience, show little interest in the aesthetic aspects of life and measure success in quantitative terms rather than the quality of goals. It has been pointed out that this is a very broad categorisation and more tried and tested personality measures (Eysenck 1985) could have been studied in relation to coronary heart disease. Nevertheless, it is the Type A construct which has entered the medical consciousness.

Friedman and Rosenman (1974) originally measured Type A using a structured interview, in which Type A behaviour was deliberately evoked and characteristics such as the tone of voice and speed of reply were noted. The structured interview is considered the 'gold standard'. However, reliable self-report measures have been developed such as the Jenkins Activity Survey (JAS, Jenkins et al 1974). Later there were calls to abandon self-report measures, especially as they do not contain items relevant to anger and hostility (Friedman and Booth-Kewley, 1988). Other conventional behavioural measures such as anxiety and depression were found to be

as good as the Type A construct in predicting coronary heart disease (Booth-Kewley and Friedman 1987). Behavioural factors have been studied extensively in relation to coronary heart disease (Rosenman et al 1975, Haynes et al 1980, and Shekelle et al 1985b) and showed that in general Type A individuals have a greater tendency to develop coronary heart disease. These studies were all based on US populations where the type A construct originated and the results in Europe have not been so consistent. For example, the Regional Heart Study found no association between Bortner Type A scores and myocardial infarction in 45-59 year old men over a 5 year period (Johnstone et al 1987).

A recent review (Evans, 1990) suggests that a core part of this relationship may be concerned with hostility. On the other hand peripheral arterial disease, although another manifestation of the process of atherosclerosis, has been less thoroughly explored. One exception by Cottier et al (1983) on a relatively small sample, found no significant association but intermittent claudicants were poorer at controlling expressions of anger compared to normal controls, suggesting that large scale research in this area would be fruitful.

5.2 Methods

5.2.1 Measurement of Personality

All the 1592 participants in the Edinburgh Artery Study (Chapter 2) completed a self-reported personality questionnaire which had been used by Cottier et al (1983). This was the Bortner Personality Inventory consisting of 14 items, each item relating to different aspects of Type A behaviour (Appendix 1). Each item consisted of a visual analogue scale from low Type A activity to extreme activity, the scores (1-24) being assessed after completion using an overlay. The Type A Bortner score was then calculated as the sum of the scores over all items. In the questionnaire the individual components of the Bortner were laid out so that the extremes of Type A behaviour did not always appear on the same side of the page.

Since hostility and anger seem to be important factors in atherosclerosis (Booth-Kewley and Friedman, 1987), subjects were also asked to complete the Personality Deviance Scale (Bedford-Foulds, 1978). This reliable and validated questionnaire consisted of three major scales - extrapunitiveness (blame others), intropunitiveness (self-blame) and dominance. Each major scale is the sum of two subscales which in turn consist of six items from the questionnaire; extrapunitiveness = hostile thoughts + denigratory towards others; intropunitiveness = lack of self-confidence + dependency; dominance = hostile acts + domineering attitude. Each question was worded to elicit responses referring to 'most of their life' in order to reduce the problem of measuring disease status and risk factor at the same time, inherent in cross-sectional methodology. The whole questionnaire was checked by a research nurse and advice given on how to complete any missing items. Peripheral arterial disease was measured using the WHO questionnaire, the ankle brachial pressure index (ABPI) and a reactive hyperaemia test. Subjects were classified into

four categories as follows : 1) intermittent claudication if positive to the WHO questionnaire; 2) major asymptomatic disease if the ABPI < 0.7 or a hyperaemic reduction in ankle pressure > 35%; 3) minor asymptomatic disease if the ABPI < 0.9 or a hyperaemic reduction > 20% ; and 4) normal , if none of the above. Mean personality scores were compared across these categories of disease severity using tests for trend.

5.2.2 Statistical Methods - Principal Components Analysis

As the Bortner inventory is composed of 14 items each potentially a factor in relation to peripheral arterial disease, one means of reducing the dimensionality of the problem is through the use of principal components analysis. This procedure transforms the observed correlated variables into a smaller number of uncorrelated linear combinations of the original variables, which account for a large proportion of the variation. No statistical model is assumed and the distribution of the variables does not have to be approximately Normal.

If we let $X^T = [X_1, X_2, ... X_p]$ for p random variables with

mean = μ and variance-covariance matrix Σ.

Then we wish to find new $Y_1, Y_2, ..., Y_p$ which are uncorrelated with variances decreasing from first to last and the Y_i are linear combinations of the X's , that is,

$$Y_j = a_{1j}X_1 + a_{2j}X_2 + ... + a_{pj}X_2 \qquad = a_j^T X$$

where $a_j^T = [a_{1j}, a_{2j}, ..., a_{pj}]$ is vector of constants

The condition $a_j^T a_j = \Sigma a_{kj}^2 = 1$ is imposed, that is, the transformation is orthogonal.

The 1st principal component Y_1 is found by choosing a_1 so that Y_1 has the largest variance. In other words, the procedure maximises $Var(a_1^T X)$ subject to the constraint that $a_1^T a_1 = 1$ and this is repeated for $a_2, a_3,.....$

$Var(a_1^T X) = a_1^T \Sigma a_1 = f(x)$

Maximise by Lagrange multipliers, with one constraint

Let $L(x) = f(x) - \lambda [g(x) - c]$

where $f(x_1,...,x_p)$ is differentiable function of p variables subject to constraint $g(x_1,....,x_p) = c$

Then $\partial L/\partial X = 0$ can be written as

$\partial f/\partial x_i - \lambda \partial g/\partial x_i = 0$ [i=1,2,...p]

$L(a_1) = a_1^T \Sigma a_1 - \lambda (a_1^T - 1)$

$\partial L/\partial a_1 = 2\Sigma a_1 - 2\lambda a_1$

Then $\partial L/\partial a_1 = 0$ implies $(\Sigma - \lambda I)\, a_1 = 0$

If $(\Sigma - \lambda I)\, a_1 = 0$ has other than 0 as a solution then $(\Sigma - \lambda I)$ must be singular

We can choose λ such that $|\Sigma - \lambda I| = 0$

A solution existing implies the λ_i are the eigenvalues of Σ and are all positive since Σ is positive semidefinite

Then $\lambda_1 > \lambda_2 > .....\lambda_p \geq 0$

and $Var(a_1^T X) = a_1^T \Sigma a_1 = a_1^T \lambda a_1 = \lambda_1$

The 1st principal component a_1 is eigenvector of Σ corresponding to the largest eigenvalue λ_1. This is then repeated for a_2 and so on.

The eigenvectors A where $A = [a_1, a_2, ...a_p]$

$Y = A^T X$

Then $\Sigma Var(Y_i) = \Sigma\lambda_i = trace(\Lambda)$

$= trace(A^T \Sigma A) = trace(\Sigma A A^T) = trace(\Sigma) = \Sigma Var(X_i)$

In other words, the sum of the variances of the original variables = the sum of variances of the principal components. So we can say that the ith PC accounts for $\lambda_i / \sum \lambda_j$ of the total variation in the original data.

The Varimax method was used which took the first few components (say m) and rotated these as a linear combination of the eigenvectors to obtain a new set of components. The loadings for each of the variables was then as close to 1 or 0 as possible to make identification of important variables easier.

Logistic regression was used to analyse the relationship between intermittent claudication (yes/no) and personality scales in the Edinburgh Cross-sectional study. In addition multiple linear regressions with the ABPI as a continuous outcome were carried out for the total cross-sectional data and for males and females separately.

5.3 Results

5.3.1 The Bortner Inventory

The principal components analysis carried out on the 14 items of the Bortner scale identified 4 principal components which accounted for 50% of the total variation in the original 14 items (Table 5.1). This is not a particularly high percentage and it could be that the 14 items have already been selected by principal components analysis from a much larger Type A inventory. The first component was highly correlated with the original Bortner score which is the sum of the 14 items.

Table 5.1 Principal components analysis and relationship to the Bortner scale

Principal Component	Cumulative Percentage	Correlation* with Bortner	Correlation* with the ABPI
1	21.9	0.80	0.02
2	33.6	0.40	0.07
3	42.9	0.21	0.11
4	50.1	0.14	-0.06

* Spearman Rank correlation

The four principal components identified could be characterised as time pressure, competitiveness, speed, and emotional expression/job recognition (table 5.2), although there is an element of subjectivity in these descriptions. The important factors for each principal component are highlighted in bold in table 5.2 represented by 'large'

component loadings.The Varimax rotation produces extremes of loading for each item, that is close to 0 or 1 to make identification of important items easier and aid interpretation.

Table 5.2 Varimax rotated principal component loadings and correlations with the ABPI

Trait	PC1	PC2	PC3	PC4	Correlation with ABPI
A 'never late'	-0.06	-0.37	**0.50**	-0.29	0.03
B 'very competitive'	-0.06	**0.71**	0.27	-0.06	0.06
C 'interrupt'	0.40	0.11	0.11	**0.44**	-0.01
D 'always rushed'	**0.74**	-0.11	0.07	-0.03	0.01
E 'impatient'	**0.57**	0.26	-0.21	0.05	0.01
F 'go all out'	**0.49**	0.15	**0.49**	0.07	0.07
G 'do many things at once'	**0.67**	0.04	0.07	0.03	0.05
H 'emphatic in speech'	0.45	0.14	0.17	**0.53**	0.01
I 'job recognition'	0.25	0.32	0.14	**-0.64**	0.08
J 'fast eat / talk'	0.26	-0.11	**0.64**	-0.01	0.06
K 'hard driving'	0.44	**0.47**	0.09	0.30	0.03
L 'express feelings'	0.03	0.32	-0.05	**0.46**	-0.01
M 'few interests'	0.16	-0.27	**-0.64**	-0.08	-0.10
N 'ambitious'	0.17	**0.70**	-0.18	0.10	0.05

Apart from the fourth principal component there is a positive relationship between the constructed dimensions and the ABPI. When considering the correlations of the individual components of the Bortner with the ABPI, it is notable that only one item 'few interests apart from work' is significantly associated with low levels of the ABPI while the remaining items, as with the first three principal components are generally positively associated with the ABPI.

The change in Bortner scores and first four principal components across categories of peripheral arterial disease are shown in table 5.3. With decreasing severity of disease to normal there are significant linear trends of increasing scores on the Bortner scale, time pressure, competitiveness, and speed which is in the opposite direction to that expected. In other words, type A characteristics are associated with a lack of disease. For the fourth principal component; emotional expression/job recognition, although there is a slight suggestion of a positive association, that is, in the expected direction, this is not significant.

Table 5.3 Mean (se) Bortner scores and first four Principal Components in relation to severity of peripheral arterial disease.

	Peripheral Arterial disease				p-value
	Intermittent claudication (n = 67)	Major (n=97)	Minor (n=226)	Normal (n=1038)	
Bortner Mean (se)	146.7 (5.4)	150.4 (3.7)	151.1 (2.4)	157.6 (1.1)	0.001
Time pressure Mean (se)	44.4 (2.3)	45.1 (1.7)	46.5 (1.1)	48.5 (0.5)	0.02
Competitiveness Mean (se)	16.5 (1.8)	17.1 (1.3)	16.5 (0.8)	19.3 (0.4)	0.007
Speed Mean (se)	16.9 (1.3)	19.6 (1.1)	20.9 (0.7)	23.3 (0.32)	0.001
Emotional expression Mean (se)	3.2 (1.1)	2.6 (1.1)	2.9 (0.6)	3.1 (0.3)	0.87

In analysing the relationship between the ABPI and individual items of the Bortner scale in multiple linear regression, males and females were treated separately, as the univariate relationships differed between males and females. In a stepwise regression procedure item M was significant (p=0.005) in females after adjusting for smoking (square root transformation of packyears), age, HDL and nonHDL cholesterol, and BMI. This negative association remained on adding known diabetes. This suggested an independent relationship between low ABPI and having few interests outside work in females. In males item M was not significant while item G, 'go all out' was significant (p = 0.01) after adjusting for age, smoking, diabetes, HDL and nonHDL Cholesterol and BMI.

5.3.2 Bedford-Foulds Questionnaire

The Bedford-Foulds measures are generally higher for cases of intermittent claudication compared to others. It is notable that dominance and its subscale components hostile acts and domineering attitude are higher in claudicants (table 5.4), although only reaching statistical significance for hostile acts. In addition, males have significantly higher levels of hostility compared to females ($p < 0.0001$).

Table 5.4 Mean levels (se) of Bedford-Foulds scales in Intermittent claudicants and others

	Intermittent Claudicants (n = 72)	Others (n=1516)	t-test p-value
EXTRAPUNITIVENESS			
Denigratory towards others	12.6 (0.3)	12.4 (0.1)	0.69
Hostile thoughts	11.8 (0.3)	12.3 (0.1)	0.09
INTROPUNITIVENESS			
Lack self-confidence	12.1 (0.4)	11.8 (0.1)	0.51
Dependency	13.5 (0.3)	13.6 (0.1)	0.82
DOMINANCE			
Hostile acts	14.6 (0.4)	13.9 (0.1)	0.03
Domineering attitude	15.0 (0.4)	14.8 (0.1)	0.55

In the logistic regression of hostile acts on intermittent claudication a significant interaction ($p = 0.01$) was found for sex and hostile acts and so the analysis was carried out separately for males and females. After adjusting for age and smoking the odds ratio was 1.40 (95% C.I. = 1.01, 1.95) for hostile acts in males, while the odds ratio for females was 1.18 which was not significant. Hostility is related to

lifetime cigarette smoking as measured by packyears (r = 0.19), but after adjusting for cumulative smoking the odds ratio for hostile acts in males hardly changed to 1.42 and remained statistically significant (Table 5.5). The odds ratios were obtained from standardised coefficients ($\beta_i s_i$) to indicate that hostile acts in males were of comparable importance as smoking as a risk factor for intermittent claudication. On adding item M in the Bortner inventory to this model hostile acts in males remained significant, while the odds ratio for item M in females was 1.50 (95% C.I. = 1.06, 1.73) and was not significant in males.

Table 5.5 Logistic regression on Intermittent Claudication

Variables (+1 SD)	Females Odds Ratio (95% C.I.)	Males Odds Ratio (95% C.I.)
Hostile acts	1.08 (0.78, 1.51)	1.42 (1.01, 2.00)
Item M 'few interests'	1.50 (1.06,1.73)	1.07 (0.76, 1.51)
Packyears (Square root)	1.70 (1.24, 2.34)	1.34 (0.94, 1.93)
Age	1.41 (1.01, 1.99)	1.52 (1.06, 2.19)

It might be thought that increased hostility and dominance may be a consequence of illness rather than antecedents. In order to address this question the asymptomatic cases were selected, that is, those cases without intermittent claudication or angina (n = 94) and compared to the controls who were free from disease (n = 149). After adjusting for age and sex, the odds ratios were for dominance (OR=1.37 , 95% CI = 1.04,1.79 , p = 0.02), for hostile acts (OR=1.24, 95% CI = 0.95,1.61 , p = 0.07), and for domineering attitude (OR=1.39, 95% CI 1.06,1.82 , p = 0.02).

5.4 Discussion

These results represent the first large scale study of personality traits and peripheral arterial disease. It was surprising that the Bortner scale and most of the individual items showed significant trends in the opposite direction to that which might be expected. Subjects with disease had lower Type A scores and lower scores on the principal components compared to normals. The most likely explanation is that the instructions for completion of the Type A inventory relate to the present and so disease status could influence responses. This is supported by the significant association of competitiveness and 'going all out' with absence of disease, aspects which would be reduced by disease. Also, it is debatable whether many of the items are relevant to a retired population of this age range. The significant finding of having 'few interests outside work' associated with disease in females could be the result of social isolation as a consequence of curtailed mobility. The Type A inventory also suffers from being self-completed since in a structured interview Type A behaviours are elicited. However, in a large cross-sectional study the cost of structured interviews for all subjects would be prohibitive. The 14-item inventory did not contain items on hostility and this is a major drawback, especially as it has been suggested (Booth-Kewley and Friedman 1987) that the expression of hostility lies at the core of the relationship between atherosclerosis and personality (Evans 1990).

The results do support the association of hostility as measured by the Bedford-Foulds Personal Deviance Scales and peripheral arterial disease. Male subjects with disease have a tendency to be more domineering and act in a hostile manner in interpersonal situations. This is consistent with the finding of aggressiveness being related to coronary heart disease (Booth-Kewley and Friedman 1987). The instructions for completion of the Bedford-Foulds questionnaire emphasised the retrospective

nature of actions, that is, respondents were asked to assess how they would behave 'over most of your life'. Respondents could then disregard present aspects such as disease status and so these scales would more likely represent trait measures. In addition, when the angina cases and intermittent claudicants were excluded from the analysis, there was still a significant relationship between hostility and major asymptomatic disease. This suggests that the personality deviance is unlikely to be a consequence of disease and so hostility may be an independent risk factor for peripheral arterial disease in the general population.

CHAPTER 6

RHEOLOGICAL FACTORS

6.1 Introduction

Blood rheology is the study of the deformation of blood and its resistance to flow or viscosity and this chapter will consider rheological factors as risk factors for peripheral arterial disease. Resistance to flow is measured by whole blood viscosity and important components of this are plasma viscosity, haematocrit and fibrinogen. Atherosclerosis tends to predominate at certain sites, with lesions occurring at bifurcations and bends, areas where flow is altered and so haemodynamic factors may play a part in atherogenesis.

Of all the rheological factors, fibrinogen has been shown to be a major cardiovascular risk factor (Wilhelmson et al 1984, Stone and Thorpe 1985, Meade et al 1987), although it is important to assess whether this is independent of smoking as smoking is known to increase fibrinogen level (Lee et al 1990).

Other important factors are leucocyte elastase, urinary fibrinopeptide A and uric acid. Leucocyte elastase, a measure of blood leucocytes, is released during early events in coagulation or other pathways that occur in parellel with coagulation (Plow 1982) and in a small hospital based study was shown to be elevated in patients with ischaemic heart disease (Dawes 1987).

Fibrinopeptide A is a measure of activation of blood coagulation. It is released from fibrinogen on conversion to fibrin and so urinary concentration reflects that of plasma (Alkjaersig and Fletcher 1982).

Plasma uric acid is thought to be related to platelet function which in turn affects thrombus formation (Winocour 1977) and hence may be a risk factor for atherosclerosis.

Previous studies have suggested that patients with intermittent claudication may have activation of blood coagulation (Donaldson et al 1987, Al-Zahrani et al 1992); increased blood viscosity due to elevation of haematocrit and plasma fibrinogen (Dormandy et al 1973, Stormer at al 1974, Blunt et al 1980), and increased leucocyte activation and rigidity (Ciuffetti et al 1988, Nash et al 1988). However these factors have not been related to peripheral arterial disease in epidemiological studies in the general population, nor have relationships with other cardiovascular risk factors, such as smoking, been taken into account.

The Edinburgh Artery Study (Fowkes et al 1991) enables study of the relationships of whole blood viscosity and its major determinants; haematocrit, plasma viscosity, and fibrinogen as well as measurements of activation of blood coagulation such as urinary fibrinopeptide A (Dawes et al 1987) and of blood leucocytes such as plasma leucocyte elastase (Greer et al 1989) to symptomatic and asymptomatic peripheral arterial disease in the general population aged 55 - 74 years.

6.2 Methods

6.2.1 Measurement of Rheological Factors

Details of the study population and recruitment are described in chapter 2. Peripheral Arterial disease was measured by the WHO questionnaire identification of intermittent claudication (Rose 1962) and the ankle brachial pressure index.

From a fasting blood sample which was taken on each patient at about 9 am, serum uric acid was estimated on a Cobas Bio analyser, using a standard kit. Fibrinogen was measured in citrated plasma by a thrombin-clotting turbidometric method in a centrifugal analyser (Lowe et al 1991a). Haematocrit was measured using a Hawksley microcentrifuge and reader. Blood viscosity was corrected to a standard haematocrit of 45%. This was calculated using the formula of Matrai et al (1987) as follows:

$$Corrected\ Blood\ vis. = \left(\frac{bloodvis}{plasvis}\right)^{\frac{45}{Haematocrit}} (plasvis)$$

Relative blood viscosity, which is corrected blood viscosity/plasma viscosity, was calculated to give a measure of red cell deformability (Chien 1975). Urinary fibrinopeptide A was measured by radioimmunoassay (Dawes et al 1987) as was plasma leucocyte elastase (Greer et al 1989). Quality control was monitored by means of blind duplicate samples taken intermittently throughout the study.

6.2.2 Statistical Methods

Tests for linear trends in the mean levels of the rheological factors across four categories of peripheral arterial disease were carried out. The categories of disease have been described in chapter 2, and the results of studies comparing the ABPI and reactive hyperaemia separately with arteriography (Fowkes 1988d) suggest that this classification has some validity. The main analysis as in previous chapters concentrates on the ABPI as a continuous measure of peripheral arterial disease giving greater power to detect associations.

Multiple linear regression was used to investigate the relationships between the ABPI and age, sex, height, blood and plasma viscosity, haematocrit, fibrinogen, leucocyte elastase and urinary fibrinopeptide A, uric acid and alcohol and smoking. The log transformed data for leucocyte elastase and urinary fibrinopeptide A were used in the analysis because of the positive skewness of their distributions. Height was included in the regression analysis because it had a positive association with the ABPI (Fowkes et al 1991). Exclusion of height affected the association with sex, tending to increase the adjusted ABPI in males. The multiple linear regressions were carried out with all of the above factors entered simultaneously, and with the subsequent insertion of total cholesterol, HDL cholesterol, triglycerides (log transformed) and diabetic status (Known vs non-diabetic). Tests for sex by rheological factors were carried out as well as a test of the three way interaction of sex by smoking by fibrinogen.

The association with smoking was modelled using the number of packyears as described in chapter 2. The distribution of packyears was highly skewed with a few very heavy smokers, and so a square root transformation was used in all the analyses. The smoking histories were considered valid because stated consumption was related to mean thiocyanate levels.

Multiple logistic regressions of age, sex, height, smoking and the rheological variables were also carried out with the outcome defined by presence/absence of intermittent claudication.

6.3 Results

Table 6.1 shows the correlations between the rheological variables. In particular, haematocrit and blood viscosity are highly correlated (r = 0.70), as are fibrinogen and plasma viscosity (r = 0.46) and blood viscosity and plasma viscosity (r = 0.45).

Table 6.1 Spearman Rank correlations between rheological variables

	Blood viscosity	Plasma viscosity	Uric Acid	Fibrin-ogen	Leucoc-yte elastase	Fibrin-opeptide A	Haem-atocrit
Blood viscosity	1	0.47 (1377)	0.31 (1398)	0.21 (1382)	0.20 (1333)	0.01 (1392)	0.70 (1398)
Plasma viscosity		1	0.18 (1506)	0.45 (1484)	0.26 (1441)	0.04 (1500)	0.18 (1506)
Uric acid			1	0.06 (1547)	0.11 (1502)	0.05 (1566)	0.29 (1536)
Fibrinogen				1	0.11 (1477)	0.04 (1542)	0.05 (1513)
Leucocyte elastase					1	0.02 (1500)	0.13 (1470)
Urinary Fibrinopeptide A						1	0.09 (1530)
Haematocrit							1

Fibrinogen increased markedly with age from a mean (se) of 2.52 (0.03) g/l in those aged 55-59 years to 2.90 (0.04) g/l in those aged 70-74 years. There were many differences between the levels of rheological variables in men and women (table 6.2), blood viscosity, haematocrit, leucocyte elastase and urinary fibrinopeptide A were significantly higher in males ($p < 0.001$) while fibrinogen was significantly higher in women ($p < 0.001$). Mean plasma viscosity did not differ significantly between men and women. The trends in rheological factors in relation to severity of peripheral arterial disease are shown in table 6.3 and 6.4.

Table 6.2 Differences in mean (se) levels of rheological variables in men and women

Rheological factor	Females (n = 778) Mean (se)	Males (n = 803) Mean (se)	p-value
Blood viscosity mPa.s	3.37(0.02)	3.78(0.02)	<0.001
Relative Blood viscosity	2.56(0.01)	2.68(0.01)	<0.001
Corrected Blood viscosity	3.42(0.01)	3.57(0.01)	<0.001
Plasma viscosity mPa.s	1.34(0.004)	1.33(0.003)	0.74
Haematocrit %	44.11(0.12)	47.39(0.13)	<0.001
Fibrinogen g/l	279.4(2.5)	264.2(2.5)	<0.001
Leucocyte elastase(ln) ng/ml	3.41(0.02)	3.59(0.02)	<0.001
Urinary fibrinopeptide A(ln) ng/ml	0.38(0.02)	0.46(0.02)	<0.001
Uric acid µmol/l	287.4(2.5)	344.3(2.6)	<0.001

Table 6.3 Trends in mean (se) of Rheological variables in females across categories of peripheral arterial disease.

Rheological factor	IC (n=73)	Major (n=106)	Minor (n=228)	Normal (n=1071)	Missing (n=114)	Total (n=1592)	p-value*
	Mean (se)	Mean (se)	Mean (se)	Mean (se)	Mean (se)	Mean (se)	
Blood viscosity mPa.s	3.63 (0.06)	3.38 (0.07)	3.38 (0.04)	3.31 (0.02)	3.58 (0.07)	3.37 (0.02)	0.0003
Corrected blood viscosity	3.55 (0.05)	3.45 (0.07)	3.44 (0.03)	3.37 (0.02)	3.60 (0.06)	3.42 (0.01)	0.004
Relative blood viscosity	2.55 (0.03)	2.54 (0.03)	2.60 (0.02)	2.55 (0.01)	2.64 (0.03)	2.56 (0.01)	0.74
Plasma viscosity mPa.s	1.39 (0.01)	1.37 (0.03)	1.33 (0.01)	1.32 (0.004)	1.37 (0.01)	1.33 (0.004)	<0.001
Haematocrit %	45.6 (0.45)	44.5 (0.70)	44.2 (0.28)	43.9 (0.14)	44.1 (0.37)	44.1 (0.12)	0.004
Fibrinogen g/l	313.9 (12.1)	304.2 (9.6)	277.2 (5.6)	270.8 (2.9)	303.8 (8.6)	279.4 (2.5)	<0.0001
Leucocyte elastase(ln) ng/ml	3.74 (0.11)	3.47 (0.11)	3.36 (0.06)	3.39 (0.03)	3.44 (0.07)	3.41 (0.02)	0.009
Uric acid µmol/l	314.7 (13.1)	301.3 (10.9)	294.9 (7.5)	279.2 (2.8)	306.6 (8.1)	287.4 (2.5)	0.0001
Urinary (ng/ml) fibrinopeptide A	2.3 (0.74)	1.9 (0.41)	1.5 (0.09)	1.7 (0.07)	1.7 (0.10)	1.7 (0.07)	0.17

* Test for linear trend in means; missing excluded.

There were highly significant linear trends ($p < 0.001$) in males of increasing blood viscosity, corrected blood viscosity, plasma viscosity, and fibrinogen with severity of peripheral arterial disease. There were also significant trends in haematocrit and leucocyte elastase with severity of disease. The results were similar in females except that there was a significant linear trend in uric acid in females (table 6.3) but not in males (table 6.4).

Table 6.4 Trends in mean (se) of Rheological variables in males across categories of peripheral arterial disease.

Rheological factor	IC (n=73)	Major (n=106)	Minor (n=228)	Normal (n=1071)	Missing (n=114)	Total (n=1592)	p-value*
	Mean (se)	Mean (se)	Mean (se)	Mean (se)	Mean (se)	Mean (se)	
Blood viscosity mPa.s	4.03 (0.13)	3.94 (0.08)	3.93 (0.06)	3.73 (0.03)	3.75 (0.10)	3.78 (0.02)	<0.0001
Corrected blood viscosity	3.75 (0.08)	3.65 (0.06)	3.65 (0.04)	3.53 (0.02)	3.57 (0.08)	3.57 (0.01)	0.0001
Relative blood viscosity	2.75 (0.05)	2.68 (0.03)	2.71 (0.02)	2.67 (0.01)	2.63 (0.05)	2.68 (0.01)	0.07
Plasma viscosity mPa.s	1.36 (0.01)	1.36 (0.01)	1.35 (0.01)	1.32 (0.004)	1.36 (0.01)	1.33 (0.003)	0.0001
Haematocrit %	47.8 (0.52)	48.9 (0.58)	47.6 (0.41)	47.2 (0.14)	47.7 (0.71)	47.4 (0.13)	0.003
Fibrinogen g/l	291.8 (13.5)	302.8 (10.1)	264.4 (5.7)	258.6 (2.9)	268.8 (11.7)	264.2 (2.5)	<0.0001
Leucocyte elastase(ln) ng/ml	3.57 (0.11)	3.74 (0.09)	3.74 (0.06)	3.55 (0.03)	3.55 (0.07)	3.59 (0.02)	0.03
Uric acid µmol/l	349.3 (13.6)	363.6 (11.1)	343.9 (6.8)	342.7 (2.9)	335.7 (17.2)	344.3 (2.6)	0.13
Urinary (ng/ml) fibrinopeptide A	3.62 (1.37)	1.64 (0.11)	1.75 (0.12)	2.14 (0.24)	2.02 (0.29)	2.11 (0.18)	0.55

* Test for linear trend in means; missing excluded.

Table 6.5 Results of Multiple linear regression of rheological factors on the ABPI

Variable	Change in mean(se) of ABPI x 100	p-value
Age (+ 10 years)	-4.2(0.8)	< 0.0001
Sex	34.5(6.8)	< 0.0001
Height (+ 10 cm)	1.3(0.7)	0.05
Packyears (square root trans.)	-1.2(0.3)	0.0001
Alcohol units (square root)	0.6(0.3)	0.03
Fibrinogen:		
Males, ever smoked(+ 100g/l)	-4.85(1.13)	<0.0001
Males, never smoked(+ 100g/l)	-3.93(1.20)	0.001
Females, ever smoked(+100g/l)	-1.71(1.15)	0.14
Females, never smoked(+ 100g/l)	-0.46(1.16)	0.69
Blood Viscosity(mPa.s):		
Males	-4.5(1.5)	0.003
Females	1.0(1.7)	0.55
Plasma viscosity(mPa.s)	-2.1(6.1)	0.73
Haematocrit(+ 10%)	-1.7(1.9)	0.70
Leucocyte elastase(ln) (+1 ng/ml)	-0.9(0.76)	0.24
Uric acid (+ 100 µmol/l)	-1.08(0.68)	0.11
Known diabetic vs non (Males)	- 13.0(4.3)	0.002
Known diabetic vs non (Females)	-3.2(4.6)	0.49

There was a significant three way interaction (p = 0.01) of sex by smoking (packyears) by fibrinogen adjusting for all two-way interactions and so separate terms were entered in the regression model for fibrinogen in males and females who ever smoked and who never smoked. This classification of smoking was used for simplicity as any cut-off point for packyears would be arbitrary. The results of the multiple linear regression on the ABPI (table 6.5) show that although univariately there were

significant relationships between rheological variables and peripheral arterial disease in women (table 6.3), these were not independent of smoking. On the other hand, in men, both fibrinogen and blood viscosity ($p = 0.003$) were significant independently of smoking, and due to a significant interaction with smoking, fibrinogen was related to a greater reduction in the mean ABPI in ever smokers ($p < 0.0001$) compared to never smokers ($p = 0.001$, table 6.5). Even in females, although fibrinogen was not significant after adjusting for smoking, the differences in the regression coefficients indicate a weak multiplicative effect of fibrinogen with smoking (table 6.5). On adding HDL ($p = 0.009$) and non-HDL cholesterol ($p = 0.002$) there was little change in the significance of the regression coefficients of blood viscosity and fibrinogen for males. There was almost a significant sex by diabetes (known vs non) interaction ($p = 0.10$) and consequently separate terms were entered for males ($p = 0.003$) and females ($p = 0.96$).

When considering the presence of intermittent claudication as the outcome many of the rheological variables were univariately related to peripheral arterial disease. There were no significant interactions of sex or smoking with rheological variables. There were also no independent significant rheological factors (table 6.6), when adjusting for age, sex, height, smoking and all other rheological factors simultaneously. Age and smoking were the only significant factors independently associated with the odds of intermittent claudication.

Table 6.6 Odds ratio of intermittent claudication unadjusted and adjusted for all factors

Variable	Unadjusted OR(95% C.I.)	Adjusted OR(95% C.I.)
Age(+ 10 years)	2.26(1.38,3.70)**	2.18(1.30,3.70)**
Female (vs male)	0.96(0.56,1.65)	0.81(0.35,1.89)
Height (+ 10 cm)	0.77(0.57,1.03)	0.77(0.50,1.16)
Blood viscosity(+ 1 mPa.s)	1.80(1.20,2.70)**	1.26(0.65,2.43)
Haematocrit(+ 10%)	2.19(1.05,4.60)*	1.58(0.53,4.76)
Plasma viscosity(+ 0.1 mPa.s)	1.62(1.23,2.13)***	1.23(0.85,1.78)
Fibrinogen(+ 10 g/l)	1.05(1.04,1.09)*	1.01(0.97,1.06)
Leucocyte elastase(ln) (+1 ng/ml)	1.46(0.99,2.15)$^{+}$	1.27(0.84,1.94)
Urinary fib. A(>1 ng/ml)	0.95(0.55,1.64)	0.88(0.50,1.55)
Uric acid (+ 10 µmol/l)	1.00(0.97,1.04)	0.94(0.98,1.02)
Packyears(square root)	1.18(1.08,1.29)***	1.16(1.03,1.31)*

+ $p < 0.1$ * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

6.4 Discussion

The results in the Edinburgh Artery Study support the hypothesis that rheological factors were independently related in an older male population aged 55-74 years to both atherosclerotic peripheral arterial disease as measured by the ABPI and to leg ischaemia, as measured by the presence of intermittent claudication on questionnaire.

Blood viscosity and its major determinants, haematocrit, plasma viscosity and fibrinogen as well as leucocyte activation as measured by plasma leucocyte elastase were found to be significantly related to increasing severity of peripheral arterial disease in males and females (tables 6.3 and 6.4). Activation of blood coagulation as indicated by urinary fibrinopeptide A showed no relationship to peripheral arterial disease. This was surprising as other studies had suggested that plasma fibrinopeptide A levels were related to several major cardiovascular risk factors (Lowe et al 1991a) as well as to angiographic coronary artery disease (Small et al 1988). However, in the Edinburgh Artery Study the assay may have been insensitive as 38% of the population had levels < 1ng/ml.

The relationship between rheological factors and the extent of atherosclerotic narrowing in the arteries to the lower limbs was assessed using the ABPI, which has been shown to be up to 95% sensitive in detecting angiogram positive peripheral arterial disease (Bernstein et al 1982) and in the Edinburgh Artery Study has been shown to be related to the severity of disease on duplex scanning (Fowkes et al 1992b). The ABPI was significantly associated with blood viscosity in men, and plasma viscosity and fibrinogen in men and women. The relationships with blood viscosity and fibrinogen were still significant after adjustment for age, sex, height and smoking in men but not significant for women. These findings suggest that blood

viscosity and fibrinogen may each have an independent role in atherogenesis in men. Several biologically plausible mechanisms have been suggested, including an effect of blood viscosity on the localisation of atherosclerotic lesions (Lowe 1987). The association between fibrinogen and the ABPI was strongly related to the amount of cigarette smoking. The significant ($p = 0.02$) smoking by fibrinogen by sex interaction may be explained as a synergistic effect of smoking predominantly in males which disturbs endothelial cells and activated platelets, and fibrinogen infiltrates the arterial wall through damaged endothelium which then promotes platelet aggregation (Lowe 1987). The result that fibrinogen became non-significant in women on adding smoking to the regression model suggested that smoking was the strongest risk factor in women which may operate via the plasma fibrinogen level.

In the logistic regression model adjusting for age, sex and cigarette smoking, the associations between the rheological variables and intermittent claudication became non-significant (table 6.6). There were no significant sex or smoking by rheological interactions. This may be related to the small number of claudicants, and also does not exclude the possibility that increases in viscosity, fibrinogen and leucocyte activation may be mechanisms whereby age and smoking promote development of peripheral arterial disease.

The results suggested that blood viscosity and fibrinogen were independently associated with the severity of atherosclerosis as measured by the ABPI in the older population of men but not independently of smoking in women. Smoking appears to have a multiplicative effect on the relationship between fibrinogen and the ABPI in men.

CHAPTER 7

ARTERIAL BLOOD PRESSURE AND RHEOLOGICAL FACTORS

7.1 Introduction

Arterial blood pressure is a risk factor for arterial disease (MacMahon et al 1990) but it cannot be directly assessed in the same way as other risk factors. Arterial blood pressure as well as a risk factor is also a component of the outcome measure, directly in the case of the ABPI, and indirectly as indicated by the presence of intermittent claudication measured by the WHO questionnaire (Rose 1962). Hence it was not possible to enter blood pressure as a variable in regression analyses with these measures of peripheral arterial disease as outcomes.

Peripheral resistance is one of the main determinants of arterial pressure and is affected by vasoconstriction of the arterioles and blood viscosity. It is not surprising then that blood viscosity has been found to be increased in patients with hypertension (Tibblin et al 1966, Letcher et al 1981, Dintenfass 1981, Isles et al 1984). This elevated blood viscosity may be due partly to haematocrit and plasma fibrinogen concentration which are also increased in hypertension (Letcher et al 1981). In the general population, arterial pressure has been shown to be related to fibrinogen levels (Karsen-Bengtsen et al 1972, Kannel et al 1985a) and to haematocrit (Mc Donough et al 1965, Kannel et al 1972, Sorlie et al 1981) but the relationship with blood viscosity is not well established, having been studied only recently in one population aged 25-64 years of age (Smith et al 1992). Thus the Edinburgh Artery Study facilitates the study of the relationship between blood viscosity and its components, plasma viscosity, haematocrit and fibrinogen, and arterial pressure in the general population aged 55-74 years.

7.2 Methods

Multiple linear regression was used to assess the independence of relationships between rheological variables and systolic and diastolic arterial pressure separately. As significant sex by blood viscosity and sex by fibrinogen interactions were found in relation to peripheral arterial disease in the previous chapter , males and females were analysed separately. In addition each regression model was adjusted for BMI, smoking as measured by packyears and alcohol. Square root transformations were used for the latter two variables because of the skewed nature of their distributions.

A generalisation of multiple linear regression is the procedure of canonical correlation analysis, originally used by Hotelling (1936). It consists of considering linear combinations of one set of Y variables with linear combinations of another set of X's. In this case diastolic and systolic blood pressure form one set while the rheological variables along with age, BMI, smoking and alcohol form the second set. The linear combinations of the X variables are known as canonical variables and the correlation between these and the linear combinations of the Y's, the second set of canonical variables, are the canonical correlations.

The procedure involves finding a linear combination of Y's -

$$c_i(y) = a_1 y_1 + a_2 y_2 + ... + a_q y_q \qquad i = 1, ...,q$$

and a linear combination of the X's -

$$c_i(x) = b_1 x_1 + b_2 x_2 + ... + b_p x_p \qquad i = 1, ...,p$$

The first canonical variables $c_1(y)$ and $c_1(x)$ are chosen such that the first canonical correlation is a maximum. The next set of canonical variables $c_2(y)$ and $c_2(x)$ are chosen to maximise the correlation between them, and also to be uncorrelated with the first canonical variables. The process is repeated until Bartlett's (1947) test is

nonsignificant for one of the eigenvalues.

The correlation matrix between the X's and the Y's can be partitioned thus :

$$R=\begin{bmatrix} R_{XX} & R_{XY} \\ R_{YX} & R_{YY} \end{bmatrix}$$

where R_{XX} is the p x p correlation matrix of the X's and R_{YY} is the qxq correlation matrix of the Y's.

The next step is to solve the eigenvalue problem :

$$R_{XY}R_{YY}^{-1}R_{YX}\beta=\lambda R_{XX}\beta$$

for eigen values $\lambda_1 \geq \lambda_2 \geq \ldots\ldots \geq \lambda_m > 0$

and $\beta_1, \ldots. \beta_m$ are the corresponding eigenvectors where m is the effective rank of the two sets of variables.

The canonical correlations are given by

$$p_i=\sqrt{\lambda_i}$$

i = 1,....,m

Initially a test of whether the two sets of variables are independent is carried out using Bartlett's (1947) test. The test statistic is

$$\phi_0^2=-[n-1/2(p+q+1)]\sum_{i=1}^{m}\ln(1-\lambda_i) \sim \chi_{pq}^2$$

where p is the number of X's , q is the number of Y's and m is the number of eigenvalues.

The number of canonical variables of practical value is less than or equal to

the smallest number of eigenvalues for which Bartlett's (1947) test for the remaining eigenvalues is nonsignificant. The test statistic for the remaining correlations after the first j have been removed is given by :

$$\phi_j^2 = -[n-1/2(p+q+1)] \sum_{i=j+1}^{m} \ln(1-\lambda_i) \sim \chi^2_{(p-j)(q-j)}$$

The standardised coefficients (mean 0, variance 1) are used to interpret the results (see for example, Chatfield and Collins 1984), large values indicating the importance of these variables to the canonical variable compared to those with low coefficients. However, this is difficult if the X's or the Y's or both are highly correlated with each other. A better procedure is to consider the correlations of each canonical variable with its component X's or Y's (Manly 1991).

In the canonical correlation analysis systolic and diastolic blood pressure were treated as the first set of variables while the second set consisted of the rheological variables plus age, BMI, smoking and alcohol. Natural logarithms or square root transformations of some variables were used in the analysis as approximate multivariate Normality is assumed for this procedure.

7.3 Results

7.3.1 Multiple regression

Females

The results in table 7.1 indicate few significant relationships between systolic blood pressure and rheological factors. Increasing age was significantly related to systolic pressure, while BMI was just significant at the 5% level (table 7.1). In general, high levels of the rheological factors were associated with high systolic pressure, but only uric acid reached statistical significance ($p = 0.04$). Blood viscosity was not significantly related to systolic pressure univariately.

Table 7.1 Multiple regression of rheological factors on systolic blood pressure in females (Adjusted R^2 = 16.5%)

Factor(+ 1 S.D.)	Regression coefficient(se)	p-value
Age	**8.69(0.95)**	**<0.0001**
Packyears(square root)	-1.09(0.96)	0.26
Alcohol(square root)	-0.58(0.97)	0.55
BMI	**2.19(1.03)**	**0.03**
Blood viscosity	0.35(1.29)	0.79
Fibrinogen	1.09(1.09)	0.29
Leucocyte elastase(ln)	0.72(0.92)	0.44
Plasma viscosity	0.89(1.14)	0.43
Haematocrit	-0.75(1.14)	0.51
Uric acid	**2.07(1.03)**	**0.04**
Urinary fib. A(ln)	-0.21(0.96)	0.82

In contrast, with diastolic pressure as outcome, uric acid was highly significantly associated with high pressure(table 7.2). Age was again strongly related to arterial pressure while high levels of smoking were associated with lower arterial pressure. BMI and Blood viscosity were highly significantly related to diastolic pressure adjusting for smoking but only approached significance at the 5% level whenever uric acid was added to the model.

Table 7.2 Multiple regression of rheological factors on diastolic blood pressure in females (Adjusted R^2 = 6.8%)

Factor(+ 1 S.D.)	Regression coefficient(se)	p-value
Age	**1.49(0.51)**	**0.003**
Packyears(square root)	**-1.25(0.51)**	**0.01**
Alcohol(square root)	0.57(0.52)	0.26
BMI	0.94(0.54)	0.09
Blood viscosity	1.27(0.69)	0.07
Fibrinogen	0.14(0.54)	0.77
Leucocyte elastase(ln)	0.86(0.50)	0.08
Plasma viscosity	-0.19(0.61)	0.76
Haematocrit	0.61(0.62)	0.32
Uric acid	**1.52(0.55)**	**0.006**
Urinary fib. A(ln)	-0.06(0.51)	0.90

Males

In males as for females, age and BMI were significantly related to systolic pressure (table 7.3). Alcohol was also positively related to systolic pressure ($p = 0.02$). Among the rheological variables, blood viscosity was highly significantly related to systolic pressure, which was not the case for females.

Table 7.3 Multiple regression of rheological factors on systolic blood pressure in males (Adjusted $R^2 = 16.4\%$)

Factor(+ 1 S.D.)	Regression coefficient(se)	p-value
Age	**7.07(0.90)**	**<0.0001**
Packyears(square root)	-1.24(0.89)	0.16
Alcohol(square root)	**2.10(0.88)**	**0.02**
BMI	**4.66(0.89)**	**<0.0001**
Blood viscosity	**4.37(1.33)**	**0.001**
Fibrinogen	-0.07(1.04)	0.93
Leucocyte elastase(ln)	-1.33(0.90)	0.14
Plasma viscosity	1.36(1.06)	0.20
Haematocrit	-1.91(1.27)	0.13
Uric acid	-0.22(0.87)	0.79
Urinary fib. A(ln)	-0.07(0.88)	0.94

The results for diastolic pressure were very different in males to systolic; age was not significant while smoking was strongly related to lower pressure (table 7.4). Blood viscosity was highly significantly associated with diastolic pressure adjusting for smoking but only approached significance at the 5% level when uric acid was added (p = 0.03). As for systolic pressure, BMI was highly significantly related to high arterial pressure. The results of the multiple linear regressions were summarised in table 7.5

Table 7.4 Multiple regression of rheological factors on diastolic blood pressure in males (Adjusted R^2 = 13.3%)

Factor(+ 1 S.D.)	Regression coefficient(se)	p-value
Age	0.46(0.46)	0.32
Packyears(square root)	**-1.33(0.47)**	**0.004**
Alcohol(square root)	0.59(0.47)	0.21
BMI	**3.25(0.46)**	**<0.0001**
Blood viscosity	1.06(0.70)	0.06
Fibrinogen	-0.42(0.54)	0.46
Leucocyte elastase(ln)	0.27(0.48)	0.57
Plasma viscosity	0.54(0.56)	0.34
Haematocrit	0.53(0.67)	0.44
Uric acid	**1.02(0.44)**	**0.03**
Urinary fib. A(ln)	0.05(0.47)	0.91

Table 7.5 Summary of Multiple Linear Regressions on systolic and diastolic pressure in males and females

Variable	Regression coefficient (s.e.) p-value			
	Arterial Pressure FEMALES		Arterial Pressure MALES	
	Systolic (Adj R^2=16.5%)	Diastolic (Adj R^2=6.8%)	Systolic (Adj R^2=16.4%)	Diastolic (Adj R^2=13.3%)
Age	**8.69(0.95)** **<0.0001**	**1.49(0.51)** **0.003**	**7.07(0.90)** **<0.0001**	0.46(0.46) 0.32
BMI	**2.19(1.03)** **0.03**	0.94(0.54) 0.09	**4.66(0.89)** **<0.0001**	**3.25(0.46)** **<0.0001**
Smoking	-1.09(0.96) 0.26	**-1.25(0.51)** **0.01**	-1.24(0.89) 0.16	**-1.33(0.47)** **0.004**
Alcohol	-0.58(0.97) 0.55	0.57(0.52) 0.26	**2.10(0.88)** **0.02**	0.59(0.47) 0.21
Blood viscosity	0.35(1.29) 0.79	1.27(0.69) 0.07	**4.37(1.33)** **0.001**	1.06(0.70) 0.06
Uric acid	**2.07(1.03)** **0.04**	**1.52(0.55)** **0.006**	-0.22(0.87) 0.79	**1.02(0.44)** **0.03**

7.3.2 Canonical Correlation

Females

Bartlett's (1947) test showed that two canonical variables (CV) were sufficient to describe the relationships between the two sets of variables.

Table 7.6 Standardised coefficients for canonical variables for systolic and diastolic blood pressure in females

	1st CV	2nd CV	Adjusted R^2	p
Systolic	1.09	-0.568	0.168	<0.0001
Diastolic	-0.187	1.22	0.068	0.006

In females the first canonical variable reflects mainly systolic blood pressure. The second CV represents mainly diastolic blood pressure, and to some extent the difference between diastolic and systolic pressure, although this was difficult to interpret.

The adjusted R^2 values (table 7.6) show that 17% of the information in the variable systolic can be accounted for by a combination of the risk factors in females while only 7% of the diastolic variable is represented. Although somewhat arbitrary, high values for the standardised coefficients of the risk factors have been highlighted in table 7.7, showing that the first canonical variable represents mainly age, BMI, and uric acid. Systolic and diastolic blood pressure represent 15% of the information in age while their combination represents 4% of uric acid. Thus systolic is mainly related to age, BMI and uric acid, while the combination of diastolic and systolic pressure as represented by the second canonical variable is related to blood viscosity, uric acid and smoking in females.

Table 7.7 Standardised coefficients of canonical variables for risk factors in females

	1st CV	2nd CV	Adjusted R^2	p
Fibrinogen	0.103	-0.033	0.02	0.003
Haematocrit	-0.113	**0.339**	0.01	0.003
Plasma viscosity(ln)	0.085	-0.164	0.01	0.003
Blood viscosity(ln)	0.009	**0.448**	0.02	0.0003
Leucocyte elastase(ln)	0.046	0.280	0.00	0.12
Uric acid	**0.164**	**0.400**	0.04	<0.0001
Urinary fib. A	-0.022	-0.010	0.00	0.94
Age	**0.851**	0.205	0.15	<0.0001
BMI	**0.193**	0.167	0.02	0.0003
Packyears (square root)	-0.067	**-0.405**	0.00	0.09
Alcohol (square root)	-0.081	0.290	0.01	0.009

ln - Natural logarithm

Males

As with females there were two canonical variables necessary to describe the relationships between the two sets of variables.

Table 7.8 Standardised coefficients for canonical variables for systolic and diastolic blood pressure in males

	1st CV	2nd CV	Adjusted R^2	p
Systolic	0.95	-0.80	0.165	<0.0001
Diastolic	0.09	1.24	0.136	<0.0001

The first canonical variable represents mainly systolic blood pressure , while the second although mainly diastolic, also represents the difference between systolic and diastolic pressure.

From table 7.9 systolic (1st CV) is related to age, BMI, and blood viscosity while the second CV (mainly diastolic, but also to some extent reflecting systolic pressure) is related to age, BMI, uric acid and haematocrit.

The results are consistent with the multiple linear regressions shown earlier (table 7.5) especially for the regressions on systolic pressure compared to the first canonical variable. It is more difficult to compare the second canonical variable which represents the difference between the measures of arterial pressure, although with greater weight towards diastolic pressure.

Table 7.9 Standardised coefficients of canonical variables for risk factors in males

	1st CV	2nd CV	Adjusted R^2	p
Fibrinogen	-0.008	-0.109	0.016	0.002
Haematocrit	-0.205	**0.311**	0.02	0.0005
Plasma viscosity(ln)	0.101	0.026	0.026	0.0001
Blood viscosity(ln)	**0.499**	-0.057	0.047	<0.0001
Leucocyte elastase(ln)	-0.117	0.205	0.00	0.11
Uric acid	-0.005	**0.320**	0.036	<0.0001
Urinary fib. A	-0.011	0.042	-0.002	0.85
Age	**0.693**	**-0.547**	0.119	<0.0001
BMI	**0.517**	**0.496**	0.106	<0.0001
Packyears (square root)	-0.147	-0.258	0.004	0.09
Alcohol (square root)	0.217	-0.026	0.00	0.42

7.4 Discussion

The Edinburgh Artery Study was the first study which related arterial pressure to blood viscosity in a random sample of a population aged 55 - 74 years. The results showed a strong relationship between systolic blood pressure and age, BMI, and blood viscosity in males. The results of the canonical correlation were similar to those of the simpler multiple linear regression, although more difficult to interpret. Canonical correlation is a useful technique when multiple outcomes are present, but in this case, the results of the multiple regression were preferable as they were consistent with the more sophisticated analysis and easier to interpret. Diastolic blood pressure was mainly related to age and uric acid in both males and females, but more strongly in females. Diastolic pressure was significantly related to blood viscosity adjusted for smoking in males and females, but not independently of uric acid.

The overall results were consistent with results from the Scottish Heart Health study (Smith et al 1992) and case-control studies on patients with hypertension (Tibblin et al 1966, Letcher et al 1981, Isles et al 1984). Although plasma viscosity, fibrinogen and haematocrit are principal determinants of blood viscosity (Lowe GDO 1987), blood viscosity made a significant contribution to systolic blood pressure in males independently of these components. Letcher et al (1981) also found that high blood viscosity in hypertensives was due only in part to increases in fibrinogen and haematocrit. Age, alcohol, and BMI were also related to systolic blood pressure. Age increases arterial pressure as does BMI.

The fact that the relationship between systolic and blood viscosity was only significant in males is difficult to explain. However, other studies (Lee et al 1990) and the previous chapter showed that there were male-female differences in the relationship of rheological factors and atherosclerotic disease and hence males and

females have been analysed separately. Almost all the women were over 55 years and so would have been post-menopausal when male/female differences in cardiovascular disease are thought to diminish, although Isles et al (1992) found that protection from coronary heart disease mortality persisted after the menopause.

Fibrinogen was not independently related to arterial pressure. Other studies (Karsen-Bengtsen et al 1972, Kannel et al 1985a) have suggested that fibrinogen is related to systolic pressure in the general population but often on adjustment for other factors the relationship became nonsignificant (Karsen-Bengtsen et al 1972). Overall, the evidence suggests that fibrinogen does not have an independent relationship with blood pressure in the general population. In a similar manner, haematocrit has been shown to be related to arterial pressure in community studies (McDonough et al 1965, Kannel et al 1972, Sorlie et al 1981). In the Edinburgh Artery Study, haematocrit was related only to diastolic pressure and was not independently related after adjustment for other risk factors and rheological variables.

The relationship between systolic blood pressure and blood viscosity in males cannot solely be explained by an increased haematocrit in which the contracted plasma volume is known to be directly related to arterial pressure and to the associated transcapillary shift of extracellular fluid (Kobrin et al 1984). Nor is it likely to be related to elevations of plasma proteins which occur in hypertensives because these increases mirror those of haematocrit and are probably due simply to plasma volume contraction (Letcher et al 1981).

The pathophysiological significance of increased blood viscosity in hypertension is also not clearly known. In theory, it should contribute to increased peripheral resistance, and this is supported by correlations with haemodynamics in vivo (Devereux et al 1984). Furthermore, the increased blood viscosity might increase

cardiac afterload and promote left ventricular hypertrophy; this is supported by a stronger correlation of left ventricular hypertrophy to blood viscosity than to any other haemodynamic factor (Chien et al 1985). This effect is however unlikely to explain the association of viscosity and systolic hypertension in the general population in which relatively few people have left ventricular hypertrophy (less than 1% of the subjects in the Edinburgh Artery study had good electocardiogram evidence in the form of large R waves and left axis deviation.)

Although blood pressure itself cannot be used as a risk factor for peripheral arterial disease as indicated by the ABPI, blood viscosity in males along with age and BMI could act as markers. In males these were strongly related to systolic pressure, 17% of the variability of which could be explained by rheological factors. Thus through the analysis in the previous chapter it can be postulated that systolic pressure was related to peripheral arterial disease through the rheological factors and especially through blood viscosity in males. In females systolic blood pressure was mainly related to age, BMI, and uric acid, while uric acid was not significantly related to peripheral arterial disease.

CHAPTER 8

FIBRINOGEN HAPLOTYPE

8.1 Introduction

The main aims of genetic analyses are to identify whether genes are associated with the occurrence of disease and if so, which genes are involved. The development of polymorphic DNA markers or restriction fragment length polymorphisms (RFLPs) has led to the identification of all chromosomal regions, which has furthered these aims.

One approach is to compare the frequency of a particular genetic marker between cases of disease and matched controls. The DNA polymorphisms within a tightly linked cluster of genes show the phenomenon of linkage disequilibrium (Bodmer 1987). Crossing over within the cluster is infrequent and so DNA markers in the group tend to be inherited en bloc as a haplotype. New markers or disease causing mutations arising within the cluster thus are associated with the haplotype of origin and only slowly over many generations lose this association. Thus it would be expected that a case-control study would reveal an excess of a particular haplotype amongst the cases of disease compared with the general population. The drawback of all such genetic analyses is that as multiple markers are usually studied, the chance of reaching statistical significance is high when multiple tests are carried out.

Genes are known to contribute to variation in populations in the levels of fibrinogen, lipids, and other risk factors, sometimes known as 'level' genes. In addition, they also contribute to setting the framework within which lifestyle or dietary factors can cause variation, known as 'variability' genes (Berg and Kerulf, 1989). Restriction fragment length polymorphisms (RFLP) allow the study of genetic variation at the fibrinogen locus in relation to plasma fibrinogen concentrations.

Increased levels of plasma fibrinogen have been shown in longitudinal studies

to be associated with an increased risk of CHD and stroke (Meade et al 1986, Wilhelmson et al 1984, Kannel et al 1987) and in epidemiological studies to be associated with peripheral arterial disease (Kannel et al 1990, Lowe et al 1991b). Plasma fibrinogen increases with age and cigarette smoking (Meade et al 1979,1987) but when corrected for these factors still emerged in the above studies as an independent risk factor. Other factors associated with high levels of plasma fibrinogen were social class (Markowe et al 1985), diabetes mellitus (Fuller et al 1979), and low alcohol consumption (Lee et al 1990). In women the use of oral contraceptives and post-menopause were associated with raised plasma fibrinogen (Lee et al 1990).

It might be expected that genetic variation would be involved in elevated levels of plasma fibrinogen which have been shown to be associated with peripheral arterial disease (chapter 6). However, controversy surrounds the role of genetic variation in affecting plasma fibrinogen levels.

Fibrinogen is synthesised in the liver from three polypeptide subunits ; alpha, beta and gamma, which occur as a linked cluster of genes on the long arm of chromosome 4. Humphries et al (1987) compared levels of fibrinogen (adjusted for age, sex and smoking) in 91 healthy volunteers genotyped at the fibrinogen alpha and beta loci and estimated that genetic variation at these loci accounted for 15% of the total variation. This is lower than the finding by Hamsten et al (1987) who, on the basis of path analysis, found that 50% of the variation of plasma fibrinogen was due to genetic factors. On the other hand, Berg and Kerulf (1989) found low heritability of levels of fibrinogen in a Norwegian twin study and found no association between fibrinogen levels and fibrinogen alpha and beta genotypes.

One drawback of these studies was that they did not examine the relationship between fibrinogen genotype and atherosclerotic disease. Genetic information was

collected in the Edinburgh case-control study, described in chapter 2, allowing the role of fibrinogen haplotytpes as risk factors for peripheral arterial disease to be examined. In addition, the role of genetic variation at the fibrinogen loci in determining the level of plasma fibrinogen was assessed.

8.2 Methods

8.2.1 Measurement of fibrinogen and fibrinogen haplotypes

Details of the methods of the case-control study are described in chapter 2. Following invitation all subjects who attended the clinic had blood samples taken in the morning in the fasting state. Samples of plasma, white blood cell pellets and DNA preparations were obtained. Plasma fibrinogen levels were measured in two ways; firstly in citrated plasma by a thrombin-clotting turbidometric method in a centifugal analyser (Lowe et al 1991b). Secondly, fibrinogen levels were assayed by heat precipitation using a nephelometric method (Stone et al 1985). Details of how DNA was extracted are described in Connor et al (1992). The DNA probes used were alpha, beta, and gamma which detect polymorphic fragments of 2.4kb and 1.6kb (TaqI), 5.3kb and 4.2kb (BclI), and 14kb and 11kb (KpnI/SacI) respectively (the appropriate restriction enzymes indicated in parentheses in each instance). Gene frequencies were calculated by gene counting and compared to Hardy-Weinberg frequencies by chi-squared tests. The expected frequencies of genotypes are x^2, $2x\ (1 - x)$, and $(1 - x)^2$ according to Hardy-Weinberg equilibrium, where x = frequency of one allele in the population.

8.2.2 Statistical Methods

As the fibrinogen concentrations measured by the clotting methods were positively skewed, a logarithmic transformation was used yielding geometric means and their confidence intervals as summary measures. Cumulative smoking was expressed in terms of packyears, that is, the average number of packs of 20 cigarettes smoked per day multiplied by the number of years of smoking. Analysis of covariance was used to assess differences in fibrinogen between genotypes or haplotypes adjusting for age, sex, and cumulative smoking. One way analysis of variance was carried out on the adjusted fibrinogen values to assess the phenotypic variability. The percentage of phenotypic variability (PV) in fibrinogen values associated with RFLP genotypes was estimated as described by Sing and Davignon (1985).

The formula used was PV = 100 x genotype variance/(genotype variance + within genotype variance), where the within genotype variance is obtained from the one way analysis of variance and the genotype variance (σ_G^2) is estimated as the sum of the observed relative frequency of the genotype multiplied by the square of the deviation of the mean of the genotype from the grand mean. This assumes that the observed relative frequency does not differ from the expected Hardy-Weinberg frequency.

The estimate of between genotype variability (σ_B^2) was also expressed as a percentage of the total variability ($\sigma_W^2 + \sigma_B^2$) obtained from the one way analysis of variance.

The between group variability was calculated as :

$$\sigma_B^2 = \frac{s_B^2 - s_w^2}{n_0}$$

where s_B = between group mean square;

s_W = within group mean square; and

$$n_0 = \frac{1}{k-1}\left(N - \frac{\Sigma n_i^2}{N}\right)$$

k = number of groups; N = total = Σn_i for unequal n_i (i = 1, ...,k). This is sensible as long as the n_i are not very different (Armitage 1971).

As well as genetic factors determining the level of plasma fibrinogen it is possible that specific haplotypes or genotypes may be more associated with disease than others, the so-called 'variability' genes (Berg and Kerulf, 1989). In order to assess this, dummy variables were created which represent each haplotype with reference to the most common haplotype (2.4/2.4, 5.3/5.3, 11/11). These were added in a multiple logistic regression model on peripheral arterial disease with adjustments for smoking as measured by packyears and current and ex-smokers, and level of clotting fibrinogen. Hence, one aim of this model is to assess the possible association of haplotypes at fibrinogen loci and peripheral arterial disease, irrespective of the level of plasma fibrinogen. Since the cases and controls were frequency matched, all analyses were adjusted for age and sex (Schlesselman , 1982). In addition, two dummy variables for the beta-locus were used to assess whether particular genotypes were associated with disease as found by Humphries et al (1987).

The main drawback of the haplotype analysis is the multiple testing involved. Humphries et al (1987) had drawn attention to a possible association of the β genotypes with fibrinogen level. Two dummy variables were created for the 5.3/4.2 and 4.2/4.2 genotypes relative to the 5.3/5.3 genotype at the β polymorphism and the

regression analyses as outlined for the haplotypes was repeated for these genotype dummy variables.

8.3 Results

8.3.1 Plasma fibrinogen and fibrinogen haplotype

Out of the 306 subjects invited to the clinic 121 cases and 126 controls attended giving an overall response rate of 81%. Of the attenders, 3 individuals did not have blood taken and a further 11 had at least 1 missing allele and so could not be classified according to haplotype. In both the cases and control groups, the attenders did not differ significantly from non-attenders in terms of age, sex, cigarette smoking, prevalence of intermittent claudication and mean ABPI. Smoking in terms of packyears was higher in the cases, while the mean age of both cases and controls was 69 years and there were no differences in the proportions of males and females (Table 8.1).

Table 8.1 Characteristics of Cases and Controls

Variable	Cases (n=114) Mean (s.e.)	Controls (n=119) Mean (s.e.)
Packyears (Square root)	4.80 (0.25)	1.92 (0.22)
Age	69.2 (0.5)	69.2 (0.5)
Sex : Females	56 (49%)	56 (47%)
Males	58 (51%)	62 (52%)

The overall frequencies for each polymorphic allele in the 247 subjects were 0.75 for the 2.4kb allele and 0.25 for the 1.6 kb allele at the alpha fibrinogen locus; 0.85 for the 5.3 kb allele and 0.15 for the 4.2 kb allele at the beta fibrinogen locus; and 0.24 for the 14 kb and 0.76 for the 11 kb alleles at the gamma fibrinogen locus. The frequencies of allele combinations (genotypes) were not significantly different from Hardy-Weinberg frequencies for all three loci (Table 8.2).

Table 8.2 Comparison of observed genotype numbers to those expected from Hardy-Weinberg equilibrium

α-locus

Genotypes	Observed	Expected
2.4/2.4	135	132.6
1.6/1.6	15	14.8
2.4/1.6	86	88.3

$X^2_1 = 0.106$ (N.S.)

β-locus

Genotypes	Observed	Expected
5.3/5.3	174	170.5
4.2/4.2	6	5.3
5.3/4.2	56	60.2

$X^2_1 = 0.451$ (N.S.)

γ-locus

Genotypes	Observed	Expected
14/14	13	13.6
11/111	139	136.4
14/11	84	86.1

$X^2_1 = 0.128$ (N.S.)

Of the 27 possible combinations of genotypes only 13 haplotypes were observed (Table 8.3).

Table 8.3 Combinations of genotypes to form haplotypes in cases and controls combined

Haplotype	α - locus enzyme Taq I	β - locus enzyme BCL I	γ - locus enzyme KPN	n (%)
1	2.4/2.4	5.3/5.3	11/11	87 (37)
2	2.4/1.6	5.3/5.3	14/11	57 (24)
3	2.4/2.4	5.3/4.2	11/11	37 (15)
4	2.4/1.6	5.3/5.3	11/11	10 (4)
5	2.4/1.6	5.3/4.2	14/11	16 (7)
6	1.6/1.6	5.3/5.3	14/14	12 (5)
7	2.4/2.4	4.2/4.2	11/11	4 (2)
8	2.4/2.4	5.3/5.3	14/11	6 (3)
9	1.6/1.6	5.3/4.2	14/14	1 (0.4)
10	2.4/1.6	5.3/4.2	11/11	1 (0.4)
11	2.4/2.4	5.3/4.2	14/11	1 (0.4)
12	1.6/1.6	5.3/5.3	14/11	2 (0.8)
13	2.4/1.6	4.2/4.2	14/11	2 (0.8)
				236*

* 5 subsets with at least 1 missing allele

Table 8.4 shows the mean fibrinogen levels adjusted for age, sex, and smoking according to the RFLPs. The fibrinogen values were calculated by analysis of covariance adjusting for age, sex, and packyears. There were no significant differences in plasma fibrinogen levels between genotypes for the RFLPs. The genotypes with the highest fibrinogen levels were 2.4/1.6 at the α-polymorphism and 5.3/4.2 at the β-polymorphism for fibrinogen measured by the clotting method.

Table 8.4 Plasma fibrinogen level by fibrinogen genotype

	Fibrinogen: Clotting method*	Fibrinogen: Nephelometric Method*
Alpha-locus	Geometric Mean(95%C.I.)	Mean (95% C.I.)
2.4/2.4	2.89 (2.76,3.00)	4.06 (3.92,4.22)
2.4/1.6	3.02 (2.88,3.16)	4.32 (4.13,4.51)
1.6/1.6	2.86 (2.56,3.21)	4.33 (3.88,4.78)
p -value	0.43	0.12
Beta-locus	Geometric mean(95%C.I.)	Mean (95% C.I.)
5.3/5.3	2.90 (2.81,3.01)	4.13 (3.99,4.26)
5.3/4.2	3.04 (2.86,3.22)	4.34 (3.60,5.07)
4.2/4.2	2.82 (2.36,3.38)	4.39 (3.65,5.12)
p-value	0.32	0.30
Gamma-locus	Geometric Mean(95%C.I.)	Mean (95% C.I.)
14/14	2.91 (2.58,3.29)	4.41 (3.92,4.90)
14/11	2.95 (2.81,3.09)	4.28 (4.09,4.47)
11/11	2.93 (2.82,3.04)	4.09 (3.95,4.25)
p-value	0.98	0.23

* Adjusted by age, sex, and smoking as measured by packyears 95% confidence intervals based on pooled standard deviation

The three loci were combined to form the 13 haplotypes (Table 8.3)and comparison of fibrinogen level (adjusted for age, sex, and smoking) according to haplotype showed no significant association (Table 8.5). The between haplotypes variability explained a negligible percentage of the total variability.

Table 8.5 Plasma fibrinogen level(adjusted by age, sex and smoking) by haplotype

	Fibrinogen-Clotting Method			Fibrinogen-Nephelometric Method	
Haplotype	Geometric Mean (95%C.I.)*		n	Mean(95% C.I.)*	n
1	2.89	(2.75,3.03)	81	4.01(3.82,4.21)	85
2	2.90	(2.73,3.08)	52	4.19 (3.94,4.44)	54
3	2.97	(2.75,3.20)	34	4.18 (3.87,4.48)	36
4	3.32	(2.83,3.89)	10	4.39 (3.75,5.04)	10
5	3.22	(2.86,3.64)	15	4.64 (4.15,5.14)	15
6	2.97	(2.49,3.54)	11	4.39 (3.79,4.99)	12
7	2.64	(1.85,3.78)	4	4.25 (2.80,5.69)	4
8	2.65	(2.09,3.35)	6	4.04 (3.07,4.97)	6
9	2.52		1	4.63 -	1
10	3.21		1	4.38 -	1
11	4.04		1	4.38 -	1
12	2.71	(1.37,5.36)	2	3.82 (1.06,5.57)	2
13	3.03	(1.53,5.99)	2	4.61 (1.86,7.36)	2
Total	2.93	(2.91,2.96)	220	4.19 (4.15,4.23)	229
p-value	0.52			0.71	
Between haplotype	0%			0% variability	
% Phenotypic variability	2.4%			3.4%	

* 95% confidence interval based on pooled standard deviation

8.3.2 Fibrinogen haplotypes as risk factors

The proportions of genotypes 2.4/1.6 for the α-locus and 5.3/4.2, 4.2/4.2 genotypes for the β-locus were higher in the cases compared to the controls (Table 8.6). The differences in proportions were only significant for the β-locus (p = 0.01).

Table 8.6 Distribution of genotypes in cases and controls

	Controls	Cases
Alpha-locus	Number (%)	Number (%)
2.4/2.4	71 (59)	64 (56)
2.4/1.6	38 (32)	47 (41)
1.6/1.6	11 (9)	4 (3)
	p = 0.11	
Beta-locus	Number (%)	Number (%)
5.3/5.3	97 (81)	74 (65)
5.3/4.2	21 (18)	35 (31)
4.2/4.2	1 (1)	5 (4)
	p = 0.01	
Gamma-locus	Number (%)	Number (%)
14/14	9 (8)	4 (4)
14/11	40 (33)	43 (37)
11/11	71 (59)	68 (59)
	p = 0.37	

The frequency of haplotypes show some differences between cases and controls, with haplotype 3 (2.4/2.4, 5.3/4.2, 11/11), haplotype 4 (2.4/1.6, 5.3/5.3, 11/11), haplotype 5 (2.4/1.6, 5.3/4.2, 14/11) and haplotype 7 (2.4/2.4, 4.2/4.2, 11/11) occurring more frequently in cases of peripheral arterial disease compared to the controls (Table 8.7). Overall, the differences in distribution of haplotypes between cases and controls was not significant (p = 0.14).

Table 8.7 Distribution of haplotypes by cases and controls

Haplotype	Controls n (%)	Cases n (%)
1	49 (57)	37 (43)
2	30 (55)	25 (45)
3	16 (43)	21 (57)
4	4 (40)	6 (60)
5	4 (25)	12 (75)
6	8 (67)	4 (33)
7	1 (25)	3 (75)
8	4 (67)	2 (33)
9	1 (100)	0
10	0	1 (100)
11	0	1 (100)
12	2 (100)	0
13	0	2 (100)

When the haplotype dummy variables were added in the logistic regression model, adjusting for age and sex alone (Table 8.8), haplotype 3 (at 10% level) and haplotype 5 (5% level) relative to haplotype 1 were significant predictors of disease. An increase in log fibrinogen units of 0.2 was associated with an odds ratio of disease of 1.82 (95% C.I. 1.38,2.41) (Table 8.8), when adjusted for age and sex alone. On adding the haplotype dummy variables the odds ratio for fibrinogen reduced to 1.77 showing little change. Clotting fibrinogen remained highly significant ($p<0.001$), and haplotypes 3 and 5 were significant predictors of disease (10% level). Smoking was positively associated with peripheral arterial disease, while haplotype 3 had the lowest proportion of smokers relative to other haplotypes. When smoking as packyears and current and ex-smokers (less than 5 years) were added to this model the odds ratio for clotting fibrinogen was reduced to 1.52 (95% C.I. 1.08,2.14)(Table 8.8), although

remaining significant. The odds ratios for haplotypes 3 and 5 increased and were significant predictors (5% level) of disease. Further analyses adjusting additionally for BMI, alcohol and social class did not alter the significance of the haplotype variables and none were independently associated with disease.

Table 8.8 Univariate and Multivariate logistic regressions of fibrinogen haplotypes, cigarette smoking and plasma fibrinogen, adjusted for age and sex on peripheral arterial disease

Haplotype (relative to haplotype 1)	Univariate Odds Ratio (95% C.I.)	Multivariate Odds Ratio (95% C.I.)
2	1.07 (0.52,2.20)	1.08 (0.44,2.69)
3	2.28 (0.95,5.44)$^{+}$	4.05 (1.33,12.38)*
4	2.05 (0.53,7.96)	2.33 (0.50,10.88)
5	4.77 (1.19,19.0)*	7.57 (1.47,39.05)*
6-13 combined	0.51 (0.13,7.96)	0.29 (0.05,1.70)
Clotting fibrinogen (ln) (+0.2)	1.82 (1.38,2.41)***	1.52 (1.08,2.14)*
Cigarette smoking[$]		
Packyears	4.30 (2.5 ,7.2)***	4.47 (2.43,8.22)***
Current	0.9 (0.3 ,2.3)	1.27 (0.42,3.85)
Ex-Smokers	0.56 (0.2 ,2.2)	0.56 (0.13,2.36)

[$] change in packyears in one standard deviation on square root scale; current and ex-smokers < 5 years are compared to non-smokers adjusted for packyears

On repeating the analyses for the two dummy variables (Table 8.9) for the β-polymorphism, the odds ratio was 3.67 (95% C.I. 1.60, 8.40) after adjusting for clotting fibrinogen, smoking and age and sex for the 5.3/4.2 genotype relative to the 5.3/5.3 genotype ($p = 0.003$). In addition, the dummy variable representing the 4.2/4.2 genotype relative to the 5.3/4.2 genotype was just significant at the 5% level, although the confidence interval was wide reflecting the low number of this particular genotype.

Table 8.9 Univariate and Multivariate logistic regressions of β-fibrinogen genotypes, cigarette smoking and plasma fibrinogen, adjusted for age and sex on peripheral arterial disease

Genotype (relative to 5.3/5.3)	Univariate Odds Ratio (95% C.I.)	Multivariate Odds Ratio (95% C.I.)
5.3/4.2	2.37 (1.24,4.52)*	3.67 (1.60,8.40)**
4.2/4.2	6.94 (0.78,61.5)+	14.65 (1.21,177.6)*
Clotting fibrinogen (ln) (+0.2)	1.82 (1.38,2.41)***	1.64 (1.19,2.26)**
Cigarette smoking$		
Packyears	4.30 (2.5 ,7.2)***	4.27 (2.42,7.51)***
Current	0.9 (0.3 ,2.3)	1.12 (0.40,3.11)
Ex-Smokers	0.56 (0.2 ,2.2)	0.62 (0.16,2.43)

$ change in packyears in one standard deviation on square root scale; current and ex-smokers < 5 years are compared to non- smokers adjusted for packyears

8.4 Discussion

These results tend to agree with those of Berg and Kerulf (1989) who found no relationship between genotype in two polymorphisms (alpha and beta) and plasma fibrinogen level. This population differs from that of Berg and Kerulf (1989) and Humphries et al (1987) which consisted of 91 healthy volunteers, so it is difficult to make comparisons. It is noticeable that the results of Humphries et al (1987) who found a significant association between plasma fibrinogen and the polymorphism detected at the beta locus were mainly due to two individuals with very high plasma fibrinogen levels. The number of key homozygous subjects in this study was small and this along with the within person variation of plasma fibrinogen level over time are the main problems in such studies. This suggests that further studies are required where a large random sample of the general population is taken rather than volunteers and followed up over a suitable period of time. On the basis of the results in the Edinburgh case-control study it appeared that genetic factors did not greatly affect the level of plasma fibrinogen.

The analysis was taken a stage further by examining the influence of fibrinogen haplotype and genotype on the odds of peripheral arterial disease. The logistic regression results are consistent with the idea of 'variability' genes (Berg and Kerulf,1989). Despite little relationship between plasma fibrinogen level and genotype, there were significant univariate associations between disease and haplotypes 3 and 5 relative to haplotype 1. This relationship was also independent of fibrinogen level, smoking, alcohol, social class and BMI which are all associated with plasma fibrinogen level. Caution should be applied in interpreting these two significant results at the 5% level with multiple dummy variables in the regression model. By concentrating on the β-polymorphism as highlighted by Humphries et al (1987), the

problem of multiple tests is reduced and a highly significant relationship ($p = 0.003$) was found. An increased odds for peripheral arterial disease of approximately three and a half times was found for the genotype 5.3/4.2 relative to the genotype 5.3/5.3, adjusting for plasma fibrinogen, smoking and age and sex. The genotype 4.2/4.2 was also found to be significantly associated with peripheral disease although there were low numbers of this genotype. This was the genotype found by Humphries et al (1987) to be associated with the highest fibrinogen levels in their study and so there was some consistency with this finding. However, the Edinburgh case-control study found the genotype 5.3/4.2 to be associated with the highest fibrinogen level and a significant independent risk factor for peripheral arterial disease Hence, the results suggested that genes at the fibrinogen loci may indicate a predisposition to peripheral arterial disease or operate on lifestyle risk factors independently of the risk factors of plasma fibrinogen level and smoking.

CHAPTER 9

LIFESTYLE AND INTERMEDIARY RISK FACTORS

9.1 Introduction

This chapter will assess combinations of the risk factors which were considered separately in the previous chapters. Social class or the closely related measure, social deprivation (Carstairs and Morris 1991), has been shown to be related to ischaemic heart disease (Pocock et al 1987, Rose and Marmot 1981) and so may be an important factor for peripheral arterial disease. Hence social class will also be considered as a risk factor and its relation to other risk factors will be discussed in this chapter.

It is useful to consider the risk factors as roughly forming two clusters which could be thought of as elements in a causal chain, that is, 'Lifestyle' factors which are related to the 'Intermediary' factors which in turn are more directly related to peripheral arterial disease. The lifestyle factors consist of diet, leisure activity, smoking, and alcohol along with personality, while the rheological variables and HDL and nonHDl cholesterol, along with triglycerides form the 'intermediary' grouping. In all the analyses adjustment for age has been carried out, and height and indication of known diabetes has been assessed initially with the second grouping. This is a tentative network which does have some face validity as lifestyle factors can be seen as preceding the more immediate blood risk factors which to a large extent they help to determine. For example, lack of exercise is associated with higher blood viscosity (Ernst 1985) which in turn is associated with higher risk of coronary heart disease (Kannel and Sorlie 1979a) and peripheral arterial disease (chapter 6). The Lifestyle factors are all related to social class, as are many of the intermediary factors.

As fibrinogen haplotype was only available for the case-control study and the power to detect associations with disease when a large number of variables is

considered is low, the analysis of fibrinogen haplotype will not be explored further.

This chapter will then assess the independence of all risk factors for peripheral arterial disease, as measured in the cross-sectional part of the Edinburgh Artery Study (Fowkes et al 1991), making the distinction between 'lifestyle' and 'intermediary' risk factors.

9.2 Methods

Many sex by risk factor interactions have been found in previous analyses (chapters 3-7) , and consequently the results have been presented for males and females separately. Lifestyle and personality factors were considered first followed by the rheological and lipids and finally all factors were analysed simultaneously.

The relationship between risk factors considered in previous chapters and social class was initially assessed by tests for linear trend in mean levels for continuous variables or proportions for categorical variables across social class. The classification of social class was I - Professional, II- Intermediate, III - Non-manual/clerical, III-Skilled, manual, IV - Semi-skilled manual, V-Unskilled, manual , VI and VII - Unknown/unclassified according to the classification of occupations (OPCS 1980). The unemployed and retired were classified according to longest held occupation, and married women were assigned to their husband's social class. The independence of risk factors was assessed using multiple linear regression with the ABPI as an indicator of peripheral arterial disease.

As there was a danger of multiple testing with such a large number of variables which can undermine statistically significant results, the regression analyses were restricted to those variables which were either approaching statistical significance or significant at the 5% level in analyses in previous chapters. Nevertheless the problem still remains and so marginally significant results were treated with caution. Since smoking as indicated by packyears has been shown to be one of the strongest risk factors for peripheral arterial disease tests for smoking by risk factor interactions were carried out.

9.3 Results

9.3.1 Lifestyle factors

Females

Social class was strongly linearly related to many dietary variable intakes with significantly lower intake of energy adjusted vitamin C, β-carotene and cereal fibre in lower social classes (Table 9.1). A similar trend is shown for α-tocopherol but this did not reach statistical significance at the 5% level. Height and alcohol intake were significantly lower in lower social classes while BMI was higher in lower social classes (all $p < 0.0001$). Cigarette smoking was also significantly ($p = 0.02$) higher in lower social classes, although not in a strongly linear fashion. Social class was significantly related to leisure activity with greater prevalence of strong/moderate activity aged 35-45 in higher social classes. Most importantly social class was not significantly related to the ABPI. This suggested that social class was not a true confounder of the relationships between other risk factors and peripheral arterial disease in females.

Table 9.1 Trend in mean (se) ABPI and lifestyle risk factors for peripheral arterial disease by social class in females

Factor	Social Class						Linear trend p-value
	I (n=74)	II (n=240)	IIIN (n=207)	IIIM (n=159)	IV (n=70)	V & VI (n=33)	
ABPI	1.02 (0.02)	1.00 (0.01)	1.00 (0.01)	1.01 (0.01)	1.00 (0.02)	1.02 (0.03)	0.89
β-carotene	8.41 (0.07)	8.17 (0.05)	8.10 (0.06)	8.02 (0.06)	8.03 (0.06)	7.51 (0.10)	<0.0001
Vitamin C	83.7 (3.3)	80.4 (1.7)	73.2 (1.8)	76.9 (2.2)	72.9 (3.6)	62.1 (4.0)	0.0001
Cereal Fibre	2.52 (0.04)	2.39 (0.03)	2.25 (0.03)	2.40 (0.04)	2.23 (0.06)	2.14 (0.07)	0.0001
α-tocopherol	2.43 (0.06)	2.35 (0.03)	2.28 (0.03)	2.40 (0.04)	2.28 (0.06)	2.20 (0.06)	0.06
Height(cm)	161.9 (0.8)	160.4 (0.4)	157.7 (0.4)	159.7 (0.5)	156.7 (0.7)	155.1 (1.1)	<0.0001
Alcohol	1.89 (0.17)	1.63 (0.09)	1.00 (0.08)	1.04 (0.09)	1.02 (0.16)	0.77 (0.20)	<0.0001
Packyears	1.92 (0.27)	1.94 (0.15)	2.38 (0.18)	2.18 (0.20)	2.85 (0.39)	2.35 (0.47)	0.02
Leisure activity(n) Str/Mod.	62.2 % (46)	55.0% (132)	23.7 % (49)	39.6 % (63)	24.3 % (17)	9.4 % (3)	<0.0001

Males

Unlike in females, there was a significant linear trend of mean ABPI across social class categories in males (table 9.2), and so social class could be considered a true confounder in males. As with females, vitamin C and cereal fibre showed strong linear trends with significantly lower mean levels in lower social classes while α-tocopherol was also highly significant ($p = 0.003$) with β-carotene, only just significant ($p = 0.02$). Height had a similar relationship in males compared to females; shorter mean height in lower social classes (table 9.2). Alcohol has a similar relationship as in females, while the actual intake is much greater in males compared to females for all social classes. Similarly, smoking levels are higher in males

compared to females and showed a stronger linear trend with higher mean packyears in lower social classes ($p < 0.0001$). Another lifestyle factor which shows a strong relationship is leisure activity with greater percentages indulging in strenuous or moderate activity aged 35-45 years in higher social classes. Mean overt hostile acts is lowest in social class I but there was no significant trend across social class categories. Hostile acts was not assessed for females as previous analyses (chapter 5) had shown no significant relationship to peripheral arterial disease.

Table 9.2 Trend in mean (se) ABPI and lifestyle and hostility risk factors for peripheral arterial disease by social class in males

Factor	Social Class						Linear trend p-value
	I (n=94)	II (n=265)	IIIN (n=245)	IIIM (n=95)	IV (n=78)	V & VI (n=32)	
ABPI	1.11 (0.02)	1.07 (0.01)	1.03 (0.01)	1.04 (0.02)	1.03 (0.03)	1.06 (0.03)	0.004
β-carotene	8.1 (0.07)	8.0 (0.05)	7.9 (0.06)	7.9 (0.09)	7.9 (0.12)	7.6 (0.23)	0.02
Vitamin C	69.3 (2.5)	66.4 (1.5)	58.7 (1.6)	58.9 (2.2)	57.5 (2.4)	50.9 (3.6)	<0.0001
Cereal Fibre	2.46 (0.05)	2.42 (0.03)	2.25 (0.03)	2.29 (0.05)	2.22 (0.05)	2.13 (0.07)	<0.0001
α-tocopherol	2.30 (0.04)	2.38 (0.03)	2.20 (0.03)	2.27 (0.05)	2.14 (0.05)	2.21 (0.07)	0.0003
Hostile Acts	13.4 (0.2)	14.2 (0.2)	14.9 (0.2)	14.2 (0.3)	14.2 (0.4)	14.3 (0.6)	0.06
Height (cm)	175.9 (0.7)	173.9 (0.4)	170.3 (0.4)	171.6 (0.7)	167.8 (0.8)	167.1 (1.4)	<0.0001
Alcohol	3.12 (0.17)	2.72 (0.13)	2.90 (0.14)	2.50 (0.23)	2.69 (0.27)	1.76 (0.31)	0.01
Packyears	2.95 (0.29)	3.34 (0.18)	4.17 (0.18)	3.93 (0.31)	4.47 (0.36)	4.32 (0.62)	<0.0001
Leisure activity(n) Str/Mod.	79.8% (75)	80.0% (212)	57.6% (141)	58.9% (56)	46.2% (36)	34.4% (11)	<0.0001

Males and Females

Previous analyses in chapter 4 had shown a significant ($p = 0.02$) three way interaction of sex by smoking by leisure activity. Consequently, multiple linear regressions were carried out separately for males and females. Smoking was the strongest lifestyle risk factor along with age in females. There were no significant smoking by lifestyle factor interactions in females.

In males there was a significant smoking by hostile acts interaction($p = 0.008$) but this became non-significant on adding a leisure activity by smoking interaction. Leisure activity was strongly correlated with hostile acts in males ($p = 0.03$) and also vitamin C intake ($p < 0.001$). Vitamin C intake was significantly higher in males ($p<0.001$) and females ($p<0.001$) in those who indulged in strenuous/moderate leisure activity aged 35-45. As shown in tables 9.1 and 9.2 the prevalence of taking part in leisure activity was lower in lower social classes, showing significant linear trends ($p < 0.0001$) in proportions of strenuous/moderate activity aged 35-45. In other words leisure activity may be a marker for various lifestyle, personality and social class characteristics and hence if added to a multiple linear regression model other lifestyle and personality factors to which it was related became non-significant. Hence hostile acts was not a risk factor independently of leisure activity and social class.

Leisure activity showed a significant increase in the ABPI for strenuous/moderate activity relative to none only in those who ever smoked. Hence it appears that taking part in leisure activity aged 35-45 goes some way to ameliorate the cumulative effects of cigarette smoking but does not compensate completely. Amongst those who never smoked there were associations also with leisure activity but these did not reach statistical significance, presumably because the benefit of

activity is relatively smaller as non-smokers already had high levels of the ABPI. Alcohol was positively related to the ABPI in males but not in females, suggesting a possible beneficial effect of alcohol.

Table 9.3 Multiple regression of Lifestyle factors on the ABPI in males and females (adjusted for all lifestyle factors)

Factor (+ 1 S.D.)	Females(n=783)	Males(n=809)
	Mean ABPIx100 (se)	Mean ABPIx100 (se)
Packyears (Square root)	-4.60*** (0.51)	-4.74*** (0.59)
Age	-3.15*** (0.57)	-3.20*** (0.67)
Alcohol units (Square root)	0.48 (0.61)	1.87* (0.80)
Leisure Activity:		
Ever smoked		
Light activity vs. none	0.9 (2.2)	4.5 (2.8)
Moderate activity vs none	0.5 (2.3)	5.8* (2.6)
Strenuous activity vs. none	1.8 (4.6)	7.1* (3.0)
Never smoked		
Light activity vs none	2.2 (2.2)	3.7 (3.8)
Moderate activity vs. none	2.1 (2.3)	2.5 (3.3)
Strenuous activity vs. none	-6.0 (5.3)	4.8 (4.0)

Social class was shown to be significantly related to the ABPI in males on simple linear regression ($p = 0.002$) but on progressively adding height, age, leisure activity and smoking it became non-significant (table 9.4), indicating that social class was an important risk factor but was not independently related to the ABPI.

Table 9.4 Relationship between Social Class and Lifestyle factors in males in multiple linear regression on the ABPI

Model	Mean ABPIx100 for Social Class	p-value
Social Class	- 1.7	0.002
Social Class + Age	- 1.6	0.002
Social Class + Age + Height	- 0.5	0.017
Social Class + Age + Height + Leisure activity	- 0.9	0.11
Social Class + Age + Height + Smoking	- 0.7	0.19
Social Class + Age + Height + Smoking + Leisure Activity	- 0.4	0.33

9.3.2 Intermediary Risk Factors

As with the lifestyle factors there were many strong relationships between intermediary risk factors and social class. In females blood viscosity and fibrinogen were significantly higher in lower social classes (table 9.5). Among the lipid measures, HDL cholesterol was highly significantly lower in lower social classes while triglycerides were significantly higher. BMI showed a highly significant trend with higher mean levels in lower social classes.

In males there was a similar picture, in that, blood viscosity and fibrinogen showed significant linear trends with higher mean levels in lower social classes. On the other hand, unlike in females, there was no significant linear trends of lipids across social class categories (table 9.6).

Table 9.5 Trend in mean (se) ABPI and intermediary risk factors for peripheral arterial disease by social class in females

Factor	Social Class						Linear trend p-value
	I (n=74)	II (n=240)	IIIN (n=207)	IIIM (n=159)	IV (n=70)	V & VI (n=33)	
ABPI	1.02 (0.02)	1.00 (0.01)	1.00 (0.01)	1.01 (0.01)	1.00 (0.02)	1.02 (0.03)	0.89
Blood viscosity (mPa.s)	3.23 (0.06)	3.31 (0.03)	3.40 (0.04)	3.43 (0.04)	3.39 (0.06)	3.50 (0.10	0.001
Uric acid (µmol/l)	288 (7.8)	279 (4.1)	293 (5.1)	288 (5.8)	285 (8.6)	303 (13.1)	0.18
Fibrinogen (g/l)	264 (6.8)	275 (4.3)	281 (5.0)	282 (5.8)	290 (7.9)	293 (12.6)	0.008
BMI (kg/m^2)	24.9 (0.5)	24.8 (0.2)	26.2 (0.3)	25.5 (0.3)	26.6 (0.6)	28.8 (1.2)	<0.0001
Known diabetic(n)	0 % (0)	0.8 % (2)	1.4 % (3)	3.1 % (5)	7.1 % (5)	0 % (0)	0.01
HDL (mmol/l)	1.74 (0.05)	1.68 (0.02)	1.58 (0.03)	1.57 (0.03)	1.53 (0.06)	1.52 (0.08)	<0.0001
NonHDL (mmol/l)	5.59 (0.15)	5.84 (0.08)	5.95 (0.10)	5.76 (0.11)	5.93 (0.16)	5.45 (0.24)	0.93
Triglyceride (ln) (mmol/l)	0.17 (0.05)	0.23 (0.03)	0.38 (0.03)	0.29 (0.03)	0.38 (0.06)	0.40 (0.09)	0.0006

ln -Natural logarithm

Table 9.6 Trend in mean (se) ABPI and intermediary risk factors for peripheral arterial disease by social class in males

Factor	Social Class						Linear trend p-value
	I (n=94)	II (n=265)	IIIN (n=245)	IIIM (n=95)	IV (n=78)	V & VI (n=32)	
ABPI	1.11 (0.02)	1.07 (0.01)	1.03 (0.01)	1.04 (0.02)	1.03 (0.03)	1.06 (0.03)	0.004
Blood viscosity (mPa.s)	3.65 (0.05)	3.74 (0.04)	3.79 (0.04)	3.86 (0.07)	3.95 (0.08)	3.93 (0.16)	0.0002
Uric acid (µmol/l)	344 (7.3)	345 (4.2)	344 (4.5)	346 (7.9)	343 (9.8)	336 (16.1)	0.60
Fibrinogen (g/l)	236 (6.3)	253 (4.0)	279 (5.0)	275 (6.7)	268 (6.8)	273 (13.1)	<0.0001
BMI (kg/m^2)	175.9 (0.7)	173.9 (0.4)	170.3 (0.4)	171.6 (0.7)	167.8 (0.8)	167.1 (1.4)	<0.0001
Known Diabetic(n)	2.1 % (2)	3.0 % (8)	3.7 % (9)	2.1 % (2)	5.1 % (4)	6.3 % (2)	0.25
HDL (mmol/l)	1.34 (0.04)	1.27 (0.02)	1.21 (0.02)	1.33 (0.04)	1.27 (0.04)	1.26 (0.06)	0.46
NonHDL (mmol/l)	5.32 (0.14)	5.44 (0.07)	5.29 (0.07)	5.45 (0.12)	5.37 (0.14)	5.47 (0.22)	0.86
Triglyceride (ln) (mmol/l)	0.37 (0.05)	0.40 (0.03)	0.41 (0.03)	0.31 (0.05)	0.40 (0.06)	0.44 (0.06)	0.93

ln - Natural logarithm

In the multiple linear regressions there were significant fibrinogen by sex ($p = 0.02$) and blood viscosity by sex ($p = 0.0007$) interactions. Both these variables had significantly stronger relationships in males compared to females (table 9.7). There was also a suggestion of an HDL cholesterol by sex interaction with a highly significant positive association only in males but this did not reach statistical significance at the 5% level. Both BMI and age were significantly associated with the ABPI in males and females.

Social class in males became non-significant as soon as blood viscosity, fibrinogen and smoking were added to the model; because of the strong correlations between social class and these variables (table 9.6)

Table 9.7 Multiple regression of Intermediary factors on ABPIx100 in males and females (adjusted for all intermediary factors)

Factor (+ 1 S.D.)	Females (n=783)	Males (n=809)	
	Mean ABPIx100 (se)	Mean ABPIx100 (se)	p-value#
Age (years)	-2.06** (0.63)	-3.2*** (0.72)	0.16
BMI (kg/m^2)	2.69*** (0.72)	2.07** (0.75)	0.91
Fibrinogen (g/l)	-1.97** (0.72)	-3.93*** (0.83)	0.02
Blood viscosity (mPa.s)	0.83 (0.89)	-2.24* (1.09)	0.0007
HDL Cholesterol (mmol/l)	0.91 (0.73)	2.15** (0.76)	0.07
NonHDL Cholesterol (mmol/l)	-1.07 (0.77)	-1.42+ (0.79)	0.56
Known diabetic vs. non	-7.4* (4.4)	-16.0** (4.5)	0.10

test of difference between unstandardised regression coeffficients for males and fenales

9.3.3 Lifestyle and Intermediary Factors

Females

There were significant sex by smoking (packyears) by fibrinogen ($p = 0.01$) and sex by blood viscosity ($p = 0.01$) interactions and sex by leisure activity by smoking ($p = 0.02$) interactions. However, if the blood viscosity three way interaction was added along with the fibrinogen and leisure activity three way interactions, it became non-significant. Consequently, to aid interpretation, the analysis of all factors was split according to sex and ever/never smoked in males. In females a test for a fibrinogen by smoking interaction was not significant.

When the lifestyle and intermediary factors were combined the most striking change was that fibrinogen was no longer significant for females (table 9.8) suggesting that smoking in terms of the packyears may be the source of the raised fibrinogen levels in women, while fibrinogen remained an independent risk factor in males

Table 9.8 shows that age and smoking in terms of packyears were the most significant factors, especially smoking and these were the only independent risk factors in women. NonHDL cholesterol approached significance at the 5% level. Figure 9.1 shows a similar trend in blood viscosity with leisure activity aged 35-45 years in females and in males, although leisure activity was not significantly ralted to the ABPI in females.

Table 9.8 Multiple regression of all factors on ABPIx100 in females (n = 783)

Factor (+ 1 s.d.)	Mean ABPIx100 (se)	p- value
Packyears (Square root)	-4.30 (0.66)	<0.0001
Age (years)	-2.57 (0.63)	0.0001
Height (cm)	0.30 (0.65)	0.65
Fibrinogen (g/l)	0.49 (0.71)	0.49
Blood viscosity (mPa.s)	0.59 (0.67)	0.38
HDL Cholesterol (mmol/l)	0.56 (0.76)	0.46
NonHDL Cholesterol (mmol/l)	-1.35 (0.77)	0.08
Known diabetic vs. non	-2.0 (4.4)	0.66
Leisure activity		
Strenuous vs none	-1.2 (3.9)	0.75
Moderate vs none	0.40 (2.1)	0.86
Light vs none	0.2 (2.0)	0.92

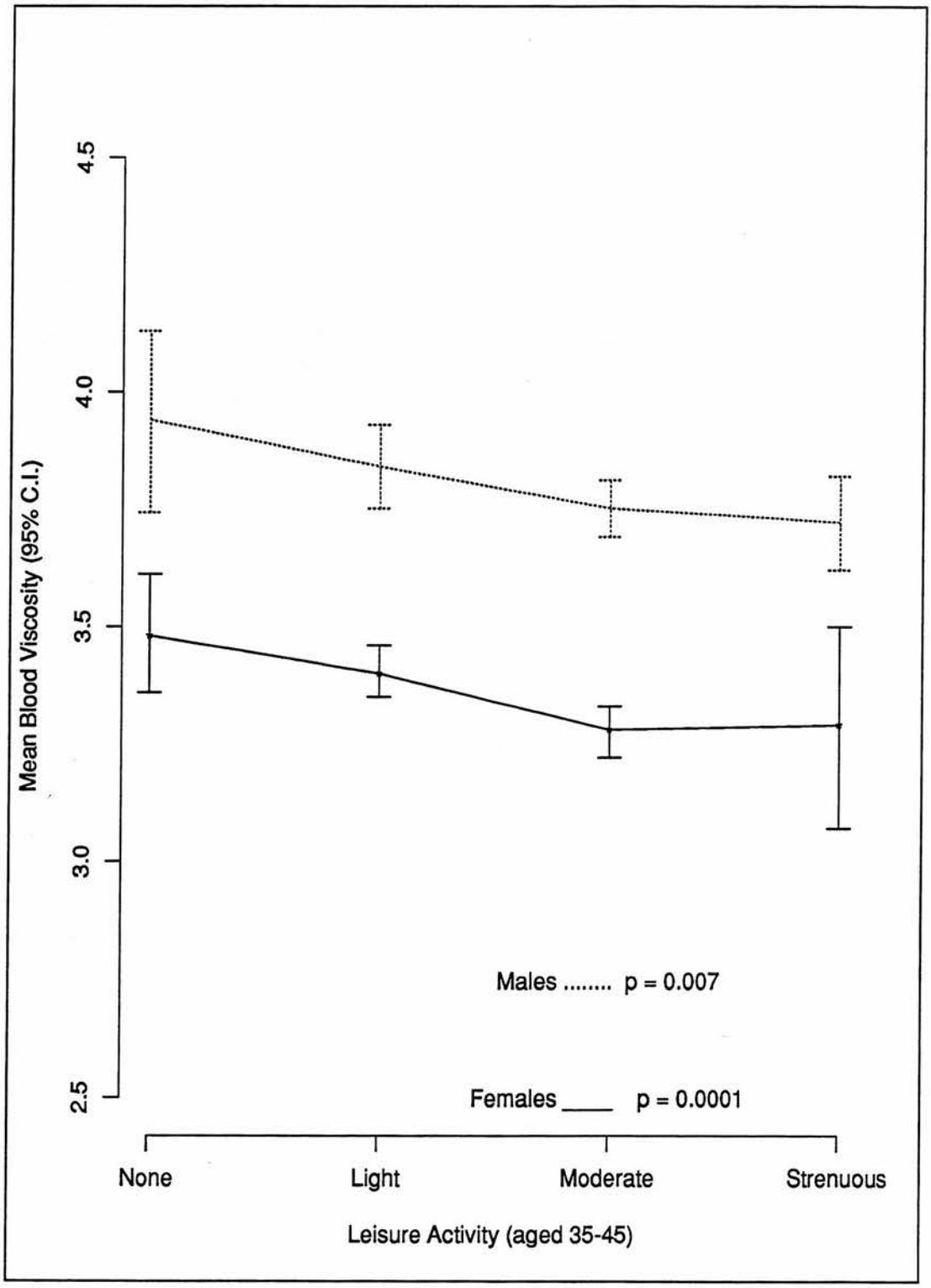


Figure 9.1 Relationship between blood viscosity and extent of leisure activity aged 35-45

Males who had ever smoked

Whenever risk factors in men who had ever smoked were considered, it was clear that smoking had a synergistic effect on many of the risk factors' associations with the ABPI (table 9.9). Smoking and age were both highly significantly related to the ABPI as were fibrinogen and blood viscosity. In fact fibrinogen appeared to be the most important risk factor ($p < 0.0001$) for men who smoke. HDL cholesterol and nonHDL cholesterol were both significant at the 5% level with independent associations with the ABPI in opposite directions. The positive association of alcohol and the ABPI found when only lifestyle factors were considered disappeared, probably due to the high correlation between alcohol and HDL cholesterol in men ($r = 0.30$). Subjects who were known diabetic had significantly lower mean ABPI relative to non-diabetics (table 9.9). Strenuous activity relative to none and moderate activity relative to none were shown to be significantly related to higher mean ABPI in those males who ever smoked when lifestyle factors were considered alone (table 9.3). On adding the intermediary factors, the leisure activity variables became less significant, although still remaining significant at the 10% level. This was mainly due to the strong relationship between leisure activity and blood viscosity in males (figure 9.1).

Table 9.9 Multiple regression of all factors on ABPIx100 in males who ever smoked (n = 568)

Factor (+ 1 s.d.)	Mean ABPIx100 (se)	p- value
Packyears (Square root)	-2.79 (0.86)	0.001
Age (years)	-2.99 (0.89)	0.0008
Height (cm)	1.01 (0.77)	0.22
Fibrinogen (g/l)	-4.26 (0.95)	<0.0001
Blood viscosity (mPa.s)	-3.42 (0.89)	0.0002
HDL Cholesterol (mmol/l)	2.31 (1.02)	0.02
NonHDL Cholesterol (mmol/l)	-2.06 (1.00)	0.03
Known diabetic vs. non	-14.5 (5.6)	0.01
Leisure activity		
Strenuous vs none	6.7 (3.5)	0.05
Moderate vs none	5.2 (3.1)	0.09
Light vs none	3.7 (3.3)	0.25

Males who never smoked

Although the power was now low to detect associations in the group of men who never smoked, table 9.10 shows that many of the risk factors were very non-significant in this group, and so could not be dismissed on that account. The strongest risk factor amongst males who had never smoked was nonHDL cholesterol ($p = 0.009$), while being a known diabetic was also significantly associated with lower mean ABPI (table 9.10). It is notable that age was not significantly related to the ABPI in male non-smokers. On removing nonHDL cholesterol from this model, of all the dietary factors dietary cholesterol approached significance ($p = 0.07$).

Table 9.10 Multiple regression of all factors on ABPIx100 in males who never smoked (n = 224)

Factor (+ 1 s.d.)	Mean ABPIx100 (se)	p- value
Age (years)	-1.20 (1.08)	0.29
Height (cm)	1.87 (1.02)	0.08
Fibrinogen (g/l)	0.83 (1.13)	0.46
Blood viscosity (mPa.s)	-1.46 (1.15)	0.20
HDL Cholesterol (mmol/l)	0.40 (1.12)	0.71
NonHDL Cholesterol (mmol/l)	-3.10 (1.17)	0.009
Known diabetic vs. non	-13.7 (5.6)	0.01
Leisure activity		
Strenuous vs none	-3.3 (4.7)	0.48
Moderate vs none	-5.8 (4.4)	0.19
Light vs none	-4.5 (4.6)	0.32

9.4 Summary

Females

Age and smoking were the most important risk factors for females, with nonHDL approaching statistical significance.

The significant relationships between the ABPI and blood viscosity and fibrinogen could be 'explained' by cigarette smoking

Social Class and leisure activity were not significantly related to the ABPI in females

Males

Age and cigarette smoking were strongly significant in males as in females.

There were significant interactions with smoking for blood viscosity and fibrinogen. In fact, in those who never smoked fibrinogen and blood viscosity were not significantly related to the ABPI. In those who ever smoked, blood viscosity and fibrinogen were significantly related to the ABPI independently of smoking.

Leisure activity factors (Strenuous and Moderate relative to none) were significantly related to the ABPI. They also had significant interactions with cigarette smoking with the greatest relative benefit to those who had ever smoked, although this may not be independent of blood viscosity.

HDL and nonHDL cholesterol were significantly related to the ABPI, nonHDL especially in male non-smokers, where it was the most important risk factor.

Social class was strongly related to the ABPI but not independently of the lifestyle factors of height, leisure activity, smoking and dietary factors, and not independently of the intermediary factors of blood viscosity and fibrinogen.

Diabetes was only a significant risk factor for males.

CHAPTER 10

POWER AND SAMPLE SIZE ESTIMATION IN EPIDEMIOLOGICAL STUDIES

10.1 Introduction - Terminology

This chapter addresses two main questions. Firstly, what is the power of tests to detect a given effect size with a fixed type I error and sample size? This question will be considered in the context of analyses of The Edinburgh Artery Study data presented in previous chapters. Secondly, at the design stage of a study, what is the required sample size to detect a given effect, with fixed type I error and power? Before discussing the methods which have been proposed to answer such questions some terminology is required.

The purpose of a statistical test is to assess the evidence against a stated null hypothesis, H_0 which it is hoped can be rejected in favour of an alternative hypothesis, H_1. In carrying out hypotheses tests there are two main types of error which can be made. The type I error (α) is the probability of achieving statistical significance (rejecting the null hypothesis) when the null hypothesis is, in fact, true. This is equal to the significance level. The type II error (β) is the probability that the null hypothesis is not rejected when it is, in fact, false. This can be summarised as follows:

	H_0 true	H_1 true
Reject H_0	type I error	Correct decision
Accept H_0	Correct decision	type II error

The type I error by convention is taken to be 5%, while $1-\beta$ the power of the test is more often considered than the type II error β. The power of a test $1-\beta$ is the

probability of rejecting the null hypothesis when the null hypothesis is false. It would be ideal to minimise both errors but a decrease in one results in an increase in the other. Low power is a problem in studies where a medically interesting effect is missed because of low sample size. Hence the need to assess the required sample size at the design stage. This will be addressed in the second part of this chapter.

10.2 Power - Continuous outcomes

10.2.1 Pearson correlation coefficient

The simplest relationship between a continuous risk factor and outcome can be described by the Pearson correlation coefficient, ρ. The sample correlation coefficient r is defined as

$$r=\frac{\sum_{i=1}^{n}(x_i-\bar{x})(y_i-\bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i-\bar{x})^2\sum_{i=1}^{n}(y_i-\bar{y})^2}}$$

or as

$$r=cov(x,y)/\sqrt{var(x)\,var(y)}$$

A test of $H_0 : \rho = 0$ is given by

$$T=r\frac{\sqrt{(n-2)}}{\sqrt{(1-r^2)}}$$

which follows a t-distribution with n-2 degrees of freedom. This assumes that the risk

factor and outcome have a joint normal distribution. This is equivalent to testing the simple linear regression coefficient (β) under $H_0 : \beta = 0$. Assuming n is large and $T \sim N(0,1)$ the power of this test is estimated by

$$z_{1-\beta} = r\sqrt{\frac{n}{(1-r^2)}} - z_{1-\alpha/2}$$

and from standard normal tables

$$Power = 1-\beta = \int_{-\infty}^{z_{1-\beta}} f(x)\,dx$$

where f follows N (0,1).

Table 10.1 shows the correlation of various risk factors from the Edinburgh Artery Study as described in earlier chapters with one continuous measure of peripheral arterial disease, the ABPI.

Table 10.1 Pearson Correlations of the ABPI and Smoking with Risk Factors for Peripheral Arterial Disease

	ABPI	Smoking Packyears	N
Age	- 0.18	0.03	1582
Smoking (Packyears)	- 0.22	-	1550
Alcohol (Square root)	0.12	0.25	1582
Fibrinogen	- 0.23	0.15	1538
Blood viscosity	- 0.11	0.23	1390
ln (Triglycerides)	- 0.12	0.15	1563
HDL	0.05	- 0.14	1557
NonHDL	- 0.15	- 0.04	1557
ln (Cereal fibre)	0.04	- 0.19	1579
α-tocopherol	0.02	- 0.15	1578
Vitamin C	0.03	- 0.19	1578
Hostile acts	- 0.01	0.16	1579
Dominance	- 0.01	0.17	1579

The power based on equation 4 to detect various population correlations (ρ) with a set of sample sizes with two-sided significance level of 5% are shown in table 10.2

Table 10.2 Power (%) to detect ρ with sample size n at $\alpha = 0.05$

$\lvert\rho\rvert$	n=100	n=250	n=500	n=1000	n=1500
0.05	7	12	20	36	49
0.075	11	22	39	66	83
0.10	17	36	61	89	97
0.15	48	67	92	99	*
0.20	53	90	99	*	*
0.25	73	98	*	*	*
0.30	88	*	*	*	*

* Power > 99.5%

The values in table 10.2 agree with those in Cohen (1988). From table 10.2 it can be seen that the Edinburgh Artery Study can detect individual $r \geq 0.07$ with power > 80%. Where the associations are weak (table 10.1) such as r = 0.05, power is inadequate with this sample size, although it is debatable whether such low correlations would be worth detecting.

10.2.2 Partial Correlation Coefficient

Table 10.1 also shows that smoking in terms of packyears is highly correlated with many other risk factors for peripheral arterial disease such as alcohol intake and blood viscosity. Clearly, smoking is a confounder of the relationship between many risk factors and the ABPI. The effect that this has on the simple correlation coefficient can be seen by consideration of the partial correlation coefficient (see for example, Kleinbaum, Kupper and Muller 1988). Define the first order partial correlation coefficient as

$$r_{yx_1 \cdot x_2} = \frac{r_{yx_1} - r_{yx_2} r_{x_1 x_2}}{\sqrt{(1 - r_{yx_2}^2)(1 - r_{x_1 x_2}^2)}}$$

From table 10.2 using some interpolation, the power to detect a population correlation coefficient of $\rho = -0.11$ is approximately 96% with n = 1400 and two-sided α. In the Edinburgh Artery Study the correlation coefficient of blood viscosity with the ABPI is -0.11. If smoking is included as a covariate (x_2) then $r_{YX2} = -0.22$ and $r_{X1X2} = 0.23$ and

$r_{YX1 \cdot X2} = -0.06$ compared with $r_{YX1} = -0.11$

and so the power is lower for a given sample size to detect an association between the ABPI and blood viscosity after adjusting for smoking. In fact, using the methods described later (Cohen 1988) the power is 66% with the presence of this covariate. On the other hand, the Pearson correlation of alcohol and the ABPI was r = 0.12, while the partial correlation adjusting for smoking was $r_{YX1 \cdot X2} = 0.18$, assuming the sample correlations represent the population values. Thus the effect of adjusting may also be to increase the power to detect associations depending on the direction of the association between risk factors and outcome and between risk factors. This can be summarised as follows

Correlation outcome and confounder r_{YX2}	Correlation risk factor and confounder r_{X1X2}	Direction effect on partial correlation relative to Pearson
Opposite sign		Positive
Same sign		Negative

It is more usual to find a reduction in power after adjusting for a covariate such as in the blood viscosity example above and in most of the factors in table 1 and when adjusting for more than one covariate. The preceding results in terms of correlations are equivalent to tests of regression coefficients in simple linear regression and when adjusting for covariates in multiple linear regression.

10.2.3 Multiple linear regression

One way of dealing with more than one covariate is to consider the multiple correlation coefficient of Y with $X_1, ..., X_p$. Assuming that the p+1 variables have a multivariate normal distribution (MVN) with a positive definite covariance matrix of

$$\Sigma = \begin{bmatrix} \sigma_y^2 & \Sigma_{yx}' \\ \Sigma_{yx} & \Sigma_x \end{bmatrix}$$

The population multiple correlation coefficient (Anderson 1984) between Y and **X**, ρ_{YX} is estimated by

$$R_{YX} = \left[\frac{\Sigma_{YX}' \Sigma_X^{-1} \Sigma_{YX}}{\sigma_Y^2} \right]^{1/2}$$

The test statistic for $H_0 : \rho_{YX} = 0$ is

$$[(N-1-p)/p] \frac{R_{yx}^2}{(1-R_{yx}^2)} \sim F_{p,N-1-p}$$

where N is the number of subjects and p is the number of variables.

Gatsonis and Sampson (1989) present a method of estimating power and sample size with two approaches: conditional and unconditional. The unconditional approach

assuming the p+1 variables have a MVN distribution is relevant to epidemiological studies where the independent variables are not fixed in advance. The unconditional power of the test is given by

$$prob\frac{[p^{-1}X_{p+2k}{}^2]}{[(N-1-p)^{-1}X^2_{(N-1-p)}]} \geq F_{p,N-1-p}$$

where k has a negative binomial distribution. Under $H_0 : \rho_{YX} = 0$ the random variable $k = 0$.

The exact power calculations require tables of the noncentral F distribution of the sample multiple correlation coefficient (MCC) but Gatsonis and Sampson (1989) provide tables based on an approximation which is very accurate. They also found reasonable agreement between their results and those of Cohen (1988). Table 10.3 shows the power of the test for various values of ρ_{YX}, n and α=0.05 based on some interpolation of the tables of Gatsonis and Sampson (1989)

Table 10.3 Approximate power (%) with number of variables p=4 from tables of Gatsonis and Sampson (1989) at 5% significance level

ρ_{YX}	n				
	100	250	500	1000	1500
0.10	< 10	20	40	70	89
0.15	20	40	77	98	*
0.20	30	74	96	*	*
0.25	50	92	*	*	*
0.30	70	98	*	*	*
0.35	85	*	*	*	*
0.40	94	*	*	*	*

* Power > 99.5%

From table 10.3 it is clear that the Edinburgh Artery Study has adequate power (>80%) to detect a $\rho_{YX} > 0.10$. The value of R for the ABPI as outcome with age, sex, alcohol and smoking in terms of packyears was R = 0.35 and on the face of it power is more than adequate. However, epidemiological studies are generally not designed to test $H_0 : \rho_{YX} = 0$.

A more interesting question is a test of $H_0 : \beta_1 = 0$ adjusting for a number of covariates where β_1 is the adjusted regression coefficient of the variable of interest. This is equivalent to testing whether the multiple partial correlation coefficient is zero. Gatsonis and Sampson (1989) handle this by partitioning the p independent variables

into groups of size p_1 and p - p_1 denoted by X_1 and X_2 respectively. The appropriate test is of an association between Y and X_1 adjusting for X_2. The simplest case is where p_1=1 , the effect of one variable adjusting for p-1 covariates. The test is then H_0: $\rho_{YX1|X2} = 0$.

In terms of the sample partial multiple correlation coefficient squared

$$r^2_{YX1|X2} = \frac{R^2_{YX1|X2} - R^2_{Y|X2}}{1 - R^2_{Y|X2}}$$

where R^2 is the percentage of variance explained by the regression model.

Using the tables of Gatsonis and Sampson (1989) with various values of the partial multiple correlation coefficient table 10.4 shows the power with $p_1 = 1$.

Table 10.4 Power (%) for partial multiple correlation coefficient with $p_1 = 1$

$\rho_{YX1\|X2}$	n				
	100	250	500	1000	1500
0.10	18	40	61	88	97
0.15	29	67	91	*	*
0.20	51	90	*	*	*

In the Edinburgh Artery Study the following results were obtained for multiple linear regression of age, sex, smoking, and alcohol on the ABPI and the same model with fibrinogen added.

Model		R^2	R
Age, sex, smoking, alcohol	X_2	0.126	0.355
Age, sex, smoking, alcohol + fibrinogen	X_1	0.142	0.377

The multiple partial correlation coefficient for fibrinogen is thus $r_{YX1|X2} = 0.14$. From table 10.4 and with a sample size of 1500, the power is greater than 99% for the case of a partial multiple correlation coefficient = 0.14. For $\rho_{YX1|X2} < 0.1$, power would be inadequate.

Cohen (1988) also gives power tables for this case by calculating λ which is a function of the squared multiple partial correlation coefficient. In addition the number of variables of interest (u) and the number of covariates (w) need to be specified. Then

$v = N - u - w - 1$ and $\lambda = f^2 (u + v + 1)$

$$where\ f^2 = \frac{r^2_{YX1|X2}}{1 - r^2_{YX1|X2}}$$

or

$$f^2 = \frac{R^2_{YX1X2} - R^2_{YX2}}{1 - R^2_{YX1X2}}$$

Note that this implies that w is swamped by N whenever N is large and w the number of covariates is really only a problem when N and w are of comparable size, for example, 50 covariates with 100 subjects.

It would be useful to look at power for a range of partial multiple correlation coefficients and table 10.5 shows this for the case of adding one variable adjusting for 4 covariates.

Table 10.5 Power (%) for partial multiple correlation coefficient for u=1 variable of interest adjusting for w=4 covariates from the tables of Cohen (1988)

$\rho_{YX1\|X2}$	n				
	100	250	500	1000	1500
0.025	<10	<10	<10	<10	12
0.05	<10	<10	18	34	50
0.075	10	20	37	65	81
0.10	14	34	61	89	97
0.15	31	65	92	*	*
0.20	51	90	*	*	*

* Power > 99.5%

The results in table 10.5 are similar to those in table 10.4, so there is consistency in the results of Cohen (1988) and Gatsonis and Sampson (1989), although the latter do not give tables for values < 0.1. In addition, the results in tables 10.4,10.5 for partial correlations are also similar to those for the simple Pearson correlation coefficient (table 10.2).

Using Cohen's tables (1988) the power of the test of fibrinogen on addition to a model containing age, sex, smoking and alcohol is also greater than 99%. However, fibrinogen is strongly related to the ABPI and it is not surprising that the

power is adequate in the Edinburgh Artery Study. When considering dietary risk factors (chapter 3) variables were entered into the regression model adjusting for age, sex, smoking and height and it would be instructive to consider the power in the case of adding vitamin C (energy adjusted), for example, to this basic model. The change in R^2 on adding vitamin C was 0.0025 or 0.25% and the partial multiple correlation coefficient was 0.053. The power from table 5 at the 5% level to detect such an effect size with 1592 subjects is 56%. The power to detect such relatively small population partial correlations is inadequate with this sample size. The question then arises as to whether such small effect sizes are meaningful. Gatsonis and Sampson (1989) do not give power for coefficients < 0.10, but it is obvious that the power for this case would also be inadequate according to their method.

10.2.4 Generalised Linear Models

The methods of Gatsonis and Sampson (1989) and Cohen (1988) only apply to continuous outcomes. The analysis of data from case-control , cohort and cross-sectional studies and randomised controlled trials can all be accommodated within the framework of generalised linear models (McCullagh and Nelder 1989). Power and sample size determination taking into account confounding variables can then be obtained directly from the Fisher information matrix within the context of generalised linear models (GLM). Such a method is outlined by Wilson and Gordon (1986). The method of Wilson and Gordon (1986) assumes an approximate MVN distribution for confounders, depends on the link function specified and does not deal with nominal or multiple outcomes. There are tests for MVN, but Cramer and Wold state that the distribution of a p-dimensional random variable **X** is completely determined by the univariate distributions of all it's linear compounds, so that if every linear compound of **X** is approximately normal <=> **X** has a MVN distribution (Chatfield and Collins, 1984)

Before discussing the method, some background theory is necessary. Let Y be the dependent variable and x_1, x_2, ...,x_p the independent variables of which x_1, say, is of most interest and the rest of the x's are covariates. The independent variables can be binary, nominal, ordinal, or continuous, although the method of Wilson and Gordon (1986) assumes MVN and so strictly only applies to continuous covariates. The distribution of the random variable Y must be from the exponential family and so can be expressed in canonical form (McCullagh and Nelder 1983).

Define $\beta^T = (\beta_0, \beta_1, ...\beta_p)$

Then $\eta_i = g(\mu_i) = \beta^T \mathbf{x}_i$ and g is known as the link function.

For the case of multiple linear regression the link function is the identity -

$$\eta_i = g(\mu_i) = \mu_i = E(Y_i)$$

For multiple logistic regression the link function is the logit, log (p/(1-p)).

If the GLM is valid, the maximum likelihood estimate β asymptotically has a MVN distribution with mean β and variance-covariance matrix $V(\beta) = [I(\beta)]^{-1}$

where $I(\beta)$ is the Fisher information matrix.

If β_1 is the variable of interest, then β_1 is approximately distributed $N[\beta_1, V_{11}(\beta)]$

Let $\beta^{(k)}$ represent the maximum likelihood estimate of β under H_k , k = 0,1.

The test of $H_0 : \beta_1 = \beta_1^{(0)}$ against $H_1: \beta_1 > \beta_{1(0)}$ at α significance level.

$$Let \quad \xi_k = [NxV_{11}(\beta^{(k)})]^{1/2}$$

$V_{11}(\beta^{(k)})$ is expressed in terms of N so that ξ_k does not involve N.

Then power is estimated from

$$z_{1-\beta} = \frac{1}{\xi_1}[\sqrt{(N)}(\beta_1^{(1)} - \beta_1^{(0)}) - z_{1-\alpha/2}\xi_0]$$

In multiple linear regression $\beta_1^{(0)} = 0$ and $\beta_1^{(1)}$ and $V(\beta_1^{(1)}) = \sigma^2 / N \text{ var } (x_1)$ are obtained from the regression model involving x_1. Returning to the power calculations for the case of adding Vitamin C to a regression model on the ABPI, adjusting for age, sex, smoking and height as described earlier for Cohen's method (1988). Using the method of Wilson and Gordon (1986) gives a power of 52.8% which is very similar to that estimated by Cohen's method (1988); a value of 54%. Post hoc power calculations are relatively easy with this method as the values of β_1 and $V_{11}(\beta)$ would be readily available.

10.3 Power - Binary Outcome

In the epidemiological literature the case-control study tends to dominate consideration in estimating sample size, even though such a design has many methodological failings (Hennekens and Buring 1987) and other methods such as cross-sectional surveys and cohort studies are often encountered. There is an extensive literature dealing with the case of a binary outcome and exposure (Walter 1977, Armstrong 1987, Schlesselman 1974, Walter 1980, Lemeshow 1988).

10.3.1 k independent 2x2 tables

The problem of confounding variables is rarely considered. One exception is Gail (1973) who presents power and sample size for a dichotomous outcome and exposure allowing for k strata, that is, k independent 2x2 tables. This method assumes constant odds ratio over all k strata and that the exposure rate in controls and cases; p_{0j} , p_{1j} respectively, lie in the interval (0.1, 0.9). The power is estimated by

$$z_{1-\beta}=\sqrt{n\Sigma f_j g_j}-z_{1-\alpha/2}$$

where f_j is the proportion of subjects in the j th stratum and

$$g_j=\frac{(\ln OR)^2}{(1/(p_{0j}(1-p_{0j}))+1/(p_{1j}(1-p_{1j})))}$$

and OR is the odds ratio assumed constant over the k strata. If only one stratum is considered this is equivalent to using the simple formula comparing two independent proportions (Schlesselman 1982)

$$z_{1-\beta}=\sqrt{\frac{n(p_1-p_0)^2}{2\bar{p}\bar{q}}}-z_{1-\alpha/2}$$

where p_0 is the proportion exposed in the controls and

$p_1 = p_0\ OR/[1 + p_0\ (OR - 1)]$ and

$$\bar{p}=\frac{1}{2}(p_0+p_1) \quad and \quad \bar{q}=1-\bar{p}$$

Gail (1973) gives a table of g_j for values of OR and p_{0j} to facilitate these calculations. Using the 8 strata from the case-control study of the Edinburgh Artery Study (Chapter 2), that is, four age groups in males and females , with the number of cases equal to 153 the estimate of power with two confounding variables can then be compared to that obtained with one stratum, that is, in the unadjusted case. Table 6 shows an example of the procedure with p_{0j} for the exposure rate to smoking (ever vs never) obtained from the case-control study of the Edinburgh Artery Study, assuming these are likely population values.

Table 10.6 Calculation of estimated sample size using Gail's (1973) method with data from the Edinburgh case-control study

Strataj	p_{0j}	f_j	g_j	$f_j g_j$
1	0.100	0.065	0.0269	0.00175
2	0.571	0.137	0.0506	0.0069
3	0.25	0.157	0.0497	0.0078
4	0.20	0.131	0.0447	0.00586
5	0.571	0.046	0.0506	0.00233
6	0.538	0.085	0.0536	0.0046
7	0.471	0.111	0.0565	0.0063
8	0.683	0.268	0.0413	0.0111
				$\Sigma f_j g_j = 0.0465$

For $\alpha = 0.05$, n=153 and OR = 2.0

$z_{1-\beta} = 0.707$, Power = 77.9%

Table 10.7 shows the power estimates with 8 strata and one stratum from Gail (1973) showing the decrease in power when adjusting for two confounders.

Table 10.7 Power (%) according to Gail (1973) for 8 strata and 1 stratum (unadjusted)

Study design		Number of cases			
		100	150	200	300
OR = 1.5	8 strata	25	36	45	61
	1 stratum (Unadjusted)	29	41	48	69
OR = 2.0	8 strata	58	75	86	96
	1 stratum (Unadjusted)	67	83	92	98

The results in table 10.7 demonstrate that the Edinburgh Case-Control study (n = 153)

is just adequate with power > 70% to detect odds ratios ≥ 2.0 when allowing for two covariates with the 8 strata identified in this study. The reduction in power is 5-8% allowing for age and sex compared to the unadjusted analysis. In the actual analysis of the case-control study (chapter 2) there was adjustment for more than 2 covariates and hence the number of strata would be greater than 8 and so the power would be even more reduced than that shown in table 10.7. These results are based on a particular study. In practice, the power would be calculated for a range of p_{0j} values and with different numbers of strata.

This method is fine for $N_j > 15$ and p_{0j} and p_{1j} lying in the interval (0.1, 0.9), which is reasonable for the Edinburgh data. It also assumes equal subjects divided between treatments or equal cases and controls and the confounders are categorical. Of course continuous covariates can also be categorised but this leads to a subsequent loss of information. This formula (Gail 1973) is based on tests such as the Mantel-Haenszel (1959) procedure. Greenland (1985) presents a method for multiway tables using the Wald statistic, of which Gail (1973) is a special case. Muňoz and Rosner (1984) also tackle the problem of power estimation by using an approximation for the asymptotic power of the Mantel-Haenszel (1959) procedure for the test of a common odds ratio in a collection of 2x2 tables.

10.3.2 Logistic regression

Logistic regession is a more versatile alternative to the Mantel-Haenszel procedure for the analysis of case-control studies, and is also appropriate in cohort and cross-sectional studies with binary outcomes. In addition, the covariates can be binary, ordinal or continuous. A more general formula for power and sample size based on logistic regression with small response probability and n covariates was proposed by Whittemore (1981). This approach is based on an approximation to the Fisher

information matrix I (θ) = E[- $\delta^2 l / \delta\theta\delta\theta'$] for the estimated parameters in a multiple logistic regression. This means that estimates are needed for the regression coefficients of each variable and the correlation matrix. Hsieh (1989) presents easy to use tables derived from Whittemore (1981) and gives an extension where the regression coefficients do not have to be given. The estimate of power with one covariate is given by

$$z_{1-\beta} = \frac{\sqrt{\frac{(n_1 p \theta^{*2})}{(1+2p\delta)}} - z_{1-\alpha/2}}{e^{(-\theta^{*2}/4)}}$$

where

$$\delta = \frac{[1+(1+\theta^{*2})\, e^{(5\theta^{*2}/4)}]}{[1+e^{(-\theta^{*2}/4)}]}$$

and p is the probability of the event at the mean values of all covariates and $\theta^* = \ln r$ (r is the odds ratio of disease corresponding to an increase in x of 1 s.d. from the mean, given the values of the remaining covariates) , that is,

$p(x) = p(y=1|x_1 \ldots x_k)$

The model expresses the logit of p as a linear combination of the x's

$$\ln[p/(1-p)] = \theta_0 + \theta_1 x_1 + \ldots + \theta_k x_k$$

The above formula only applies to the case of one covariate and Hsieh (1989) provides an upper bound for sample size

$$n_m = \frac{n_1}{(1-\rho^2)}$$

where ρ is the multiple correlation coefficient of x_1, with a number of covariates x_2, ...x_k. Hence n_1 can be substituted by n_m $(1 - \rho^2)$ in the power formula, where each of the x_i have been normalised with mean 0 and variance 1. The tables (Hsieh 1989) are easy to use and suitable for high or low event probabilities.

Assuming a case-control study with equal numbers of cases and controls then the probability of being a case with a mean level of the variable of interest is 0.5. Table 10.8 shows the power to detect odds ratios of 1.5 and 2.0 for a subject with a 1 S.D. increase in fibrinogen for various numbers of cases. In order to detect the same odds ratios adjusting for age, sex, and smoking, which has a multiple correlation of 0.29 with fibrinogen n_1 is replaced with n_m $(1 - \rho^2)$ in the power formula (table 10.8).

Table 10.8 Power (%) for odds ratios of 1.5 and 2.0 from tables of Hsieh (1989)

Model		Number of cases			
		100	150	200	300
OR = 1.5	Fibrinogen	48	65	78	92
	Fibrinogenl age, sex,smoking	44	61	74	89
OR = 2.0	Fibrinogen	84	96	99	*
	Fibrinogen I age, sex,smoking	80	94	98	*

Table 10.8 shows that the power is only reduced by about 4% after adjustment for age, sex, and smoking suggesting that the multiple correlation coefficient needs to be relatively large to give a substantial reduction in power. As before, with many covariates the calculations would be repeated for likely values of the population multiple correlation coefficient between the $x_1,....x_p$.

Whittemore (1981) showed that inclusion of covariates which are correlated with x_1 but independent of the event (i.e. $\theta_2 = \theta_3 =...=\theta_k = 0$) leads to a loss of power when testing for association of x_1 with the event. This suggests that adjustment should only be made for true covariates, that is, factors which are related both to the outcomes and the risk factor being considered.

An adjustment of the overall p-value for multiple significance testing may be needed when several covariates are of interest. Having looked at the power for various postulated population statistics for continuous and binary outcomes and with specific examples from the Edinburgh Artery Study the next section considers the problem of estimating sample size for a given power at the design stage of a study.

10.4 Sample size - Continuous outcomes

10.4.1 Pearson Correlation Coefficient

As outlined earlier when considering power for continuous outcomes the test statistic for the population Pearson correlation coefficient ρ is

$$T = r\frac{\sqrt{(n-2)}}{\sqrt{(1-r^2)}}$$

which follows a t-distribution with n-2 degrees of freedom. Assuming n is large and T ~ N (0,1) we can estimate sample size by

$$n = (z_{1-\alpha/2} + z_{1-\beta})^2 \frac{(1-r^2)}{r^2}$$

Table 10.9 shows the sample sizes required for various values of ρ with power from 80%-95%.

Table 10.9 Sample size estimation for Pearson Correlation Coefficient (ρ)

for α= 0.05 and power = 80%, 90% and 95%

ρ	95%	90%	80%
0.05	5171	4188	3128
0.075	2298	1856	1396
0.10	1283	1039	776
0.15	563	456	341
0.20	311	252	188
0.25	194	157	118
0.30	131	106	79
0.35	93	75	56
0.40	68	55	41

Clearly, in cross-sectional studies where the association with a quantitative outcome is weak, such as r = 0.05, for example, large numbers would be required, in fact 3128 to give 80% power.

10.4.2 Multiple Linear Regression

As shown when considering power, if a covariate is correlated with the outcome and the risk factor of interest the partial correlation will generally be lower than the Pearson Correlation Coefficient and so the sample size needed to detect significant correlation when adjusting for covariates would be higher. With more covariates the need for a larger sample is increased depending on the precise nature of the relationship between the variables in terms of their correlations.

Gatsonis and Sampson (1989) present a method for assessing sample size in multiple linear regression with tables for p independent variables, dependent variable y and multiple correlation coefficient ρ_{YX}. From tables (Gatsonis and Sampson, 1989) with p = 20 and various values of R the sample sizes calculated are given in table 10.10

Table 10.10 Sample size estimates from Gatsonis and Sampson (1989) with p = 20

Multiple Correlation coefficient ρ_{YX}	Power		
	95%	90%	80%
0.15	1356	1156	1020
0.20	759	648	522
0.25	482	412	334
0.30	333	285	231
0.40	183	156	130

The number of variables is important and p = 20 would not be exceptional in an observational study. This method appears limited in that the $H_0 : \rho_{YX} = 0$ is generally

not of great interest. The more usual case is of testing one variable while adjusting for a number of covariates. In order to address this question Gatsonis and Sampson(1989) partition X into two sets X_1 and X_2 where X_1 is the set of variables of interest and X_2 is the set of covariates. By replacing ρ_{YX} by $\rho_{YX1|X2}$ in the tables (Gatsonis and Sampson 1989) and p_1 = number of variables of interest, the appropriate sample size can be assessed (table 11).

Table 10.11 Sample size with one variable of interest ($p_1 = 1$) adjusting for 20 covariates ($p - p_1 = 20$), $\alpha = 0.05$

$\rho_{YX1\|X2}$	Power		
	95%	90%	80%
0.10	1313	1066	802
0.15	691	482	366
0.20	339	278	213

In practice partial multiple correlations < 0.10 will often be encountered but unfortunately Gatsonis and Sampson (1989) do not give tables for this situation. In addition, more than one test would be carried out and so the type I error would be adjusted to a lower value α/n where n is the expected number of tests to be carried out.

Cohen (1988) gives sample size tables in terms of the noncentrality parameter λ which is a function of the squared partial multiple correlation coefficient. The parameters λ and v are functions of n , which is to be determined, and so the assessment involves some iteration using an initial value of v.

The sample size is given by

$$N=\lambda\frac{(1-r^2_{YX1|x2})}{r_{YX1X2}{}^2}+w$$

where w is the number of covariates and $r_{YX1|X2}$ is the sample partial multiple correlation coefficient.

Table 10.12 Sample size for various $\rho_{YX1|X2}$ adjusting for w = 20 covariates with u = 1 variable of interest, with significance level α.

$\rho_{YX1\|X2}$	α	Power		
		95%	90%	80%
0.025	0.05	20807	16809	12492
	0.01	28482	23845	18728
0.05	0.05	5207	4209	3132
	0.01	7122	5965	4688
0.075	0.05	2308	1868	1393
	0.01	3153	2642	2079
0.10	0.05	1307	1059	792
	0.01	1782	1495	1178
0.15	0.05	579	471	355
	0.01	785	660	523
0.20	0.05	332	272	207
	0.01	447	378	301

The results in table 10.12 are similar to those of Gatsonis and Sampson (1989) in table 11. Cohen's method (1988) shows how the sample size increases dramatically with $\rho_{YX1|X2} < 0.1$ and such values are not unusual as shown by the Edinburgh Artery Study. Of course many such t-tests of the regression coefficients would be undertaken and one way of dealing with this is to reduce the α to 0.01, if say 5 such tests were

carried out. Thus, the sample sizes given for $\alpha = 0.01$ are probably more relevant to the actual analysis carried out and table 12 shows that the sample size increases considerably in this case.

10.4.3 Generalised Linear Models

Rearranging the formula for power for the method of Wilson and Gordon (1986) sample size is estimated by

$$N = \left[\frac{(z_{1-\alpha/2}\xi_0 + z_{1-\beta}\xi_1)}{(\beta_1^{(1)} - \beta_1^{(0)})} \right]^2$$

Assuming the population regression coefficient and variance are similar to that for vitamin C in the example used earlier the sample sizes required to give 80%, 90%, and 95% power are 3018, 4041, and 4988 respectively. The power to detect Vitamin C on adding it to a model with four covariates was about 53% with a sample size of 1592. Clearly, the sample size of 1592 for the Edinburgh Artery Study gives inadequate power to detect such effect sizes as that of vitamin C, adjusting for four covariates.

The main drawback is in specifying β_1 and the $V_{11}(\beta)$, which is possible once a study or pilot study is completed, but difficult at the design stage with little prior information. The advantage of this method lies in the generalisability of linear models for different outcomes, through the use of the link function.

10.5 Sample size- Binary Outcome

10.5.1 k 2x2 independent tables

In case-control studies and hence binary outcomes with k strata the method of Gail (1973) can be used to assess sample size. Rearranging the power equation given earlier the number of cases can be estimated by

$$n=\frac{(z_{1-\alpha/2}+z_{1-\beta})^2}{\Sigma f_j g_j}$$

where f_j is the proportion of subjects in the j th stratum and

$$g_j=\frac{(\ln OR)^2}{(1/(p_0j(1-p_0j))+1/(p_1j(1-p_1j)))}$$

where OR is the odds ratio assumed constant over the k strata. The values of p_{0j} , the exposure rates in the controls needs to be specified for each stratum. Assuming the p_{0j} reflect likely population values the exposure rates to smoking (ever vs never) from the Edinburgh case-control study from 8 strata have been used to give sample sizes for odds ratios of 1.5 and 2.0 in table 10.13. The total sample size required is 2 x n assuming an equal number of controls.

Table 10.13 Comparison of estimated sample size unadjusted and adjusted for two covariates

Study design		Power		
		95%	90%	80%
OR = 1.5	8 strata	750	624	470
	Unadjusted	647	522	388
OR = 2.0	8 strata	278	225	170
	Unadjusted	226	183	136

The increase in sample size is approximately 100 cases and 100 controls when adjusting for these two covariates to detect an odds ratio of 1.5. The increase when adjusting for covariates is less when trying to detect odds ratios of 2 or greater. These calculations were based on values from the Edinburgh case-control study and the calculations would be repeated for different values of p_{0j} and j.

10.5.2 Logistic Regression

Hsieh (1989) presents easy to use tables derived from Whittemore (1981) and gives an extension where the regression coefficients for the logistic regression do not have to be given. The estimate of sample size is given by

$$n_1 = [z_{1-\alpha/2} + z_{1-\beta} e^{(-\theta^{*2}/4)}]^2 \frac{(1+2p\delta)}{(p\theta^{*2})}$$

where p is the probability of the event at the mean values of all covariates and θ^* = ln r (r is the odds ratio of disease corresponding to an increase in x of 1 s.d. from the mean, given the values of the remaining covariates).

Hsieh (1989) provides an upper bound for sample size

$$n_m = \frac{n_1}{(1-\rho^2)}$$

where ρ is the multiple correlation coefficient of x_1, with x_2, ...x_k.

The tables (Hsieh 1989) are easy to use and suitable for high or low event probabilities and for any type of study with a binary outcome.

Table 10.14 Estimates of sample size for logistic regression (Hsieh1989)

(α= 5% one-tailed)

Power		Probability of event p			
		0.01	0.05	0.10	0.20
OR=1.2	80%	18889	4086	2236	1311
	90%	26120	5651	3092	1813
	95%	32967	7132	3903	2288
OR=1.5	80%	3751	823	457	274
	90%	5154	1131	628	376
	95%	6478	1421	789	473
OR=2.0	80%	1237	285	166	106
	90%	1674	385	224	144
	95%	2084	480	279	179

To obtain sample size for multiple logistic regression, the number in table 10.14 is divided by a factor of 1 - ρ^2 (table 10.15).

Table 10.15 Estimates of sample size for logistic regression (Hsieh 1989) with OR=1.5 and range of values of MCC of the covariates.

	ρ	Power		
		80%	90%	95%
Probability of event p 0.05	0.25	878	1206	1516
	0.50	1097	1508	1895
	0.75	1881	2585	3248
Probability of event p 0.10	0.25	487	670	842
	0.50	609	837	1052
	0.75	1045	1435	1803

For example, fibrinogen has a multiple correlation coefficient with age, sex, and smoking of 0.29, so that if a model is fitted with these four variables, the sample size in the tables should be increased by a factor of $1/(1 - \rho^2) = 1.092$.

10.6 Sample size and measurement error in dietary studies

As discussed in chapter 3 measurement error in assessment of dietary intake can effect the estimates and precision of regression coefficients. An additional effect is that measurement error leads to reduced power and the need for larger sample sizes. This problem has been shown for cohort and case-control studies investigating diet-cancer links (Prentice et al 1989, Freudenheim et al 1988, and Hebert et al 1988) and suggested conventionally sized studies which show non-significant associations could be explained in terms of loss of power. Freedman et al (1990) suggests a simple model of measurement error could be incorporated into the calculation of sample size. The model is :

$$Y = X + \varepsilon \quad \text{where } \varepsilon \text{ follows } N(0, \sigma^2)$$

and Y is the measured nutrient intake and X represents the true intake. If Y and X are normally distributed then they have the same mean and the 'true' standard deviation can be expressed as

$$SD_X = \rho\, SD_Y \quad \text{where } \rho \text{ is the correlation coefficient between Y and X}$$

Under this model the measured nutrient values have no bias but greater variance than the true value. The result of this greater variance is a reduction in power. Freedman et al (1990) then incorporate this model into the sample size calculations for a test of linear trend in a cohort study (Breslow and Day, 1987). For a correlation coefficient of 0.65 between observed and true percentage fat intake the sample size was inflated by 6-8 times compared to no measurement error in assessing the relative risk for colorectal cancer. This method requires a validation study to be incorporated into the overall design and in terms of nutrient intake this implies a weighed intake study for a subsample of the full study which uses a food frequency questionnaire (chapter 3).

10.7 Discussion

This chapter has surveyed the main methods of estimating power and sample size for epidemiological studies. The results have shown that reliance on simple methods for two-group comparisons underestimate the sample size and overestimate the power for the case of numerous confounders, a situation which is often encountered in observational studies. At the design stage the methods outlined are fine if some information on the correlation matrix of variables involved and some idea of the likely effect size is known. In this case the methods lend themselves to a form of sensitivity analysis where a likely range of correlations can be imputed into the formulae to calculate power and sample size. An example of this was outlined in this chapter on various partial multiple correlation coefficients. However, often in a new study prior information is sparse and it is difficult to assess the likely correlation structure. This is another area in which a pilot study can contribute to the design of a study.

10.7.1 Continuous outcomes

If the outcome is continuous, there is a loss of power in transforming to a binary outcome. The method of Wilson and Gordon (1989) is initially theoretically attractive as GLM encompass most situations, including binary outcomes. However, it is necessary that the covariates should be MVN. In reality, binary, ordinal and nominal data are often encountered , so this restriction is difficult to meet. Cohen's method (1988) relies on the change in R^2 on adding covariates of whatever type to the regression model and seems simpler to use in practice. Although Cohen's tables (1988) were based on approximations, they gave results similar to the more exact methods and so seem adequate, especially since sample sizes are only approximate guides in any case.

10.7.2 Binary outcomes

For binary outcomes, the tables of Hsieh (1989) are easy to use and based on logistic regression, which is a common form of analysis. This method allows sample size estimates to be made for a range of multiple correlation coefficients of the covariates and probabilities of the outcome.

10.7.3 Multiple testing

Most of the calculations assumed a single test at the 5% level whereas in reality many tests are usually carried out. A simple way of allowing for this is to calculate power or sample size at significance level of 0.05/k where k is the number of tests. This gives an overall significance level of 0.05 for each test. This was discussed for Cohen's method (1988), and the results showed a large increase in sample size when allowing for multiple testing in this way. Even in studies which explain their sample size calculations, these are usually based on one two-group comparison at the 5% significance level, and then numerous tests are carried out in the analysis (Sulaiman et al 1988).

Measurement error, especially in dietary intake studies can also reduce the power of tests and so allowance for this can be made by incorporating a simple error model in the sample size calculations.

10.7.4 Software

EPIINFO was designed to analyse proportions and it gives sample size and power estimates for the estimation of a single proportion and for comparing two proportions based on simple formulae, for example from Fleiss (1981). The programs do not take confounding into account in the calculations or multiple testing nor for studies involving continuous outcomes. Clearly there is scope for the development of such power and sample size estimation packages.

More recently, the software EGRET SIZ was launched based on the method of Self et al (1992). This covers conditional and unconditional logistic regression models, Poisson regression and Cox proportional hazard models. The method of Self et al (1992) uses GLM and is based on an approximation to the distribution of the likelihood ratio statistic. The program allows various plots and simulations to be carried out and gives estimates of power and sample size allowing for up to 10 variables per regression with 1 exposure variable and up to 9 confounder and matching variables. Although all the variables have to be categorical, and these are specialised models, this software does provide a useful addition to sample size/power estimation in planning epidemiological studies.

CHAPTER 11

DISCUSSION

11.1 Introduction

This chapter will summarise the analysis of individual groups of risk factors considered in the previous chapters, followed by an assessment of all risk factors together as described under the groupings of lifestyle factors and lipid/rheological risk factors in chapter 9. Then, the implications for prevention and treatment of peripheral arterial disease are discussed, based on the foregoing analysis of the Edinburgh Artery Study and evidence from other studies. Finally, recommendations for future research are presented.

11.2 Analysis of individual risk factors

11.2.1 Diet

Fibre

Despite the methodological drawbacks of Food Frequency Questionnaire derived nutrient intake, significant associations were found with the ABPI. Higher energy adjusted cereal fibre was significantly associated with higher levels of the ABPI in men only. In general men have a lower proportion of fibre in their diet compared to women (Gregory et al 1990), and so could benefit most from an increase in their dietary intake. This result was also consistent with a Greek hospital based case-control study which showed a significant association of low crude fibre with peripheral arterial disease (Katsouyanni et al 1991).

Alcohol

The positive association of alcohol with the ABPI in men reflects previous results where moderate alcohol consumption was associated with a decrease in the risk of coronary heart disease and ischaemic stroke in women (Stampfer et al 1988) and reduced cardiovascular mortality in middle-aged men (Marmot et al 1981), which has led to the widely held belief that moderate intake of alcohol may be protective for atherosclerosis. Such results have led to the consideration of a U-shaped curve for mortality from coronary heart disease and alcohol intake (Shaper et al 1988). However, in the British Regional Heart Study of men aged 40-59, Shaper et al (1988) concluded that the U-shaped relationship could be explained by other factors such as social class, and the movement of ex-drinkers with pre-existing disease into the non-drinking category. Later studies have addressed these methodological problems, such as in a study of New Zealand men and women, which showed that both moderate drinkers and ex-drinkers had lower risk of non-fatal myocardial infarction compared to total abstainers (Jackson et al 1991).

The Edinburgh results suggest a linear relationship with morbidity in men rather than a U-shaped relationship, indicating that the higher the alcohol intake the greater the benefit in terms of the ABPI. This seems unlikely, given that high alcohol intake is associated with increased risk of high blood pressure (Klatsky et al 1977), along with the attendant health and social problems (Smith 1981). On adjusting for social class and cigarette smoking in the Edinburgh Artery Study this linear association was considerably reduced (Jepson et al in press). The higher alcohol intake in social classes I and II was mostly due to wine rather than beer, and it has been suggested that wine may be beneficial for coronary heart disease in France despite high intake of saturated fat (Renaud and de Longeril 1992). In the Edinburgh

Artery Study the wine effect could be explained by the higher alcohol intake in the social classes I and II who tended also to have healthier diets, and smoke less than lower social classes. There is thus little evidence from the Edinburgh Artery Study data of a U-shaped relationship between alcohol and the ABPI and the linear relationship that was found could be explained by cigarette smoking and social class, so there was little evidence of any protective effect of alcohol (Jepson et al in press). This was also the conclusion reached by Shaper et al (1988) in their assessment of the U-shaped relationship with cardiovascular mortality. Even if alcohol were protective, this would have to be balanced against the fact that increasing alcohol intake would increase the mean level of intake in the population (Rose and Day 1990) and hence increase the prevalence of problem drinkers. Further analysis considering mortality in the follow-up of the Edinburgh population is planned and it will be interesting to assess the possible relationship of mortality and cardiovascular events to alcohol intake measured at the cross-sectional stage.

Vitamins

The associations of vitamins with the ABPI were consistent with previous studies concerned with atherosclerosis. Smoking reduces plasma vitamin levels of β-carotene, vitamin C and α-tocopherol (Stryker 1988, Shectman 1989), so it would be expected that smokers would benefit most from vitamin supplementation, if unwilling or unable to give up smoking. Unfortunately in a Bristol survey it was shown that supplements were more likely to be taken by already healthier individuals, that is, women and social classes I and II (Gregory et al 1990). The association with α-tocopherol, although weak, is consistent with studies showing a link between low vitamin E and mortality from coronary heart disease (Gey and Puska 1989) and

increased risk of angina pectoris (Riemersma et al 1991). Indeed vitamin E has been used as a treatment for intermittent claudication (Livingstone 1958, Haegar et al 1974, Williams et al 1971, Pinsky 1980) and angina (Gillilan et al 1977). This link is clearly worth investigating, especially as vitamin E intake is lower in lower social classes, who also have higher cigarette consumption and lower mean ABPI values.

11.2.2 Leisure Activity

Men

The greatest benefit of taking part in leisure time activity when aged 35-45 on present disease status (time of the survey) occurred in males, and especially in males who had ever smoked. This does not indicate that exercise was of no benefit to those males who never smoked, but that the relative benefit was greater in those who had ever smoked. This is consistent with other findings, which suggest positive associations between high risk of coronary heart disease and lack of leisure time activity (Kannel et al 1979a, Paffenbarger et al 1978, Morris et al 1973, Morris et al 1980).

The proportions indulging in moderate/strenuous exercise were significantly higher in higher social classes in both males and females. This was consistent with a study of Swedish women which showed lower cardiovascular disease and total mortality in higher socioeconomic groups who indulged in more physical activity (Lapidus and Bengtsson 1986). Taking part in leisure activity when aged 35-45 years was also positively related to hostile acts in males and vitamin C intake, but remained significant after adjustment for these factors in the multiple regression analysis.

Mechanism

The effects of exercise are well known on blood rheology and include reduction of blood and plasma viscosity (Charm et al 1979, Ernst 1985). In one study, exercise was shown to increase pain-free walking distances in intermittent claudication patients (Edzard et al 1987) and this was achieved by increased 'fluidity' of blood. Hence part of the benefit from regular exercise in the general population may be related to increased blood fluidity indicated by lower blood viscosity. Other known effects of physical activity are reductions of nonHDL cholesterol and increased HDL cholesterol (Haskell 1984).

As leisure activity tends to reduce blood viscosity (Ernst 1985) so it would be expected that when rheological factors were added, leisure activity would no longer have a significant independent effect on the ABPI. However, in males who ever smoked there was still some indication of a positive association for strenuous/moderate activity at the 10% level, allowing for other lifestyle and rheological factors. So it appeared that blood viscosity was not the sole mechanism by which leisure activity could have had a beneficial effect on the ABPI.

Women

Although taking part in leisure activity was associated with social class in women, it was not significantly related to the ABPI. This may in part be due to the low number of women aged 35-45 years who took part in strenuous activity, or the questionnaire design is biased towards activities which men are more likely to indulge in. It was notable that activities such as childminding, gardening and general housework were not covered by leisure activity categories or occupational categories; and these would tend to involve many women aged 35-45 over long periods of time.

Certainly, the questionnaire seems more likely to pick up activities involving organised sport rather than physical activity in general. There could also be a sex difference in the recall of leisure activity, although Blair et al (1991) found no evidence for such a bias.

Occupational Activity

The lack of an association between occupational activity and atherosclerosis was surprising, given that coronary heart disease has been shown to be related to occupational activity (Morris et al 1953). However, the sample surveyed in the Edinburgh Artery Study did not include a large proportion of manual classes, so the occupational activity classification may have been less relevant. Also the classification of occupational activity was relatively crude (Appendix 1).

11.2.3 Personality

The results on personality showed a weak significant relationship between hostile acts adjusting for age and cigarette smoking and the odds of having intermittent claudication in males but not in females. This is consistent with results suggesting impatience and the expression of hostility are linked to atherosclerosis (Evans 1990, Sykes et al 1992).

The weak association between hostile acts in men and the ABPI became non-significant on adjusting for lifestyle factors such as diet and leisure activity when aged 35-45. Overt hostile acts in males were also highly correlated with cigarette smoking ($r = 0.14$) and triglyceride levels ($r = 0.13$) (Fowkes et al 1992c). The mechanism of how hostility might operate via triglyceride levels may be similar to the hypothesis that lower serum cholesterol lowers brain serotonin which in turn reduces

the suppression of aggressive behaviour (Engelberg 1992, Zuckerman 1991).

The Edinburgh results suggest that hostility should be assessed rather than the type A construct which may not be as relevant to British or European populations as the U.S population or if a type A inventory is used it should incorporate items on hostility. A recent comparison of three European countries did show a relationship between mortality from ischaemic heart disease and the personality dimensions of hard-driving and impatience (Sykes et al 1992). The Edinburgh results also suggested that hostility was related to other risk factors and may not be an independent risk factor for peripheral arterial disease. A synergism between smoking and hostility has also been suggested by other studies (Epstein and Perkins 1988, Grossarth-Maticek et al 1988) and there was a significant interaction (chapter 9), although this was not independent of the leisure activity by smoking interaction.

The Edinburgh type A inventory (Bortner 1969) did not show strong associations with peripheral arterial disease possibly because it did not contain items on hostility, and also it may not have been relevant to a largely retired population, who presumably show less competitiveness and time pressure than younger populations. It was also self-completed rather than derived from a structured interview and hence may have been influenced by symptoms of disease. The latter explanation, a problem of the cross-sectional methodology was suggested as a possible reason for the association between type A behaviour and prevalent heart disease in the Caerphilly study (Gallacher et al 1988). The Bedford-Foulds Inventory (Bedford and Foulds 1978) which assessed hostility, domineering attitude and overdependency made some attempt to overcome this drawback by careful phrasing of the initial statement in the questionnaire; respondents were asked how they would behave over 'most of your life'.

11.2.4 Rheological Factors

Fibrinogen and blood viscosity

In men blood viscosity and its components, especially fibrinogen were important risk factors for peripheral arterial disease in the Edinburgh Artery Study. Plasma fibrinogen is a well known risk factor for peripheral atherosclerosis (Fowkes et al 1991) and high levels of fibrinogen have been shown to be strongly related to smoking (Lee et al 1990). As fibrinogen is a component of blood viscosity it was perhaps surprising that each had strong and independent associations with the ABPI in men. There was a synergistic effect of smoking on the relationship between fibrinogen and the ABPI. The effect of smoking could be via damage to the endothelial cells of arteries, which then allows fibrinogen to infiltrate the arterial wall. This, in turn, promotes platelet aggregation a process which is consistent with raised levels of von Willebrand factor and cross-linked fibrin degradation products which are found in cases of peripheral arterial disease (Smith et al 1993).

In women, there were also strong associations of blood viscosity and fibrinogen with the ABPI, but these could be explained by smoking in terms of the packyears. This evidence suggests that raised fibrinogen and blood viscosity levels in women with peripheral arterial disease may be largely due to heavy cigarette smoking, and thus cigarette smoking was the main independent risk factor for peripheral arterial disease in women.

Arterial Blood Pressure and rheological factors

The relationship of arterial pressure to peripheral arterial disease was assessed in an indirect way, since it is a component of the ABPI, by relating rheological factors

to both systolic and diastolic blood pressure (chapter 7). Fibrinogen was not independently related to systolic or diastolic arterial pressure and this was consistent with other studies in the general population (Karsen-Bengtsen et al 1972). Blood viscosity was significantly associated with systolic blood pressure in males, so that blood pressure can be indirectly related to peripheral arterial disease via blood viscosity in men. In women, systolic pressure was related to uric acid, but uric acid was not significantly related to peripheral arterial disease. This was another difference in the role of risk factors for peripheral arterial disease between men and women.

Even though an association was found between blood viscosity and systolic pressure and by implication to disease, it was not possible to assess whether the relationship was causal. A longitudinal study would be necessary to distinguish whether high systolic pressure was a risk factor for or a result of peripheral arterial disease. The 26-year follow-up of the Framingham study (Kannel and McGee 1985b) suggested an increased risk of intermittent claudication for systolic pressures $\geq$ 180 mmHg, so there is some evidence that systolic pressure is a risk factor for atherosclerosis.

Fibrinogen Haplotype

Fibrinogen haplotype was not found to be significantly associated with plasma fibrinogen level in the case-control study (chapter 8). Perhaps surprisingly fibrinogen haplotype was related to peripheral arterial disease independently of fibrinogen and smoking. Thus genetic factors are clearly worth investigating further, but it was not possible to extend the analysis to the whole cross-sectional data at present.

11.2.5 Diabetes

It is well known that diabetes is related to microvascular disease in the lower limbs, although it is not clear whether it is a risk factor for peripheral arterial disease or whether arterial disease promotes diabetes (Strandness et al 1964).

Although the test for a sex by diabetes (known vs non-diabetic) interaction was not significant, when the analysis was split by males and females, having known diabetes was significantly associated with a low ABPI in males only. This suggested that diabetes increases the risk of disease, only in men. This was the opposite result to that found for coronary heart disease in women in the Framingham Study (Kannel et al 1985a). On the other hand, the incidence of intermittent claudication was higher for diabetics compared to non-diabetics in the Framingham study (Kannel and McGee 1979b) and in Finland (Uusitupa et al 1990) and these were consistent with the Edinburgh results.

11.2.6 Cholesterol

Dietary cholesterol was not significantly related to the ABPI in the total population, which may in part be due to the underestimation of nutrients from food frequency questionnaire data. Also dietary cholesterol was not correlated with serum total cholesterol ($r = -0.01$). However, saturated fat intake was correlated with serum total cholesterol ($r = 0.13$) and nonHDL cholesterol ($r = 0.11$). The only link found between dietary cholesterol and serum nonHDL cholesterol was in male non-smokers. On removing nonHDL cholesterol from the regression model dietary cholesterol approached significance ($p = 0.07$) for this group. Thus, there was a suggestion that in male non-smokers the most important risk factor was nonHDL cholesterol which

was influenced by dietary cholesterol intake or dietary saturated fat intake (Figure 11.1). However, caution should be exercised as this came from a subgroup analysis with relatively weak associations.

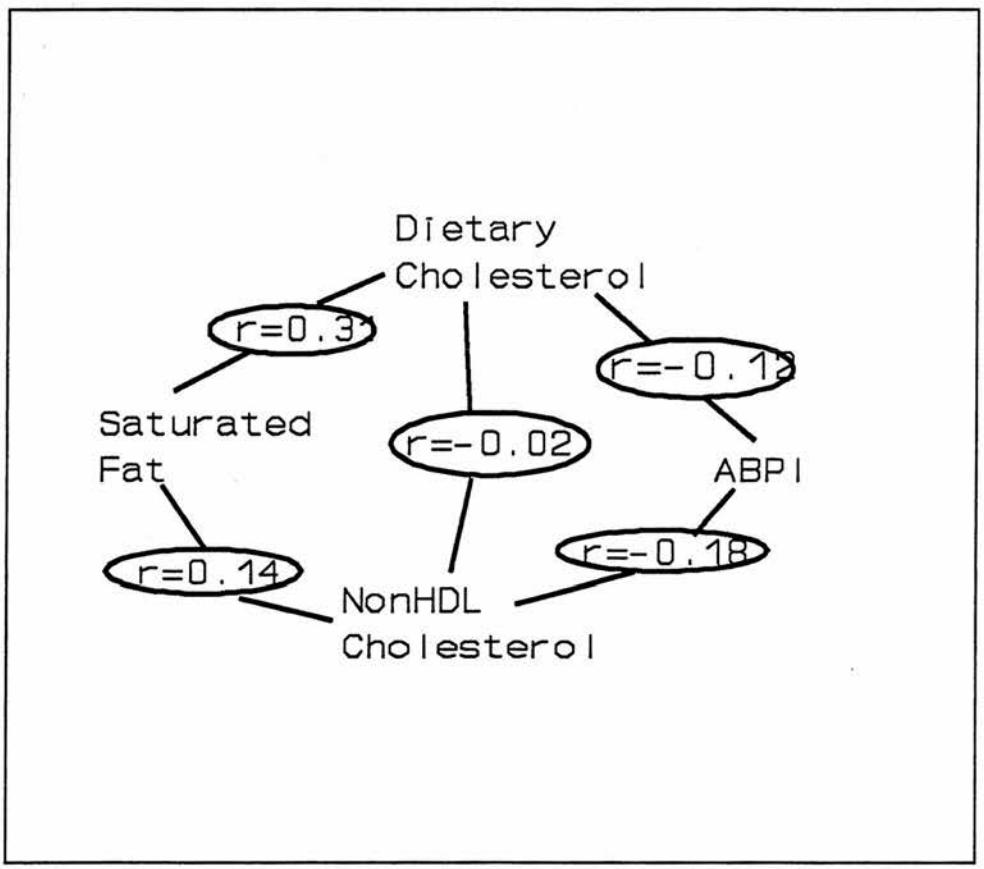


Figure 11.1 Relationships between Dietary Cholesterol, Saturated fat, and the ABPI in male non-smokers

Lowering total cholesterol has been shown to reduce risk of coronary heart disease (Kannel et al 1971, MRFIT 1982, Frick et al 1987). Reductions of serum cholesterol have also shown reductions in total mortality in epidemiological studies (Anderson et al 1987), although some studies suggest an increase in mortality from other causes (Muldoon et al 1990). The Oslo clinical trial study showed significant reduction in mortality (Hjermann et al 1986) due to dietary lipid lowering. However, a reduction in total mortality may not be the best indicator of the value of lowering serum cholesterol; quality of life could be improved by a reduction of suffering for large numbers of people with CHD, whether they live longer or not (LaRosa et al 1990). Dietary reduction of cholesterol has also been shown to reduce the risk of coronary heart disease and total mortality, after 15 years of follow-up of the Seven Countries Study (Keys et al 1986) and after 19 years of follow-up in the Western Electric Study (Shekelle et al 1981). Despite these results there have also been conflicting results and Oliver (1991) suggests that population lipid-lowering is still controversial.

MacRury et al (1992) showed an inverse relationship between serum cholesterol and leucocyte vitamin C. Low levels of anti-oxidants such as vitamin C may lead to oxidative modification of LDL cholesterol by free radicals which in turn increases the likelihood of atheromatous lesions (Steinberg et al 1989). NonHDL cholesterol was significantly negatively associated with the ABPI in the whole population, especially in male non-smokers where it was the strongest risk factor. This is consistent with the results of a meta-analysis of lipid lowering trials showing that LDL cholesterol rather than total cholesterol was the major risk factor for coronary heart disease (Yusuf et al 1988).

The finding that nonHDL cholesterol was associated with the ABPI in women

as well as men, although weakly, is consistent with studies on coronary heart disease. The Edinburgh Artery Study consisted of women aged 55-74, so most would be postmenopausal (approximately 95%). After the menopause oestrogen levels would be reduced and this leads to an increase in nonHDL cholesterol levels. In the Edinburgh Artery Study the mean nonHDL cholesterol level was higher in women (mean = 5.82 mmol/l se = 0.05) compared to men (mean = 5.38 mmol/l se = 0.04). However, men develop atherosclerotic disease earlier in their 50s and 60s compared to women (Lerner and Kannel 1986) and so it is perhaps not surprising that there was still greater risk in men because of their lifetime exposure to risk factors. There were large differences in risk factors between males and females for peripheral arterial disease in this population, apart from nonHDL cholesterol and smoking. Indeed, cigarette smoking in terms of packyears was the strongest risk factor for women.

HDL cholesterol was only positively significantly related to the ABPI in male smokers but not independently of nonHDL cholesterol in other groups.

11.2.7 Social Class

In chapter 9, when social class was considered as a risk factor for peripheral arterial disease, it was significantly related to the ABPI only in males. This was perhaps not surprising as it has been shown that social class as determined by a woman's occupation was not a good indicator of disease or mortality (McDowall 1983). Among the alternatives to social class, social deprivation (Carstairs and Morris 1991) is a related measure based on the area of residence rather than occupation and may be a more appropriate measure for women. However, deprivation was highly correlated with social class (Males r = 0.46, Females r = 0.35) and the associations with the ABPI were similar for males and females for both measures. Social class has

also been shown to be a strong indicator of disease in studies of coronary heart disease (Rose and Marmot 1981, Pocock et al 1987) and so this thesis has concentrated on social class as a measure of socio-economic status. Of course, individuals can change social class over their lifetimes, but this was less of a problem in the Edinburgh Artery Study, since social class was defined by the longest held occupation (chapter 2).

There were significant linear trends of low levels of dietary factors such as vitamins E and C, β-carotene and cereal fibre with lower social classes in males and females. The lower social classes also indulged less in strenuous or moderate leisure activity when aged 35-45 compared to higher social classes. Lower social classes were also on average shorter which may be an indication of poorer nutrition at an earlier age. Walker et al (1989) in the British Regional Heart Study found that the risk of heart attack was significantly greater for shorter men compared to taller men, and this became less significant on adjusting for social class, among other confounders. The cumulative lifetime cigarette smoking in terms of packyears was significantly greater in lower social classes, especially for men, while alcohol intake was greater in higher social classes. This is consistent with a study of ischaemic heart disease where Pocock et al (1987) found that the high risk of ischaemic heart disease events in manual workers during 6 years of follow-up was due to cigarette smoking, higher blood pressure, greater obesity and less physical activity in leisure time.

11.3 Analysis of All Risk Factors

From the analyses of individual groups of risk factors, it was clear that there were a number of significant smoking and sex interactions. Consequently, when considering all factors it was appropriate to consider males and females separately, and never smokers and ever smokers separately in males.

11.3.1 Females

Cigarette smoking and age were the most important risk factors for women (figure 11.2). Although a raised fibrinogen level was significantly associated with the ABPI, this could be explained by cigarette smoking. On adding BMI which was surprisingly positively related to the ABPI, nonHDL was inversely related to disease. No lifestyle or rheological factors were independently related to peripheral arterial disease.

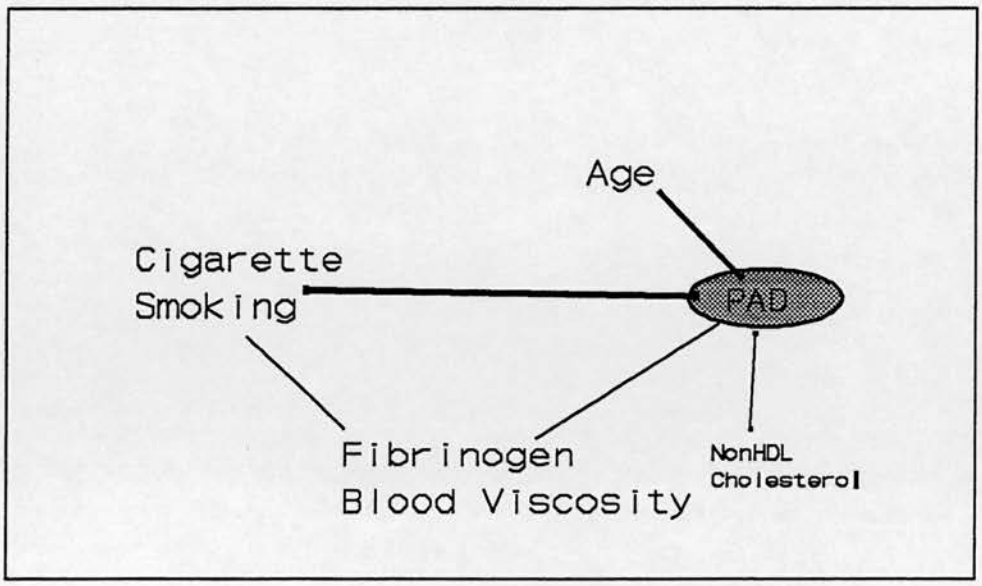


Figure 11.2 Risk factors for women in the Edinburgh Artery Study

11.3.2 Males who never smoked

The strongest independent risk factor was nonHDL cholesterol in those who never smoked. Known diabetes was the only other independent risk factor in this group.

11.3.3 Males who had ever smoked

Cigarette smoking was of course an important risk factor in this group. Synergistic effects of smoking with fibrinogen and blood viscosity were found and these rheological factors also had significant associations with the ABPI, independently of smoking. Thus smoking was not the only factor involved in raised levels of fibrinogen and blood viscosity.

Leisure activity was related to blood viscosity and after adjustment for this, moderate and strenuous activity when aged 35-45 years relative to none was only just significant at the 10% level.

NonHDL cholesterol and HDL cholesterol had significant independent associations with the ABPI in opposite directions. Known diabetes was a significant risk factor in those who had ever smoked as well as for non-smokers.

The multiple regression analysis of lifestyle and personality risk factors on the ABPI showed that the relative benefit of moderate or strenuous leisure activity aged 35-45 years was greatest for men who had ever smoked. As leisure activity, cigarette smoking and height were strongly related to social class in males, when these were entered into the regression model, social class became non-significant. Also on adding the rheological factors as well as the lifestyle risk factors, social class was not significantly associated with the ABPI. However, social class was not a true confounder in the sense of being only related to the outcome (ABPI) and the variables of interest, because social class could be considered as the first component in a causal chain linked to the lifestyle factors and the rheological factors through to peripheral arterial disease (figure 11.3). Hence social class remains an important risk factor for peripheral arterial disease while taking part in leisure activity aged 35-45 years, height, and cigarette smoking are behavioural and nutritional markers associated with social class.

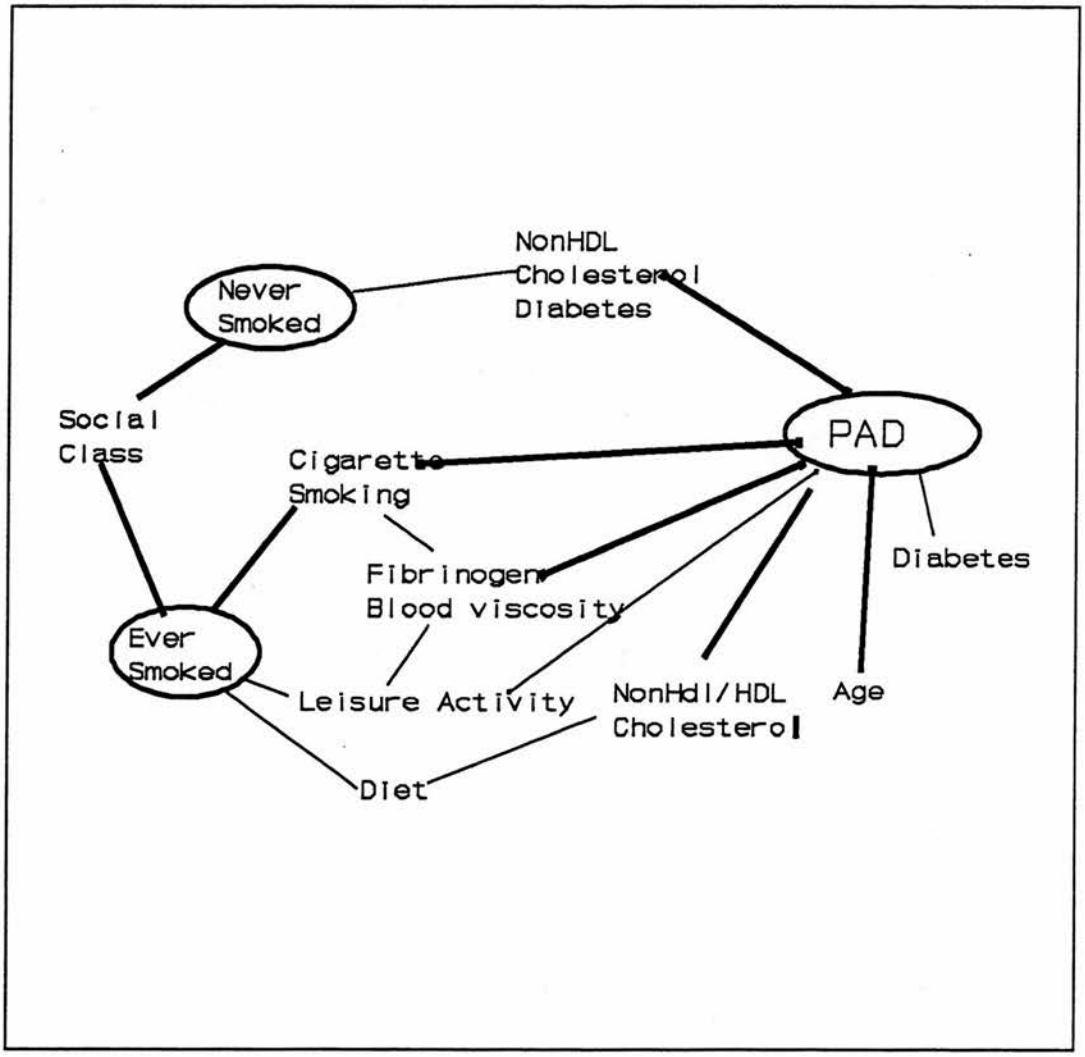


Figure 11.3 Relationships between risk factors for Peripheral Arterial Disease in males who had ever and who had never smoked

Mechanism

It was clear that smoking was the major risk factor for peripheral arterial disease and there was some indication that this was the main difference between risk factors for peripheral arterial disease compared to ischaemic heart disease (Fowkes et al 1992a). In non-smokers, nonHDL cholesterol was the strongest risk factor.

The mechanism by which smoking increases peripheral arterial disease is complex. Evidence suggests this may be via damage to the arterial wall (Smith et al 1993) indicated by increased levels of von Willebrand factor and cross-linked fibrin degradation products. Smoking also increased fibrinogen level and hence blood viscosity and these were higher in subjects with peripheral arterial disease. In women smoking 'explained' the higher levels of fibrinogen and blood viscosity while in men, although this was also true to some extent, there remained a significant residual relationship with disease independently of smoking. Smoking acts in other more direct ways which may increase the risk of arterial disease. Smoking depletes vitamin C and E levels (Stryker 1988, Shectman 1989), both of which are antioxidants reducing oxidation of nonHDL cholesterol involved in the growth of atheromatous lesions (Gey et al 1989, Riemersma et al 1991). Oxidised NonHDL cholesterol has been recognised as present in human atherosclerotic plaques (Steinberg et al 1989). These processes are summarised in figure 11.4.

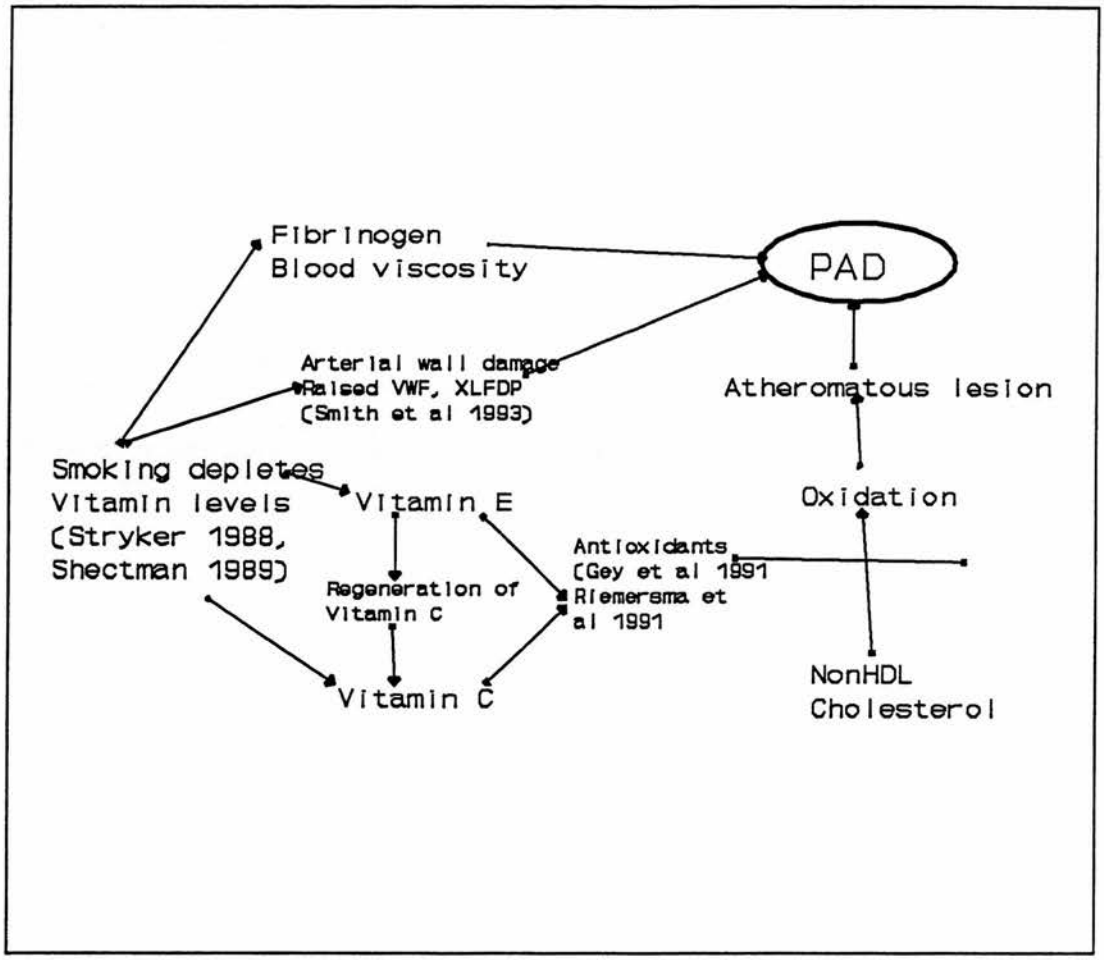


Figure 11.4 Possible mechanism of relationships between Cigarette smoking, Vitamin levels, Arterial wall damage, nonHDL cholesterol and Peripheral Arterial Disease

11.4 Recommendations

11.4.1 Questionnaire design

The questionnaires for diet, physical activity and personality were not validated for the Edinburgh Artery Study itself. Rather reliance was placed on validation by the authors of these questionnaires, or on validity studies of similar questionnaires.

In studies of dietary risk factors a subsample assessed by weighed intake as well as food frequency questionnaire is recommended, which allows measurement error to be taken into account in the analysis (Rosner et al 1990, Rosner et al 1992). The Edinburgh Artery Study used a Food Frequency Questionnaire (FFQ) with decreasing frequencies of consumption of food items and with some items, portion size was added. In a Swedish study of women attending mammography screening different types of FFQ were compared and it was found that the reported mean frequencies of consumption of fat, milk fat and vegetables, which are all important in relation to atherosclerosis, were significantly reduced if portion sizes were added to the questionnaire (Kuskowska-Wolk et al 1992). In addition, decreasing categories of response were associated with increased responses for bread and vegetables. Thus the design of FFQs will affect the results of dietary studies.

The physical activity questionnaire was assessed in the Welsh Heart Health Study (1985b) against physiological measures such as forced expiratory volume. The Edinburgh Artery Study used a similar but modified version of the questionnaire and so a further validation study might have been appropriate.

In studies of atherosclerosis and personality one of the main problems has been the number of different instruments used to measure Type A behaviour pattern, which may partly explain the inconsistent results. It would be worth targeting hostility rather

than general personality traits, as there appears to be a consensus that impatience and hard-driving, aspects of hostility, are the core elements of the Type A behaviour which are linked to coronary heart disease (Evans 1990, Sykes et al 1992). It would also be worth considering structured interviews rather than self-completed questionnaires, the former having the advantage that note can be taken of the tone and speech behaviour of the interviewee as well as the content of the replies. Rosenman et al (1975) used this instrument in the follow-up of the Western Electric Study and it is considered the gold standard for measures of Type A behaviour.

11.4.2 Sample Size and Power

At the design stage sample size calculations are often based on two-sample comparisons which may be appropriate for clinical trials, but are probably not realistic in an observational study. For example, multiple testing will be carried out in a cross-sectional study and therefore it would be better to calculate sample size based on a more realistic type I error of 0.1% say, rather than 5%. Calculations should also take into account adjustment for numerous confounders in observational studies. The methods of Cohen (1988), Hsieh (1989), Wilson and Gordon (1986) and Self et al (1992), could be used depending on whether the outcome was binary or continuous.

There were many differences in the relationships between risk factors and peripheral arterial disease for males and females and this has been noted for coronary heart disease (Lerner and Kannel 1986). Hence sub-group analyses are likely in observational studies and the power is reduced to detect associations with disease and sample size should be increased to compensate for this.

11.4.3 Within-person measurement error

Measurement error in clinical factors such as serum cholesterol and serum glucose have been shown to greatly attenuate the estimates of regression coefficients (Rosner et al 1992) leading to underestimation of their importance in relation to disease. Other factors such as BMI, age and smoking were relatively more accurate and therefore less of a problem (Rosner et al 1992). The solution would be to incorporate reproducibility substudies within the design of a study for factors which are known to vary in time, such as dietary factors, serum cholesterol and fibrinogen, and adjust the regression coefficients according to the degree of within-person variability.

11.5 Future Research

11.5.1 Implications for prevention

The advice to 'stop smoking and keep walking' was offered to cases of intermittent claudication (Housley 1988). The analysis of risk factors in the Edinburgh Artery Study suggest that this advice should also be applied to the general population where asymptomatic and symptomatic peripheral arterial disease was prevalent (26%). To this advice could be added that one should also try to change social class or more practically adopt behaviours associated with higher social class. These are reduce cigarette smoking, reduce dietary saturated fat intake, increase vitamin intake, that is, as fruit and vegetables or as supplements, and increase participation in leisure activity.

Smoking

The Edinburgh Artery Study results and studies on ischaemic heart disease (Pocock et al 1987) have implications for health education programs, in that campaigns to reduce smoking should perhaps be targeted at manual social classes. Although mortality from all causes and coronary heart disease and presumably peripheral arterial disease have declined in time, the relative improvement has been greater in non-manual social classes, and the proportionate decline in smoking has been greater in non-manual classes (Marmot and McDowall 1986). It would appear that these messages have already got through to higher social classes, or at least they are in a more favourable position to act upon the message.

Since smoking appears to be the main risk factor for women and is especially prevalent in lower social classes, this is clearly a group which could be targeted. In the 1982 General Household Survey it was found that only 12% of women with university degrees were smokers compared to 42% of those with no educational qualifications (OPCS, 1990). It is known that lower social class lone mothers are well aware of the health risks, but see their habit as a luxury and relief from stress (Graham 1987). Hence the smoking cessation message needs to be appropriate to the social class and gender of the recipients.

Saturated fat intake

On the other hand, in terms of Health Education programs, reduction of nonHDL cholesterol in the whole population would be a worthwhile aim. This has been reinforced by studies in China which suggested that even in distributions which are shifted to lower levels than those found in the West there was still a dose-response

relationship of coronary heart disease and plasma cholesterol (Chen et al 1991).There was no social class gradient in the Edinburgh Artery Study for nonHDL cholesterol unlike that found for smoking. Meta-analyses of lipid-lowering trials have suggested an unexpected increase in deaths from accidents/suicide (Muldoon et al 1990), but analysis of the Edinburgh Artery Study showed no significant relationship between nonHDL cholesterol and hostility measured by the Bedford-Foulds (1978) scales but did indicate a positive relationship between overt hostile acts and triglyceride levels (Fowkes et al 1992c). It will be interesting to see whether high triglyceride or low cholesterol are associated with deaths from accidents/suicide in the follow-up of the Edinburgh Artery Study. Recent analysis of the Whitehall study (Davey Smith et al 1992) speculated that low cholesterol level was not the cause of the increased risk for other diseases.

Thus large population programmes are indicated for some factors, such as cholesterol, to lower the mean of the distribution of a risk factor but for other factors such as smoking, targeting subgroups may be more appropriate. Attention needs to be paid to 'the shape as well as the position of the distribution and how the distribution varies among subgroups of the population' (Marmot 1993).

Of course all of these prevention programmes apply equally to reducing risk of coronary heart disease, although smoking appears to be a stronger risk factor for peripheral arterial disease (Fowkes et al 1992a). Although symptomatic peripheral arterial disease was relatively rare in the Edinburgh population, secondary prevention could relieve suffering of millions with coronary heart disease and/or PAD throughout the world (LaRosa et al 1990).

11.5.2 Implications for Treatment

In terms of evaluation of interventions the gold standard is the randomised controlled trial (RCT). This method could be applied to smoking cessation programs, including counselling, and nicotine patches. Cholesterol lowering trials either by drug treatment or dietary advice would be worth exploring, although it has been suggested that cholesterol-lowering drugs should only be used for those at high risk (Davey Smith et al 1993). Amongst the possible trials concerned with dietary change would be vitamin supplementation or dietary advice to increase intake of vitamin C and E containing foods, high fibre and low saturated fat. Finally, advice or programs to increase leisure activity could be implemented and evaluated.

11.6 Summary

The results suggest that men and women should be considered separately and possibly non-smokers/smokers in the analysis of risk factors for arterial disease. Cross-sectional studies can demonstrate associations but there is a need for longitudinal studies to show causative relationships. Although measures of lifetime risk such as packyears for smoking alleviate this problem somewhat, these in turn are liable to recall bias. Ideally intervention studies to alter risk factors are needed to demonstrate causation.

Sample size calculations based on two group comparisons tend to give an underestimate in epidemiological studies, where multiple confounders are likely and multiple tests tend to be carried out. Methods which take confounding into account would give more realistic estimates of sample size.

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APPENDIX 1 : The Edinburgh Artery Study Questionnaire

E D I N B U R G H A R T E R Y S T U D Y

QUESTIONNAIRE

THE INFORMATION IN THIS QUESTIONNAIRE IS HIGHLY CONFIDENTIAL AND IS PART OF A MEDICAL RESEARCH STUDY

The information you give in this personal health record will be treated as strictly confidential and will be available only to your own doctor and the study team. The results of the research will appear only in the form of general statistics from which it will be impossible to identify you as an individual.

Please complete the following:

SURNAME: .....

FORENAMES: .....

DATE: .....

If you have any difficulties in answering some questions you will have a chance to discuss these later with a member of the study team.

THANK YOU FOR YOUR CO-OPERATION IN THIS STUDY. THE FINDINGS WILL HELP TO IMPROVE HEALTH IN SCOTLAND.

IT IS IMPORTANT TO ANSWER ALL THE QUESTIONS CAREFULLY. PLEASE TAKE YOUR TIME.

There is some evidence of a relationship between health and other factors such as exercise, occupation, education, diet etc. In order to compare our data with national figures and other research work, we are interested to have the following details about yourself.

PERSONAL HISTORY

1. Please tick one box: Male ☐ 1 Female ☐ 2

2. Enter your date of birth: Day ☐☐ Month ☐☐ Year ☐☐

3. Please tick the box showing your present marital status:

Married (or equivalent)	☐ 1
Single	☐ 2
Widowed	☐ 3
Divorced or separated	☐ 4

EDUCATION

4. What is the HIGHEST level of education you and your spouse or ex-spouse have completed? Please tick boxes as appropriate.

	Yourself	Spouse or Ex-spouse
University/college degree course	☐ 1	☐ 1
Other professional or technical qualification after leaving school	☐ 2	☐ 2
Secondary School	☐ 3	☐ 3
Primary School	☐ 4	☐ 4

PAID EMPLOYMENT

5. What is your employment status at the moment? Please tick boxes as appropriate.

Employed, full-time	☐ 1
Employed, part-time	☐ 2
Unemployed	☐ 3
Retired	☐ 4
A housewife (full-time)	☐ 5
Other, please specify	☐ 6

Please complete questions 6 and 7 as appropriate for yourself and your spouse or ex-spouse.

6. YOURSELF | YOUR SPOUSE or EX-SPOUSE

(a) Please give the name of your present job and describe what you do as fully as possible. If unemployed or retired, do not complete this question, BUT PROCEED TO QUESTION 7.

..... | .....

..... | .....

..... | .....

..... | .....

(b) What business or industry is this in?

..... | .....

(c) In this job are you?

self-employed	☐	foreman	☐	self-employed	☐	foreman	☐
manager	☐	other	☐	manager	☐	other	☐

(d) In this job do you supervise/employ?

25 or more people	☐	25 or more people	☐
fewer than 25 people	☐	fewer than 25 people	☐
no-one	☐	no-one	☐

7. YOURSELF | YOUR SPOUSE or EX-SPOUSE

(a) Please give the name of the job you have done for the longest period of your life, and describe what you did as fully as possible. (If the answer is the same as in Question 6 above, write SAME)

..... | .....

..... | .....

..... | .....

..... | .....

(b) What business or industry was this in?

..... | .....

(c) In this job were you?

self-employed	☐	foreman	☐	self-employed	☐	foreman	☐
manager	☐	other employee	☐	manager	☐	other employee	☐

(d) In this job did you supervise/employ?

25 or more people	☐	25 or more people	☐
fewer than 25 people	☐	fewer than 25 people	☐
no-one	☐	no-one	☐

FOR OFFICE USE ONLY

S.C.

SMOKING

Smoking has been linked with many health problems. It is important that you answer the following section as accurately as possible. Please tick appropriate boxes.

8(a) Do you smoke at present? Yes ☐ No ☐

IF NO, PROCEED TO QUESTION 8(f)

(b) What do you usually smoke now?

cigarettes	Yes ☐	No ☐
pipe	Yes ☐	No ☐
cigars	Yes ☐	No ☐

(c) How many do you usually smoke now?

cigarettes per day	.....	cigarettes
oz. tobacco per week	.....	oz.
cigars per week	.....	cigars

(d) For how many years during your life have you smoked cigarettes? ..... years

(e) How many cigarettes have you smoked on average per day during the period you have smoked? ..... cigarettes

NOW PROCEED TO QUESTION 8(k)

(f) Have you ever smoked regularly? Yes ☐ No ☐

IF NO, PROCEED TO QUESTION 8(k)

(g) What did you usually smoke?

cigarettes	Yes ☐	No ☐
pipe	Yes ☐	No ☐
cigars	Yes ☐	No ☐

(h) How much did you smoke on average while you were a smoker?

cigarettes per day	.....	cigarettes
oz. tobacco per week	.....	oz.
cigars per week	.....	cigars

(i) For how many years did you smoke cigarettes? ..... years

(j) If you smoked cigarettes, how long is it since you finally gave up? ..... years ..... months

(k) Is any other member of your household a smoker? Yes ☐ No ☐

MEDICAL HISTORY

We should now like to ask you questions about your health, illnesses you have had in the past, and how you are feeling now. Please tick appropriate boxes.

9. Have you ever been told by a doctor that you have or have had any of the following?

	Yes	No
Hardening of the arteries in the legs	☐	☐
Angina	☐	☐
Heart attack (coronary thrombosis, myocardial infarction)	☐	☐
High blood pressure	☐	☐
Stroke	☐	☐
Diabetes (sugar disease)	☐	☐
Bronchitis	☐	☐
Tuberculosis	☐	☐
Asthma	☐	☐

10. Are you on any regular medical treatment from a doctor as follows?

	Yes	No
Drugs to lower blood pressure	☐	☐
Diuretics (water tablets)	☐	☐
Insulin injections	☐	☐
Tablets for diabetes	☐	☐
Other treatments? Give names if possible.	☐	☐

.....

.....

.....

.....

CHEST PAIN

11(a) Do you ever get pain or discomfort in your chest?
IF NO, PROCEED TO QUESTION 12

Yes ☐ No ☐

(b) Do you get this pain or discomfort when you walk uphill or hurry?
IF NO, PROCEED TO QUESTION 11g

Yes ☐ No ☐

(c) Do you get it when you walk at an ordinary pace on the level?

Yes ☐ No ☐

(d) When you get any pain or discomfort in your chest what do you do?

Stop ☐

Slow down ☐

Continue at the same pace ☐

(e) Does it go away when you stand still or sit down?

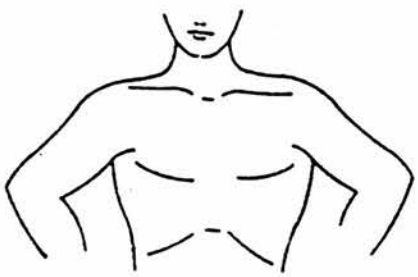
Yes ☐ No ☐

(f) How soon?

10 minutes or less Yes ☐ No ☐

more than 10 minutes Yes ☐ No ☐

(g) Where do you get this pain or discomfort? Mark the place(s) with 'X' on the diagram.

RIGHT  LEFT

12(a) Have you ever had a severe pain across the front of your chest lasting for half an hour or more?

Yes ☐ No ☐

(b) What was the cause? .....

FOR OFFICE USE ONLY A: GRADE MI:

COUGH

13(a) Do you usually cough several times first thing in the morning in the winter? (Ignore clearing throat or single cough)

Yes ☐ No ☐

(b) Do you usually cough during the day or night in winter? (Ignore the occasional cough)

Yes ☐ No ☐

(c) If yes to (a) or (b), do you cough on most days for at least three months each winter?

Yes ☐ No ☐

PHLEGM (SPIT)

14(a) Do you usually bring up any phlegm (spit) from your chest first thing in the morning in the winter?

Yes ☐ No ☐

(b) Do you usually bring up any phlegm from your chest during the day, or at night, in the winter?

Yes ☐ No ☐

(c) If yes to (a) or (b), do you bring up phlegm like this on most days for as much as three months each year?

Yes ☐ No ☐

BREATHLESSNESS

15(a) Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill?

IF NO, GO TO QUESTION 16

Yes ☐ No ☐

(b) Do you get short of breath walking with other people of your own age on level ground?

Yes ☐ No ☐

(c) Do you have to stop for breath when walking at your own pace on level ground?

Yes ☐ No ☐

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C

P

B GRADE

WHEEZING

16(a) Have you had attacks of wheezing or whistling in your chest at any time in the last 12 months?

Yes ☐ No ☐

(b) Have you ever had attacks of shortness of breath with wheezing?

Yes ☐ No ☐

(c) If yes to (b), is/was your breathing absolutely normal between attacks?

Yes ☐ No ☐

(d) Have you at any time in the last 12 months been woken at night by an attack of shortness of breath?

Yes ☐ No ☐

LEG PAIN

17(a) Do you get a pain in either leg on walking?
IF NO, GO TO QUESTION 18

Yes ☐ No ☐

(b) Does this pain ever begin when you are standing still or sitting?

Yes ☐ No ☐

(c) Do you get this pain in your calf (or calves)?

Yes ☐ No ☐

(d) Do you get it when you walk uphill or hurry?

Yes ☐ No ☐

(e) Do you get it when you walk at an ordinary pace on the level?

Yes ☐ No ☐

(f) Does the pain ever disappear while you are still walking?

Yes ☐ No ☐

(g) What do you do if you get it when you are walking?

Stop 1 ☐

Slow down 2 ☐

Continue at same pace 3 ☐

(h) What happens to it if you stand still?

Usually continues for more than 10 minutes 1 ☐

Usually disappears in 10 minutes or less 2 ☐

18. Have you ever had surgery on the arteries of your legs other than fo varicose veins?

Yes ☐ No ☐

Please specify .....

19. Have you ever had surgery to remove

toes? Yes ☐ No ☐

leg below the knee? Yes ☐ No ☐

leg above the knee? Yes ☐ No ☐

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I.C. GRADE

OTHER MEMBERS OF YOUR FAMILY

20. Please tick the appropriate boxes for other members of your family if they have been diagnosed as having any of the illnesses below:

Illnesses	Father	Mother	Any brother or sister	Any son or daughter
Angina	☐	☐	☐	☐
Stroke	☐	☐	☐	☐
High blood cholesterol level	☐	☐	☐	☐
Diabetes (sugar disease)	☐	☐	☐	☐
Hardening of the arteries in the leg/claudication	☐	☐	☐	☐
Thrombosis/embolism	☐	☐	☐	☐
High blood pressure	☐	☐	☐	☐
Heart attack	☐	☐	☐	☐
If died from heart attack, at what age?	....Yrs	....Yrs	....Yrs	....Yrs

PHYSICAL ACTIVITY

The following section gives examples of the sort of activities you might do or may have done REGULARLY.

LIGHT activity	MODERATE activity	STRENUOUS activity
Ballroom dancing	Badminton	Basketball
Bowling	Cricket	Competitive cycling
Light do-it-yourself	Cycling (include to and	Competitive swimming
Light gardening	from work, to shops etc)	Competitive running
Horse riding	Heavy do-it-yourself	Field sports (such as
Sailing	Golf	rugby, soccer, hockey)
Walking (include to and	Jogging	Training for strenuous
from work, to shops etc)	Swimming	sport
Yoga	Tennis	Squash
And other activities of similar intensity. Please specify others you have done.	And other activities of similar intensity. Please specify others you have done.	And other activities of similar intensity. Please specify others you have done.
.....	.....	.....
.....	.....	.....

21. In a typical week during the last year, on how many occasions would you take part FOR MORE THAN 20 MINUTES EACH TIME:
Insert 'None' if appropriate

in LIGHT physical activity? in summer ..... times

in winter ..... times

in MODERATE physical activity? in summer ..... times

in winter ..... times

in STRENUOUS physical activity? in summer ..... times

in winter ..... times

22. In a typical week, when you were 35-45 years old, on how many occasions would you take part, FOR MORE THAN 20 MINUTES EACH TIME:
Insert 'None' if appropriate

in LIGHT physical activity? in summer ..... times

in winter ..... times

in MODERATE physical activity? in summer ..... times

in winter ..... times

in STRENUOUS physical activity? in summer ..... times

in winter ..... times

23. Which of the following best describes your daily work or other daytime activity at the present time?
Please tick one box only.

I am usually sitting during the day and do not walk about much	☐	eg. office workers, drivers
I stand or walk about quite a lot during the day, but do not have to carry or lift things very often	☐	eg. housewives, shop assistants
I usually lift or carry light loads and have to climb stairs and/or hills often	☐	eg. postmen, packers
I do heavy work and carry heavy loads	☐	eg. building, mining workers, agricultural workers

24. Which of the following best described your daily work or other daytime activity WHEN YOU WERE 35-45 YEARS OLD?

I usually sat during the day and did not walk about much	☐	eg. office workers, drivers
I stood or walked about quite a lot during the day, but did not have to carry or lift things very often	☐	eg. housewives, shop assistants
I usually lifted or carried light loads and had to climb stairs and/or hills often	☐	eg. postmen, packers
I did heavy work and carried heavy loads	☐	eg. building, mining heavy workers, agricultural workers

LIFE STYLE AND ATTITUDES

25. Each of us belongs somewhere along the line between two extremes. For example, most of us are neither the most competitive nor the least competitive person we know. What we would like you to do is to make a vertical line where you think you belong between these two extremes.

For example:

Always tidy ——————|—— Never tidy

(a)	Never late	_____	Casual about appointments
(b)	Not competitive	_____	Very competitive
(c)	Anticipate what others are going to say (nod, interrupt, finish for them)	_____	Good listener, hear others out
(d)	Always rushed	_____	Never feel rushed, even under pressure
(e)	Can wait patiently	_____	Impatient when waiting
(f)	Go "all out"	_____	Casual
(g)	Take things one at a time	_____	Try to do many things at once, think about what to do next
(h)	Emphatic in speech (may pound desk or chair))	_____	Slow, deliberate talker
(i)	Have wanted good job recognised by others	_____	Only care about satisfying myself no matter what others may think
(j)	Fast (eating, walking etc.)	_____	Slow doing things
(k)	Easy going	_____	Hard driving
(l)	Hide feelings	_____	Express feelings
(m)	Many interests	_____	Few interests (outside work)
(n)	Am/was satisfied with job	_____	Ambitious

26. The next section contains descriptions of how you may have felt, thought, or acted during most of your life. Below each statement there are four words or phrases; choose the one which best describes you for most of your life and draw a circle round it.

EXAMPLE

(a) I have enjoyed being with other people.

Nearly always (Often) Seldom Never

The first example would mean that most of your life you have often enjoyed being with other people.

(1) I would have liked to get my own back on someone.

Very often Often Seldom Never

(2) I have been content to act in a very humble way.

Never Seldom Often Nearly always

(3) I have thought that people will tell the truth, even if it gets them into trouble.

Nearly always Often Seldom Never

(4) I have felt as capable as other people.

Never Seldom Often Nearly always

(5) When I've wanted to have a row with someone, I have done so.

Nearly always Often Seldom Never

(6) I have preferred to take a lot of advice before doing anything.

Never Seldom Often Nearly always

(7) I have felt like telling people to go to blazes.

Nearly always Often Seldom Never

(8) When in a group I have been content to be led.

Never Seldom Often Nearly always

(9) When someone has been particularly helpful, I've wondered what real reason lay behind it.

Nearly always Often Seldom Never

(10) I have had confidence in myself.

Never Seldom Often Nearly always

(11) When I've disliked someone, I have shown it.

Nearly always Often Seldom Never

(12) I have wanted plenty of support from people.

Never Seldom Often Nearly always

(13) I have felt the urge to smash things.

Very often Often Seldom Never

(14) I have been content to be dominated by someone else.

Never Seldom Often Nearly always

(15) I have believed that people are pretty reliable.

Nearly always Often Seldom Never

(16) I have been very unsure of myself.

Never Seldom Often Nearly always

(17) When I've been angry with someone, I've bottled it up.

Nearly always Often Seldom Never

(18) I have liked to be told what needs doing.

Never Seldom Often Nearly always

(19) I have wanted to give someone a piece of my mind.

Very often Often Seldom Never

(20) I have preferred to let people have their own way.

Never Seldom Often Nearly always

(21) I have felt that people would tell lies to get ahead.

Nearly always Often Seldom Never

(22) I have given up doing something because I thought too little of my own ability.

Never Seldom Often Very Often

(23) Even when crossed, I've let people get away with it.

Nearly always Often Seldom Never

(24) I have been content to lean on other people for emotional support.

Never Seldom Often Nearly always

(25) I would have liked to pick a quarrel with someone.

Very often Often Seldom Never

(26) I have been happy to play second fiddle.

Never Seldom Often Nearly always

(27) I have felt that people are out for what they can get.

Nearly always Often Seldom Never

(28) I have felt that even when difficulties were piling up I would overcome them.

Never Seldom Often Very often

(29) When I've thought I was justified in losing my temper, I have done so in no uncertain terms.

Very often Often Seldom Never

(30) I have preferred to find out for myself what's to be done.

Never Seldom Often Nearly always

(31) I have felt like blaming others when things have gone wrong.

Nearly always Often Seldom Never

(32) I have preferred to stay in the background.

Never Seldom Often Nearly always

(33) I have thought one can safely trust people.

Nearly always Often Seldom Never

(34) I have felt pretty useless.

Never Seldom Often Nearly always

(35) When I've felt like blaming someone to their face for something that has gone wrong, I have done so.

Nearly always Often Seldom Never

(36) I have needed a lot of help from other people.

Never Seldom Often Very often

DIET

The following questions are about the food that you usually eat. Please give the number of days each week on which you usually eat the various foods.

EXAMPLE

Ring the appropriate answer like this:	Number of days each week
If you eat a food every day, then ring the 7:	(7) 6 5 4 3 2 1 M R
If you eat a food 3 days each week ring the 3:	7 6 5 4 (3) 2 1 M R
If you eat a food less than one day a week (but more than once a month), then ring M (Monthly):	7 6 5 4 3 2 1 (M) R
If you eat a food rarely, or never, then ring R (Rarely):	7 6 5 4 3 2 1 M (R)

Please complete every line even if you do not take the food in question.

27. BREAD

			How many slices or rolls per day	Please tick whether slices (or rolls) are:
(a)	White Bread	7 6 5 4 3 2 1 M R	.....	Thick Medium Thin
(b)	Brown Bread	7 6 5 4 3 2 1 M R	.....	Thick Medium Thin
(c)	Wholemeal	7 6 5 4 3 2 1 M R	.....	Thick Medium Thin
(d)	Bread Rolls (all types)	7 6 5 4 3 2 1 M R	.....	Large Medium Small
(e)	Crispbread Ryvita, cream crackers etc. Please specify type:	7 6 5 4 3 2 1 M R	.....	

28. BREAKFAST CEREALS

(a)	Grapenuts, Porridge, Ready Brek, Rice Crispies, Special K, Sugar Puffs	7 6 5 4 3 2 1 M R
(b)	Cornflakes, Muesli, Shredded Wheat, Sultana Bran, Weetabix	7 6 5 4 3 2 1 M R
(c)	Bran Flakes or Puffed Wheat	7 6 5 4 3 2 1 M R
(d)	All bran or Wheat Bran	7 6 5 4 3 2 1 M R
(e)	Other cereals.	7 6 5 4 3 2 1 M R

Please specify .....

29. MEAT

(a)	Beef (including minced beef, beefburgers)	7	6	5	4	3	2	1	M	R
(b)	Lamb	7	6	5	4	3	2	1	M	R
(c)	Pork, bacon or ham	7	6	5	4	3	2	1	M	R
(d)	Chicken, Turkey,, or other poultry	7	6	5	4	3	2	1	M	R
(e)	Tinned meat (all types, eg.corned beef)	7	6	5	4	3	2	1	M	R
(f)	Pork sausages	7	6	5	4	3	2	1	M	R
(g)	Beef sausages	7	6	5	4	3	2	1	M	R
(h)	Meat pies or pasties	7	6	5	4	3	2	1	M	R
(i)	Liver or kidney or heart	7	6	5	4	3	2	1	M	R

30. FISH

(a)	White fish (cod, haddock, hake, plaice, or fish fingers etc.)	7	6	5	4	3	2	1	M	R
(b)	Kippers, herrings, pilchards, tuna, sardines, salmon or mackerel (including tinned)	7	6	5	4	3	2	1	M	R

31. VEGETABLES

(a)	Potatoes:boiled, baked or mashed	7	6	5	4	3	2	1	M	R
(b)	Potatoes:(a) chips or fried (from shop)	7	6	5	4	3	2	1	M	R
	(b) chips or fried (cooked at home)	7	6	5	4	3	2	1	M	R
(c)	Green vegetables or salad	7	6	5	4	3	2	1	M	R
(d)	Carrots	7	6	5	4	3	2	1	M	R
(e)	Parsnips, swedes, turnips, beetroot, and other root vegetables	7	6	5	4	3	2	1	M	R
(f)	Baked or butter beans, lentils, peas, sweetcorn	7	6	5	4	3	2	1	M	R
(g)	Onions, (cooked, raw or pickled)	7	6	5	4	3	2	1	M	R
(h)	Spaghetti and other pasta	7	6	5	4	3	2	1	M	R
(i)	Rice (all types except pudding rice)	7	6	5	4	3	2	1	M	R

32. BISCUITS/PUDDINGS

(a)	Digestive biscuits or plain biscuits	7	6	5	4	3	2	1	M	R
(b)	Sweet biscuits, sweeties, or jellies	7	6	5	4	3	2	1	M	R
(c)	Ice cream, sweet yoghurts or chocolate	7	6	5	4	3	2	1	M	R
(d)	Fruit cake, sponge cake, scones or buns	7	6	5	4	3	2	1	M	R
(e)	Fruit tart, jam tart, fruit crumble, trifle	7	6	5	4	3	2	1	M	R
(f)	Milk pudding(eg. rice, tapioca custard)	7	6	5	4	3	2	1	M	R
(g)	Tinned fruit	7	6	5	4	3	2	1	M	R

33. FRESH FRUIT

(a) Number of apples eaten per week .....

(b) Number of pears eaten per week .....

(c) Number of oranges or grapefruit eaten per week .....

(d) Number of bananas eaten per week .....

34. EGGS

How many eggs do you eat per week? .....

35. MILK

(a) How much milk do you drink per day in tea or coffee, in milky drinks, with cereals etc.?

(Please tick one)

- None at all ☐ 0
- Half-pint or less ☐ 1
- Between half and one pint ☐ 2
- More than one pint ☐ 3

(b) What kind of milk do you usually use?

(Please tick one)

- Full-fat milk, fresh or dried ☐ 1
- Semi-skimmed milk ☐ 2
- Fully-skimmed milk, fresh or dried ☐ 3
- Other kinds or milk, eg. condensed, evaporated ☐ 4

36. FATS

(a) What do you usually eat on bread?
(Please tick one or more)

Butter	1	☐
Soft margarine (specify type)	2,3,4	☐
Hard margarine	5	☐

(b) How often do you eat home-fried food (including chips) cooked with:

Lard, dripping or solid vegetable oil	7	6	5	4	3	2	1	M	R
Specify brand									
Liquid Vegetable oil	7	6	5	4	3	2	1	M	R
Specify type									

37. DRINKS

(a) How many cups of coffee do you have **per day**? .....

(b) How many teaspoons of sugar do you take per cup? .....

(c) How many cups of tea do you have **per day**? .....

(d) How many teaspoons of sugar do you take per cup? .....

(e) How many days each week do you drink fruit juices and squashes? 7 6 5 4 3 2 1 M R

Are these usually? (please tick one or more)

Natural juices	☐ 1
Squashes	☐ 2
Fizzy drinks	☐ 3

38. How much of the following foods does the **whole family** use on average per week?

Butter	.... lbs .... ozs
Margarine (all types)	.... lbs .... ozs
Lard and solid vegetable oil	.... lbs .... ozs
Liquid vegetable oil	.... lbs .... ozs
Cheese (excluding cottage cheese)	.... lbs .... ozs
Cream	.... lbs .... ozs
Sugar	.... lbs .... ozs

39. SALT

(a) How much salt is added in your cooking? (please tick one)

None	1 ☐
A little	2 ☐
A lot	3 ☐

(b) Do you add salt to your meals at the table? (please tick one)

No	1 ☐
When the food is not salty enough	2 ☐
Almost always before tasting	3 ☐

(c) Is, in your opinion, ready-made food compared to home-made food? (please tick one)

Less salty than at home	1 ☐
As salty as at home	2 ☐
More salty than at home	3 ☐

40. How many persons normally eat in your household?

Number of adults (including yourself)	.....
Number of children 5 to 16 years	.....
Number of children 1 to 4 years	.....
Number of babies under 1 year of age	.....

41. Are you on a special diet? (Please tick one)

None	0 ☐
Slimming diet suggested by your doctor	1 ☐
Slimming diet prescribed by yourself	2 ☐
Diabetic diet	3 ☐
Other "medical" diet, please specify	4 ☐
.....	
Vegetarian diet	5 ☐
Vegan diet	6 ☐

ALCOHOL

42(a) Think back carefully over the last seven days. Please write in each column exactly the number of alcoholic drinks you have consumed on each day during the past week. If none consumed write '0' in the boxes.

Try to remember where you were and who you were with on each day. This may help you remember what you had to drink.

	Pints of beer, lager cider, etc	Single Glasses of whisky, vodka gin etc.	Single Glasses of martini, sherry, etc.
Monday	☐	☐	☐
Tuesday	☐	☐	☐
Wednesday	☐	☐	☐
Thursday	☐	☐	☐
Friday	☐	☐	☐
Saturday	☐	☐	☐
Sunday	☐	☐	☐

(b) Would you say that last week was fairly typical of what you usually have to drink in a week?

Yes ☐ No ☐

(c) If last week was not typical, would you normally drink more or less in a week?

1 ☐ More 2 ☐ Less

43. Think about the last time you had a drink. Exactly how much did you drink on that occasion? Please insert the numbers in the boxes.

How many pints of lager, cider, shandy, stout etc. did you drink? ..... Pints

How many single glasses of whisky, vodka, gin, rum or other spirits did you drink? ..... Glasses

How many single glasses of Martini, port, sherry or wine did you drink? ..... Glasses

THANK YOU VERY MUCH FOR YOUR CO-OPERATION IN COMPLETING THIS QUESTIONNAIRE.

YOU WILL HAVE AN OPPORTUNITY TO DISCUSS ANY PROBLEMS WITH THE QUESTIONNAIRE WITH A NURSE AT THE END OF THE MORNING.

APPENDIX 2: Published Papers

(Permission to reproduce the papers has been obtained from each relevant journal)

Diet as a risk factor for peripheral arterial disease in the general population: The Edinburgh Artery Study[1-3]

Peter T Donnan, Marjory Thomson, F Gerald R Fowkes, Robin J Prescott, and Edward Housley

ABSTRACT The Edinburgh Artery Study included a cross-sectional survey of 1592 men and women (aged 55–74 y). One aim was to examine relationships between an indicator of peripheral arterial disease, the ankle brachial pressure index (ABPI), and dietary factors. Nutrient intake was derived from a food-frequency questionnaire. Higher frequency of consumption of fiber-containing foods was associated with greater mean ABPI in males and higher consumption of meat and meat products were significantly associated with low mean ABPI in males and females. In a multiple linear regression with ABPI as outcome and energy-adjusted nutrients as predictors, cereal fiber ($P = 0.02$) and alcohol ($P = 0.04$) were positively associated with the ABPI in males but not in females. Dietary vitamin E(α-tocopherol) intake was positively associated with ABPI ($P = 0.04$) independently of smoking and other nutrients. Dietary vitamin C intake was significantly related to ABPI ($P = 0.006$) only among those who had ever smoked. *Am J Clin Nutr* 1993;57: 917–21.

KEY WORDS Atherosclerosis, peripheral arterial disease, diet, dietary fiber, vitamin E, vitamin C

Introduction

The underlying mechanism of peripheral arterial disease and coronary heart disease is atherosclerosis and similarities in dietary risk factors for these two conditions would be expected. Most research has concentrated on the relationship between diet and coronary heart disease.

Epidemiological evidence suggests that high consumption of fish oil (1) and dietary fiber (2) reduces the risk of coronary heart disease, whereas smoking is associated with a higher incidence of coronary heart disease (3). Dietary interventions to reduce cholesterol have been shown to lower the risk of coronary heart disease and cardiovascular mortality (4).

In contrast to coronary heart disease the relationship between diet and peripheral arterial disease has not been studied extensively. In a hospital-based case-control study in Greece (5) dietary intake of saturated fatty acids, proteins, and cholesterol were associated with increased risk of peripheral arterial disease, whereas a high intake of polyunsaturated fatty acids and crude fiber reduced risk. In dietary-intervention studies on patients with peripheral arterial disease, clinical improvement has been demonstrated in patients on low-fat, high-fiber diets (6–8). Fish-oil (eicosapentaenoic acid) supplementation produced a reduction in blood viscosity (9).

This limited research on diet and peripheral arterial disease has been conducted only on hospital patients. In this study our aim was to determine the relationships between diet and a wide spectrum of peripheral arterial disease in a representative sample from the general population of men and women aged 55–74 y surveyed in the Edinburgh Artery Study.

Methods

The Edinburgh Artery Study carried out in 1988 consisted of a cross-sectional survey of 1592 men and women aged 55–74 y, randomly selected from the age-sex registers of 10 general practices. These general practices had catchment populations spread geographically and socioeconomically throughout the city. The subjects were invited to a university clinic to complete a questionnaire and have a comprehensive medical examination. This examination included measurement of the left and right ankle brachial pressure index (ABPI), which is the ratio of the ankle systolic pressure to the brachial systolic pressure. The ABPI is related to the extent of peripheral arterial disease in the general population (10), low values being indicative of more extensive disease.

The response rate to the invitation to participate in the study was 65% and follow-up of a sample of nonresponders did not show any significant bias in terms of age, sex, smoking, and extent of peripheral arterial disease. Details of the study population, recruitment, and prevalence of peripheral arterial disease were described previously (11). The questionnaire included validated questions on social class, cardiovascular history, intermittent claudication and angina [World Health Organization questionnaire (12)], smoking history, and alcohol consumption (13).

In the Edinburgh Artery Study, a version of a food-frequency questionnaire used in a previous study (14) was used to obtain nutrient information. This consisted of a list of $\approx$60 food items and respondents were asked to rate the frequency of consumption

[1] From the Wolfson Unit for Prevention of Peripheral Vascular Diseases and the Medical Statistics Unit, Department of Public Health Sciences, University of Edinburgh, and the Department of Dietetics, Eastern General Hospital, Edinburgh, UK.

[2] Supported by the British Heart Foundation.

[3] Address reprint requests to PT Donnan, Wolfson Unit for Prevention of Peripheral Vascular Disease, Department of Public Health Sciences, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, UK.

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(rarely/monthly or 1–7 times/wk) for each item. This was completed by all who attended the clinic, and a nurse was available to go through the questionnaire if there were any problems of interpretation. Calculation of energy and nutrient intakes for individuals was estimated by multiplying the nutrient content of a typical portion size of the specified food item by the frequency of consumption and summing over all food items. Even if participants failed to complete all questions, nutrients were still calculated, provided a response had been given regarding those foods that are major sources of a specific nutrient. Consequently, some nutrients may have been slightly underestimated.

The study was approved by the National Health Service Lothian Health Board Committee on Ethics of Medical Research.

Statistical methods

The results of the food-frequency questionnaire in terms of nutrients have been expressed as medians along with the upper and lower quartiles because many of the nutrients had positively skewed distributions. The Mann-Whitney test was used to compare differences in absolute nutrient intakes between males and females. In addition, the relationships between the frequency of consumption of individual foods and peripheral arterial disease were explored by testing for linear trends in the mean ABPI by using analysis of variance across the food-frequency categories: rarely/monthly, 1–3 times/wk, and 4–7 times/wk. These analyses were carried out for males and females separately and were adjusted for age.

Multiple linear regression was used to assess the independence of associations between dietary factors and the minimum ABPI of the two limbs, expressed as a continuous outcome. Tests of sex by nutrient and smoking by nutrient interactions were carried out. The statistical package *BMDP* was used for all analyses (15). The proportion of diabetics, known and positive after a glucose tolerance test, was small (5.7%) and were excluded from all regression analyses. Subjects with symptomatic coronary heart disease were not excluded because this would have also excluded many subjects with peripheral arterial disease, resulting in a nonrepresentative sample and a reduction in power.

It is important in epidemiological analyses to use measures of nutrient intake that are independent of total energy intake, especially whenever energy is related to disease (16). Consequently, energy-adjusted nutrient intakes were obtained from the residuals of the regression of each of the nutrients with total energy intake as predictor, applying the usual assumptions of regression. The residuals were added to the expected nutrient intake for the mean total energy intake of the population to give more meaningful measures of nutrient intake. This procedure gave relatively uncorrelated measures of nutrient intake, allowing easier interpretation of the results of multiple-linear-regression models, with the ABPI as a continuous outcome.

Initially, a regression model for each energy-adjusted nutrient was used to assess the independent association of each dietary factor from other factors known to be associated with the ABPI, such as age, sex, height, and cigarette smoking. Height was used rather than body mass index (BMI) because it was found that the measurement of the ABPI was related to height, whereas BMI was not significantly associated with the ABPI. Smoking history was defined in two ways. First, consumption was estimated in terms of pack-years, which is a measure of the amount smoked and the number of years as a smoker. A square-root transformation of pack-years was used in the models to give a more symmetrical distribution for the analyses. Second, when interactions with smoking were found, the population was divided into those who had never smoked and those who were ex-smokers or current smokers (ever smokers).

Finally, a regression model with all the energy-adjusted nutrients, allowing for age, sex, height, total energy, and smoking, was used to assess the independent effect of each nutrient, allowing for all other nutrients in relation to the ABPI.

Results

The absolute values of nutrient intakes and as a percentage of energy for men and women in the population of the Edinburgh Artery Study are shown in **Table 1.** Most amounts of nutrient consumption were higher in males, except for vegetable fiber and vitamin C, which were significantly higher in females. In addition, females had significantly greater intakes of protein and polyunsaturated fatty acids as a percentage of total energy compared with males. **Table 2** shows the trend in mean ABPI in females across three categories of frequency of food consumption: rarely/monthly, 1–3 times/wk, and 4–7 times/wk. There were significant reductions in mean ABPI in females, with increasing consumption of low-fiber breakfast cereals, meat pies and pastries, and pork, bacon, and ham, and significant trends in mean ABPI with increasing frequency of consumption of onions and biscuits/sweets. Among males there were significant linear reductions in mean ABPI with increasing consumption of white bread and meat pies and pastries, whereas significant increases in mean ABPI were associated with increasing consumption of whole-meal bread, high-fiber breakfast cereals, and pears (**Table 3**). Increased consumption of lard in males and females was associated with lower mean ABPIs, but not significantly lower. Caution should be applied in interpreting these results because with many tests at the 5% level, 1 in 20 would be expected to be significant by chance alone. In fact the number of significant tests is greater than would be expected by chance. The significant linear trends (Tables 2 and 3) provide stronger evidence of cause and effect than do the more general tests for association.

In the multiple-linear-regression models with ABPI as a continuous outcome, the core model consisted of age, height, sex, smoking, and total energy, to which each energy-adjusted nutrient was added separately (**Table 4**).

Finally, to assess the independent effects of nutrients, all nutrients were added simultaneously to this core model. The results for the nutrients did not differ greatly for these two procedures, the main difference being that cereal fiber in males was less significant (Table 4) when other nutrients were not adjusted for compared with when all nutrients were simultaneously adjusted for (**Table 5**).

For the variables in the core model (Table 5) there were significant relationships between the ABPI and age ($P < 0.001$), height ($P = 0.08$), and smoking (ever vs never, $P < 0.001$). Sex ($P = 0.71$) and total energy intake were also added to this model ($P = 0.69$).

The energy-adjusted intake of alcohol had the strongest association with the ABPI, and a significant interaction with sex was found in the regression analysis at the 10% level. Alcohol was positively associated with the ABPI in males ($P = 0.04$) when all variables were adjusted for (Table 5), whereas there was no significant association in females. Similarly, cereal fiber intake had a significant interaction with sex ($P = 0.05$) and was significantly related to the ABPI in males ($P = 0.02$) but not in

TABLE 1
Medians and upper and lower quartiles of absolute nutrient intake and as a percentage of energy in males and females aged 55–74 y in the Edinburgh Artery Study

	Females (n = 783)			Males (n = 809)		
	Median	Lower quartiles	Upper quartiles	Median	Lower quartiles	Upper quartiles
Total energy (kJ)	7425	6245	8748	8538*	7245	10 250
Total fat (% energy)	38.1	34.4	42.0	38.0	33.6	44.6
Carbohydrate (% energy)	44.2	40.3	47.8	45.2*	41.5	49.5
Protein (% energy)	17.4	15.9	19.1	16.8*	15.5	18.3
Saturated fatty acids (% energy)	16.5	14.0	19.5	16.2	13.9	18.8
Polyunsaturated fatty acids (% energy)	4.9	3.7	6.7	4.5†	3.5	6.4
P:S‡	0.31	0.20	0.46	0.30	0.19	0.44
Alcohol (g)	1.2	0.0	6.2	9.5*	1.2	21.9
Cereal fiber (g)	8.9	6.2	12.8	9.7*	6.9	14.0
Vegetable fiber (g)	12.2	9.5	15.0	10.5*	7.8	13.7
Vitamin C (mg)	63.5	46.3	85.4	48.3*	35.0	67.5
Cholesterol (mg)	309.4	238.2	396.4	328.2*	258.8	433.6
Retinol (μg)	646.7	404.6	956.9	657.9	427.3	985.3
β-Carotene (mg)	3.3	1.8	5.8	3.3	1.8	4.8
α-Tocopherol (mg)	8.1	6.1	12.2	8.2	6.1	12.7
Linoleic acid (g)	7.2	5.1	11.7	7.7§	5.4	11.9

*†§ Significantly different from females (Mann-Whitney test): *$P < 0.001$, †$P < 0.01$, §$P < 0.05$.
‡ Ratio of polyunsaturated to saturated fatty acids.

females. A highly significant interaction ($P = 0.006$) was found between vitamin C and smoking (ever vs never). In ex-smokers and current smokers vitamin C was significantly related to the ABPI ($P = 0.006$) but this association was not significant in lifelong nonsmokers (Table 5). There were no other significant sex-by-nutrient or smoking-by-nutrient interactions. Finally, α-tocopherol (vitamin E) intake was significantly associated ($P = 0.04$) with an increase in the ABPI, after adjustment for all variables. Vegetable fiber and β-carotene were univariately related to high ABPIs ($P < 0.1$), although not significantly so. In the multiple-regression analysis these along with the other nonsignificant nutrients were not independently related to the ABPI.

Discussion

Although food-frequency questionnaires have often been used in epidemiological studies of cardiovascular disease, few validation studies have been carried out. Nevertheless, those few attempts at validation (17, 18) on questionnaires similar to the one used in the present study have shown reasonable agreement between nutrient intakes, especially when expressed as a percentage of total energy, derived from food-frequency questionnaires and weighed intake. In particular, good agreement has been shown for alcohol, when expressed as a percentage of total energy.

TABLE 2
Age-adjusted ankle brachial pressure index, by frequency of individual food consumption in females in the Edinburgh Artery Study*

	Rarely/monthly	1–3/wk	4–7/wk	P†
White bread	1.00 ± 0.01	1.01 ± 0.02	1.01 ± 0.01	0.91
Whole-meal bread	1.01 ± 0.01	1.01 ± 0.01	1.01 ± 0.01	0.93
Low-fiber breakfast cereals (eg, porridge)	1.02 ± 0.01	0.98 ± 0.02	0.98 ± 0.02	0.04
High-fiber breakfast cereals (eg, bran flakes)	1.01 ± 0.01	1.01 ± 0:03	1.01 ± 0.02	0.75
Pork, bacon, ham	1.03 ± 0.01	1.00 ± 0.01	0.94 ± 0.03	0.002
Meat pies and pastries	1.01 ± 0.01	0.98 ± 0.01	0.84 ± 0.24	0.03
Kipper, herrings, tuna, mackerel	1.02 ± 0.01	1.00 ± 0.01	0.94 ± 0.06	0.06
Carrots	0.99 ± 0.02	1.02 ± 0.01	0.99 ± 0.01	0.71
Onions (raw, cooked)	1.00 ± 0.01	1.00 ± 0.01	1.03 ± 0.01	0.04
Sweets, biscuits, jellies, etc	0.99 ± 0.01	1.01 ± 0.01	1.04 ± 0.01	0.002
Lard, drippings, solid vegetable oil	1.01 ± 0.01	1.00 ± 0.01	0.97 ± 0.04	0.09
Apples	0.99 ± 0.01	0.99 ± 0.01	1.03 ± 0.01	0.11
Pears	1.01 ± 0.01	1.01 ± 0.01	1.02 ± 0.03	0.46

* $\bar{x}$ ± SE; n = 783.
† Linear trend.

TABLE 3
Age-adjusted ankle brachial pressure index, by frequency of individual food consumption in males in the Edinburgh Artery Study*

	Rarely/monthly	1–3/wk	4–7/wk	P†
White bread	1.08 ± 0.01	1.05 ± 0.02	1.04 ± 0.01	0.0008
Whole-meal bread	1.05 ± 0.01	1.08 ± 0.02	1.07 ± 0.01	0.03
Low-fiber breakfast cereals (eg, porridge)	1.06 ± 0.01	1.04 ± 0.02	1.06 ± 0.02	0.97
High-fiber breakfast cereals (eg, bran flakes)	1.05 ± 0.01	1.08 ± 0.03	1.10 ± 0.02	0.02
Pork, bacon, ham	1.05 ± 0.01	1.05 ± 0.01	1.06 ± 0.03	0.80
Meat pies and pastries	1.07 ± 0.01	1.04 ± 0.01	1.05 ± 0.07	0.03
Kipper, herrings, tuna, mackerel	1.05 ± 0.01	1.04 ± 0.01	0.99 ± 0.09	0.29
Carrots	1.03 ± 0.02	1.05 ± 0.01	1.07 ± 0.01	0.07
Onions (raw, cooked)	1.04 ± 0.01	1.06 ± 0.01	1.08 ± 0.02	0.08
Sweets, biscuits, jellies, etc	1.05 ± 0.01	1.06 ± 0.01	1.07 ± 0.01	0.22
Lard, drippings, solid vegetable oil	1.07 ± 0.01	1.04 ± 0.01	1.04 ± 0.03	0.06
Apples	1.05 ± 0.01	1.05 ± 0.01	1.08 ± 0.01	0.14
Pears	1.05 ± 0.01	1.08 ± 0.01	1.13 ± 0.03	0.004

* $\bar{x}$ ± SE; n = 809.
† Linear trend.

There were many cases (n = 297) of symptomatic coronary heart disease in the study sample, which if excluded would have also excluded many subjects with peripheral arterial disease. We included all cases of peripheral arterial disease in the analysis, thus our results are representative of the general population. Exclusion of cases of symptomatic coronary heart disease would also unduly reduce the power to detect dietary associations with the ABPI.

The total energy intake and nutrient intakes (Table 1) in males were slightly lower than might be expected from a previous survey in Edinburgh (19), although time trends in consumption indicate some consistency with this finding (20). The exception to the general decrease is a slight increase in cereal fiber in the Edinburgh Artery Study. However, comparisons are difficult because the Edinburgh Artery Study population is older.

When the frequencies of consumption of individual foods were considered, low ABPI was significantly associated with high frequency of consumption of low-fiber–containing foods (white bread in males, low-fiber breakfast cereals in females) and high frequency of consumption of meat and meat products in both

TABLE 4
Multiple linear regressions of age, sex, height, smoking, total energy, and each energy-adjusted nutrient separately on the ankle brachial pressure index (ABPI) in the Edinburgh Artery Study*

Energy-adjusted nutrients (+1 unit)	Value†	P
Vitamin C		
Never smoked	−0.02 (0.03)	0.53
Ever smoked	0.08 (0.02)	0.0004
Cereal fiber (ln)		
Males	2.43 (1.40)	0.08
Females	0.37 (1.40)	0.79
Alcohol (ln)		
Males	1.00 (0.48)	0.04
Females	0.02 (0.59)	0.97
Retinol (ln)	0.10 (0.82)	0.90
α-Tocopherol (ln)	1.85 (0.97)	0.05
β-Carotene (ln)	0.69 (0.54)	0.20
Carbohydrate (ln)	−0.02 (0.01)	0.17
Vegetable fiber	0.16 (0.11)	0.14
Protein (ln)	0.13 (3.26)	0.97
Saturated fatty acid (ln)	1.96 (1.90)	0.30
Polyunsaturated fatty acid (ln)	0.48 (1.15)	0.67
Linoleic acid (ln)	0.48 (0.99)	0.63

* ln, Natural logarithm. Diabetics excluded from the analysis. n = 1501.
† Change in ABPI × 100; SEE in parentheses. Adjusted for age, sex, height, smoking, and total energy.

TABLE 5
Multiple linear regression of age, sex, height, smoking, total energy, and all energy-adjusted nutrients simultaneously on the ankle brachial pressure index (ABPI) in the Edinburgh Artery Study*

	Value†	P
Sex (1 = female; 2 = male)	−1.91 (5.19)	0.71
Age (+10 y)	−4.87 (0.83)	< 0.001
Height (+10 cm)	1.21 (0.70)	0.08
Cigarette smoking (ever vs never)	−14.10 (2.64)	< 0.001
Total energy intake (+1000 kJ)	0.31 (0.79)	0.69
Energy-adjusted nutrients (+1 unit)		
Vitamin C		
Never smoked	−0.02 (0.03)	0.57
Ever smoked	0.07 (0.03)	0.006
Cereal fiber (ln)		
Males	3.79 (1.61)	0.02
Females	1.10 (1.52)	0.47
Alcohol (ln)		
Males	1.22 (0.59)	0.04
Females	0.05 (0.63)	0.94
Retinol (ln)	−0.79 (0.98)	0.42
α-Tocopherol (ln)	4.27 (2.17)	0.04
β-Carotene (ln)	0.24 (0.69)	0.73
Carbohydrate (ln)	−0.02 (0.02)	0.50
Vegetable fiber	0.01 (0.17)	0.95
Protein (ln)	−1.66 (3.96)	0.67
Saturated fatty acid (ln)	2.42 (3.13)	0.44
Polyunsaturated fatty acid (ln)	−2.64 (8.62)	0.76
Linoleic acid (ln)	−1.48 (7.76)	0.85

* ln, natural logarithm; diabetics excluded from the analysis. n = 1501.
† Change in ABPI × 100; SEE in parentheses.

males and females. The significant tests for trend indicate a dose-response relationship and provide strong evidence of cause and effect. The results are consistent with the general consensus concerning a healthy diet, ie, high fiber intake and a low intake of saturated fatty acids.

The relationships found between the frequency of consumption of individual foods, especially those containing fiber, and the ABPI were also reflected in the relationships between the calculated nutrient intakes and the ABPI in the regression analyses. In a multiple linear regression model, high intake of cereal fiber in males was significantly associated with high ABPIs, independent of smoking. This finding was consistent with the recent case-control study in Greece, in which it was found that a significant relationship existed between intake of crude fiber, which presumably included cereal fiber, and peripheral arterial disease, independently of smoking (5). In addition, in the Edinburgh Artery Study alcohol intake in males was associated with an increase in the ABPI. Alcohol intake in males was significantly higher than in females, and it is possible that this effect was mediated through recreational and sporting activity rather than a direct effect of alcohol, although there is some evidence that high wine consumption may be beneficial for coronary heart disease (21).

Food supplements contribute to the intake of nutrients, especially vitamin intake. Although this was not measured in the present study, a British survey (22) suggests that the frequency of food supplementation is low: 16% in the age range 50–64 y compared with that in the United States (23), 21%–40% in the age range 55–74 y. Thus our measures of vitamin intake may be underestimated, but despite this we still found significant relationships between ABPI and vitamin intake.

It is interesting that dietary α-tocopherol intake had a significantly positive effect on the ABPI ($P = 0.04$), independently of smoking. This finding is consistent with population studies in which a low plasma vitamin E concentration was correlated with ischemic heart disease (24) and angina (25). The association of dietary α-tocopherol intake and the ABPI occurred despite the fact that vitamin E measures derived from food-frequency questionnaires are not as accurate as blood measures (24). The other main dietary antioxidant, vitamin C, which is also thought to be related to atherosclerosis, showed some positive relationship to the ABPI, but this was only significant for those who had ever smoked. Nonsmokers already had significantly higher ABPIs and dietary vitamin C intake than did smokers; thus a high intake of vitamin C would have little effect in nonsmokers. By contrast, high dietary vitamin C intake may partly compensate for the reduction of vitamin C through smoking, although never reaching the amounts in nonsmokers. Hence, smoking remained one of the strongest risk factors for peripheral arterial disease.

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ORIGINAL ARTICLES

Fibrinogen genotype and risk of peripheral atherosclerosis

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There is conflicting evidence about the influence of fibrinogen genotype on plasma fibrinogen concentrations, and the relation between genotype and atherosclerotic disease has not been studied. In a population-based case-control study we aimed to find out whether certain fibrinogen genotypes are associated with an increased risk of peripheral atherosclerosis.

121 subjects with peripheral arterial disease and 126 healthy controls matched for age and sex were selected from a random population sample aged 55–74 years in the Edinburgh Artery Study. Mean fibrinogen concentrations were higher in cases than in controls (3·12 [95% confidence interval 2·99–3·26] *vs* 2·75 [2·64–2·85], $p < 0·001$). A greater proportion of cases than controls were homozygous or heterozygous for an allele at the β fibrinogen locus (4·2 kb allele, *Bcl* I digestion); the allele frequency was 0·197 in cases and 0·097 in controls ($p < 0·005$). Extended haplotypes for 4·2 kb heterozygotes were also associated with an increased risk of peripheral arterial disease. However, haplotype had only a small effect on the association of plasma fibrinogen concentration with disease, and the relation of haplotype with disease was independent of age, sex, social class, smoking status, plasma fibrinogen, alcohol consumption, body mass index, and diabetes mellitus.

We conclude that variation at the β fibrinogen locus is associated with an increased risk of peripheral atherosclerosis. The influence is not mediated simply by way of increased fibrinogen concentrations but could be due to a structurally variant fibrinogen or linkage disequilibrium with a neighbouring gene.

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Introduction

Fibrinogen concentration in the blood is believed to be important in the pathogenesis of atherosclerosis. High plasma concentrations are associated with increased risks of ischaemic heart disease,[1-4], stroke,[1-4] and arterial disease in the legs.[5-7] In population surveys, cigarette smoking has been related to fibrinogen concentrations,[8-13] and after smoking cessation the concentration falls.[14] Other factors that may influence fibrinogen concentrations include age, social class, obesity, serum cholesterol, diabetes mellitus, alcohol consumption, intake of cereal fibre, use of oral contraceptives, and the menopause.[4,8,9,11-15]

Attention has focused lately on the extent to which fibrinogen concentrations are genetically determined. There are conflicting results: variation at the β fibrinogen locus related to fibrinogen concentrations was seen in selected populations in the UK[16,17] but not in Norway.[18] These studies, however, did not include the relation between fibrinogen genotype and atherosclerotic disease. We aimed, in this study, to find out the extent to which fibrinogen genotypes are associated with an increased risk of peripheral

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arterial disease, taking account of cigarette smoking and other cultural factors.

Subjects and methods

The design was a population-based case-control study. Cases and controls were selected from the 1988 Edinburgh Artery Study, a cross-sectional survey of peripheral arterial disease in the leg. Men and women aged 55–74 years were randomly selected from the registers of ten general practices. 1592 (65% response) attended for a clinical examination. Follow-up of a random sample of non-responders revealed no substantial bias. The severity of peripheral arterial disease was assessed by means of the World Health Organisation questionnaire on intermittent claudication,[19] the ankle brachial pressure index (ABPI), and a reactive hyperaemia test. A history of angina and myocardial infarction was taken,[19] and a 12-lead resting electrocardiogram (ECG; Minnesota coded) was done.[20] Social class was coded according to the method of the Registrar General.[21] The subjects were asked about alcohol consumption, and height, weight, and glucose tolerance were measured. Further details have been published elsewhere.[22]

153 subjects were classified as cases, defined as having ABPI of 0·7 or below, hyperaemic reduction in ankle pressure of at least 35%, or two or more of the following—intermittent claudication, ABPI of 0·9 or lower, and hyperaemic reduction in ankle pressure of at least 20%. The cut-off points were based on validity studies in hospital patients.[23,24] Subsequent duplex scanning of subsamples of the cases and controls confirmed the validity.[22] Exclusion criteria were pain at rest, gangrene, ulcer, or previous arterial surgery.

The controls met none of the inclusion criteria and had no evidence of angina pectoris, previous myocardial infarction, or ischaemia on ECG. They were matched to the cases by sex and by 5-year age bands. They were not matched for smoking history, which was elicited by questionnaire and correlated with thiocyanate concentrations measured in the community survey.

The cases and controls were invited in March, 1989, to attend a clinic at the University Health Centre, Edinburgh. Non-responders were reminded by a second letter and by telephone. Subjects unable to travel but willing to take part were visited at home. Subjects completed a questionnaire on medical history, current smoking status, and medication. After the subject had rested supine for 5 min, blood was sampled from the antecubital vein without stasis.

Plasma fibrinogen was assayed by a clotting method (modified Clauss assay with a 'Coag-A-mate' coagulometer and Organon Teknika reagents and standards; Organon Teknika, Cambridge, UK) and by heat precipitation with a nephelometric method.[2] Plasma glucose was measured on a Cobas-Bio autoanalyser (Roche Products, Welwyn Garden City, UK). DNA was extracted from white blood cells and digested with appropriate restriction enzymes. DNA fragments were separated by electrophoresis in 0·8% agarose gels and transferred to a DNA binding filter ('Hybond-N', Amersham) by Southern blotting. Hybridisation was carried out under standard conditions.[25] Autoradiographs were developed at −70°C with Kodak 'Exomat' films and super-rapid intensifying screens for 1–7 days. The probes used were cDNAs for the alpha (pFA1), beta (pFB5), and gamma (pFG1) fibrinogen loci, which detect polymorphic fragments of 2·4 kb and 1·6 kb (*Taq* I), 5·3 kb and 4·2 kb (*Bcl* I), and 14 kb and 11 kb (*Kpn* I/*Sac* I), respectively.

Data were analysed on the University of Edinburgh mainframe computer with the BMDP statistical package.[26] Subjects were classified as current smokers, ex-smokers (<5 years), or non-smokers; life-time consumption in pack years was also studied because it is also associated with the risk of disease.[27] The square root of pack years was used, so as to reduce the influence of a few very heavy smokers. Diabetic status was classified by WHO criteria.[28] Alcohol consumption was analysed separately for men and women. Dummy variables were created to assess the association with disease of each haplotype in relation to the commonest haplotype. Multiple logistic regression on peripheral arterial disease was used to determine the independent effect of each factor on the odds of disease in relation to fibrinogen concentration. The logistic regressions were adjusted for age and sex.[29]

TABLE I—PLASMA FIBRINOGEN AND CIGARETTE SMOKING

—	Cases (n=121)	Controls (n=126)	p
*Mean fibrinogen concentration (g/l)**			
Clotting method	3·12 (2·99–3·26)	2·75 (2·64–2·85)	<0·001
Nephelometric method	4·48 (4·31–4·65)	3·91 (3·74–4·06)	<0·001
Cigarette smoking			
% current smokers	44	20	<0·001
% ex-smokers	42	30	
% non-smokers	14	50	
Mean $\sqrt{\text{pack}}$ years (SE)	4·82 (0·22)	2·04 (0·20)	<0·001

*95% confidence interval in parentheses.

TABLE II—FIBRINOGEN GENOTYPE AND HAPLOTYPES

—	Cases (n=115)	Controls (n=120)	p*	p†
Genotype α				
2·4 2·4	56	59	0·11	..
2·4 1·6	41	32		
1·6 1·6	4	9		
Genotype β				
5·3 5·3	65	82	0·01	..
5·3 4·2	31	18		
4·2 4·2	4	1		
Genotype γ				
11 11	60	59	0·37	..
14 11	37	33		
14 14	4	8		
Haplotype				
1: α2·4/2·4; β5·3/5·3; γ11/11	42	58	0·14	0·18
2: α2·4/1·6; β5·3/5·3; γ14/11	29	36		0·56
3: α2·4/2·4; β5·3/4·2; γ11/11	24	19		0·30
4: α2·4/1·6; β5·3/5·3; γ11/11	7	5		0·47
5: α2·4/1·6; β5·3/4·2; γ14/11	14	5		0·03
6: α1·6/1·6; β5·3/5·3; γ14/14	5	10		0·27
Others	10	10		0·73

*Significance of overall association between genotype/haplotype and peripheral arterial disease.
†Significance of difference between each haplotype and every other haplotype.

Results

121 cases and 126 controls of 153 selected in each group took part in the study (overall response rate 81%). In neither group did responders differ from the subjects selected in age, sex, smoking status, frequency of intermittent claudication, or mean ABPI. The mean age of cases and controls was the same (67 years) and there were approximately equal numbers in each group of men and women. Among the cases, 21% had claudication and the mean ABPI was 0·7.

Mean fibrinogen concentrations were significantly higher in subjects with peripheral arterial disease than in controls (table I). Significantly more of the cases than of the controls had ever smoked (table I). Alcohol consumption was lower in cases than in controls (median 2·0 *vs* 4·0 units per week; $p = 0·06$). There was no difference between the groups in body mass index or frequency of diabetes mellitus (both $p = 0·7$). The social-class distribution differed slightly; there were fewer cases than controls in classes I and II and more cases than controls in classes III–V ($p = 0·07$).

The genotype frequencies among the 247 subjects were compared with those predicted from the Hardy-Weinberg equilibrium; no significant differences were found. The frequencies of allele combinations at the α, β, and γ fibrinogen loci in cases and controls are shown in table II. There were significant excesses ($p < 0·01$) at the β locus of cases compared with controls heterozygous or homozygous for 4·2 kb but no significant differences between cases and controls in allele combinations at the α and γ loci. Frequencies of the individual alleles at the β locus reflected differences in allelic combinations; there were highly

TABLE III—UNIVARIATE AND MULTIVARIATE REGRESSIONS OF FIBRINOGEN HAPLOTYPES, CIGARETTE SMOKING, AND PLASMA FIBRINOGEN WITH PERIPHERAL ARTERIAL DISEASE

	Odds ratio (95% CI) of peripheral arterial disease	
—	Univariate	Multivariate
Haplotype (compared with type 1)		
Type 2	1·1 (0·5–2·2)	1·1 (0·4–2·7)
Type 3	2·3 (1·0–5·4)†	4·1 (1·3–12·4)‡
Type 4	2·1 (0·4–8·0)	2·3 (0·5–10·9)
Type 5	4·8 (1·2–19·0)‡	7·6 (1·5–39·1)‡
Type 6	0·5 (0·1–8·0)	0·3 (0·1–1·7)
*Cigarette smoking**		
√pack years	4·3 (2·5, 7·2)¶	4·5 (2·4, 8·2)¶
Current smokers	0·9 (0·3, 2·3)	1·3 (0·4, 3·9)
Ex-smokers	0·6 (0·2, 2·2)	0·6 (0·1, 2·4)
Fibrinogen (+0·2 g/l)	1·82 (1·38, 2·41)¶	1·52 (1·08, 2·14)‡

All analyses adjusted for sex and age group.
*Change in pack years is one unit on square root scale; current and ex-smokers < 5 years are compared with non-smokers and are adjusted for pack years.
†$p < 0·10$; ‡$p < 0·05$; ¶$p < 0·001$.

significant ($p < 0·005$) differences between cases and controls in the frequencies of the 5·3 kb (0·802 *vs* 0·903) and 4·2 kb (0·197 *vs* 0·097) alleles.

Variation at the β locus was also shown in the haplotype analysis; types 3 and 5 were more common in cases than in controls, although the difference in type 3 was small and not significant ($p = 0·3$). A finding of one significant difference (type 5, $p = 0·03$) is more than would be expected by chance with multiple tests. Overall, the haplotype distribution among cases and controls did not differ significantly ($p = 0·14$), but the differences between type 3 or type 5 and type 1 were sufficient to be associated in univariate analysis with an increased risk of disease (table III).

To examine the relation between fibrinogen concentrations and vascular disease, concentrations measured by the clotting method were chosen for further analysis. An increase in fibrinogen concentration of 0·2 g/l was associated with an odds ratio of disease of 1·82 (table III). Cigarette smoking, adjusted for lifetime consumption in pack years, current smoking, and ex-smoking, reduced the odds ratio to 1·68 ($p < 0·01$). Adjustment for haplotype alone had little effect on the odds ratio for fibrinogen (1·77, $p < 0·001$). This limited effect may have been related partly to the lower prevalence of smoking in subjects with type 3 haplotype than in the whole study sample (50% *vs* 65% ever smoked). With adjustment for both smoking and haplotype, the odds ratio for fibrinogen fell to 1·52, but it was still significant (table III). The odds ratios after separate adjustment for body mass index, diabetes mellitus, and alcohol consumption were 1·80, 1·79, and 1·76, respectively, with no change in significance. Social class had a stronger effect, lowering the odds ratio to 1·70, but it was related to social-class differences in cigarette smoking.

The factors significantly associated with peripheral arterial disease (haplotype, cigarette smoking, and fibrinogen concentration) were included in a multivariate analysis (table III); both haplotype and cigarette smoking retained significant associations with disease independently of fibrinogen concentration. Inclusion of social class, alcohol consumption, body mass index, and diabetes mellitus had little effect; none of the latter factors was associated independently with disease.

Discussion

The influence of fibrinogen genotype on plasma fibrinogen concentrations in the general population is not well established. In a UK study of three restriction-fragment length polymorphisms,[16] the strongest association of fibrinogen concentrations was with polymorphism detected by means of the β-fibrinogen probe and *Bcl* I. Genetic variation at the fibrinogen locus accounted for 15% of total phenotypic variance in fibrinogen concentration. In a larger subsequent study, variation in the promoter region of the β gene (*Hae* III polymorphism) explained 3·1% of variance in fibrinogen concentrations.[17] By contrast, a study in Norway[18] found no association between plasma fibrinogen concentrations and genotype in two polymorphisms (at the α and β loci). We too have found in the whole population of our study that polymorphisms at the α, β, and γ loci are not significantly related to fibrinogen concentrations.[30] Furthermore, estimates of the heritability of plasma fibrinogen concentrations have varied from 27%[18] to 51%.[31]

We have examined the possible influence of fibrinogen genotype on the risk of atherosclerotic disease. The main finding was that at the fibrinogen β locus the 4·2 kb allele was over-represented in cases compared with controls. Variation at the β fibrinogen locus might well affect fibrinogen concentrations, because the β gene controls formation of the Bβ chain, the rate-limiting step in fibrinogen synthesis. Interestingly, however, the higher odds of disease related to β-locus genotype seemed not to be attributable solely to the present plasma fibrinogen concentrations; they were also independent of other likely factors. The fact that the association between genotype and disease was not strong is not surprising, because atherosclerosis is a heterogeneous trait influenced by many environmental and genetic factors. The association may also have been affected by variation in the measurement of fibrinogen concentration.

Berg and Kierulf have pointed out[18] that genes may be important in causing disease without having any direct effect on the extent of a risk factor. The significant effect of fibrinogen haplotype on disease was retained after adjustment for fibrinogen concentration: thus there may be a mechanism of pathogenesis other than the effect of haplotype on fibrinogen. The polymorphic variant might be in a linkage disequilibrium with a mutation within the β fibrinogen gene or a neighbouring gene that has a direct role in atherogenesis. Mutations in the β fibrinogen locus could produce a protein with abnormal physicochemical characteristics and hence increased atherogenic potential. Also, there might be mechanisms by which genes can help to set the framework for lifestyle and cultural characteristics to cause variations in risk factors. We noted, for example, a slight but unexplained relation between haplotype and smoking, although this may have been a chance finding. The association between fibrinogen genotype and atherosclerosis needs to be investigated in different populations, and the interaction with other risk factors should be analysed.

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Voluntary dehydration and heat intolerance in cystic fibrosis

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Although exercise may be beneficial in cystic fibrosis (CF), patients' low tolerance to climatic heat stress means that physical exertion can increase morbidity and mortality. We postulated that the high salt content of CF patients' sweat and the consequent absence of body-fluid hyperosmolality during a long episode of sweating might deprive such patients of a thirst stimulus.

Eight children with CF (four boys, four girls; aged 9·5–14·1 years) and eight controls, matched for age and sex, attended two randomly ordered sessions of exercise (cycling) in a chamber at 31–33°C, relative humidity 43–47%. 20 min bouts of exercise (at 45% of predetermined maximum oxygen uptake) were interspersed with 25 min rest periods. At one session, chilled water was given every 15–20 min to replace fluid lost; at the other, drinking was guided by the child's thirst. At the thirst-guided session, CF patients drank much less than the controls did (0·80% *vs* 1·73% initial body weight) and lost twice as much fluid (1·57% *vs* 0·78% initial body weight). The recovery of heart rate after exercise was slower in CF patients, but there were no other signs of heat strain. The groups did not differ in any variable during the forced drinking session.

We conclude that children with CF underestimate their fluid needs and undergo excessive dehydration during extended exposure to hot conditions.

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Introduction

Interest in exercise prescription for patients with cystic fibrosis (CF) has increased during the past few years,[1-3] but physical exertion has two potentially detrimental effects in CF: arterial oxygen desaturation, mostly in patients with advanced disease during high-intensity exercise;[1] and low tolerance to climatic heat stress, which increases morbidity and mortality.[4-6] No deficiency in the ability of CF patients to dissipate heat during exercise in hot conditions has been

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Genetic variation at fibrinogen loci and plasma fibrinogen levels

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Abstract

In view of the controversy regarding genetic variation at the fibrinogen loci and plasma fibrinogen levels, we have analysed DNA polymorphisms at the alpha (*Taq*I), beta (*Bcl*I and *Hae*III), and gamma (*Kpn*I/*Sac*I) fibrinogen loci in 247 subjects whose plasma fibrinogen was determined by clotting and nephelometric assays. Strong linkage disequilibrium was found between the alpha/*Taq*I and gamma/*Kpn*I/*Sac*I markers and between the beta/*Bcl*I and beta/*Hae*III markers. A lesser association was found between the alpha/*Taq*I and beta/*Bcl*I loci, beta/*Bcl*I and gamma/*Kpn*I/*Sac*I markers, alpha/*Taq*I and beta/*Hae*III markers, and the gamma/*Kpn*I/*Sac*I and beta/*Hae*III markers. This is consistent with the known physical order of these loci and suggests a relative excess of recombination in the alpha/gamma to beta interval. Plasma fibrinogen levels, by either assay method, when corrected or uncorrected for age, sex, and smoking habit, did not show any statistically significant associations with the four fibrinogen polymorphisms examined at the alpha, beta, and gamma fibrinogen loci either singly or when analysed as a haplotype.

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Plasma levels of fibrinogen rise acutely in response to infection, injury, or other trauma and in population surveys are associated with age, smoking, obesity, cholesterol, alcohol, and the menopause.[1] Raised levels of fibrinogen have been a consistent risk factor for atherosclerosis and this has led to studies to see if plasma levels of fibrinogen are influenced by genetic variation at the fibrinogen loci. Humphries *et al*[2] performed DNA analysis for the fibrinogen alpha and beta loci on 91 healthy English subjects and concluded that genetic variation at these loci accounted for 15% of the total variance. In contrast, Berg and Kierulf[3] could detect no association in 118 Norwegian subjects between levels of plasma fibrinogen and the fibrinogen alpha and beta genotypes. More recently, Thomas *et al*[4] analysed the *Hae*III polymorphism at the 5' end of the beta fibrinogen gene in 292 healthy subjects and found a significant association with fibrinogen levels which explained 3·1% of the variance. We therefore undertook clotting and nephelometric plasma fibrinogen assays and analysis of polymorphic variation at the fibrinogen gamma locus in addition to polymorphisms at the alpha and beta loci in 247 subjects to help understand the role of genetic variation at the fibrinogen loci in determination of plasma fibrinogen levels.

Subjects and methods

The subjects (121 cases and 126 age and sex matched controls) were recruited from the 1988 Edinburgh Artery Study for a case-control study of peripheral arterial disease.[5] After five minutes rest in the supine position, a specimen of venous blood was obtained from the antecubital vein usually without stasis. In the laboratory, fibrinogen was assayed using two methods: firstly, a clotting method (modified Clauss assay using a Coag-A-Mate X2 coagulometer and reagents and standards from Organon Teknika) and, secondly, heat precipitation by a nephelometric method.[6] DNA was extracted from white blood cells and digested with the appropriate restriction enzyme. DNA fragments were then separated by electrophoresis in 0·8% agarose gels and transferred to a DNA binding filter (Hybond-N, Amersham) by Southern blotting. Hybridisation was performed with ^{32}P labelled probes in 2 × Denhardts, 4·5 × SSC, 0·1% SDS, 6% w/v PEG at 65°C overnight followed by several high stringency washes in 1 to 0·05 × SSC/0·1% SDS at 65°C. Autoradiographs were developed at −70°C using Kodak Exomat films and intensifying screens for one to seven days. The probes used were all cDNAs, pAF1 (alpha), pFB5 (beta), and pFG1 (gamma) which detect polymorphic fragments of 2·4 kb and 1·6 kb (*Taq*I), 5·3 kb and 4·2 kb (*Bcl*I), and 14 kb and 11 kb (*Kpn*I/*Sac*I) respectively (appropriate restriction enzymes indicated in parentheses in each instance). The *Hae*III/beta polymorphism (alleles of 958 bp/575 bp + 383 bp) was shown after PCR amplification and DNA digestion.[4]

Gene frequencies were calculated by gene counting. χ^2 analysis using Yates's correction was applied for comparison of allele frequencies, the standardised disequilibrium coefficients (delta values) were calculated according to Chakravarti *et al*,[7] and probability values were calculated according to Fisher's exact probabilities. As the fibrinogen concentrations measured by the clotting method were positively skewed, a logarithmic transformation was used, yielding geometric means and their confidence intervals as summary measures. Fibrinogen levels were adjusted by age, sex, and cumulative smoking using multiple linear regression. Cumulative smoking was expressed in terms of packyears: the average number of packs of cigarettes smoked per day × number of years as a smoker. One way analysis of variance was carried out on the

Table 1 Mean fibrinogen values adjusted for age, sex, and smoking habit in groups with different fibrinogen genotypes at the alpha, beta and gamma loci.

	Clotting method	Nephelometric method
	Mean* (95% CI)	Mean (95% CI)
Alpha locus (*Taq*I)		
2·4/2·4	2·94 (2·90,2·99)	4·19 (4·13,4·24)
2·4/1·6	2·94 (2·89,2·98)	4·20 (4·13,4·27)
1·6/1·6	2·90 (2·80,3·00)	4·17 (4·03,4·32)
	p=0·83	p=0·95
Beta locus (*Bcl*I)		
5·3/5·3	2·94 (2·90,2·97)	4·2 (4·15,4·25)
5·3/4·2	2·91 (2·86,2·98)	4·15 (4·06,4·23)
4·2/4·2	2·99 (2·83,3·17)	4·25 (4·00,4·48)
	p=0·67	p=0·48
Beta locus (*Hae*III)		
575+383/575+383	2·92 (2·80,3·05)	
575+383/958	2·99 (2·76,3·24)	
958/958	2·68 (1·94,3·04)	
	p=0·58	
Gamma locus (*Kpn*I/*Sac*I)		
14/14	2·92 (2·80,3·04)	4·19 (4·03,4·36)
14/11	2·93 (2·88,2·98)	4·19 (4·12,4·26)
11/11	2·94 (2·90,2·97)	4·19 (4·14,4·25)
	p=0·94	p=0·99

* Mean for clotting assay is the geometric mean as the distribution is positively skewed.

adjusted fibrinogen values to assess differences between genotypes.

Results

The overall frequencies for each polymorphic allele in the 247 subjects were 0·75 for the 2·4 kb (A) allele and 0·25 for the 1·6 kb (a) allele at the alpha fibrinogen locus; 0·84 for the 5·3 kb (B) and 0·16 for the 4·2 kb (b) alleles at the beta fibrinogen locus; 0·82 for the 575 bp + 383 bp (H1) and 0·18 for the 958 bp (H2) alleles at the 5′ end of the beta fibrinogen locus; and 0·24 for the 14 kb (D) and 0·76 for the 11 kb (d) alleles at the gamma fibrinogen locus. The overall genotype frequencies were compared with those predicted from the Hardy-Weinberg equilibrium and no significant differences were noted.

Strong linkage disequilibrium was found between the alpha/*Taq*I and gamma/*Kpn*I/*Sac*I markers and between the beta/*Bcl*I and beta/*Hae*III markers which was highly significant at any level (delta values of 0·6981 and 0·462, $p < 0·001$ and $p < 0·001$ respectively). A lesser association was found between the alpha/*Taq*I and beta/*Bcl*I loci (delta value of −0·1423, $p < 0·001$); beta/*Bcl*I and gamma/*Kpn*I/*Sac*I markers (delta value of −0·1450, $p < 0·01$); alpha/*Taq*I and beta/*Hae*III markers (delta value of −0·174, $p < 0·001$); and the gamma/*Kpn*I/*Sac*I and beta/*Hae*III markers (delta value of −0·188, $p < 0·001$).

Table 1 indicates the mean fibrinogen values adjusted for age, sex, and smoking history analysed by the clotting and nephelometric assays for different fibrinogen genotypes at the alpha, beta, and gamma fibrinogen loci. No trend is apparent and no statistically significant differences were noted. Similarly no statistically significant differences were apparent in an analysis using fibrinogen levels uncorrected for age, sex, and smoking history or if cases and controls were considered separately (data not shown).

Table 2 indicates the mean fibrinogen values adjusted for age, sex, and smoking history analysed by the clotting and nephelometric assays for different fibrinogen haplotypes. No statistically significant differences are apparent, nor were they present in an analysis using fibrinogen levels uncorrected for age, sex, and smoking history or if cases and controls were considered separately (data not shown).

Discussion

Plasma fibrinogen is synthesised in the liver from three polypeptide subunits (A alpha, B beta, and gamma) whose structural genes occur as a cluster at 4q31.[8] These genes show considerable homology and are believed to have arisen by duplication of an ancestral gene with subsequent divergence.[9] Each gene is approximately 10 kb in size and as all three are found on a 50 kb stretch of DNA, linkage disequilibrium between these loci would be expected. In this study strong linkage disequilibrium was found between the two polymorphisms at the beta fibrinogen locus and between the polymorphisms at the alpha and gamma loci with a weaker association between beta and alpha or beta and gamma polymorphisms. This is consistent with previous reports on linkage disequilibrium for these loci[2 10 11] and with the physical order of gamma-alpha-beta.[9] The relative excess of recombination in the gamma/alpha to beta interval cannot be explained by physical distance and recombination in this interval has also been implicated in the evolution of these loci to account for the reverse orientation of transcription of the beta gene relative to the alpha and gamma fibrinogen genes.[9]

In the present study, in cases or controls or both combined, the plasma level of fibrinogen assayed by the clotting and nephelometric methods when corrected or uncorrected for age, sex, and smoking showed no association with the fibrinogen genotype at the alpha, beta, or gamma loci. This is consistent with the experience of Berg and Kierulf[3] using the

Table 2 Mean fibrinogen levels assayed by the clotting and nephelometric methods corrected for age, sex, and smoking habit for different fibrinogen haplotypes.

					Fibrinogen, clotting	Fibrinogen, nephelometric
Haplotype	Alpha	Beta	Gamma	%	Mean* (95% CI)	Mean (95% CI)
1	2·4/2·4	5·3/5·3	11/11	37	2·94 (2·90,2·99)	4·22 (4·15,4·29)
2	2·4/1·6	5·3/5·3	14/11	21	2·93 (2·87,3·00)	4·19 (4·09,4·28)
3	2·4/2·4	5·3/4·2	11/11	16	2·91 (2·83,2·99)	4·12 (4·00,4·24)
4	2·4/1·6	5·3/5·3	11/11	4	2·91 (2·80,3·03)	4·16 (3·97,4·36)
5	2·4/1·6	5·3/4·2	14/11	7	2·93 (2·84,3·02)	4·19 (4·07,4·32)
6	1·6/1·6	5·3/5·3	14/14	5	2·94 (2·82,3·06)	4·23 (4·08,4·38)
Others				7		

* Mean for clotting assay is the geometric mean as the distribution is positively skewed.

alpha (*Taq*I) and beta (*Bcl*I) polymorphisms, but differs from the findings of Humphries *et al*[2] and Thomas *et al*.[4] Humphries *et al*[2] found a statistically significant rise of plasma fibrinogen levels in homozygotes for the rarer beta/*Bcl*I polymorphism but not with the beta/*Ava*II (which is in strong linkage disequilibrium with the beta/*Bcl*I polymorphism[2]) or alpha/*Taq*I polymorphisms. Thomas *et al*[4] also found a statistically significant rise of plasma fibrinogen in homozygotes for the rarer beta/*Hae*III polymorphism when smokers and non-smokers were combined. Although numbers in these studies were large (91 in Humphries *et al*,[2] 118 in Berg and Kierulf,[3] 292 in Thomas *et al*,[4] and 247 in the present study) the numbers of key homozygous subjects were small (4 bb homozygotes in Humphries *et al*,[2] 2 bb homozygotes in Berg and Kierulf,[3] and six in the present study; 11 H2H2 homozygotes in Thomas *et al*[4] and five H2H2 homozygotes in the present study). This, rather than population differences, is probably the main problem for these studies together with the considerable within person variation of fibrinogen values over time.[12]

Berg and Kierulf[3] also analysed the monozygotic twin intraclass correlation coefficient in respect of plasma fibrinogen levels and gave heritability estimates of 0·27 in the total series and 0·29 in non-smokers alone. Hamsten *et al*,[13] using path analysis in 170 nuclear families (half with a proband with premature myocardial infarction), suggested that 51% of the variance in plasma fibrinogen levels was because of genetic heritability after adjusting for cultural factors such as smoking and obesity. These studies thus suggest that there are genetic determinants of plasma fibrinogen levels although the magnitude of this effect and the importance of the fibrinogen genotype in this respect is unresolved. These studies have been hampered by the marked within individual variation which occurs for plasma fibrinogen levels, which in one study accounted for 27% of the sample variance.[12] Longitudinal studies of plasma fibrinogen levels in genotyped subjects would thus be of value in helping to resolve the role of fibrinogen and other genes in determination of plasma fibrinogen levels.

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Blood Viscosity, Fibrinogen, and Activation of Coagulation and Leukocytes in Peripheral Arterial Disease and the Normal Population in the Edinburgh Artery Study

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Background. **Increased blood and plasma viscosity, hematocrit, fibrinogen, and activation of coagulation and leukocytes have been reported in patients with claudication; however, their associations with symptomatic and asymptomatic peripheral arterial disease have not been reported in an epidemiological study.**

Methods and Results. **Blood and plasma viscosity, hematocrit, fibrinogen, urinary fibrinopeptide A, plasma leukocyte elastase, and uric acid were measured in a random sample of 1,581 men and women aged 55–74 years in Edinburgh, Scotland, and related to peripheral arterial stenosis (ankle–brachial systolic pressure index, ABPI) and to lower limb ischemia (intermittent claudication and reactive hyperemia test). Each variable (except fibrinopeptide A) was significantly related to prevalent symptomatic and asymptomatic peripheral arterial disease. On multivariate analysis, blood viscosity ($p<0.05$) and fibrinogen ($p<0.01$) were independently associated with peripheral arterial narrowing (ABPI); a positive interaction was found between fibrinogen and smoking in the association with ABPI. Plasma viscosity was associated with claudication in the presence of a given degree of arterial narrowing (odds ratio of claudication in top quintile compared with bottom quintile of plasma viscosity, 3.35; 95% CI, 1.32, 8.51). Leukocyte elastase and uric acid were each associated with reactive hyperemia independently of arterial narrowing ($p<0.01$).**

Conclusions. **Blood rheological factors and leukocyte activation as well as arterial narrowing are associated with lower limb ischemia in the general population and may be implicated in its pathogenesis. (*Circulation* 1993;87:1915–1920)**

KEY WORDS • **plasma viscosity** • **fibrinopeptide A** • **atherosclerosis** • **claudication**

Chronic ischemia of the lower limbs may result from not only atherosclerotic stenoses but also from thrombotic occlusions[1] and rheological abnormalities such as increased blood viscosity and impaction of activated leukocytes in the nutritive microcirculation.[2,3] Previous studies have suggested that patients with intermittent claudication may have activation of blood coagulation,[4,5] increased blood viscosity caused by elevation of hematocrit and plasma fibrinogen,[2,6–9] and increased leukocyte activation and rigidity.[10,11] However, these factors have not been related to peripheral arterial disease in epidemiological studies in the general population, nor have relations with other cardiovascular risk factors (e.g., smoking) been taken into account.

The Edinburgh Artery Study is a cross-sectional survey of 1,592 men and women aged 55–74 years residing in Edinburgh, Scotland. A high prevalence of symptomatic and asymptomatic peripheral arterial disease has been identified in this population[12] as well as associations with conventional cardiovascular risk factors.[13] We now report the relations of whole blood viscosity and its major determinants (hematocrit, plasma viscosity, fibrinogen) as well as measurements of activation of blood coagulation (urinary fibrinopeptide A)[14] and of blood leukocytes (plasma leukocyte elastase)[15] to symptomatic and asymptomatic peripheral arterial disease in this population. We also tested the hypothesis that, for a given degree of atherosclerotic arterial narrowing (measured by the ankle–brachial pressure index, ABPI), leg ischemia (measured by the World Health Organization [WHO] intermittent claudication questionnaire[16] and by a reactive hyperemia test[12]) was related to viscosity[7] and to leukocyte activation.[10]

Methods

In this cross-sectional survey,[12] 1,592 men and women aged 55–74 years were selected from the age/sex regis-

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ters of 10 general practices with catchment area populations spread geographically and socioeconomically throughout the city. The sample was selected randomly within sex-specific 5-year age groups to produce equal numbers in each group and an adequate sample size to conduct a future cohort study. Subjects attended a university clinic to complete a questionnaire and have a comprehensive medical examination. The response rate was 65%, and follow-up of a sample of nonresponders did not show any significant bias. Details of the study population, recruitment, and prevalence of peripheral arterial disease are described elsewhere.[12] The questionnaire included validated questions on cardiovascular history, intermittent claudication and angina (WHO questionnaire[16]), and smoking history. A 12-lead ECG was taken and coded independently by two observers using the Minnesota code.[17] Arm blood pressure was taken supine after 10 minutes of rest using a random zero sphygmomanometer. Peripheral pulses were palpated, and ankle systolic pressures were then measured using a Sonicaid Doppler probe and random zero sphygmomanometer with the patient supine. The ABPI was calculated as a measure of arterial narrowing in the lower limb.[12]

A reactive hyperemia test was then carried out in which ankle systolic pressures were measured 15 seconds after the release of a cuff occluding arterial flow for 4 minutes above the knee at 50 mm Hg above systolic pressure.[12] Reactive hyperemia tests have been shown to have adequate validity in detecting angiogram-positive disease in hospital patients.[18,19] In preliminary studies, we found that our technique detected the greatest hyperemic response and had adequate reproducibility.[20] The main purpose of this test was to detect those with substantial peripheral atherosclerosis who might have had a normal ABPI, as may occur in diabetics. Details of the results of the reactive hyperemia test in this population are published elsewhere.[21]

From a fasting blood sample taken on each patient at about 9:00 AM, serum uric acid was estimated on a Cobas Bio analyzer, using a standard kit. Fibrinogen was measured in citrated plasma by a thrombin-clotting turbidometric method in a centrifugal analyzer.[22] Blood and plasma viscosity were measured from a blood sample anticoagulated with dry dipotassium edetate (EDTA, 1.5 mg/mL) at high shear rates (over 300 sec^{-1}) in a Coulter-Harkness viscometer at 37°C.[23] Hematocrit was measured using a Hawksley microcentrifuge and reader. Blood viscosity was corrected to a standard hematocrit of 45% using the formula of Matrai et al.[24] Relative blood viscosity (corrected blood viscosity/plasma viscosity) was calculated as a measure of red cell deformability.[3,25] Urinary fibrinopeptide A was measured by radioimmunoassay as previously described,[14] using reagents from IMCO (Stockholm). Plasma leukocyte elastase was also measured by radioimmunoassay as previously described.[15] Quality control was monitored by means of blind duplicate samples taken intermittently throughout the study.

Data were analyzed on the Edinburgh University mainframe computer using SPSSX and BMDP statistical packages. In the univariate analysis, the population was divided for descriptive purposes into four categories of peripheral arterial disease[12]: intermittent claudication (WHO questionnaire positive and ABPI ≤0.9 or reactive hyperemia >20%), major asymptomatic disease (ABPI ≤0.9 and reactive hyperemia >20% or ABPI ≤0.7 or reactive hyperemia >35%), minor asymptomatic disease (ABPI ≤0.9 or reactive hyperemia >20%), and normal (none of the above). Because these categories have not been used in other studies, the validities were unknown, but results of studies comparing the ABPI and reactive hyperemia separately with arteriography[26] suggest that the classification has adequate face validity. The main analysis, however, concentrated on the ABPI because it was almost completely recorded and is a continuous measure, thus giving more power to detect associations. The minimum ABPI in the two legs was used because disease often occurs unilaterally. The minimum of two measurements induced slight negative skewness caused by random variation between legs, but this was not sufficient to justify transformation.

Multiple linear regression was used to investigate the relations between the ABPI and age, sex, blood and plasma viscosity, hematocrit, fibrinogen, leukocyte elastase, urinary fibrinopeptide A, uric acid, and smoking. Logarithmic transformations[27] were carried out on the values for leukocyte elastase and urinary fibrinopeptide A because of positive skewness in their distributions. Height was also included in the regressions because a positive association with the ABPI was demonstrated.[12] This may have been due to the widening of pulse pressure, as blood flows through arteries leading to a relatively high systolic pressure at the ankle in taller individuals.[28] Exclusion of height would have affected the association with sex, tending to increase the ABPI in male subjects. The multivariate analysis was carried out with all of the above factors estimated simultaneously, with the subsequent insertion of total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, and diabetic status. (The prevalence of diabetes was 10% in claudicants and 6% in those without evidence of peripheral arterial disease.[13])

The association with smoking was modeled using number of "pack-years" with additional variables for current smokers, recent ex-smokers (stopped within the last 5 years), and smokers of pipe/cigars only. The additional variable for current smokers, for example, measures the difference between ABPI in a current smoker and an ex-smoker who gave up more than 5 years ago but who had smoked the same amount of pack-years. The distribution of pack-years was highly skewed with a few very heavy smokers, therefore the square root of the pack-years was used to reduce the influence of these few individuals. The smoking histories were considered reasonably valid because the stated amount was related to mean thiocyanate levels, another measure (albeit imperfect) of cigarette consumption. Only smoking history is reported in this article.

Multiple regressions of age, sex, height, smoking, and the rheological factors were also carried out on intermittent claudication, the reactive hyperemia test, and three separate measures of heart disease: 1) subject recall of physician's diagnosis of angina or heart attack, 2) WHO questionnaire positive for angina or previous myocardial infarction, and 3) ECG evidence of ischemia.

Results

The mean levels of the rheological factors in the total population of 1,581 men and women from whom blood

TABLE 1. Distribution of Hemostatic and Rheological Factors by Category of Peripheral Arterial Disease and Normal Subjects

	Intermittent claudication (n=45)	Major asymptomatic (n=105)	Minor asymptomatic (n=238)	Normal (n=1,096)	Missing (n=97)	Total (n=1,581)	p*
	Mean (standard error of mean)						
Blood viscosity (mPa)	3.90	3.67	3.66	3.53	3.63	3.58	<0.0001
	(0.1)	(0.1)	(0.04)	(0.02)	(0.06)	(0.01)	
Hematocrit (%)	47.2	46.7	46.0	45.7	45.2	45.8	0.0003
	(0.5)	(0.5)	(0.3)	(0.1)	(0.3)	(0.1)	
Corrected blood viscosity (mPa)	3.65	3.55	3.55	3.46	3.59	3.49	<0.0001
	(0.05)	(0.04)	(0.02)	(0.01)	(0.05)	(0.01)	
Relative blood viscosity	2.65	2.61	2.65	2.61	2.64	2.62	0.26
	(0.03)	(0.02)	(0.01)	(0.01)	(0.03)	(0.01)	
Plasma viscosity (mPa)	1.39	1.37	1.34	1.32	1.37	1.33	<0.0001
	(0.01)	(0.02)	(0.01)	(0.002)	(0.01)	(0.002)	
Fibrinogen (g/L)	3.03	3.03	2.70	2.65	2.97	2.72	<0.0001
	(0.11)	(0.07)	(0.04)	(0.02)	(0.07)	(0.02)	
Uric acid (μmol/L)	331.5	332.8	320.4	313.0	314.7	316.3	0.0008
	(9.6)	(8.3)	(5.3)	(2.3)	(7.6)	(1.9)	
	Geometric mean (95% confidence interval)						
Urinary fibrinopeptide A (ng/mL)	1.66	1.46	1.45	1.52	1.58	1.52	0.62
	(1.39, 1.99)	(1.33, 1.61)	(1.36, 1.54)	(1.49, 1.55)	(1.46, 1.71)	(1.49, 1.55)	
Leukocyte elastase (ng/mL)	40.8	36.6	35.2	32.1	32.8	33.1	0.002
	(32.5, 51.3)	(31.9, 41.9)	(31.9, 38.0)	(30.9, 33.4)	(29.9, 36.7)	(32.1, 34.2)	

*Test for linear trend across categories of peripheral arterial disease and normal subjects.

was obtained are shown in Table 1. Analysis by age and sex showed that blood viscosity, hematocrit, urinary fibrinopeptide A, and leukocyte elastase were significantly higher in men ($p<0.001$), whereas fibrinogen was higher in women ($p<0.001$). Fibrinogen increased markedly with age from 2.52 g/L (SEM, 0.03) in those aged 55–59 years to 2.90 g/L (SEM 0.04) in those aged 70–74 years ($p<0.001$). A significant increase with age also occurred with plasma viscosity ($p<0.001$) and leukocyte elastase ($p<0.05$). Substantial positive correlations were present between many of these factors and also with cigarette consumption (measured by pack-years). The highest correlations were between hematocrit and blood viscosity ($r=0.69$), fibrinogen and plasma viscosity ($r=0.46$), and blood viscosity and plasma viscosity ($r=0.45$).

Table 1 also shows the mean levels of the rheological factors according to categories of peripheral arterial disease. Blood viscosity, hematocrit, hematocrit-corrected blood viscosity, plasma viscosity, fibrinogen,

TABLE 2. Univariate and Multivariate Regressions of Hemostatic and Rheological Variables on Intermittent Claudication and the Ankle–Brachial Pressure Index

	Odds ratio (95% CI) of intermittent claudication		ABPI coefficient difference×100 (SEM)	
Change in risk factor	Univariate	Multivariate	Univariate	Multivariate
Age (+10 years)	1.90 (1.09, 3.28)†	2.20 (1.06, 4.58)†	−5.3 (0.9)§	−4.7 (0.9)§
Female (vs. male)	1.12 (0.61, 2.05)	0.79 (0.26, 2.45)	4.9 (0.9)§	6.8 (1.5)§
Height (+10 cm)	0.75 (0.54, 1.05)*	0.57 (0.33, 1.00)†	3.3 (0.5)§	1.3 (0.7)*
Blood viscosity (+1 mPa)	2.06 (1.24, 3.44)‡	1.40 (0.59, 3.33)	−3.0 (0.9)§	−2.7 (1.3)†
Hematocrit (+10%)	3.41 (1.29, 9.04)†	1.86 (0.46, 7.55)	−2.1 (1.4)	−0.8 (1.9)
Plasma viscosity (+0.1 mPa)	1.36 (1.07, 1.74)‡	1.18 (0.57, 1.78)	−2.8 (0.6)§	0.7 (0.7)
Fibrinogen (+0.1 g/L)	1.67 (1.05, 2.65)†	1.01 (0.57, 1.80)	−0.6 (0.1)§	−0.3 (0.1)‡
Urinary fibrinopeptide A (>1 ng/mL)	1.05 (0.51, 2.19)	0.87 (0.40, 1.88)	−0.2 (1.0)	−0.2 (0.9)
Uric acid (+10 μmol/L)	1.21 (0.77, 1.90)	0.93 (0.55, 1.56)	−0.03 (0.07)	−0.08 (0.07)
Leukocyte elastase (+1 ng/mL)	1.61 (0.97, 2.67)*	1.42 (0.82, 2.45)	−1.8 (0.8)†	−1.5 (0.8)*
Current cigarette smoker	2.39 (1.30, 4.42)‡	1.06 (0.40, 2.76)	−7.7 (1.1)§	−3.5 (1.4)‡
Ex-smoker <5 years	4.29 (1.99, 9.22)§	2.42 (0.75, 7.89)*	−6.2 (2.1)‡	−4.6 (2.1)†
$\sqrt{}$ Pack-years (+1)	1.32 (1.19, 1.47)§	1.29 (1.10, 1.52)§	−1.4 (0.2)§	−1.06 (0.2)§

ABPI, ankle–brachial pressure index.
*$p<0.1$, †$p<0.05$, ‡$p<0.01$, §$p<0.001$.

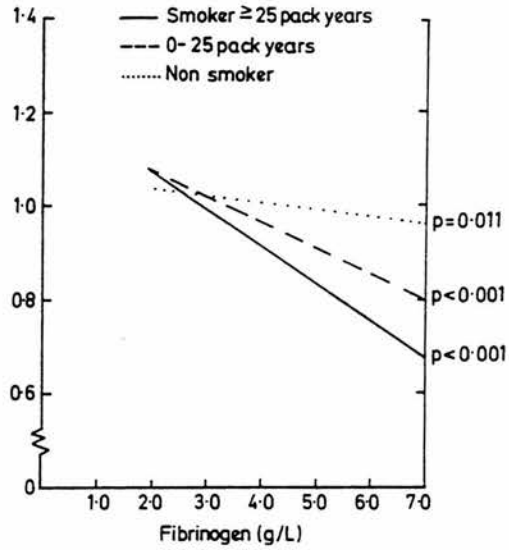

FIGURE 1. *Graph of regression of fibrinogen on ankle–brachial pressure index (APBI) at different levels of lifetime cigarette consumption.*

leukocyte elastase, and uric acid were each related significantly to the severity of the disease. Urinary fibrinopeptide A levels were higher in claudicants than in the other groups, but there was no significant trend across all categories of peripheral arterial disease ($p=0.62$). Relative blood viscosity (a measure of red cell deformability)[3,25] was unrelated to peripheral arterial disease ($p=0.26$).

Multiple logistic regression on claudication and multiple linear regression on the ABPI are shown in Table 2. Blood viscosity and fibrinogen remained significantly independently related to the ABPI but not to claudication (although this may have been related to the small number of claudicants). Plasma viscosity was related to both claudication ($p<0.01$) and the ABPI ($p<0.001$) on univariate analysis, but this disappeared on multivariate analysis including blood viscosity and fibrinogen. Blood viscosity and fibrinogen remained significantly associated with the ABPI on the inclusion of other vascular risk factors (diabetes mellitus, cholesterol, HDL cholesterol, and triglycerides). Conversely, these vascular risk factors did not lose their independent relations with the ABPI on the inclusion of fibrinogen and viscosity.

Analysis of possible interactions among age, sex, cigarette smoking, fibrinogen, and blood viscosity with the ABPI showed that the relations between both fibrinogen and blood viscosity and the ABPI occurred predominantly in male subjects ($p<0.01$) and were less marked and nonsignificant in female subjects. Figure 1 shows that the association between fibrinogen and the ABPI was strongly related to the amount of cigarette smoking, with the slope of the graphs increasing at higher levels of smoking. No interactions were found among blood viscosity, smoking, and the ABPI.

Multiple logistic regressions of age, sex, height, smoking, and the rheological factors on separate measures of ischemic heart disease showed that fibrinogen was associated only with a history of heart disease on the WHO questionnaire ($p<0.01$). Blood viscosity was not related to any measure of heart disease, although plasma viscosity was associated with recall of a physician's diagnosis of heart disease ($p<0.1$). A major difference from the findings in peripheral arterial disease was that uric acid remained independently related to all measures of heart disease, namely ECG evidence of ischemia ($p<0.001$), WHO questionnaire positive for heart disease ($p<0.01$), and recall of physician's diagnosis of heart disease ($p<0.1$).

To study the possible impact of rheological factors on ischemic symptoms and on reaction to vascular stress in the presence of a given degree of arterial narrowing, multiple logistic regressions were carried out separately on intermittent claudication and the reactive hyperemia test, with the inclusion of the ABPI as a measure of arterial narrowing. Plasma viscosity was the only factor that was independently related to claudication after adjustment for the ABPI: Figure 2 shows that the odds of having claudication in the top quintile of plasma viscosity were 3.35 times that in the bottom quintile (95% CI, 1.32, 8.51). Leukocyte elastase and uric acid were the only factors associated with the results of the reactive hyperemia test independently of the ABPI and other factors ($p<0.01$).

Discussion

Blood rheological factors (viscosity, hematocrit, fibrinogen, activated leukocytes) may contribute to ischemia by

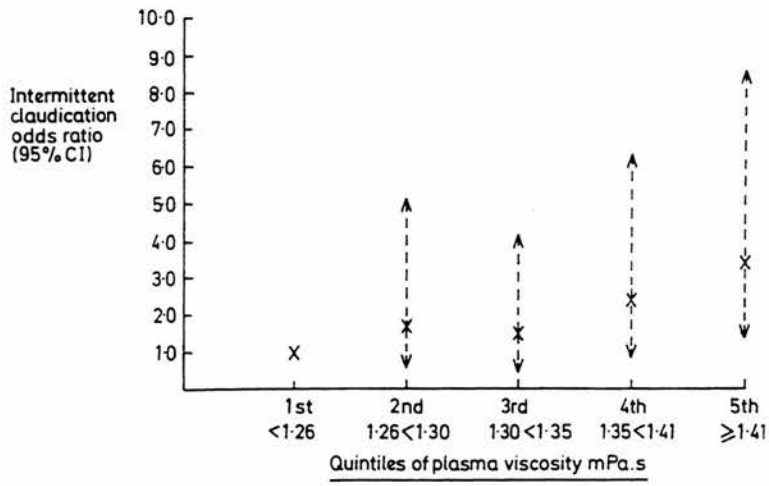

FIGURE 2. *Graph of odds ratios of intermittent claudication by quintiles of plasma viscosity independent of the ankle–brachial pressure index.*

promoting atherosclerosis, thrombosis, or obstruction to microcirculatory flow distal to atherosclerotic stenoses.[2,3] The results of our study support the hypothesis that rheological factors are related in an older population aged 55–74 years to both atherosclerotic peripheral arterial disease (measured by the ABPI) and to leg ischemia (measured by the presence of intermittent claudication on questionnaire or an abnormal reactive hyperemia test).

Blood viscosity and its major determinants (hematocrit, plasma viscosity, and fibrinogen) as well as leukocyte activation (plasma leukocyte elastase) were found to be significantly related to increasing severity of peripheral arterial disease within the population (Tables 1 and 2). No such relation was evident for activation of blood coagulation (urinary fibrinopeptide A), but this may have been related to insensitivity of the assay because 38% of the population had levels <1 ng/mL. Indeed, plasma fibrinopeptide A levels have been related to several major cardiovascular risk factors[22] as well as to angiographic coronary artery disease[29] and peripheral arterial disease.[4]

In the Edinburgh Artery Study, peripheral arterial disease has been related to conventional risk factors such as age, cigarette smoking, systolic blood pressure, and HDL and non-HDL cholesterol.[13] Several rheological factors were also associated with these risk factors, particularly age and cigarette smoking, as previously reported.[2,3,23] On multivariate analyses including age, sex, and cigarette smoking, the associations between the rheological factors and intermittent claudication became nonsignificant (Table 2). However, this finding may be related to the small number of claudicants and does not exclude the possibility that increases in viscosity, fibrinogen, and leukocyte activation may be mechanisms whereby age and smoking promote development of peripheral arterial disease.

Are rheological factors related to the extent of atherosclerotic narrowing in the arteries to the lower limbs? The latter was assessed using the ABPI, which at a level of 0.9 has been shown to be up to 95% sensitive in detecting angiogram positive peripheral arterial disease[30] and in the Edinburgh Artery Study has been shown to be related to the severity of disease on duplex scanning.[31] The ABPI was significantly associated with blood viscosity, plasma viscosity, and fibrinogen, and the relations with blood viscosity and fibrinogen persisted after multivariate analysis including conventional risk factors (Table 2). These findings suggest that blood viscosity and fibrinogen each may have an independent role in atherogenesis. Several biologically plausible mechanisms have been suggested, including an effect of blood viscosity on the localization of atherosclerotic lesions.[2,3] Fibrinogen levels may influence infiltration of fibrinogen into the arterial wall, platelet aggregation, and fibrin formation as well as increasing plasma and blood viscosity.[2,3] Interestingly, the association between fibrinogen and the ABPI was strongly related to the amount of cigarette smoking (Figure 1). Possible explanations include a synergistic effect of smoking (which disturbs endothelial cells and activates platelets) and fibrinogen (which infiltrates the arterial wall through damaged endothelium and promotes platelet aggregation).[2,3] An interaction between cigarette smoking and plasma fibrinogen has also been reported in the prediction of occlusion of femoropopliteal grafts in peripheral arterial disease.[32]

In the presence of a given degree of atherosclerotic narrowing (ABPI), do rheological factors predispose to leg ischemia? If blood viscosity is reduced by lowering the hematocrit, blood flow in the leg increases,[33] but this may reflect vasodilation caused by changes in oxygen carriage and blood volume rather than blood viscosity.[34] However, lowering plasma fibrinogen also reduces plasma and blood viscosity and increases leg blood flow, including nutritive skin flow,[35] and this cannot be ascribed to changes in oxygen carriage or blood volume. The importance of plasma viscosity has been shown in the present study, in which higher levels significantly increased the likelihood of symptomatic intermittent claudication at a given level of arterial narrowing (Figure 2). It is likely that this association reflects a direct effect of plasma viscosity on leg muscle blood flow distal to arterial stenosis, not only from theoretical considerations[36] but also because reductions in plasma viscosity after exercise training,[37] cessation of cigarette smoking,[22,38] or treatment with some pharmacological agents[39] are accompanied by improvements in claudication that are quite consistent with the relation shown in Figure 2. Indeed, the relation shown in Figure 2 between plasma viscosity in the population and prevalent symptomatic leg ischemia is very similar to the recently described relation between plasma viscosity in the male population and incident coronary heart disease.[40] It is therefore possible that plasma viscosity may also promote myocardial ischemia distal to coronary arterial stenoses, although measurement of the latter in population studies is more problematical than measurement of lower limb arterial stenoses using the ABPI.

We have also shown that leukocyte elastase was related to leg ischemia as measured by the reactive hyperemia test. Again, it is possible that this is a direct effect: Elastase release is a measure of leukocyte activation, which is also associated with production of oxygen metabolites that cause skeletal muscle vasodilatation.[41] Uric acid was also related to leg ischemia as measured by the reactive hyperemia test as well as to ischemic heart disease. Because of the importance of the uric acid/xanthine oxidase pathway in reactive oxygen metabolism, these associations may also be relevant to oxygen metabolite–related ischemia. Leukocyte activation may play a role in atherosclerosis and thrombosis as well as in ischemia.[3,42] However, an overall interpretation of the findings of the present study is that, in the presence of a given degree of arterial narrowing, determinants of microcirculatory blood flow (plasma viscosity, leukocyte activation) may be important determinants of leg ischemia.[2,3] Conversely, reduction of plasma viscosity (for example, by reduction of plasma fibrinogen or lipoproteins) and inhibition of leukocyte activation may be rational approaches to prevention and treatment of ischemia.[39]

Conclusions

Our findings suggest that rheological factors are associated with both the severity of atherosclerosis in the older population and with the presence of leg ischemia for a given degree of arterial occlusion. We are currently assessing the predictive value of rheological factors for arterial events in the limbs, heart, and brain

in both this and other cohorts as well as the effects of interventions on blood rheology and prognosis in peripheral arterial disease.

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