Linoleic acid and sudden cardiac death

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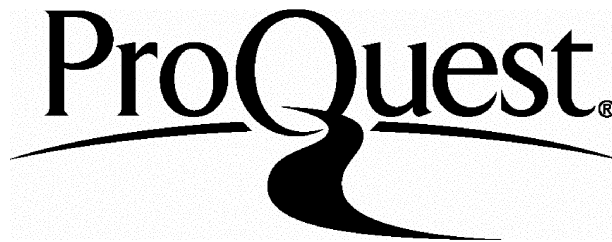
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Linoleic acid and sudden cardiac death

Abstract

Dietary linoleic acid consumption, as reflected by adipose tissue triglyceride fatty acid percent composition, has been shown in between population studies, cross sectional studies and population case control studies to be inversely related to the risk of angina pectoris, first acute myocardial infarction and mortality due to coronary heart disease (CHD). Death that occurs within 24 hours of symptoms starting is not only a common mode of death from CHD but is frequently the mode of presentation of this disease. These deaths are commonly referred to as sudden cardiac deaths. This thesis describes a population case control study designed to address the hypothesis that dietary linoleic acid is inversely related to the risk of sudden cardiac death (SCD) due to coronary heart disease in men under the age of 65 years. Over a period of eighteen months, 66 samples of adipose tissue were obtained from 84 male cases of SCD. From 357 age matched male controls, 229 samples of adipose tissue were obtained from disease free controls. The mean percentage (SEM) composition of adipose tissue linoleic acid in the cases was 11.16% (0.41) compared to 12.96% (0.27) in the controls. The estimated relative risk of SCD for the lowest quintile of the frequency distribution of linoleic acid in the controls was 5.75 (95% confidence interval 1.84 to 18.0). Multivariate analysis revealed that low linoleic acid was independently related to the risk of SCD when treated hypertension, diabetes and age were included. Smoking, however, displaced all other variables and remained the only significant factor related to SCD. Dietary adjustment to increase the relative amount of linoleic acid present in the diet is recommended for populations at risk from CHD.

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INTRODUCTION

"Eats first, then morals"

Bertolt Brecht *"The Threepenny Opera"* 1928

The relative importance of fat in the diet of man, or woman, has generated debate for many years. The finding of cholesterol in atheromatous plaques at post mortem in the nineteenth century set off a train of investigation continuing to the present day. Atheromatous disease of the coronary arteries is one of the commonest causes of death and morbidity in many countries. If a dietary factor, or factors influenced susceptibility to this disease it would have important implications for individual health. Even small changes in the diet of individuals can have major implications for populations. It could also have implications for health care spending.

Over sixty years has elapsed since the first studies suggesting that certain species of fat molecules are obligatory factors in the diet of mammals. These essential fatty acids were not initially considered to be an important factor in the diet of humans. Absolute deficiency, although recorded in humans, is rare. The optimal levels of these fats in the diet of man has been more difficult to quantify.

The most abundant polyunsaturated fatty acid in the human diet is linoleic acid. It is also an essential fatty acid. Increasing the proportion of this fat in the diet of man has been known to lower total serum cholesterol levels for almost fifty years. However, it has been only recently demonstrated that increasing proportions of this fatty acid in the diet are associated with a lower risk of coronary heart disease (CHD) in the general population.

Of the total number of individuals who die from CHD in any given period of time, many will die within a day of symptoms starting. Any study of this disease can not be complete without a description of this important group. They are generally termed sudden cardiac deaths (SCD). Previous studies of linoleic acid and CHD have been performed on individuals who have survived an acute myocardial infarction or who have had chronic stable angina.

This study is an attempt to define what relation, if any, there is between dietary consumption of linoleic acid and SCD due to coronary artery disease.

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Linoleic acid: an essential fatty acid

History and physical chemistry of linoleic acid

Derived from the Latin name for flax, and the oil produced from this plant, linoleic acid is a carboxylic, or fatty, acid. It was identified from linseed oil and so named as a distinct chemical entity by Sacc in 1844[1]. The chemical formula was determined by two groups between 1886 and 1887 and its stereo chemical structure was initially studied by Rollett in 1909[1]. Chemical synthesis was first achieved in 1950[2]. The systematic chemical name for linoleic acid is 9(*cis*), 12(*cis*)-Octadecadienoic acid[1]. The basic formula is that of a chain of eighteen carbon atoms with a methyl group at one end and a carboxyl group at the other. The short hand notation is C18:2, indicating that it has two carbon-carbon double bonds. The site for these double bonds in linoleic acid, as indicated by the full systematic chemical name, is at the ninth and twelfth carbon atoms counting from the carboxyl end. The geometric configuration of both these double bonds in linoleic acid is in the *cis* configuration. The numbering for biological, medical and nutritional purposes is now from the methyl end of the molecule and then the double bonds become sited at the n-6 and n-9 carbon atoms. As it possesses more than one double bond it is referred to as a polyunsaturated or polyethenoic fatty acid; it is one of the n-6 series of polyunsaturated fatty acids. This nomenclature is used interchangeably with the notation ω6 to denote the position of the first double bond in carboxylic acids. It is one of the most abundant fatty acid components of seed oil triglycerides. These are obtained from many plants that are consequently of major commercial importance being used for the manufacture of margarines and other hydrogenated fat materials used in the food industry. These include safflower, sunflower, soybean and cottonseed, in addition to linseed. The characteristics of this compound are that it is a yellow liquid at room temperature, has a melting point of -5°C and is highly susceptible to oxidation[3]. This last property is the basis for much of its industrial value[4] and may have relevance to role of unsaturated fats in the pathophysiology of atheroma[5]. Seed oils in their natural state, however, have an abundance of antioxidant material to help to prevent oxidation, free radical formation and polymerisation of unsaturated fats[3].

History of the evidence for the essential nature of linoleic acid

Linoleic acid is a member of the lipid class of organic chemical compounds, a group more commonly referred to as fats and oils. Work on the chemical composition of foods, and fats in particular, has been attributed to Scheele, Chevreul, Prout and Magendie during the eighteenth and nineteenth centuries[6]. The basic chemical, nutritional and metabolic properties of each class of foodstuff in mammalian nutrition, that is the carbohydrates, proteins and fats, together with the vitamins and trace elements, have been studied extensively since the early nineteenth century.

The drive for the discovery of new vitamins and the elucidation of their chemical structures heightened in the years between the two world wars of the first half of this century. A contemporary review of the vitamins lists patents for the various vitamins owned by chemical companies[7]. Competition between research groups was the overt reason for this activity but the potential exploitation of the findings for commercial purposes really determined the agenda. Attitudes to the importance of these compounds in human nutrition have been largely coloured by claims of efficacy usually not supported by the facts available.

In the early part of this century fat was thought nutritionally important only for its value as a "fuel", or its calorific value[8], although one worker at the turn of the century had suspected a physiological role for unsaturated fat in the liver[9]. Evidence that fats had a more subtle role to play in nutrition came initially in 1927 and 1928 when Evans and Burr from Berkeley in California published data on the effect of excluding fat from the diet of rats[10-12]. Burr extended this work after leaving Evans' laboratory. He moved to Minnesota and in 1930 established by exclusion that it was the lack of the fatty acid portion of triglyceride in fat that was responsible for the pathological changes observed in rats[13]. He and his wife subsequently established by feeding oils and fats of different fatty acid composition that linoleic acid was likely to be the essential component[14].

Depriving rats of dietary fat, whilst keeping all other known nutrients constant, affected the growth of the animals as measured by their daily weight. Fat deficient animals developed skin and tail lesions and were frequently noted to have haematuria. Fluid balance was also noted to be disturbed by analysis of urine production and water

consumption. Adding fat to the diet restored the health of these animals. They gained weight and lost their skin lesions. It was the efficacy of specific fats in restoring health to fat deficient rats that was the initial assay procedure for testing various fatty acids for their "essential" properties. Subsequently, other fatty acids were demonstrated by Burr, and others, to have "essential fatty acid activity". In particular, linolenic acid (C18:3 ω3), arachidonic acid (C20:4 ω6) and docosahexaenoic acid (C22:6 ω3) were found to be effective[15, 16]. Arachidonic acid was found to be the most potent compound, followed by linoleic acid, then linolenic, then docosahexaenoic.

The essential fatty acids were initially designated as being vitamin F or H by Evans and Burr in 1927[10], not because of the association with fat but because of the natural alphabetical progression from vitamin E as the essential factor was thought to be another vitamin[17]. This term has now lapsed, although at least one modern dictionary still uses this designation for linoleic acid[18] and it was used by Sinclair in a relatively recent publication[19]. The minimum amount of an essential fatty acid required in the diet of animals has been calculated from feeding experiments in rats to be approximately 1% of caloric intake. It was thought that on this basis, taking account of the average fat intake in the western world, that essential fat deficiency was not likely to occur in isolation in Western society. Cases documenting essential fatty acid deficiency in humans have been reported[20, 21], but these reports have been of individuals in special situations and isolated essential fatty acid deficiency occurring in a free living individual has not been reported. Feeding experiments on humans have also been carried out[22], but the optimum requirement for essential fatty acids in the human diet has not been formally defined. Data from rat feeding experiments suggest there is an optimum proportion of the diet that should be consumed as essential fatty acids for growth[23] but extrapolation to the human diet may not be justified.

The tedious methods of biochemical analysis prior to the advent of gas liquid chromatography made this a time consuming and difficult area to study. Precisely which chemical properties of linoleic acid, and the other fatty acids, were responsible for curing the features of fat deficiency was not understood at the time they were discovered but subsequent investigations in diverse fields have clarified the picture since the 1930's. The

only source of these fatty acids for many animals, including man, is from diet [24].

Epidemiological evidence for the relation of dietary linoleic acid to coronary heart disease

1. Clinical studies

The fat intake of free living individuals can take many days to measure accurately [25] and so surrogates for linoleic acid intake have been used by many investigators. Adipose tissue fatty acid composition is, however, a reliable indicator of long term dietary intake of linoleic acid[26]. It has also been demonstrated in man that increasing the intake of dietary linoleic acid will be reflected in the adipose tissue composition[27].

James et al in 1957 reported the fatty acid composition of blood lipids in twelve patients with CHD and found no difference the percentage of phospholipid fatty acids represented by linoleic acid between these cases and the controls they selected[28]. Subsequently, others have found that there are lower levels of linoleic acid in the blood lipids of cases compared to controls [29-35]. The composition of adipose tissue from cases of atherosclerotic disease was looked at by others [36-38] but although these studies found that the adipose tissue linoleic acid was lower in cases of atherosclerotic arterial disease than controls, this difference was not statistically significant. Kirkeby and colleagues then found that linoleic acid was significantly lower in cases of atherosclerotic arterial disease compared to controls but only where there was no previous history of CHD[39, 40].

The percent composition of platelet membrane phospholipids with respect to linoleic acid have also been studied. With one exception [41], all have reported that there is a lower level of linoleic acid in platelet membrane phospholipids following acute myocardial infarction[42, 43]. The composition of erythrocyte membrane phospholipids have also been shown to have lower linoleic acid percent composition in survivors of myocardial infarction[44, 45].

2. Cross cultural and between population comparisons

Comparing the composition of adipose tissue between populations in search of differences that might help to explain variation in CHD event rates were initially performed on small, highly selected populations and found little difference in the fat composition[30, 46-49]. A comparison of the adipose tissue from Korean and American soldiers eating their national diet, and then consuming one another's national diet, was

made in the early 1960's and showed that the composition of the diet could influence the adipose tissue level of linoleic acid[50]. A study of samples taken from Japanese and American men at post mortem examination also demonstrated that American men had a higher proportion of saturated fatty acids and lower linoleic acid, and other long chain polyunsaturated fatty acids than Japanese men[51].

Comparisons of the adipose tissue fatty acid composition taken from individuals living in Scotland, Sweden, Finland and Italy, where there are different CHD event rates, using samples taken from otherwise healthy men, have been performed[52, 53]. Both these studies have shown that percent composition of adipose tissue triglyceride fatty acids taken up by linoleic acid is consistently lower in the areas where the event rate from CHD is high.

Tavendale and colleagues, as part of the Scottish Heart Health Study, obtained adipose tissue samples from over 3000 individuals, men and women, from different areas in Scotland[54]. The different areas from which the samples were taken were known to have differing CHD mortality rates and they have related the mortality rates to the adipose tissue linoleic acid composition. They have found that for both men and women, there is an inverse relation between the linoleic acid percent composition and CHD standardised mortality ratio for each district.

3. Post mortem studies

Studies of the adipose tissue composition of samples taken from post mortem examinations have previously found no association between death from CHD and linoleic acid levels. Thomas and Scott[55] studied samples taken from various areas in England and Wales, but information on the prior diagnostic status of these cases is not given in their paper. Strong and colleagues compared the composition of adipose tissue from white and black men who had died from CHD and that of those men who had died accidentally[56]. Again they found no difference in linoleic acid levels.

4. Within population studies

In a cross sectional survey of men in Scotland by Wood and colleagues, those men who responded positively to the WHO chest pain questionnaire or who were found to have had a definite myocardial infarction on their resting electrocardiogram (and had

not been told by a doctor that they had CHD) had lower levels of linoleic acid in their adipose tissue compared to their healthy peers[57].

5. Population case control studies

A population case control study was undertaken in the Edinburgh area to estimate the relative risk for CHD in relation to the adipose tissue and platelet membrane fatty acid composition[58]. A postal questionnaire of 6000 men aged 35 to 54 years of age in the Edinburgh area identified new cases of angina pectoris by a positive response to the WHO chest pain questionnaire. New cases of acute myocardial infarction were identified by monitoring the admissions to coronary care units in the city during the period of a year. Adipose tissue samples were taken from the cases so identified and from age matched healthy controls drawn from the sample of 6000 men. Adipose tissue linoleic acid was lower in both the angina pectoris cases and the acute myocardial infarction cases compared to the healthy controls. Estimates of the relative risks of being a case of angina pectoris or acute myocardial infarction were calculated relative to that for the highest quintile of linoleic acid for the control population. There was an inverse relation between adipose tissue linoleic acid and both angina pectoris and acute myocardial infarction with the highest risk for each class of CHD event occurring in the lowest quintile of linoleic acid. No attempt was made in this study to obtain samples from those men who died outside hospital or within 24 hours of admission to hospital; that is the SCD cases.

6. Prospective studies

Follow up of subjects whose blood, or adipose tissue, fatty acid composition had been characterised was first reported by Kingsbury and colleagues[59]. They found that there was an inverse relation between the incidence of myocardial infarction and death due to vascular causes and the percentage of dienoic (mainly linoleic acid) fatty acids in plasma. They later reported that only linoleate distinguished patients with fatal and non-fatal myocardial infarctions from those who did not suffer such events[60].

A formal prospective study of blood lipid fatty acid composition, particularly linoleic acid, was performed by Miettinen and colleagues[61]. They followed up 1222 middle aged men who were initially free of clinical CHD for 7 years. From this cohort 33 men experienced a fatal or non fatal myocardial infarction, or SCD, during the period of

study. Controls were selected from the remaining 1189 men and were matched for age, blood pressure, cholesterol and triglyceride concentrations, smoking habit and glucose tolerance at the start of follow up. The fatty acid composition of phospholipids, cholesterol esters and triglycerides was determined from a fasting serum sample taken on entry to the study. A significantly lower linoleic acid level was found in the phospholipid fraction of the cases compared to the controls. As all the controls were matched for other risk factors, this can be considered to be an independent finding.

A similar study was carried out in Prague by Valek and colleagues[62]. They followed up 107 survivors of acute myocardial infarction and measured the fatty acid composition of serum phospholipids and triglycerides at the start of follow up. After 5 years there had been 23 vascular deaths. Compared to the survivors the percent composition of linoleic acid in those who had died was lower.

Coronary heart disease presenting as sudden cardiac death

Introduction

The time interval between the onset of symptoms of atherosclerotic coronary heart disease and eventual death resulting from the complications of this disease is extremely variable [63, 64]. Those who present with angina of effort may have a restricted lifestyle and they are certainly more prone to further manifestations of CHD but they can live for many years from the start of their symptoms[65]. Acute myocardial infarction due to coronary thrombosis is another prime manifestation of CHD[66-68], but the majority of these patients who reach hospital alive will live for more than 24 h following admission. Their subsequent risk of dying suddenly or developing heart failure depends on the extent of infarction, as measured by left ventricular ejection fraction[69]. The remainder of incident cases of CHD are those who have but a short time between the onset of their first declared symptoms and their death. Some will have no declared symptoms before death, or will be found dead. Such cases are referred to generally as sudden cardiac deaths (SCD).

Firstly one must consider the definition of SCD. Cross cultural, between population and within population evidence from surveys on the incidence of this manifestation of CHD exist. Several post mortem series of the pathological findings in those who succumb in this manner are also available. These papers will acknowledged but caution should be exercised in drawing conclusions from these patients as they represent highly selected groups.

Definitions

Surveys including an inventory of death due to any disease are in part, or in whole, retrospective. Information not gained from the victim of SCD is, of course, lost entirely. Attempts to minimise the effect that such practical considerations have when surveys of CHD have been performed have varied. This subsequently makes direct comparisons between surveys not strictly comparable. The opinion of the Nomenclature Committee of the International Federation of Societies of Cardiology is that there should be an "operational" definition of SCD based on local circumstances[70]. Death, they argue, is due principally to failure of the circulation resulting from primary cardiac arrest and a definite time interval from the onset of symptoms to eventual death that would classify a death as being "sudden" is left open. Establishing the exact time of death is a problem as a large number of deaths go unwitnessed. The precise time of the onset of symptoms preceding such deaths is also subject to similar constraints. Such information is usually based on the report of relatives or witnesses interviewed by investigating officers of the coroner or medical examiner. Because of these sources of possible bias some studies have only admitted those deaths that have been witnessed to the class of SCD.

Table 1. sets out the definitions used in the surveys that have looked at this matter. It is apparent from this table that there is a great deal of diversity encompassed in the term sudden cardiac death.

Not only have the time intervals varied from study to study but the rigour with which the actual cause of death was persued has also varied. Total reliance, in some surveys, on death certificate data contrasts sharply with the 80% post mortem rate in others. Establishing the cause of death as being due to cardiac failure resulting from a deficiency of the coronary circulation is sometimes presumptive in the absence of full clinical and post mortem evidence. In the light of post mortem information diagnoses based on clinical suspicions can occasionally be substantially modified[71]. There is a long list of pathological processes that can lead to sudden death and may mimic SCD due to coronary artery disease, especially in a young population[72].

The purpose of many of these surveys was to establish the magnitude of the problem in public health terms, i.e. what was the absolute rate of death occurring within

the population studied. The other purpose was to establish whether there are any distinguishing features between those who remain healthy and the victims of SCD, and between the victims of SCD and those who present with other manifestations of CHD. These two questions need to be addressed with fundamentally different surveys. Retrospective surveys of SCD can only supply information on the proportion of all deaths attributable to SCD due to CHD over a certain period of time and give death rates for each age group. This is providing the population from which the deaths are taken is accurately defined.

Prospective cohort studies, such as the Framingham study and the Honolulu Heart Program, are required to answer other questions. However, these surveys are still limited to a certain extent by the initial measurements that were done at baseline to provide comparison between groups.

While there have been calls for a more standardised approach to the definition of SCD[73-75] it is evident that the classification of deaths from coronary heart disease can be a complicated affair[76]. That is perhaps why the Nomenclature Committee referred to above decided that decisions on what exactly is, or was, SCD should be left to be decided on a study by study basis.

Table 1. Definitions of sudden cardiac death

Reference	Time interval from onset of symptoms to death	Inclusion of unwitnessed deaths?
Pell & D'Alonzo 1964[77]	Within 24 h	Not stated
Kuller et al 1967[78]	Within 24 h, Subdivided into less than 2 h and 2-24 h	Yes: only if known to be alive and well 24 h before body found
McNeilly & Pemberton 1968[79]	Deaths divided into less than 12 h or greater than 12 h. Those under 12 h subdivided into 0<15 min and 15 min to 12 h	Yes
Chiang et al 1970[80]	Within 1 h	Not stated
Wikland 1971[81]	Medically unattended	Yes
Reinis et al 1971[82]	Less than 1 h	Not stated
Hagstrom et al 1971[83]	Less than 24 h	Not stated
Gordon & Kannel 1971[84]	Within 1 h	No
Armstrong et al 1972[85]	Deaths subdivided into several time intervals from less than 1 h to less than 4 weeks	Not stated

Table 1.(Continued) Definitions of sudden cardiac death

Reference	Time interval from onset of symptoms to death	Inclusion of unwitnessed deaths?
Fisher & Tyroler 1973[86]	Less than 24 h subdivided into less than 1 h and 1-24 h	Not stated
Romo 1973[87]	Within 1 h	No
Stamler 1975[88]	Less than 1 h	Not stated
Tunstall-Pedoe et al 1975[89]	Deaths within 28 days subdivided into less than 15 min, less than 1 h and within 4 h	Not stated
Kannel et al 1975[90]	Within 1 h	No
Bekker & Grunfeld 1976[91]	Less than 1 h	Not stated
Gillum et al 1976[92]	Less than 1 h	No
Doyle et al 1976[93]	Less than 1 h	No
Januskevichius et al 1977[94]	Within 6 h	Not stated
Ulrich et al 1977[95]	Less than 24 h subdivided into up to 1 h, 1 h to 12 h and 12 to 24 h	Yes

Table 1. (Continued) Definitions of Sudden Cardiac Death

Reference	Time interval from onset of symptoms to death	Inclusion of unwitnessed deaths?
Tunstall-Pedoe 1978[97]	Within 24 h	Not stated
Klatsky et al 1979[98]	Less than 24 h subdivided into less than 1 h and "instantaneous"	Not stated
Elveback et al 1981[99]	No time interval described in paper	Not stated
Beard et al 1982[100]	No time interval described in paper	Not stated
Rabkin et al 1982[101]	Less than 24 h	Not stated
MRFIT Study 1982[102]	Less than 24 h	Not stated
Gillum et al 1983[103]	No time interval given in paper	Not stated
Schatztkin et al 1984[104]	Less than 1 h	No
Madsen 1985[105]	Within 24 h	Yes: If death likely to have occurred within 24 h of symptoms starting
Kuller et al 1986[106]	Within 24 h	Not stated
Elveback et al 1986[107]	No time interval given in paper	Not stated

Table 1. (Continued) Definitions of sudden cardiac death

Reference	Time interval from onset of symptoms to death	Inclusion of unwitnessed deaths?
Furberg et al 1977[96]	Less than 24 h subdivided into less than 1 h and 1 to 24 h	Not stated
McIlwaine et al 1986[108]	All deaths up to 28 days after onset of symptoms included. Subdivisions into several temporal categories, from less than 4 minutes up to 28 days from the onset of symptoms	Yes
Beard et al 1986[109]	No time interval given in paper	Not stated
Willich et al 1987[110]	less than 1 h	No
Yano et al 1987[111]	Less than 24 h subdivided into less than 1 h, 1 to 3 h, less than 3 h, 3 to 6 h, less than 6 h and 6 to 24 h	Only if known to be alive within 24 h of being found
Abdalla et al 1987[112]	Within 1 h	No

Table 1. (Continued) Definitions of sudden cardiac death

Reference	Time interval from onset of symptoms to death	Inclusion of unwitnessed deaths?
Folsom et al 1987[113]	"Out of hospital" deaths subdivided into "definite sudden death due to coronary heart disease" and "others". No time intervals specified in paper	Not stated
Thomas et al 1988[114]	Within 6 h	Not stated
Kagan et al 1989[115]	Less than 24 h subdivided into less than 1 h, 1 to 3 h, less than 3 h, 3 to 6 h, less than 6 h and 6 to 24 h	Not stated
Gillum 1989[116]	"Out of hospital death". No time interval given in paper	Not stated
Gillum 1990[117]	"Out of hospital death". No time interval given in paper	Not stated
Lanti et al 1990[118]	Less than 1 h	No

Table 1. (Continued) Definitions of sudden cardiac death

Reference	Time interval from onset of symptoms to death	Inclusion of unwitnessed deaths?
Drory et al 1991[119]	Within 24 h of the onset of symptoms, subdivided into less than 6 h and 6 to 24 h	Not stated

Epidemiological surveys of sudden cardiac death

Surveys of SCD of have to be referred to a population if meaningful rates of disease are to be calculated and conclusions drawn. Kuller's review in 1966 of the epidemiology of SCD drew attention to the dearth of accurate information at that time about this manifestation of CHD[120].

The first study to show that SCD was a major part of the overall burden of CHD mortality in an urban population was by Kuller and colleagues[121]. By taking a subsample of all deaths in the Baltimore area between 1964 and 1965 and classifying them into those that were probably and possibly "sudden" they identified those definitely due to CHD and those due to other causes. They thereafter made adjustments by multiplication taking into account the way in which the sample had been drawn; this survey makes assumptions that the sample was representative of the population as a whole. In this survey, SCD due to CHD accounted for 31.5% of all deaths in Baltimore in the period studied. A similar study was performed in Belfast in 1966[79] where all deaths certified as being due to arteriosclerotic heart disease or disease involving the coronary arteries were identified. They found that of the men in whom the fatal attack was their first event 29% were dead within 15 min and 66% were dead within 12 h. Bekker & Grunfeld[91] reviewed all the death certificates from the whole of Denmark for September 1972. The proportion of all SCD, male and female, that were known to be dead within 1 hour of symptoms starting was 57%.

The first study to identify the rate of death attributed to acute myocardial infarction within 24 hours of the onset of first symptoms and refer this to a known population was from the du Pont company in the United States[77]. Over four fifths of the deaths attributed to acute CHD events in the employees of this company occurred within 24 h of symptoms starting. Other insured populations studied were found to have similar proportions of early deaths in those afflicted with acute CHD[98].

In the late 1960's and early 1970's the World health Organisation (WHO) helped to set up Myocardial Infarction Community registers which operated in 21 population areas, figures for 16 of which are available[122]. A strict definition of SCD was not adopted but "every effort" was made to establish the time of death in relation to the onset of

symptoms. For deaths occurring in men in the hour following the onset of symptoms the absolute annual rates of death per 1,000 individuals in the population ranged from 0.19 in Gothenburg to 1.59 in Helsinki. However, when considered as a proportion of all incident individual cases of CHD, SCD as presenting manifestation of CHD is relatively constant between populations.

This indicates that while there may be substantial differences between populations as far as susceptibility to CHD is concerned, there will always be those individuals who may succumb rapidly. However, it is not clear from these figures whether this applies to those in whom this was the first and sole manifestation of CHD. The proportion of attacks in people who already had angina pectoris, for example, instance is not given.

The prospective surveys of cardiovascular disease in Honolulu, Puerto Rico and Framingham have been performed in the same manner. There was a standard definition of SCD in all these surveys and regular exchange of material for standardisation purposes. When the levels of the classical risk factors were examined, such as serum cholesterol and blood pressure, whilst there was a consistent relationship within these populations with regard to CHD event risk, the magnitude of the differences in these factors *between* the populations was insufficient to explain the difference in overall CHD incidence between the populations. Smoking was related to CHD incidence in Honolulu and Framingham but not in Puerto Rico. However, this factor was not solely able to account for the different incidence rates. As in the WHO surveys there is a considerable difference between the populations in the overall attack rate of CHD but there is a substantial proportion in each population who present as SCD. It is worth noting that approximately half of those dying from CHD in these populations die suddenly. However, those having developed angina or having had a clinical myocardial infarction had a substantially increased risk of SCD. Yet the factors analysed could not differentiate between the SCD victims and those suffering other forms of CHD.

Death certificate surveys similar to those cited above, CHD registers and prospective surveys of CHD, such as Framingham and the Honolulu Heart Program have all identified that SCD is a major manifestation of CHD. In all of them the major risk factors that help to distinguish those who experience any form of CHD from their peers

do not appear to distinguish those who die suddenly from those who have other manifestations of CHD with the exception perhaps of left ventricular hypertrophy[84, 90, 93, 104, 110, 111, 115, 123-126].

The mechanism for such deaths has been shown to be most commonly ventricular fibrillation in 24 h recordings[127] in those dying suddenly during such recordings. Electrocardiographic recordings on the arrival of emergency teams have confirmed this rhythm disturbance as the most common form of terminal arrhythmia[128]. The underlying pathological event is now thought to be an acute thrombotic event within the coronary arteries[68, 129]. The ischaemia within the myocardium as a result of this coronary thrombosis predisposing to arrhythmias[130, 131].

Hypothesis statement

That the risk of being a case of sudden cardiac death due to coronary artery disease in men under the age of 65 is inversely related to the dietary consumption of linoleic acid as measured by the percentage composition of linoleic acid in adipose tissue triglycerides. No specific mechanism of death, such as ventricular fibrillation, is required for the definition of sudden death; all cases are to be included coming under the definition to be specified below.

If linoleic acid consumption is lower in victims of sudden cardiac death due to coronary artery disease then further exploration of the mechanism and the implications thereof in terms of population dietary advice, agricultural and food policy could be justified.

METHODS

Study design outline

To test the above hypothesis, a population incident case control study design was chosen. The aim was to identify all men, between the ages of 25 and 65 years, who had died as a result of coronary heart disease within 24 h of symptoms starting. The diagnosis was made after post mortem examination to establish that the death was due to coronary artery disease. Those men who had been diagnosed by a doctor that they had angina or had a "heart attack" or who had coronary artery surgery were excluded from the sample.

Controls for the cases were selected from the same General Practice Registers with which the cases were registered. All controls were male and within 2 years of age of the case for which they were being matched. Four controls were selected by this process for each case and invited to attend for interview. At interview, if they did not have a history of CHD, they were asked to consent to have a sample of adipose tissue taken, under local anaesthetic, from the subcutaneous fat of their abdomen.

An overview of the method is shown in Figure 1.

Ethical approval

Ethical approval for this study was obtained from the Southampton and South West Hampshire District Health Authority Joint Ethics Sub-Committee on the 20th October 1987; submission number 103/87.

Figure 1. Population case control study of sudden cardiac death in men: study outline

Population of Southampton & South West Hants Area Health Authority

Sudden cardiac death cases	Male controls
Consecutive post mortems Men aged 25 to 64	Drawn from the same GP registers as cases
Coronary artery disease likely cause of death	age matched
No history prior to death	No history of coronary disease
Outside hospital or < 24 h after admission	

Female partners of sudden death cases interviewed	Female partners of controls interviewed

Approval of Her Majesty's Coroner

Discussions were held between Professor D.A. Wood, Dr P.J. Gallagher and Mr R.N. MacKean, H.M. coroner for the Southampton and New Forest District concerning the collection of samples for this study.

Under section 1 of the Human Tissues Act 1961, the person lawfully in possession of a body after death may authorise the removal of parts of the body for medical research only in the following circumstances:-

a)   the deceased had previously given consent in writing or orally before two witnesses;

b)   if (a) does not apply, and if after having made such reasonable enquiry as may be practicable, he has no reason to believe that:

     (i)   the deceased expressed an objection to his body being so dealt with after his death and had not withdrawn it; or

     (ii)   that the surviving spouse or any surviving relatives of the deceased objects to the body being so dealt with.

The matter of ascertaining whether the deceased had ever objected to having any part of his body used for research purposes in his lifetime was delegated to myself after consideration by the coroner of the fact that adipose tissue was taken and stored as a matter of routine. A consent form was then drawn up to be signed by the contactable surviving relative of the case identified. This was to ascertain that the deceased had not expressed such wishes as outlined above and also to seek permission to analyse the adipose tissue by gas liquid chromatography.

Definition of sudden cardiac death

Sudden Cardiac Death is defined for this study as death due to coronary artery disease within 24 h of the onset of symptoms of the final episode of illness, or if it could be reasonably supposed that the deceased was alive and well at least 24 h prior to the presumed time of death. All cases fitting this description had a post mortem examination and evidence on such examination of coronary artery disease; this was to be the only identifiable cause of death at post mortem. An additional requirement was that there was to be no known clinical history of angina pectoris or acute myocardial infarction prior to the final illness.

Definitions used for the purposes of classification in results

Social class

All social class codes are taken from the Registrar General's Classification of Occupations 1980.

Angina

This is defined by the standard chest pain questionnaire from Rose et al 1982[132]. Angina is chest pain that is related to effort and relieved after exertion stops. The degree of exertion required to bring on symptoms is classified into two groups: grade 1 angina is brought on by strenuous activity at high work loads; grade 2 is brought on by moderate work loads.

Alcohol consumption

A light drinker is one who consumes alcohol once or twice a month or less often. A moderate drinker is one who consumes up to 6 drinks of an alcoholic beverage (a drink being the equivalent of a half pint of beer, a glass of wine or a sixth of a gill single spirit measure) most days of the week, weekends or less than this often. A heavy drinker is defined as one who consumes more than 3 drinks daily or most days or more than 6 drinks daily at weekends. A "problem drinker" from the coroner's officer information is defined as one for whom the reporting officer states that the deceased was a regular heavy drinker or uses words similar to this to describe the habitual alcohol consumption of the deceased. For the information received from the case spouse, the control and his spouse a

"problem drinker" is defined as one for whom there was at least one positive answer to the following questions:

Have you felt at any time that he was drinking too much for his own good health?

Has he ever attended a meeting of alcoholics anonymous?

Has he ever lost friends because of his drinking?

Has he ever got into trouble at work because of drinking?

Has he ever neglected his obligations, his family or his work for more than two days in a row because he was drinking?

Has he ever had delirium tremens, severe shaking, hearing voices or seen things that were not there after heavy drinking?

Has he ever gone to anyone for help about his drinking?

Has he ever been in hospital because of drinking?

Has he ever been arrested for drunk driving or driving after drinking?

Has he ever had an accident requiring a visit to a casualty department before which he had been drinking?

Has he ever been charged with any offence which involved drinking alcohol?

Collection of case data

Case identification, post mortem data and sampling arrangements

To ensure that case ascertainment and sampling were as high as possible during the period of study it was necessary to have a method of surveillance that did not rely solely on the judgement of one individual. Before the period July 1989 to December 1989, consultant pathologists were briefed about the study by Dr P. J. Gallagher, Reader in Pathology. I also made a presentation of the study at a meeting of the Pathology Department. The Pathologists performing post mortem examinations were asked to instruct the technicians to take adipose tissue samples from cases which fitted the inclusion criteria. They were specifically asked to exclude those with a history of CHD, those whose bodies were severely decomposed and those with coincident longterm illnesses, e.g. cancer, which could have influenced the diet of the deceased. The inclusion and exclusion criteria are given in Table 2.

Simultaneously, the Research Nurses involved with the study collected the names and details of all men thought to be under the age of 65 coming to post mortem in the Southampton area. This was done by direct visits to all three mortuaries in the Southampton and South West Hants Health District. They also obtained photocopies of the post mortem reports from the Pathology Department offices in the Southampton General Hospital. These reports were then reviewed by myself and information from these reports abstracted to a computer codable form. Each post mortem report was given a code number and all records pertaining to those cases thereafter contained that number. During this review I also had access to photocopied reports of the coroner's investigating officer. The information given on this form was exactly that available to the pathologist performing the post mortem examination. I made a judgement as to which cases I could expect to receive samples of adipose tissue from.

The samples collected from the cases actively designated by the pathologists involved were stored in a central freezer at -70°C labelled by their surname and post mortem number. Meetings were then held approximately monthly to match the sample records to those whom I had recorded as fitting the criteria for entry into the study and

therefore to have samples taken. We were then able to identify which patients had not had samples taken and ascertain for what reason the sample had not been taken.

At the end of the first six months of surveillance 24 cases of SCD fitting our inclusion criteria had been identified by myself. During that period only 12 of these cases had adipose tissue samples stored. Two of the original 24 were severely decomposed, so the sampling rate of those actually eligible was 50%. However, this was thought to be a rather poor performance and so a more active method of post mortem room surveillance was instituted from the 1st of January 1990. This involved a direct visit every weekday morning to the post mortem room in Southampton General Hospital where the majority of coroner's post mortem examinations are performed. This duty was performed by Dr P. J.Gallagher or, in his unavoidable absence, by myself. The collection of all post mortem reports continued and were reviewed as before. Monthly review thereafter of the sample identity and matching of the eligible cases gleaned from post mortem report review indicated that the sample rate had improved.

Table 2. Inclusion and exclusion criteria

Inclusion criteria

Male.

Greater than 25 years old, Less than 65 years old.

Principal Cause of death coronary heart disease (other definitions such as

ischaemic heart disease, atherosclerotic coronary artery disease accepted as CHD

if included as primary cause of death).

No known history of clinical CHD prior to final event.

Absence of medical or pathological condition likely to profoundly affect the

habitual diet, e.g. oesophageal carcinoma.

Absence of severe decomposition.

Exclusion criteria

Females.

Males younger than 25 years old or greater than 65 years old.

Pre existing clinical CHD.

Presence of medical or pathological condition likely to profoundly affect the

habitual diet, e.g. oesophageal carcinoma.

Severe post mortem decomposition.

Collection of data on the case from the nearest surviving relative of the case

Information on the last illness of the cases of SCD was partly available from the report of the coroner's officer. However, in order to obtain further information about the presence of the major risk factors for CHD attempts were made to interview the nearest surviving relative (NSR) of the deceased.

On identification of a SCD case by myself from the post mortem report surveillance, the General Practitioner(GP) of the deceased was identified. The GP concerned was then approached, initially by a standardised letter and reply form . He was asked if he would know, or enquire, whether the NSR, usually the spouse, of the deceased had any objections to being interviewed directly about the circumstances surrounding the death of her husband. If he responded positively, a standard letter was sent to the NSR together with a reply form. If the NSR responded positively, I made contact by telephone to arrange a time for myself to visit and conduct an interview with them.

Conduct of the interview was semi-structured. In order not to upset the NSR unnecessarily I elected to ask them, initially, open ended questions about the deceased. This generally yielded information about the final illness which I could then code specifically for my purposes on a pre-printed form. Thereafter, I proceeded to conduct a structured interview using a standardised questionnaire which was designed to gather information on the nationality, birthplace, social class and marital status of the deceased. The questionnaire then proceeded to the known past medical history of the deceased, any medication he was taking on a regular basis, any history of chest pain using the Rose Questionnaire, the deceased's smoking history, his family history, alcohol consumption and finally an estimate of the deceased's height and weight at death and whether he was a habitual snorer.

I then asked the NSR, if necessary, for consent to be able to analyse the adipose tissue sample by gas liquid chromatography. Their signature was required if they agreed and this was witnessed by myself. All NSR's that I interviewed kindly agreed to give their consent for the laboratory analysis to proceed.

For those GPs who initially felt that the NSR required a certain period of time to grieve sufficiently for the deceased a follow up telephone call was made by myself to ask

whether the GP could raise the matter with the NSR or would indeed allow us to approach the NSR directly. Those who did not reply to the initial approach, or whom the GP felt it unwise for us to interview, were not contacted again. Those NSRs whom I could not directly interview after at least two attempts at contact had been made were assumed to have not actively expressed the wish that the adipose tissue could not be used for analysis.

If a GP could not be identified for the SCD case, contact was made directly with the NSR named in the coroner's officer's report. This only occurred in one case; the NSR did not reply to our letters.

Control selection, data collection and sampling arrangements

Control selection

To obtain controls for each of the SCD cases, the Research Nurses first obtained the GP's name with which the case was registered. This was generally available from the coroner's officer's report. However, if this proved inadequate, the name, address and date of birth of the case was taken to the central register of the Hampshire Family Practitioner Committee(FPC) in Winchester. They were given access to the computer terminals to identify the deceased individuals concerned and the GP with which they were registered. On identifying the GP they then searched the register for male patients who were within two years of age of the deceased. This was done using the automatic search criteria available with the software used on the FPC computer. On producing the search list of names, they then took the first four names together with their dates of birth and addresses. The names were arranged in alphabetical order. These men were to then act as the controls for that particular case. The names of the chosen controls were then sent to the GP concerned who was asked specifically to indicate those who were known to him to be unsuitable for invitation as controls. That is, those who had died or moved from the practice, or who were suffering from illnesses that precluded their eligibility as controls (terminal or severe psychiatric disease).

If a GP could not be identified for the case then the nearest General Practice to the deceased's address was used as a source for controls using the senior partner's list.

After receiving the approved names the Research Nurses then used a standard letter to invite each control and their respective spouses to attend a clinic. They were all given a specific date and time as a personal appointment.

No reply forms were sent with these invitations to reduce postage costs. A number of controls did either telephone to confirm, or refuse, or to rearrange their appointment to suit their needs.

Controls were not specifically asked to fast prior to attendance at the clinic.

A number of invitations were returned marked "no longer at this address". It was thus noted that the GP records as kept by the Hampshire Family Practitioner Committee were not up to date. If at least 2 controls for each case attended then no further controls

were drawn for that case. If no controls for a particular case attended or invitations were returned for all four, then four more controls were drawn in the same way at a separate visit to the FPC computer.

The number of controls invited together with the numbers considered unsuitable by the GP, those whose invitations were returned as "not known at this address", the refusals and the numbers of controls and their spouses attending are given in Table 4.

Table 3. Control numbers

Total number of controls drawn from GP Lists	711	Rate of response
Considered unsuitable by GP	25	3.5%
Invitations returned as "not known at address"	51	7.2%
Refusals	61	8.6%
Controls attended	357	50.2%
Controls attended with spouse	287	40.4%

Control clinics and data collection

Each control was invited together with his wife, or partner, to attend the Preventive Cardiology Clinic at the Royal South Hants Hospital in Southampton. Clinics were usually arranged for the morning and afternoon of three days a week. Evening clinics were also run for three hours from 7pm for an experimental period of approximately two months but it was found that this did not increase the yield of controls. Therefore no further evening clinics were arranged.

On arrival, the couple would be met by myself, or if I was interviewing another control, by one of the Research Nurses. A brief explanation was given as to why they had been invited, what was going to happen to them whilst they were in our department and what we were going to do with the information we obtained. An opportunity was given for them to refuse and to leave before data collection was commenced. No controls who arrived decided to leave.

An overview of the data collection schedule is given in Figure 2.

Figure 2 Overview of data collection from controls

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┌─────────────────────────┐        ┌─────────────────────────┐
│                         │        │    Female partner of    │
│      Male control       │        │      Male control       │
│                         │        │                         │
└────────────┬────────────┘        └────────────┬────────────┘
             │                                  │
┌────────────┴────────────┐        ┌────────────┴────────────┐
│        Interview        │        │        Interview        │
│                         │        │                         │
│ Birthplace              │        │   about male partner    │
│ Occupation              │        │                         │
│ Marital history         │        │  on the same subjects as the │
│ Medical history & medication │   │                         │
│ Chest pain history      │        │      Male control       │
│ Smoking history         │        │                         │
│ Family history          │        │                         │
│ Alcohol consumption     │        │                         │
│                         │        │                         │
└────────────┬────────────┘        └────────────┬────────────┘
             │                                  │
┌────────────┴────────────┐        ┌────────────┴────────────┐
│       Examination       │        │       Examination       │
│  Height in m            │        │      Height in m        │
│  Weight in Kg           │        │      Weight in Kg       │
│  Waist circumference in cm │     │  Blood pressure in mmHg │
│  Hip circumference in cm │       │ Reflotron total cholesterol │
│  Blood pressure in mmHg │        │                         │
│  Reflotron total cholesterol │   │                         │
│  Electrocardiogram      │        │                         │
│  Adipose tissue sample  │        │                         │
└─────────────────────────┘        └─────────────────────────┘
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Questionnaire

The control and his spouse were then interviewed in separate rooms within the department. As far as possible the control and his partner were interviewed at the same time so that there was no opportunity for them to confer on the questions that were asked.

The control was interviewed by one of the Research Nurses using a standardised questionnaire aimed to obtain information on the nationality, birthplace, social class and marital status of the control. The questionnaire then proceeded to the control's past medical history, the medication he was taking on a regular basis, whether there was a history of chest pain using a modified Rose Questionnaire, the control's smoking history, his family history, and finally alcohol consumption.

Whilst the control was being interviewed I interviewed the spouse using a structured questionnaire similar to that administered to the spouse of the case. All questions referred to the control and included the nationality, birthplace, social class and marital status of the control. The questionnaire then proceeded to the known past medical history of the control, any medication he was taking on a regular basis, any history of chest pain using a modified Rose Questionnaire, the control's smoking history, his family history, alcohol consumption and finally an estimate of the control's height and weight at the time of interview and whether he was a habitual snorer.

Measurements

1. Blood pressure

The blood pressure of the control was then measured in the left arm whilst the subject was seated. Two measurements were made approximately five minutes apart to within 2mmHg using a Hawksley Random Zero Sphygmomanometer (Hawksley, England).

2. Anthropometric

On removal of heavy outer clothing and shoes the control was then weighed to within 0.1Kg and his height measured to within 0.01m using a Seca Model 707 electronic scales and measuring stick. Measurements were then made of the left arm circumference of the control at a point midway between the olecranon and the acromion process. This was measured with an ordinary household tape measure (Woolworth & Co.) to 0.1cm.

Measurements were also made of the waist size to 0.1cm and of the maximum

circumference at the hips to 0.1cm using the same tape measure.

3. Venous blood

After the above measurements had been made samples of blood were drawn by the

Research Nurse from an antecubital fossa vein, with minimum venous stasis, using the

Vacutainer® (Becton Dickinson VACUTAINER Systems Europe, BP No. 37-38241

MEYLAN CEDEX, France) system. Blood was drawn into one 10 ml plain tube and 6 ml

into two 3 ml EDTA tubes. A micropipette sample of blood was taken from the EDTA

sample to be used for total cholesterol analysis using a Boehringer Mannheim Reflotron®

I dry chemistry analyser. This gave a value for total cholesterol in 120seconds, which was

recorded to the nearest 0.01mmol/l. The blood was spun at the end of the clinic at 2,200g

for 20 min. The supernatant from the plain tubes were then separated into two 1ml aliquots

and stored at -70°C as was the supernatant from the EDTA tubes.

Whilst the Research Nurse was taking and recording the measurements for the

spouse of the control I took the control to a separate room and if there was no record of

him ever having been told that he had angina or a heart attack by a doctor I explained the

procedure of taking a sample of adipose tissue to him. It was stressed that the control was

under no obligation to agree to have a sample of adipose tissue taken and every

opportunity was given to allow the subjects to refuse. If they did no attempt was made to

persuade them to recant. If he consented verbally then a standard 12-lead

electrocardiogram was performed by myself using a Hewlett Packard HP 4760 Pagewriter

electrocardiogram (Hewlett Packard Company, Medical Products Group, Andover

Division 3000 Minuteman Road, Andover, Massachusetts 01810-1099, USA). This was

followed by a 60s rhythm strip recording of standard lead II.

If the control did not consent to have a sample of adipose tissue taken then he was

asked to dress. If the control had been told by a doctor that he had angina or had had a

heart attack no electrocardiogram or sample were taken.

4. Adipose tissue

The details of the procedure for taking the sample of adipose tissue are as follows:-

The abdominal skin adjacent to the navel (approximately 5cm to the right of the midline) was prepared with a Medi-Swab® pre-injection swab containing 70% v/v Isopropyl alcohol BP (Smith & Nephew Medical Limited, Hull, England). The area was then anaesthetised locally by infiltration of 1 to 2ml of 1% w/v lignocaine hydrochloride (Antigen Pharmaceuticals Ltd, Rosecrea, Ireland) delivered via a 25G needle from a 2 ml syringe (syringe and needle from Terumo® Europe N.V., 3030 Leuven, Belgium). The lignocaine was infiltrated into the subcutaneous layer at a level of 2 to 3cm below the skin at an angle to the skin of approximately 20°. A wheal was raised in the dermis and epidermis as the needle was withdrawn and the lignocaine continuously infiltrated. The needle was then withdrawn and approximately 30 to 60s given for the anaesthetic to work. During this time approximately 5ml of washing solution. The washing solution was made up of 2% vitamin C (L-Ascorbic acid, BDH Chemicals Ltd., Poole, England) w/v in 0.9% sterile Sodium Chloride (Baxter-Travenol, UK) prepared by myself in the haematology laboratory of the Royal South Hants Hospital. This was poured into a 60ml sterile plastic gallipot (Wessex Sterile Supply Department, Queen Alexandra Hospital, Portsmouth). A 16G 1½ in needle (PrecisionGlide®, Becton Dickinson & Co., Rutherford, New Jersey 07070, USA) was then fitted to a 30ml Terumo® syringe. The needle was then introduced into the skin at an angle of approximately 20° through the wheal in the abdominal skin and advanced into the subcutaneous adipose tissue applying maximum suction. This action, moving in and out through the subcutaneous layer, was performed two to four times, still applying maximum suction, or until a globule of adipose tissue could be seen in the hub of the needle.

Once this had been achieved the needle was withdrawn and firm pressure applied over the site of the puncture with a sterile swab of cotton wool (Wessex Sterile Supply). The control was asked to continue to apply pressure with a finger as I prepared the adipose tissue sample. The sample of adipose tissue was then expelled from the syringe using several washings from the solution in the gallipot. The floating adipose tissue was then picked out from the solution, using the needle and syringe used to infiltrate the lignocaine, and placed in a 2ml microtube (Sarstedt Ltd., 68 Boston Road, Leicester LE4 1AW, UK). This was then labelled with the code for the control, recorded on the log of samples and in

the data collection form for the control, and then stored in a -70°C storage facility within the department (Gallenkamp Supercold 85, Fisons Scientific Equipment, Bishops Meadow Road, Loughborough, Leicestershire LE11 0RG, UK).

Once this had been performed and I was satisfied that the puncture site had stopped bleeding I placed a small plaster (Band-Aid®, Johnson & Johnson, Consumer Products, Skillman, New Jersey 08558-9418, USA) on the site and asked the control to dress.

The spouse was then asked to state her date of birth and then she was asked to allow the Research Nurse to measure her blood pressure, height and weight using the same protocol and instruments as were used for the control. No blood was taken from the spouse apart from a finger prick capillary sample to be used to measure her total cholesterol using the Reflotron® I dry chemistry analyser.

I then discussed the findings of the questionnaire, blood pressure recordings, cholesterol, body mass index (weight in Kg divided by height in m²) and electrocardiogram with the control and his spouse, together with the results of her measurements. General advice about the relevance of the findings to the future health of the controls and their spouse was given to all. All smokers were encouraged to stop. Specific advice, together with a recommendation to contact their GP, was given to those with a systolic blood pressure recording of greater than or equal to 160 mmHg and or a diastolic blood pressure reading of 100 mmHg was recorded. Similar recommendations for with Reflotron® cholesterol readings of greater than or equal to 7.0 mmol/l were made. All subjects were given the opportunity to ask questions about the study and any matter which arose from the discussion.

GPs were not notified of the findings routinely unless the control or spouse specifically requested this. Notification was always given when a systolic blood pressure greater than 160mmHg was found and a diastolic of greater than 100mmHg or both were found. General Practitioners were also automatically informed if the Reflotron® cholesterol was greater than 7.0mmol/l. Any control giving a positive history of exertional chest pain relieved by rest or having evidence on their electrocardiogram of a definite healed myocardial infarction was notified and contact was made, either by telephone or by letter, with their GP.

Social class coding

All cases and controls were classified into the Registrar General's social class coding using the Classification of Occupations 1980, Office of Population Census and Surveys.

The information for social class coding from the coroner's officer was present in the majority of cases but was inadequate in respect of detail.

Information from the NSR was excellent in detail but incomplete.

Adipose tissue sample management

All adipose tissue from the SCD cases were transferred to the -70°C freezer from the pathology department freezers as soon as practicable after the post mortem examination had been carried out. After delivery to the -70°C facility, an aliquot of the post mortem sample approximately similar in size to the samples routinely obtained from the controls was placed in a 2ml microtube. This tube was then marked with a code and returned to the freezer. All samples from the cases and controls were coded in a similar manner so that the microtube numbers were consecutive. The microtubes containing the adipose tissue samples were then delivered by myself by air in a domestic plastic coolbox containing 1 to 2 Kg of solid carbon dioxide (National Blood Transfusion Laboratory, Wessex Branch, Southampton General Hospital) to the freezers of the Cardiovascular Research Laboratories of the University of Edinburgh. There, gas liquid chromatography was carried out on all samples provided by Ms Karin Lyall and Ms Jacqui Lawrence under the direction of Dr Rudolph Riemersma, Senior Lecturer in Cardiac Biochemistry.

Management of plasma and serum samples

All serum samples from the controls were coded with identification numbers and transferred at the same time as the adipose tissue samples to the Cardiovascular research Laboratory in the University of Edinburgh. The samples were stored in their -70°C freezer facility. Analysis of sera was performed by Mrs May Walker for total cholesterol, HDL cholesterol and non-fasting triglycerides under the direction of Dr Rudolph Riemersma.

Plasma samples have been stored for later analysis.

Laboratory procedures

Adipose tissue analysis

After thawing, the adipose tissue samples were rinsed in saline. Lipids from the samples were then extracted into redistilled heptane and the extract was washed with isopropanol with 0.5%(w/v) potassium hydroxide, to remove non-esterified fatty acids and phospholipids. The neutral lipid extract was reduced to dryness under a vacuum and then dissolved in dry toluene. Fatty acid methyl esters were prepared by direct transmethylation (10min at 50°C with 0.5M sodium methoxide in methanol). The fatty acid esters were washed with acidified water and re-extracted into hexane; the hexane layer was evaporated under vacuum. The methyl esters were redissolved in 40 μl redistilled chloroform, ready for analysis on a Pye Chromatograph 204, fitted with a 1.5m column, packed with GP 10% SP-2330 on 100/120 mesh "Chromosorb WAW" (Supelco). The peaks were identified with a flame ionisation detector and an electronic integrator was used to calculate a continuous output for the particular run. This data was then transferred to an IBM PS/2 computer for viewing data.

The peaks in the chromatograms were identified by thin-layer chromatography and by comparison of retention times with those of commercially supplied fatty acids of known molecular structure. The coefficient of variation in determining the percentages of the individual fatty acids varied with the heights of the peaks but for linoleic acid this was less than 3%.

Serum lipid analysis

Cholesterol and triglycerides were analysed enzymatically on the thawed serum using Boehringer-Mannheim "Monotest" for cholesterol and "Merck" test for triglycerides by means of a "Cobas-Bio" centrifugal analyser. High density lipoprotein cholesterol was measured using the same kits after precipitation of the very low and low density lipoprotein by magnesium dextran. The coefficient of variation for a pooled cholesterol sample using this method was 1.7%. The coefficient of variation for triglyceride for a pooled sample was 2.0% and the coefficient of variation for the high density lipoprotein cholesterol was 1.9%.

Data management

All data collection forms were checked for completeness, coding inconsistencies and for clarification and insertion of codes. This procedure was performed by myself, Sr Frances Watts, Sr Tricia Elliot and Sr Ruth MacFarlane. After this had been performed all data collection forms were despatched to an independent computer data entry facility (Sunlight Computer Services Ltd., Hollybrook Road, Shirley, Southampton, UK). At this facility the data from the forms was entered on to a format which was compatible with the computer of Southampton University (IBM 3090). All forms were dual entered. That is, all data from each form was entered by two persons on separate occasions. The two records were then compared electronically to ensure that no keystroke errors had occurred. The data was then read into the IBM 3090. The data was then checked again by Mrs Fiona Lampe, Medical Statistics and Computing Department, Southampton General Hospital. Interrogation of this data set was possible through a land link from an IBM compatible PC AT terminal to the IBM 3090. All data review and cleaning was performed via this link.

The data from the Cardiovascular Research Laboratories in Edinburgh was sent directly to Mrs Lampe on 3.5in floppy disc. These contained the raw data output from the chromatographs and from the Cobas-Bio analyser. Matching of data and transformation of the raw data was performed by Mrs Lampe via the PC mainframe link.

Statistical methods

Statistical estimate

At the time of writing the protocol, an estimate was made of the numbers of cases and controls required to be able to confirm, or refute the hypothesis with reasonable confidence. From tables[133], 75 cases and 150 controls would give a power of 80% to detect a true difference of 0.34 of a standard deviation at the p<0.05 level of significance using a two-sided test. Taking the mean percentage composition of linoleic acid of adipose tissue of men in Edinburgh, as a reference example, which was 8.94% with a standard deviation of 2.29% a difference of 0.78% in the relative linoleic acid content could be detected with the above probabilities.

Statistical procedures

All statistical procedures were carried out on raw and transformed data after checking by Mrs Fiona Lampe. These were performed using a PC AT link to an IBM 3090 mainframe computer running SPSS X statistical software (SPSS Inc., 444 N. Michigan Avenue, Chicago, Illinois 60611, USA). Further analysis was performed by myself on an IBM compatible PC using SPSS/PC+ version 4.0.

For analysis of dichotomous variables the χ^2 statistic was used. For continuous variables that were normally distributed the two sample t test was used. The majority of fatty acid distributions were not normally distributed therefore a two sample Mann-Whitney U test was employed. Analysis of variance was used in the analysis of continuous variables across multiple categories.

For examination of the validity of proxy responses from the relatives of the cases and controls the Kappa coefficient was used. This provides an estimate of the agreement between two methods of measurement allowing for an element of chance[134].

Multiple logistic regression was applied to dichotomous variables using a stepwise procedure (forwards and backwards) or by forced entry using the maximum likelihood ratio estimate at the 0.05 level as a determinant for further inclusion. If continuous variables were being examined, linear regression modelling was used.

Conditional logistic regression analysis was performed by myself using the clogit

procedure on an IBM compatible personal computer using STATA statistical software

version 3.1.

RESULTS

Characteristics of sudden cardiac death cases

Post mortem findings

During the period of the study 333 men resident in the Southampton area, dying within the age range specified came to post mortem. Of these, 84 (25.2%), were sudden cardiac death(SCD) cases according to our inclusion criteria. The attributed causes of death after post mortem of the other 249 are given in the table below.

Table 4. Classification of cause of death for all post mortems

Cause of death	Number(n)	Percent of total(%)
Sudden cardiac death	84	25.2
Other death from coronary heart disease	52	15.6
Other cardiovascular death	15	4.5
Pulmonary embolus	10	3.0
Aortic stenosis	4	1.2
Other valvular heart disease	2	0.6
Endocarditis	2	0.6
Cardiomyopathy	3	0.9
Myocarditis	1	0.3
Cerebrovascular disease	11	3.3
Respiratory disease	21	6.3
Carcinoma	28	8.4
Gastrointestinal	14	4.2
Renal	2	0.6
Trauma	28	8.4
Suicide	40	12.0
Epilepsy	7	2.1
Other miscellaneous	4	1.2
Uncertain	5	1.5

A summary of the post mortem findings in the SCD cases is given in the table below. There was one case for which it was not possible to estimate from the post mortem report what degree of severity of coronary artery atheroma was present. At least 95.2% of cases had moderate or severe coronary artery atheroma. Ante mortem thrombus was present in 41.7% of cases in at least one coronary artery. A fresh regional acute myocardial infarction was visible to the naked eye in 32.1% of cases. There was an old regional myocardial scar in 26.2% of cases despite there being no history of CHD prior to death. The pathologist performing the post mortem considered the left ventricle to be hypertrophied in 53.6% of cases and to be dilated in 8.3%. Rupture of the free wall of the left ventricle had occurred through the site of a necrotic infarct in 3.6% of cases. There were no cases where an acquired ventriculoseptal defect had occurred. Pericarditis was observed to be present in 2.4% of cases and a pericardial effusion present in 8.3%. Of the SCD cases, 1 was considered to have right ventricular hypertrophy and dilatation in conjunction with pulmonary atheroma. Aortic atheroma was noted to be present in 85.7% but aneurysmal change in the abdominal aorta was noted in only 1.2% of cases. The mean heart weight was 438.05g.

Table 5. Summary of post mortem findings in 84 sudden cardiac death cases

Finding at post mortem	n (%)
Mild coronary artery atheroma	3 (3.6)
Moderate coronary artery atheroma	21 (25.0)
Severe coronary artery atheroma	59 (70.2)
Coronary artery thrombus	35 (41.7)
Coronary artery occlusion	9 (10.7)
Acute myocardial infarction	27 (32.1)
Old regional myocardial scarring	22 (26.2)
Left ventricular hypertrophy	45 (53.6)
Left ventricular dilatation	7 (8.3)
Left ventricular rupture (free wall)	3 (3.6)
Pericarditis	2 (2.4)
Percardial effusion	7 (8.3)
Left atrial dilatation	1 (1.2)
Right ventricular hypertrophy	1 (1.2)
Right ventricular dilatation	1 (1.2)
Pulmonary artery atheroma	1 (1.2)
Aortic atheroma	72 (85.7)
Abdominal aortic aneurysm	1 (1.2)
Heart weight g (standard deviation)	438.05 (85.41)

The mean age at death of the 84 cases of sudden cardiac death was 54.95 years (standard deviation 7.35 years; range 33 to 64 years).

Of the 84 cases identified, 66 had samples of adipose tissue taken and stored at post mortem. The reasons for non-sampling are given in the table below.

Table 6. Identification of sudden cardiac deaths 1st January 1990 to 31st July 1991

Total number of sudden cardiac deaths identified	84
Too severely decomposed for sample	4
Other medical conditions precluding sample*	3
Sample not taken for miscellaneous reasons**	11
Total number of adipose tissue samples	66

* 1 case of carcinoma of the stomach found at post mortem

1 case with history of recurrent oesophageal strictures

1 case of known hyperlipidaemia and therefore excluded inadvertently

** 1 case with incorrect date of birth given to pathologist

1 case thought to have had symptoms for more than 24 h but on review of records this was found not to be the case

1 case known to have a peripheral circulatory condition and therefore inadvertently excluded

5 cases missed because of a misunderstanding that known hypertension was a criterion for exclusion

3 cases not sampled due to oversight

Information from the Coroner's officer

A report from the Coroner's officer was available with the post mortem report in 96.4% of cases. The remaining 3 cases had died in hospital. The hospital notes were available for review in all three cases.

Social class distribution

Of the 81 cases on whom there was Coroner's officer information 1.2% were in social class I, 11.1% were in social class II, 4.9% were in social class III (non-manual), 44.4% were in III (manual), 17.3% were in social class IV and 6.2% were in social class V. The other 14.8% could not be classified due to their long term unemployment status.

Table 7. Social class classification from the 81 Coroner's officer reports

Social Class coding	n (%)
I	1 (1.2)
II	9 (11.1)
III (non manual)	4 (4.9)
III (manual)	36 (44.4)
IV	14 (17.3)
V	5 (6.2)
Others	2 (6.5)
Unclassifiable	10 (12.3)
Total	81

Marital status

Almost two thirds, 61.7%, of cases were married at the time of death and had only been married once. Bachelors made up 12.3% of cases. Those who were married at the time of death but who had been married more than once comprised 7.4% of cases. There were 7.4% of cases who could not be classified as being single or married and who were co-habiting with a common law wife. A further 11.1% of cases were divorced and were living alone. One case could not be classified from the information available.

Table 8. Marital status according to the 81 Coroner's officer's reports available

Marital status	n (%)
Married once	50 (61.7)
Married more than once	6 (7.4)
Divorced	9 (11.1)
Other co-habiting	6 (7.4)
Single	10 (12.3)
Data missing	1 (1.2)
Total	81

Medical history

A limited medical history was available from review of the Coroner's officer reports. In 11.1% of cases a history of high blood pressure was recorded, diabetes mellitus in 4.9%, high cholesterol in 1.2% and a history of stroke in 2.5%. Prescribed medication of any sort was being taken before the time of death by 40.7% of cases. Cigarette smoking was recorded as a habit of the case in 25.9% of cases. Habitual heavy intake of alcohol was remarked upon by the Coroner's officer in 14.8% of the cases.

Table 9. Medical history from 81 Coroner's officer reports

Medical history	n (%)
High blood pressure	9 (11.1)
High cholesterol level	1 (1.2)
Diabetes	4 (4.9)
Stroke	2 (2.5)
On any prescribed medication	33 (40.7)

Table 10. Smoking and drinking habits of 81 SCD cases from Coroner's officer reports

Class of information	n (%)
Cigarette smokers	21 (25.9%)
Heavy intake of alcohol	12 (14.8)

Data concerning last illness

According to the report of the Coroner's officer the final loss of consciousness was actually witnessed in 45.7% of cases. There was a history obtained from a witness, or relative, of the case having complained of chest pain in 44.4% of cases. The time from the onset of any symptoms to death was interpreted from the Coroner's officer's report as being less than 60 min in 33.3%, 60 min to less than 6 h in 12.3%, 6h to less than 12 h in 8.6%, and 12 h to 24 h in 3.7% of cases. In 11.1% of cases there was a report of symptoms compatible with a cardiac condition having been reported to be present for more than 24 h before death.

Table 11. Information from the Coroner's officer regarding the final illness of 81 SCD cases

Class of information	n = 81 (%)
Witnessed death	37 (45.7)
Record of chest pain	36 (44.4)

Table 12. Time from onset of symptoms (if any) to death from Coroner's officer of 81 SCD cases

Time from symptom onset to death	n = 81(%)
Less than 60 min	27 (33.3)
60 min to less than 6 h	10 (12.3)
6 h to less than 12 h	7 (8.6)
12 h to less than 24 h	3 (3.7)
More than 24 h	9 (11.1)

Information from the Nearest Surviving Relative (NSR) of the case

Characteristics of the NSR

The coroner's officer had interviewed a relative, bystander or neighbour in 96.4% (81/84) of cases and a relative was identified for interview in 94.1% (79/84). A living spouse was identified in 64 cases, 33 of whom agreed to be interviewed by myself after the GP had given his approval. However, 2 wives withdrew their permission to be interviewed. In 15 cases the wife refused to be interviewed after the general practitioner had approved my approach. In 12 cases the GP refused permission for interview with the wife. In one case the case was not registered with a GP as far as we are aware; the wife did not respond to our approach. In 3 cases the deceased was divorced and his ex wife uncontactable.

In 5 cases a sibling was available for interview, in 2 cases the mother of the case was available, in 2 cases the daughter of the case was interviewed and in one case the long term friend was interviewed. In one case where the ex wife could not be contacted the landlady of the case was interviewed. In 5 cases no relatives were known either to the general practitioner or neighbours of the case who had been interviewed by the coroner's officer.

The number and class of NSR eventually interviewed about the SCD case is given in the table below.

Table 13. Class of NSR interviewed

Relationship of NSR to sudden cardiac death case	n = 42(%)
Wife	31 (73.8)
Other relative	9 (21.4)
Other person	2 (4.8)
Total	42

Information concerning the case from the NSR

1. Nationality

The deceased was reported as having British nationality, and Caucasian, in 95.2% of cases. The other 2 (4.8%) were from the Indian subcontinent and therefore of Asian origin.

Table 14. Nationality of cases from information received from NSR

Nationality	n = 42 (%)
British	40 (95.2)
Other	2 (4.8)
Total	42

2. Social class

The occupation of the case given by the NSR classified the cases into social class I in 2.4%, II in 14.3%, III (non manual) in 7.1%, III (manual) in 35.7%, IV in 21.4%, V in 11.9% and the armed forces in 7.1%.

Table 15. Social class of 42 SCD cases from information received from NSR

Social class coding	n = 42 (%)
I	1 (2.4)
II	6 (14.3)
III (non manual)	3 (7.1)
III (manual)	15 (35.7)
IV	9 (21.4)
V	5 (11.9)
Armed forces	3 (7.1)
Total	42

3. Marital status

The case was reported by the NSR to have been married once only in 59.5%. The deceased was a lifelong bachelor in 9.5% of cases. Divorcees made up 4.8% and other co-habitees made up 9.5%. Those that had been married more than once comprised 16.7%.

Table 16. Marital status of 42 SCD cases from information of NSR

Marital status	n = 42 (%)
Married once only	25 (59.5)
Married more than once	7 (16.7)
Divorced	2 (4.8)
Single	4 (9.5)
Other	4 (9.5)
Total	42

4. Medical history

A history of recall of a doctor's diagnosis of high blood pressure being made was present in 23.8% of cases. A doctor's diagnosis of high cholesterol was recalled by 9.5% of NSR. Only 2.4% of NSR's recalled a diagnosis of diabetes. A history of peptic ulceration was recalled in 11.9%, gallbladder disease in 4.9%. Liver disease and "hardening of the arteries in the legs" were recalled in 2.4% respectively. Almost three quarters (73.8%) of NSR's recalled that the case had had an admission to hospital at some point in their lives.

Table 17. Medical history of 42 SCD cases from NSR information

Medical history	n = 42 (%)
High blood pressure	10 (23.8)
High cholesterol level	4 (9.5)
Diabetes	1 (2.4)
"Hardening of the arteries in the legs"	1 (2.4)
Liver disease	1 (2.4)
Gall bladder disease	2 (4.8)
Peptic ulceration	5 (11.9)
Any admission to hospital	31 (73.8)

5. Medication

Over half of the cases were taking prescribed medications (52.4%). In addition, 7.1% were taking over the counter preparations and 9.5% were taking regular vitamin preparations. Of the 23.8% where there was a recall of a doctor's diagnosis of high blood pressure, 60% (6/10) were actually prescribed antihypertensive medication. None of the cases where a diagnosis of a high cholesterol had been mentioned were taking, or had taken, any medication for this condition.

Table 18. Medication taken by 42 SCD cases recorded from NSR

Class of information	n = 42 (%)
On any prescribed medication	22 (52.4)
On any other over the counter preparation	3 (7.1)
Taking vitamin preparations regularly	4 (9.5)
Receiving medication for high blood pressure	6 (14.3)

6. Smoking history

The majority of NSR's reported that the case was a regular smoker at some point in his life (90.5%). However, 16.7% had quit smoking at some point prior to death and 9.5% were lifelong non-smokers. Of the 31 cases who were smoking until the time of death, 28 were reported to be cigarette smokers. The 3 others were pipe smokers or cigar smokers, or both.

Table 19. Smoking habit of 42 cases as reported by NSR

Smoking status at time of death	n = 42 (%)
Cigarette smoker only	28 (66.7)
Pipe or cigar or both	3 (7.1)
Quit smoking prior to death	7 (16.7)
Never smoked	4 (9.5)
Total	42

7. Alcohol consumption

From the report of the NSR in response to the standard questionnaire about alcohol consumption 42.9% were classed as light drinkers, 18.6% drank moderately and 23.8% were heavy consumers of alcohol. Those who could be classed as "problem drinkers" made up 9.5% of the cases. The NSR felt unable to classify the alcohol consumption of the case in 2 instances.

Table 20. Alcohol consumption of 42 SCD cases reported by NSR (Data missing in 2)

Class of alcohol consumption†	n = 42 (%)
Light or non drinker	18 (42.9)
Moderate	12 (18.6)
Heavy	10 (23.8)
Total	40
Problem drinkers	4 (9.5)

†A light drinker is one who consumes alcohol once or twice a month or less often. A moderate drinker is one who consumes up to 6 drinks of an alcoholic beverage (a drink being the equivalent of a half pint of beer, a glass of wine or a sixth of a gill single spirit measure) most days of the week, weekends or less than this often. A heavy drinker is defined as one who consumes more than 3 drinks daily or most days or more than 6 drinks daily at weekends. A "problem drinker" from the coroner's officer information is defined as one for whom the reporting officer states that the deceased was a regular heavy drinker or uses words similar to this to describe the habitual alcohol consumption of the deceased. For the information received from the case spouse, the control and his spouse a "problem drinker" is defined as one for whom there was at least one positive answer to the following questions:

Have you felt at any time that he was drinking too much for his own good health?

Has he ever attended a meeting of alcoholics anonymous?

Has he ever lost friends because of his drinking?

Has he ever got into trouble at work because of drinking?

Has he ever neglected his obligations, his family or his work for more than two days in a row because he was drinking?

Has he ever had delirium tremens, severe shaking, hearing voices or seen things that were not there after heavy drinking?

Has he ever gone to anyone for help about his drinking?

Has he ever been in hospital because of drinking?

Has he ever been arrested for drunk driving or driving after drinking?

Has he ever had an accident requiring a visit to a casualty department before which he had been drinking?

Has he ever been charged with any offence which involved drinking alcohol?

8. Family history

There was a history from the report of the NSR of heart disease in a first degree relative of the case under the age of 60 years in 35.7% of cases.

9. Medically prescribed diets

Two of the cases from which there were reports from NSR were on a medically prescribed diet. In both cases they had been diagnosed as having high cholesterol by their doctor.

10. Snoring

Habitual snoring was reported by the NSR in 15 of 32 cases for whom the data were available.

Table 21. Snoring habits of 32 SCD cases from the NSR

Class of information	n = 32 (%)
No snoring reported	5 (15.6)
Reported as being occasional snorers only*	15 (46.9)
Reported as being habitual snorers*	12 (37.5)

* Data not available in 8 cases

11. Information regarding final illness

In just under half of the cases (47.6%) the NSR reported that the case died within 60 min of symptoms starting. In 35.7% of cases death had occurred from 60 min to within less than 6 h of symptoms starting. Symptoms lasted over 6 h in 11.9% and of these 5 cases, 2 NSR's reported that symptoms had actually been present for more than 24 h. In 2 cases the NSR was uncertain as to the duration of symptoms.

From information received from NSR's 78.5% (33/42) of cases had at least one symptom prior to death. In 23.8% (10/42) this was chest pain only. At least one other symptom apart from chest pain was reported in 38.1% (16/42) and 16.7% (7/42) had at least one other symptom but no chest pain. Of the cases who had chest pain and at least

one other symptom, 9 had one other symptom, 2 had 2 other symptoms, 2 had 3 other symptoms and 3 had 4 other symptoms. Of the 7 who had symptoms other than chest pain, 4 had one symptom only, 2 had 2 and one had 3 symptoms. The frequency of symptoms other than chest pain from all cases was epigastric or abdominal pain in 12, general malaise and fatigue in 8, other miscellaneous symptoms in 6, shortness of breath in 5, vomitting and nausea in 4, anxiety in 2 and diarrhoea in 1 case.

Table 22. Time from onset of symptoms to death in 42 cases of SCD from the report of NSR

Time from symptom onset to death	n = 42 (%)
Less than 60 min	20 (47.6)
60 min to less than 6h	15 (35.7)
6 h to less than 12 h	3 (7.1)
12 h to less than 24 h	0
More than 24 h	2 (4.8)
Unknown	2 (4.8)

Table 23. Class of symptoms prior to death

Class of symptom	n = 42 (%)
At least one type of symptom	33 (78.6)
Chest pain only	10 (23.8)

A history of exertional chest pain defined as angina from the WHO questionnaire remote from the period of the final illness was elicited for 1 case from the 42 relatives interviewed. Seventeen other relatives reported that the SCD case had complained of chest pain at some time prior to the final illness but this was not thought to be related to exertion.

Table 24. Recall of a report of chest pain made by the deceased remote from period before death in 18 NSRs

Chest pain classification	n = 18 (%)
Angina grade 1	1 (2.4)
Angina grade 2	0
Other chest pain	17 (40.5)

The death, or loss of consciousness immediately prior to death, was actually witnessed in 61.9% of cases. The wife of the deceased was the witness in 23.8%. Basic cardiopulmonary resuscitation was attempted initially by the wife in 14.3% of cases, by a relative in 7.1%, a bystander in 21.4% and by police or ambulance personnel in 35.7%. CPR was attempted by at least one person in 78.6% of cases.

Table 25. Witness of final loss of consciousness in 26 SCD cases

Witness status	n = 26 (%)
Wife	10 (23.8)
Other	16 (38.1)
Total	26 (61.9)

Table 26. Attempted resuscitation in 33 cases of SCD

Resuscitation performed by whom	n = 33 (%)
Wife	6 (14.3)
Other relative	3 (7.1)
Bystander	9 (21.4)
Police	1 (2.4)
Ambulance personnel	14 (33.3)
Total	33 (78.6)

Comparison between information from the Coroner's officer and the NSR

To estimate the degree of confidence that can be placed on the accuracy of the responses of the spouse as a proxy for interviewing the SCD case himself the Kappa statistic was calculated for responses from the nearest surviving relative (NSR) and the Coroner's officer. The gold standard was the response of the NSR.

For coronary heart disease history

The value of Kappa was 1.0 for this comparison indicating absolute agreement.

NSR

		Yes	No
Coroner's officer	Yes	0	0
	No	0	40

Kappa = 1.0

For smoking history at time of death

The value of Kappa for this response of the NSR was 0.29, indicating very poor agreement.

NSR

		Yes	No
Coroner's officer	Yes	11	1
	No	15	13

Kappa = 0.29

The sensitivity of the Coroner's officer for the presence of smoking prior to death is 42.3%, the specificity was 92.8%. The systematic error was 46.2%. The positive predictive value was 91.7% with a negative predictive value of 46.4%.

For being described as a problem drinker

The Kappa value for this response was 0.72 which indicates reasonable agreement between the two responses.

NSR

		Yes	No
Coroner's officer	Yes	3	1
	No	1	35

Kappa = 0.72

The sensitivity of the Coroner's officer response according to the NSR's response was 75%. The specificity was 97.2%. The systematic error was 100%. The positive predictive value was 75% with a negative predictive value of 97.2%.

For other Coroner's officer information the Kappa values are listed below. Agreement on the diagnosis of diabetes mellitus was absolute but the agreement on the diagnosis of high blood pressure, on the witnessing of death and the presence of chest pain prior to death, while not being totally opposite, were not good.

Table 27. Kappa values for information from Coroner's officer and NSR

Class of information	Kappa
High blood pressure	0.66
Diabetes mellitus	1.0
Witnessed death	0.60
Chest pain before death	0.50

Agreement between Coroner's officer information and NSR for marital status and

social class

The agreement, with a Kappa value of 0.92 , for this response was very good.
For marital status (married yes/no)

NSR

	Yes	No
Coroner's officer Yes	32	1
No	0	7

Kappa = 0.92

For Social Class

(Social Class I and II are recoded to 1, III non manual and III manual to 2, and IV and V to 3)

The agreement between these classifications is poor, the Kappa value being 0.30.

		NSR		
		1	2	3
Coroner's officer	1	2	2	3
	2	4	13	3
	3	1	2	7

Kappa = 0.30

Information concerning control from spouse of control

Status of control spouse (CS)

Of the 293 controls that were invited and whose spouse attended the CS was the control's wife in 96.6% of cases. In 0.7% another relative of the control acted as CS and 2.7% of controls the CS was the common law wife or live-in girlfriend of the control. There were 6 spouses who attended without the control partner.

Table 28. Status of control spouse (CS)

Relationship of CS to control	n = 293(%)
Wife	283 (96.6)
Other relative	2 (0.7)
Other person	8 (2.7)
Total	293

Information from control spouse concerning control

1. Nationality of controls

According to the CS the control was of British nationality in 98.3% of cases and of other nationality in 1.7% of cases. Racial origin was almost entirely Caucasian with only one West Indian of African origin attending as a control. No Asians attended as controls.

Table 29. Nationality of 293 controls according to CS

Nationality of control	n = 293(%)
British	288 (98.3)
Other	5 (1.7)
Total	293

2. Social class of controls

From information given by the CS the controls were classified by occupation. Of the 292 controls 7.5% were in social class I, 15.7% in social class II, 13.0% in social class III (non manual), 48.1% in social class III (manual), 8.5% in social class IV and 2.4% in social class V. There were 4.8% classified as being in the armed forces.

Table 30. Social class of 293 controls according to report of CS

Social class coding	n =293 (%)
I	22 (7.5)
II	46 (15.7)
III (non manual)	38 (13.0)
III (manual)	141 (48.1)
IV	25 (8.5)
V	7 (2.4)
Armed forces	14 (4.8)
Total	293

3. Marital status of controls

According to the CS the control had been married once only in 82.9% of cases and more than once in 13.7%. Of the others, 0.7% were classed as being single, 1.4% as divorced and 1.4% classed the control as being a common law husband.

Table 31. Marital status of 293 controls

Marital status	n = 293 (%)
Married once only	243 (83.2)
married more than once	40 (13.7)
Divorced	4 (1.4)
Single	2 (0.7)
Other	4 (1.4)
Total	293

4. Medical history of controls

The CS reported the controls to have had rheumatic fever in 2.4% of cases. CHD had been diagnosed in 10.2% of controls according to the CS. Valvular heart disease of any kind was reported in 0.7% of cases. Other types of heart disease, usually rhythm abnormalities (not all confirmed by hospital diagnoses) were reported in 2.7% of cases. A doctor's diagnosis of high blood pressure was reported to have been made at some time for the control in 24.2% of cases. Just over half (54.9%) of these, however, had been reported as taking regular medication for this condition. A doctor's diagnosis of a high cholesterol level in the controls was known to the CS in 7.5% of cases. Only 18.2% of these men had ever received medical therapy for this condition. There were 3.8% diagnosed as diabetic and 1% had been told they had suffered a stroke at some time. "Hardening of the arteries in the legs" had been diagnosed in 2% of cases. Liver disease and pancreatitis had been diagnosed in 2.4% and 0.7% respectively and gall bladder disease in 3.8%. Peptic ulceration had been diagnosed in 10.2% of controls. Over three quarters (75.8%) had been admitted to hospital at some time in their lives.

Table 32. Medical history of 293 controls from CS

Medical history reported	n 293 (%)
Rheumatic fever	7 (2.4)
Coronary heart disease	30 (10.2)
Valvular disease of the heart	2 (0.7)
Other heart disease	8 (2.7)
High blood pressure	71 (24.2)
High cholesterol level	22 (7.5)
Diabetes	11 (3.8)
Stroke	3 (1.0)
"Hardening of the arteries in the legs"	6 (2.1)
Liver disease	7 (2.4)
Pancreatitis	2 (0.7)
Gall bladder disease	11 (3.8)
Peptic ulceration	30 (10.2)
Any admission to hospital	222 (75.8)

5. Medication taken by controls

Just under forty percent of the controls were taking prescribed medication of any kind according to the CS. Just over 15% were taking over the counter preparations, some in addition to their prescribed medication. Almost a quarter were taking what were interpreted by the CS as vitamin preparations. The vitamin preparations were often in addition to prescribed and other purchased medication. The proportions taking, or having taken at any time, medication for specific conditions such as high blood pressure are listed in the table below.

Table 33. Regular medication being taken by 293 controls at time of interview: report of CS

Class of medication	n = 293 (%)
On any prescribed medication	116 (39.6)
Any other over the counter preparation	45 (15.4)
Regular vitamin preparations	70 (23.9)
Ever received medication for high blood pressure	39 (10.7)
Ever received medication for high cholesterol	4 (1.1)
On oral hypoglycaemic agents	8 (2.2)
On insulin	1 (0.3)

6. Chest pain recall by the control spouse

On classification of the chest pain questionnaire, 3.8% of controls according to their spouses had angina of effort; of these a fifth reported that chest pain occurred on at least moderate exertion (grade 1 Angina). However, another 30.7% of CS reported that the control had complained of chest pain not consistently related to exertion at some time in the past. This was central chest pain or pain with a radiation typical of ischaemic pain in the majority of cases.

Table 34. Chest pain recall by the CS

Chest pain classification	n = 293 (%)
Angina grade 1 (on severe exertion)	9 (3.1)
Angina grade 2 (on moderate exertion)	2 (0.7)
Chest pain not angina	90 (30.7)
Central chest pain or of typical radiation but not angina	80 (27.3)
Other chest pain	10 (3.4)

7. Smoking habit of controls

A quarter (25.3%) of the CS reported that the control had never been a regular smoker. Three quarters (74.7%) had been regular smokers at some time during their lives and almost two thirds of these controls had become ex-smokers. Of the regular smokers the vast majority (86.4%) were cigarette smokers, the others being pipe smokers or cigar smokers, or both.

Table 35. Reported smoking habit of controls from CS

Smoking status	n = 293 (%)
Ever a regular smoker	219 (74.7)
Quit smoking	138 (37.9)
Never smoked	74 (25.3)
Current cigarette smoker	70 (23.9)
Only smoking pipe or cigar or both	11 (3.8)

8. Family history of controls

From the history given and coded from the CS, there was a history of heart disease in a first degree relative of the control under the age of 60 years in 10.2% of cases.

9. Alcohol consumption of controls

The CS reported that the control drank lightly or not all in 38.6% of cases, moderately in 40.6% of cases and heavily in 20.8% of cases. Over a quarter of the light to moderate drinkers were reported to have drunk more heavily on a regular basis, at some point in the past, than their present consumption. Those that were classed as problem drinkers made up 5.8% of all controls.

Table 36. Alcohol consumption of control reported by CS

Class of consumption	n = 293 (%)
Light or none	113 (38.6)
Moderate	119 (40.6)
Heavy	61 (20.8)
Problem drinkers	17 (5.8)

10. Dieting behaviour

Almost 6% of the total were acknowledged by the CS to be on a medically prescribed diet at the time of interview.

11. Snoring habits of controls

The CS was asked about the snoring habits of the control in 214 cases (73.3%). This was due to an initial oversight in the preparation of the original questionnaire. Over three quarters (79.4%) snored at least occasionally at night. Over a third of these controls (37.7%) were reported to snore habitually.

Table 37. Snoring habits of 214 Controls

Snoring at least occasionally	170 (79.4%)
Habitual snorers	64 (29.9%)

Information obtained from the control

1. Nationality of control

A total of 357 controls attended for interview from the 711 invited originally. Only 4 (1.1%) were not of British nationality according to their report. Almost all were of Caucasian racial origin. Only one was West Indian of African origin. There were no Asians.

Table 38. Self reported Nationality of controls

Nationality	n = 357 (%)
British	353 (98.9)
Other	4 (1.1)
Total	357

2. Social class of control

The coding of social class was performed on the information given by the control without reference to the information from the CS. Just over 8% of the controls were classed as being in social class I, 15.6% were in social class II, 14.5% in social class III (non manual), 43.3% in social class III (manual), 10.9% in social class IV and 3.1% in social class V. The others, comprising 4.2%, had spent most of their working lives in the armed forces or they could not be classified from the information given.

Table 39. Self reported social class of 357 controls

Social class coding	n = 357 (%)
I	30 (8.4)
II	56 (15.6)
III (non manual)	52 (14.5)
III (manual)	155 (43.3)
IV	39 (10.9)
V	11 (3.1)
Armed forces	14 (4.2)
Total	357

3. Marital status of control

Over three quarters (77.7%) of the controls had been married only once according to their report. Just under 10% had been married more than once. Life long batchelors comprised 7% and those who classed themselves as being divorced made up 3.4%. The others who were co-habiting made up 2.8%.

Table 40. Self reported Marital status of 357 controls

Marital status	n = 357 (%)
Married once only	278 (77.7)
Married more than once	33 (9.2)
Single	25 (7.0)
Divorced	12 (3.4)
Other	9 (2.8)
Total	357

4. Medical history of controls

A history of rheumatic fever was reported by the controls in 2.8% and valvular heart disease in 0.6%. CHD being diagnosed by a doctor was reported by 9.8%. The presence of a doctor's report of other heart disease was made in 2.5%. The control recalled being told by a doctor that his blood pressure was high in almost a quarter of cases (22.6%) and recalled being told that cholesterol level was high in 6.4%. Just over 3% of the controls reported that they were diabetic. A stroke had been diagnosed at some point in the past in 2% and a similar proportion had been told that they had "hardening of the arteries in the legs". Just 5% had suffered from some form of liver disease; usually infective jaundice. Gall bladder disease had occurred in 4.5%, pancreatitis only in 1.1%. Peptic ulceration had been diagnosed as being present at some time in the past by 10.9% and over 80% had been admitted to hospital at some time in their lives.

Table 41. Self reported Medical history of 357 controls

Medical history	n = 357 (%)
Rheumatic fever	10 (2.8)
Valvular disease of the heart	2 (0.6)
Coronary heart disease	35 (9.8)
Other heart disease	9 (2.5)
High blood pressure	81 (22.6)
High cholesterol level	23 (6.4)
Diabetes	12 (3.4)
Stroke	7 (2.0)
"Hardening of the arteries in the legs"	7 (2.0)
Liver disease	18 (5.0)
Gall bladder disease	16 (4.5)
Pancreatitis	4 (1.1)
Peptic ulceration	39 (10.9)
Any admission to hospital	297 (83.0)

5. Medication reported by controls

Over a third (38.3%) of controls reported that they were taking prescribed medication on a regular basis. Another 8.1% reported taking over the counter preparations and 21.8% reported taking vitamin preparations regularly. Of the 22.6% of controls who recalled ever having been told that they had high blood pressure approximately half had ever taken any medication for this condition. A diagnosis of a high cholesterol had been made in 6.5% of the controls; just under a quarter of these had ever been prescribed medication for this condition.

Table 42. Self reported Medication taken by the 357 controls

Class of information	n = 357 (%)
On any prescribed medication	136 (38.3)
On any over the counter medication	29 (8.1)
Regular vitamin preparation	78 (21.8)
Ever received medication for high blood pressure	43 (12.0)
Ever received medication for high cholesterol	6 (1.7)
On oral hypoglycaemic agents	8 (2.2)
On insulin	2 (0.6)

6. Chest pain reported by controls

Almost a third (32.7%) of controls reported that they had ever had chest pain. This pain was classed as angina of effort in 7.5%. Of these controls with angina of effort, just under half had angina on only moderate exertion (grade 1 Angina). Over a quarter (25.1%) of controls had chest pain that was not classed as angina of effort. In the cases which were not defined as angina by response to the Rose questionnaire, central chest pain or pain of a typical radiation of cardiac ischaemia was described in almost 90%.

Table 43. Chest pain reported by controls

Chest pain classification	n = 357 (%)
Angina of effort grade 1 (on severe exertion)	15 (4.2)
Angina of effort grade 2 (on moderate exertion)	12 (3.4)
Chest pain but not angina	90 (25.1)
Central chest pain or of typical radiation but not angina	78 (21.7)
Other chest pain	12 (3.4)

7. Smoking habit of controls

Just over three quarters (75.1%) of controls reported that they had ever been regular smokers. A quarter of controls (25.1%) were cigarette smokers at the time of interview; 5% were pipe smokers or cigar smokers, or both, and 41.3% were ex cigarette smokers. Almost a quarter (24.9%) were lifelong non smokers. A small percentage were ex pipe or cigar smokers.

Table 44. Self reported Smoking status of 357 controls

Smoking category	n = 357 (%)
Current cigarette smoker	90 (25.1)
Current pipe or cigar or both	18 (5.0)
Ex cigarette smoker	148 (41.3)
Ex pipe or cigar or both	13 (3.6)
Lifelong non smoker	89 (24.9)

8. Family history of controls

From the report of the controls there was a history of heart disease in a first degree relative under the age of 60 in 12.9%.

9. Alcohol consumption of the controls

Approximately 40% described themselves as being light drinkers, and just under a half were moderate drinkers. Controls who consumed alcohol heavily by our definition made up 13%. In 5 cases the data were missing. However, of the light and moderate consumers at the time of interview a half admitted to having consumed more on a regular basis at some time in the past. Problem drinkers by our definition comprised 7.3% of controls (data missing in 2).

Table 45. Self reported Alcohol consumption of controls

Class of consumption	n = 357 (%)
Light or none	140 (39.7)*
Moderate	167 (47.3)*
Heavy	46 (13.0)*
Problem drinker	26 (7.3)**

* Data missing for 5 controls

** Data missing for 2 controls

10. Medically prescribed diets

Only 7.3% were taking a medically prescribed diet at the time of interview.

Exclusion of controls stated by control spouse to have coronary heart disease and those without adipose tissue samples

Of the 357 controls who attended, 35 stated that they had been diagnosed as having CHD. 30 of the controls who had attended with their spouses were stated to have CHD by their wives. 229 controls remained who had no history of CHD, either from the wife or by their own report, and who had provided a sample of adipose tissue. Subsequent analyses are performed on the data from these 229 controls.

Age, anthropometry, blood pressure and lipid results for controls

The results tabulated for all 229 controls reported by their spouses to be free of CHD are given in the table below.

Table 46. Age anthropometry, blood pressure, and lipid results

(n = 229 unless stated otherwise)

Class of measurement	Mean	SD	Min	Max
Age at interview years	56.4	7.1	32	67
Body mass index Kg/m^2	26.8	3.7	18.7	39.4
Waist/hip ratio (n = 216)	0.926	0.063	0.773	1.205
Systolic blood pressure mmHg	137.6	19.8	99	203
Diastolic blood pressure mmHg	85.2	11.5	58	119
Serum total cholesterol mmol/l (n = 219)	5.93	1.20	1.60	9.13
Serum HDL mmol/l (n = 219)	1.14	0.30	0.16	2.04
Serum nonfasting triglycerides mmol/l(n = 217)	2.26	1.34	0.50	8.16

Correlations between continuous data for controls

A correlation matrix for the continuous variables obtained from the controls was constructed. This was performed for age, body mass index, waist hip ratio, systolic blood pressure, diastolic blood pressure, total serum cholesterol, HDL cholesterol and non fasting triglycerides.

There were significant positive correlations between age and systolic blood pressure and between age and total cholesterol.

Significant positive associations were observed between body mass index and waist hip ratio and between body mass index and both systolic and diastolic blood pressure. Non fasting triglycerides were also positively related to body mass index.

The waist hip ratio was positively related to both systolic and diastolic blood pressure and also to non fasting triglycerides.

Systolic and diastolic blood pressure were highly correlated. There was also a positive relation of total cholesterol to systolic blood pressure.

A positive relation was seen between total cholesterol and HDL cholesterol . Weaker positive relations were seen between age and HDL cholesterol, waist hip ratio and total cholesterol, systolic blood pressure and non fasting triglycerides, diastolic blood pressure and non fasting triglycerides and total cholesterol and non fasting triglycerides.

A negative association was seen between HDL cholesterol and non fasting triglycerides. A weaker negative association was observed between body mass index and HDL cholesterol.

Table 47. Correlation matrix for Control data

DATA	BMI	WHR	SBP	DBP	TCHOL	HDL	TG
Age	0.0185	0.0619	*0.3131**	0.1241	*0.2398**	0.1612	-0.0588
BMI		*0.5051**	0.3217*	*0.4549**	0.0866	-0.1851*	*0.2723**
WHR			0.2846*	*0.2630**	0.1803*	-0.0848	*0.3820**
SBP				*0.6249**	*0.2282**	0.0735	0.1796*
DBP					0.1382	-0.0211	0.1873*
TCHOL						*0.2814**	0.2005*
HDL							*-0.4280**

* p < 0.01

Italicised correlation coefficients indicate p < 0.001

Comparison of data from nearest surviving relative and control spouse

Comparison of data from case nearest surviving relative and control spouse

1. Nationality

The nationality of the cases as stated by the Nearest surviving relative and that of the controls as stated by the Control spouse was British in the majority. There was no significant difference between cases and controls with respect to the frequency of other nationalities.

Table 48. Comparison of Nationality

Nationality of Case or Control	Nearest surviving relative	Control spouse
British	40 (95.2)	288 (98.3)
Other	2 (4.8)	5 (1.7)

$$\chi^2 = 1.67622 \quad p = 0.1943$$

2. Social class

The distribution of cases and controls with respect to social class was significantly different. There were proportionately more controls than cases in social class I, II and III Non-manual and fewer controls than cases in social classes IV and V.

Table 49. Comparison of Social class data

Social Class of Case or Control	Nearest surviving relative (%)	Control spouse (%)
I	1 (2.4)	22 (7.5)
II	6 (14.3)	46 (15.7)
III Non manual	3 (7.1)	38 (13.0)
III Manual	15 (35.7)	141 (48.1)
IV	9 (21.4)	25 (8.5)
V	5 (11.9)	7 (2.4)
Armed forces	3 (7.1)	14 (4.8)
Total	42	293

$$\chi^2 = 19.39755 \quad p = 0.0035$$

3. Marital status

The proportion of cases that had been married once only was significantly lower than that of all controls. Divorced and single men were more common amongst the cases also as were other co-habitees.

Table 50. Comparison of marital status

Marital status	All Sudden Cardiac Death Cases (%)	All Controls (%)
Married once only	47 (56.0)	267 (73.6)
Married more than once	11 (13.1)	41 (11.3)
Divorced	10 (11.9)	16 (4.4)
Single	11 (13.1)	25 (6.9)
Other	5 (5.9)	14 (3.9)
Total	84	363

$$\chi^2 = 13.75947 \quad p = 0.00810$$

4. Medical history

There were no significant differences between cases and controls with respect to the frequencies of any of the common medical conditions asked about in the questionnaire. Specifically, a doctor's diagnosis of hypertension was slightly more common in the controls as in the cases as was a history of diabetes. The frequency of a diagnosis of high cholesterol was slightly higher in the cases than in the controls. A diagnosis of peripheral vascular disease seemed to be just as common in the controls as in the cases. liver disease was just as common in the cases as in the controls. Gall bladder disease was proportionately more common in the cases than in the controls as was peptic ulceration. However, none of these frequencies differed significantly from one another.

Excluding the 30 controls whose spouse declared that they had been told by a doctor that they had been diagnosed as having CHD did not alter the outcome of this analysis.

Table 51. Comparison of frequencies of medical diagnoses

Medical History	Nearest surviving relative (%)	Control spouse (%)
High blood pressure	10 (23.8)	71 (24.2)
High cholesterol level	4 (9.5)	22 (7.5)
Diabetes	1 (2.4)	11 (3.8)
"Hardening of the arteries in the legs"	1 (2.4)	6 (2.1)
Liver disease	1 (2.4)	7 (2.4)
Gall bladder disease	2 (4.8)	11 (3.8)
Peptic ulceration	5 (11.9)	30 (10.2)
Any admission to hospital	31 (73.8)	222 (75.8)

5. Medication history

The cases were reportedly taking prescribed medications more commonly than the controls but his was not so for other over the counter preparations or vitamin preparations. The frequency with which they had ever been prescribed antihypertensive medication was the same as that for the controls. The differences between the cases and controls for these frequencies was only statistically significant for the frequency with which regular vitamin preparations were consumed by the controls. This analysis was performed after excluding those 30 controls who were reported by their spouses to have had CHD. This did not alter the result of the analysis.

Table 52. Comparison of medication history

Class of information	NSR (%)	CS (%)
On any prescribed medication	22 (52.4)	116 (39.6)
On any other over the counter preparation	3 (7.1)	45 (15.4)
Taking vitamin preparations regularly	4 (9.5)	70 (23.9) *
Receiving medication for high blood pressure	6 (14.3)	39 (13.3)

* $\chi^2 = 4.40575$ p = 0.03582

6. Chest pain history

From the answers given to the Rose chest pain questionnaire by the nearest surviving relative and the control spouse there was one case classified as having angina, and 11 controls. This frequency was higher in the controls, but not significantly so. The frequency of any reported chest pain was higher in the cases but this was not significantly higher. Repeating this analysis after exclusion of the 30 controls who were reported as having CHD did not alter the outcome of this analysis.

Table 53. Comparison of frequency of reported chest pain

Chest pain classification	Nearest surviving relative (%)	Control spouse (%)
Angina grade 1	1 (2.4)	9 (3.1)
Angina grade 2	0	2 (0.7)
Other chest pain	17 (40.5)	90 (30.7)

7. Smoking history

The cases were reported to have used tobacco products much more frequently than the controls. Just over nine tenths of the cases were reported to have smoked regularly at some time in their lives by the nearest surviving relative. Just under three quarters of the controls had done so. The frequency if successful abandonment of smoking was more frequent in the controls than in the cases. Almost three times as many cases were current smokers at the time of death compared to the smoking habits of the controls at the time of interview. These differences in distribution of frequencies between the cases and controls is highly significant. Excluding the 30 controls who were reported to have CHD at the time of interview did not affect this analysis.

Table 54. Comparison of smoking habit

Smoking status	NSR (%)	CS (%)
Ever a regular smoker	38 (90.5)	219 (74.7)
Quit smoking	7 (16.7)	138 (47.1)
Never smoked	4 (9.5)	74 (25.3)
Current cigarette smoker	28 (66.7)	70 (23.9)
Only smoking pipe or cigar or both	3 (7.1)	11 (3.8)

$$\chi^2 = 35.97909 \quad p = 0.00000$$

8. Alcohol consumption

Reported alcohol consumption by the cases and controls was different in the classes defined for this study. The cases were reported to be light drinkers more frequently than the controls and the controls were more frequently moderate drinkers. Problem drinkers were more proportionately more common in the cases. These frequency differences were not, however, statistically significant. This remained so after exclusion of the 30 controls who had been reported to have been told they had CHD.

Table 55. Comparison of alcohol consumption

Class of consumption	NSR (%)	CS (%)
Light	18 (42.9)	113 (38.6)
Moderate	12 (28.6)	119 (40.6)
Heavy	10 (23.8)	61 (20.8)
Problem drinkers	4 (9.5)	17 (5.8)

9. Family history

A history of heart disease in a first degree relative under the age of 60, fatal or not, was three times as common in the cases as the controls. This difference in frequency is highly significant. This analysis was not altered in its significance by excluding the 30 controls who were reported to have had CHD diagnosed.

Table 56. Comparison of Family history

Class of information	NSR (%)	CS (%)
History of heart disease in a first degree relative	15 (35.7)	30 (10.2)

$$\chi^2 = 20.50182 \quad p = 0.00001$$

10. Medical advice on diet

Controls were more commonly reported to be on a medically prescribed diet. However, this difference was small and not significant. Exclusion of the 30 controls reported to have CHD did not affect this analysis.

Table 57. Comparison of reported dieting

Class of information	NSR (%)	CS (%)
On a medically prescribed diet	2 (4.8)	17 (5.8)

Adipose tissue triglyceride fatty acid data

The following tables give the descriptive data for individual fatty acid species obtained by gas liquid chromatography.

Fatty acid data from cases

Adipose tissue samples were obtained from 66 of the 84 identified cases. The tabulations of the descriptive statistics for each individual fatty acids are for all of these cases.

1. Saturated fatty acids

The largest percentage of saturated fat in the adipose tissue triglycerides is represented by Palmitic acid. This is followed by Stearic acid and then Myristic acid.

Table 58. Saturated fatty acids in case samples

Fatty acid	Mean	SD	Min	Max
Myristic C14:0	3.36	0.84	2.00	6.12
Palmitic C16:0	21.45	2.29	16.98	29.07
Stearic C18:0	4.65	1.33	2.39	7.68

2. Monounsaturated fatty acids

Oleic acid is the most abundant monounsaturated fatty acid in the adipose triglycerides and usually makes up approximately half of all adipose tissue triglyceride fatty acids. Palmitoleic acid is the next most common monounsaturated fatty acid followed by eicosaenoic acid.

Table 59. Monounsaturated fatty acids in case samples

Fatty acid	Mean	SD	Min	Max
Palmitoleic C16:1	6.79	1.93	3.20	12.12
Oleic C18:1	46.86	2.42	38.07	52.05
Eicosaenoic C20:1	2.30	0.08	1.52	4.77

3. Polyunsaturated fatty acids

Of the many polyunsaturated fatty acids (PUFA) in adipose tissue triglycerides, linoleic acid is the most abundant and is thereby the most abundant polyunsaturated fatty acid in this tissue compartment. All other PUFA represent under one percent of all fatty acids in triglycerides residing in adipose tissue.

Table 60. Polyunsaturated fatty acids from case samples

Fatty acid	Mean	SD	Min	Max
Linoleic C18:2ω6	11.16	3.32	4.40	23.90
α linolenic C18:3ω3	0.73	0.24	0.28	1.45
Eicosatrienoic C20:3ω9	0.001	0.007	0	0.053
Eicosatrienoic C20:3ω6	0.133	0.066	0	0.329
Arachidonic C20:4ω6	0.318	0.083	0.144	0.525
Eicosapentaenoic C20:5ω3	0.041	0.068	0	0.516
Docosatetraenoic C22:4ω6	0.089	0.043	0.007	0.207
Docosapentaenoic C22:5ω6	0.001	0.005	0	0.040
Docosapentaenoic C22:5ω3	0.187	0.076	0.055	0.458
Docosahexaenoic C22:6ω3	0.128	0.065	0.017	0.355

4. Totals of each class of fatty acid and polyunsaturated: saturated ratio

The total percent represented by each class of fatty acid in adipose tissue triglycerides are shown below. Monounsaturates are the most abundant followed by saturates and then polyunsaturates.

The polyunsaturated: saturated fat (P:S) ratio is a ratio of the two totals.

Table 61. Totals and ratios for case samples

Class of fatty acid data	Mean	SD	Min	Max
Total saturated fatty acids	28.47	3.61	25.20	42.06
Total monounsaturated fatty acids	55.96	3.91	43.70	65.56
Total polyunsaturated fatty acids	13.07	0.45	5.58	25.85
P:S ratio	0.455	0.149	0.190	0.983

Fatty acid data from controls

The following data is for the total of 229 adipose tissue samples that were obtained from the controls whose spouse attended and were not reported to have CHD by their spouse during the course of the study.

1. Saturated fatty acids

As in the cases palmitic acid is the most common saturated fat followed by stearic acid and myristic acid.

Table 62. Saturated fatty acids for control samples

Fatty acid	Mean	SD	Min	Max
Myristic C14:0	3.30	0.69	1.37	5.29
Palmitic C16:0	22.00	2.15	14.77	28.35
Stearic C18:0	4.94	1.05	1.74	9.58

2. Monounsaturated fatty acids

As for the cases, oleic acid represent a large proportion of all fatty acids in this tissue compartment. Palmitoleic and eicosaenoic acid are found in smaller proportions.

Table 63. Monounsaturated fatty acids for control samples

Fatty acid	Mean	SD	Min	Max
Palmitoleic C16:1	5.82	1.45	3.04	13.43
Oleic C18:1	45.74	2.35	37.02	52.19
Eicosaenoic C20:1	2.02	0.43	0.94	4.14

3. Polyunsaturated fatty acids

The most common polyunsaturated fat in adipose tissue triglycerides in the controls is linoleic acid as in the cases. All other PUFA represent under one percent of all fatty acids in adipose tissue triglycerides.

Table 64. Polyunsaturated fatty acids from controls

Fatty acid	Mean	SD	Min	Max
Linoleic C18:2ω6	12.96	4.05	6.40	31.01
α linolenic C18:3ω3	0.77	0.23	0.33	1.83
Eicosatrienoic C20:3ω9	0.00	0.01	0	0.11
Eicosatrienoic C20:3ω6	0.13	0.07	0	0.35
Arachidonic C20:4ω6	0.29	0.09	0.10	0.59
Eicosapentaenoic C20:5ω3	0.05	0.05	0	0.27
Docosatetraenoic C22:4ω6	0.08	0.04	0	0.20
Docosapentaenoic C22:5ω6	0	0.00	0	0.01
Docosapentaenoic C22:5ω3	0.18	0.06	0.05	0.38
Docosahexaenoic C22:6ω3	0.13	0.07	0.02	0.47

4. Totals of each class of fatty acid and polyunsaturated: saturated ratio

The totals for each class of fatty acid in adipose tissue triglycerides are represented here. The polyunsaturated: saturated ratio (P:S ratio) is calculated from the ratio of the totals for each class.

Table 65. Totals and ratio for controls

Class of data	Mean	SD	Min	Max
Total saturated fatty acids	30.24	2.97	19.51	39.51
Total monounsaturated fatty acids	53.58	3.39	43.25	63.17
Total polyunsaturated fatty acids	14.83	4.21	7.82	33.26
P:S ratio	0.50	0.19	0.20	1.54

Analysis of adipose tissue triglyceride fatty acids with respect to case information

1. Nationality

There were 36 cases with adipose tissue data for whom nearest surviving relative information on nationality was known. Of these 36, 35 were known to be of British nationality and Caucasian. The other was of Asian origin.

2. Social class

There were no significant differences in the distribution of any adipose tissue triglyceride fatty acids between the social classes of the cases. This was performed for the social class defined from the response of the nearest surviving relative and the occupation obtained from the coroner's information.

3. Marital status

The marital status of the cases was not significantly related to the distribution of any of the fatty acids including linoleic acid. There was no difference between those known to be married once only, those married more than once, those known to be single, divorced and other co-habitees.

4. Medical history

All adipose tissue triglyceride fatty acids were analysed with respect to positive nearest surviving relative responses to the presence of a doctor's diagnosis of high blood pressure, high cholesterol, diabetes, "hardening of the arteries in the legs", liver disease, gall bladder disease and peptic ulceration. Only the following difference was found between the positive and negative responders for all the fatty acid percent compositions.

Table 66. Differences for cases with medical conditions

Peptic ulceration	Yes	No	p (ANOVA)
C22:5 ω6	0.008 (0.008)	0.0	0.0106

ω6 docosapentaenoic acid was significantly higher in those who had been told that they had peptic ulceration at any time prior to death compared to those who had not.

5. Medication history

On analysis by nearest surviving relatives response to the taking of prescribed, over the counter and vitamin preparations there were no significant differences between those cases taking medications of any kind and those not with respect to any fatty acid percentage.

6. Smoking history

The various classifications of smoking history were condensed into smokers of any tobacco product at the time of death and those who were non smokers at the time of death. The majority were cigarette smokers. The smoking data and fatty acid data was available for 36 cases.

Table 67. Differences for cases with respect to current cigarette smoking

Fatty acid	Yes	No	p (ANOVA)
C16:1	7.26 (0.33)	5.38 (0.48)	0.0052
C18:0	4.33 (0.22)	5.33 (0.44)	0.0348
C18:1	47.62 (0.39)	45.17 (0.76)	0.0045
C18:2 ω6	10.76 (0.47)	14.30 (1.78)	0.0100
C20:5 ω3	0.039 (0.007)	0.013 (0.004)	0.0487
Total monounsaturates	57.24 (0.57)	52.70 (1.22)	0.0007
Total polyunsaturates	12.59 (0.52)	16.17 (1.83)	0.0138

There were several fatty acid species that were found to have be significantly different between the smokers and non-smokers. palmitoleic acid, oleic acid and total monounsaturates were significantly higher in the smokers compared to the non-smokers. Stearic acid, linoleic acid, ω3 eicosapentaenoic acid and total polyunsaturates were higher in the non-smokers compared to the smokers.

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7. Chest pain history

Analysis of all fatty acid percent composition by the response of the nearest surviving relative to the question "did the deceased ever complain of chest pain" was performed. The results for all chest pains so defined are presented below for those differences that were significant.

Table 68. Differences in fatty acids for cases with chest pain

Fatty acid	Yes	No	p (ANOVA)
C14:0	2.86 (0.15)	3.65 (0.20)	0.0054
Total monounsaturates	57.58 (1.02)	55.06 (0.69)	0.0413

Myristic acid was significantly lower in those cases who had complained at some point prior to the final illness of chest pain and total monounsaturates were higher.

There was only one case definitely classified as having angina by questionnaire.

8. Alcohol consumption

The nearest surviving relative was able to give an alcohol consumption history in 40 of the 42 interviews. There was fatty acid data on 34 of these cases and the results classified by alcohol consumption group are given below for those analyses which were significantly different between groups.

Table 69. Differences in case fatty acids with respect to alcohol consumption

Fatty acid	Low (15)	Medium (10)	High (9)	p (ANOVA)
C14:0	3.76 (0.27)	2.96 (0.11)	3.05 (0.26)	0.0419
C16:1	6.24 (0.21)	5.68 (0.46)	8.32 (0.67)	0.0006
C18:0	5.18 (0.25)	4.76 (0.38)	3.82 (0.35)	0.0175
C18:2	11.19 (0.76)	14.41 (1.38)	10.05 (0.89)	0.0194
C20:1	2.35 (0.10)	1.99 (0.09)	2.54 (0.25)	0.0481
C22:4ω6	0.06 (0.01)	0.10 (0.01)	0.09 (0.02)	0.0358
Total monounsaturates	55.39 (0.56)	53.76 (1.31)	59.02 (1.07)	0.0029
Total polyunsaturates	12.88 (0.83)	16.40 (1.39)	11.97 (0.99)	0.0213

A "U-shaped" pattern of fatty acid percentages is seen in this data for myristic acid, palmitoleic acid, stearic acid and total monounsaturates that is significant by analysis of variance. There is an "inverted U-shaped" pattern for linoleic acid, ω6 docosahexaenoic acid and total polyunsaturates.

9. Family history

There were 36 cases on whom there was fatty acid data and information about the presence or absence of heart disease in a first degree relative. There were no differences between the cases who had a positive family history and those who did not with respect to any of the fatty acid percentages.

10. Medical advice on diet

Of the 36 cases with fatty acid data and information from the nearest surviving relative only one had been placed on a diet by a doctor.

11. Information regarding final illness

There were no significant differences in the percent composition of any adipose tissue fatty acids with respect to time to death.

Analysis of adipose tissue triglyceride fatty acids with respect to control information

This information is based on the adipose tissue data obtained from the 229 controls whose spouses also attended and for whom there was no reported history of CHD.

1. Nationality

Of the 229 controls there were 225 British nationals and 4 other controls who were of other nationalities.

The following differences between the British controls and those of the other nationalities were found to be significant.

Table 70. Differences for fatty acids between nationalities for controls

Fatty Acid	British	Other nationalities	p ANOVA
C14:0	3.31 (0.05)	2.53 (0.5)	0.0243
C20:4ω6	0.29 (0.01)	0.38 (0.08)	0.0425

2. Social class

There was only a significant difference in the means of each social class for oleic acid only.

Table 71. Oleic acid means by social class for controls

Social class	Mean C18:1 (SEM)
I	44.87 (0.70)
II	45.22 (0.33)
III Non manual	45.30 (0.39)
III Manual	46.12 (0.22)
IV	46.38 (0.60)
V	47.17 (0.90)
Armed forces	44.26 (0.56)

p = 0.016 by Analysis of Variance for differences between means.

3. Marital status

Analysed by cohabitation with a female partner, married or otherwise, or living alone, single or divorced. Significant differences between palmitic, α linolenic and arachidonic acid were found.

Table 72. Marital status and fatty acids for controls

Fatty acid	Yes	No	p (ANOVA)
C16:0	22.14 (0.15)	21.30 (0.36)	0.0279
C18:3 ω3	0.76 (0.02)	0.83 (0.04)	0.0491
C20:4ω6	0.29 (0.01)	0.32 (0.02)	0.0475

4. Medical history

Fatty acid percentages were analysed by the control spouses response to the presence or absence of a doctor's diagnosis of high blood pressure, high cholesterol, diabetes mellitus, "hardening of the arteries of the legs", liver disease, gall bladder disease and peptic ulceration.

(a). High blood pressure

Univariate analysis of the fatty acid percentages for those controls with and without a diagnosis of hypertension revealed that α linolenic acid was lower in those with hypertension.ω6 eicosatrienoic acid and ω6 docosatetraenoic acid were higher.

Table 73. Fatty acids and diagnosed high blood pressure in controls

Fatty acid	Yes	No	p (ANOVA)
C18:3 ω3	0.71 (0.03)	0.79 (0.02)	0.0198
C20:3 ω6	0.15 (0.01)	0.12 (0.01)	0.0137
C20:4ω6	0.31 (0.01)	0.29 (0.01)	0.0430
C22:4 ω6	0.09 (0.01)	0.08 (0.003)	0.0023

(b). High cholesterol

The report of a diagnosis of high cholesterol was associated with the following significant differences in fatty acid percentages. Palmitoleic acid ,oleic acid were lower in those who were reported to have been diagnosed as having high cholesterol as were total monounsaturates. Linoleic and ω6 eicosatrienoic acid were higher in those with a report of high cholesterol. Total polyunsaturates and the P:S ratio were also higher in those reported to have been told they had high cholesterol.

Table 74. Fatty acids and high cholesterol in controls

Fatty acid	Yes	No	p (ANOVA)
C16:1	4.87 (0.35)	5.90 (0.10)	0.0067
C18:1	43.69 (0.72)	45.90 (0.15)	0.0003
C18:2 ω6	16.30 (1.52)	12.71 (0.23)	0.0006
C20:3 ω6	0.16 (0.22)	0.12 (0.01)	0.0355
Total monounsaturates	50.38 (0.99)	53.82 (0.22)	0.0001
Total polyunsaturates	18.27 (1.56)	14.57 (0.27)	0.0006
P:S Ratio	0.64 (0.08)	0.49 (0.01)	0.0035

(c). Diabetes mellitus

Univariate analysis of all fatty acids in respect to the reported diagnosis of diabetes mellitus in the controls revealed that α linolenic acid was significantly lower in those with a diagnosis of diabetes than those without. ω6 Docosatetraenoic acid was significantly higher in diabetics.

Table 75. Fatty acids and diabetes mellitus in controls

Fatty acid	Yes	No	p (ANOVA)
C18:3 ω3	0.59 (0.04)	0.77 (0.02)	0.0496
C22:4 ω6	0.11 (0.02)	0.08 (0.00)	0.0479

(d). Hardening of the arteries of the legs

Univariate analysis of all fatty acids with respect to the spouses report of a doctor's diagnosis of "hardening of the arteries of the legs" showed that only oleic acid was significantly different between the two groups.

Table 76. Fatty acids and reported "hardening of the arteries of the legs"

Fatty acid	Yes	No	p (ANOVA)
C18:1	42.45 (2.28)	45.78 (0.15)	0.0147

(e). Liver disease

Univariate analysis of individual fatty acids with the reported presence of a diagnosis of liver disease revealed that ω3 docosapentaenoic acid and ω3 docosahexaenoic acid were higher in those with such a diagnosis.

Table 77. Fatty acids and liver disease in controls

Fatty acid	Yes	No	p (ANOVA)
C22:5 ω3	0.25 (0.02)	0.18 (0.00)	0.0085
C22:6 ω3	0.25 (0.06)	0.13 (0.01)	0.0067

(f). Gall bladder disease

Univariate analysis of all fatty acids with the presence of a reported diagnosis of gall bladder disease revealed that stearic acid was significantly higher in those reported to have had gall bladder disease as was ω3 docosahexaenoic acid.

Table 78. Fatty acids and gallbladder disease in controls

Fatty acid	Yes	No	p (ANOVA)
C18:0	5.70 (0.55)	4.91 (0.07)	0.0276
C22:6 ω3	0.19 (0.03)	0.13 (0.01)	0.0243

(g). Peptic ulceration

There were no significant differences for any fatty acid between those stated to have peptic ulceration and those not.

5. Medication history

This analysis was performed from the report of the control spouse and analysed separately for the taking of any prescribed medication, any over the counter medication and any vitamin preparation.

(a). Any prescribed medication

Univariate analysis of all fatty acids against the presence or absence of a report of prescribed medication being taken by the control at the time of interview revealed that only ω3 eicosapentaenoic acid was significantly different between the two groups.

Table 79. Fatty acids with respect to prescribed medication in controls

Fatty acid	Yes	No	p (ANOVA)
C20:5ω3	0.06 (0.01)	0.05 (0.00)	0.0359

(b). Any over the counter preparation

The univariate analysis of all fatty acids with respect to the reported taking of any over the counter medication revealed that ω6 eicosatrienoic acid and stearic acid were significantly different in the group reportedly taking over the counter medications.

Table 80. Fatty acids and over the counter medication

Fatty acid	Yes	No	p (ANOVA)
C18:0	4.62 (0.16)	5.00 (0.08)	0.0497
C20:3ω6	0.15 (0.01)	0.12 (0.01)	0.0266

(c). Any vitamin preparation

Analysis of each fatty acid against the reported taking of any vitamin preparation by the control resulted in the following differences between those taking vitamins and those not. Oleic acid and total monounsaturates were significantly lower in those reported to be taking vitamins. Linoleic acid, α linolenic acid, ω3 docosahexaenoic acid and total polyunsaturates were higher in those taking vitamins than those not. The P:S ratio was also higher in those taking vitamins compared to those not.

Table 81. Fatty acids and vitamin taking in controls

Fatty acid	Yes	No	p (ANOVA)
C18:1	44.87 (0.29)	46.02 (0.18)	0.0014
C18:2 ω6	14.22 (0.60)	12.56 (0.29)	0.0080
C18:3 ω3	0.82 (0.04)	0.75 (0.02)	0.0476
C22:6 ω3	0.16 (0.01)	0.12 (0.01)	0.0005
Total monounsaturates	52.41 (0.42)	53.95 (0.26)	0.0033
Total polyunsaturates	16.18 (0.63)	14.41 (0.30)	0.0062
P:S Ratio	0.56 (0.03)	0.49 (0.01)	0.0170

6. Smoking history

On analysis of all fatty acids with regard to the reported smoking habits of the controls only those known to be cigarette smokers at the time of interview were considered to be smokers. Each fatty acid was analysed with respect to smokers and non smokers. Palmitoleic acid, oleic acid and total monounsaturates were higher in the smokers compared to non smokers. Linoleic acid, docosadienoic acid, ω6 eicosatrienoic acid, ω3 docosapentaenoic acid, ω3 docosahexaenoic acid and total polyunsaturates were higher in the non smokers compared to the smokers. The polyunsaturated: saturated ratio was higher in the non smokers.

Table 82. Fatty acids and current smoking in controls

Fatty acid	Yes	No	p (ANOVA)
C16:1	6.28 (0.19)	5.67 (0.11)	0.0057
C18:1	46.46 (0.31)	45.51 (0.17)	0.0080
C18:2 ω6	11.72 (0.59)	13.36 (0.29)	0.0081
C20:2	0.21 (0.01)	0.25 (0.01)	0.0230
C20:3 ω6	0.10 (0.01)	0.14 (0.01)	0.0011
C22:5 ω3	0.16 (0.01)	0.19 (0.01)	0.0141
C22:6 ω3	0.11 (0.01)	0.14 (0.01)	0.0033
Total monounsaturates	54.81 (0.47)	53.18 (0.25)	0.0017
Total polyunsaturates	13.42 (0.61)	15.29 (0.30)	0.0037
P:S Ratio	0.46 (0.03)	0.52 (0.01)	0.0290

7. Chest pain history

(a). For any chest pain

There were no significant differences for any fatty acid between those controls whose spouse had recalled a complaint of any chest pain.

(b). For angina

Those controls whose spouse had responded to the chest pain questionnaire in such a manner to define the control as having angina pectoris were identified. Each fatty acid was compared between the groups of controls who had angina pectoris and those who did not. The following differences were found.

Palmitoleic acid and total monounsaturates were higher in those who had angina pectoris compared to those did not; ω6 eicosatrienoic acid was lower.

Table 83. Fatty acids and angina in controls

Fatty acid	Yes	No	p (ANOVA)
C16:1	7.19 (0.26)	5.78 (0.10)	0.0188
C20:3 ω6	0.07 (0.01)	0.13 (0.01)	0.0255
Total monounsaturates	56.62 (0.99)	53.5 (0.23)	0.0256

8. Alcohol consumption

The distribution for palmitoleic, stearic, arachidonic, ω6 docosatetraenoic, ω3 docosapentaenoic, ω3 docosahexaenoic acids and total monounsaturates were found to be significantly different between the alcohol consumption groups. Palmitoleic acid and total monounsaturates being lowest in the moderate consumption group and stearic acid being highest in this group. The other fatty acids seeming to have a gradual increase in percentage with increasing alcohol consumption.

Table 84. Fatty acids and alcohol consumption in controls

Fatty acid	Low	Moderate	High	p
C16:1	5.70 (0.14)	5.55 (0.13)	6.57 (0.26)	0.0002
C18:0	4.88 (0.10)	5.13 (0.11)	4.68 (0.15)	0.0403
C20:4ω6	0.27 (0.01)	0.30 (0.01)	0.31 (0.01)	0.0414
C22:4ω6	0.07 (0.004)	0.08 (0.003)	0.09 (0.007)	0.0038
C22:5ω3	0.16 (0.01)	0.19 (0.01)	0.19 (0.01)	0.0017
C22:6ω3	0.11 (0.01)	0.14 (0.01)	0.16 (0.01)	0.0002

All by ANOVA

9. Family history

There were significant differences between those controls reported to have a positive family history of CHD for ω3 eicosapentaenoic, arachidonic and ω6 docosatetraenoic acids.

Table 85. Fatty acids and Family history of controls

Fatty acid	Yes	No	p (ANOVA)
C20:3ω6	0.17 (0.02)	0.12 (0.01)	0.0044
C20:4ω6	0.33 (0.02)	0.29 (0.01)	0.0287
C22:4 ω6	0.10 (0.01)	0.08 (0.00)	0.0177

10. Medical advice on diet

Those men reported to be on a medically prescribed diet had significant differences from those men not on a diet for eicosaenoic and arachidonic acids.

Table 86. Fatty acids and reported diet in controls

Fatty acid	Yes	No	p (ANOVA)
C20:1	1.76 (0.14)	2.03 (0.03)	0.0316
C20:4 ω6	0.35 (0.03)	0.29 (0.01)	0.0181

Correlations between adipose tissue triglyceride fatty acids and other continuous variables for controls

The correlation matrix for the continuous variables available in the data from the controls resulted in significant associations for the following variables.

Systolic blood pressure was associated positively with ω6 docosatetraenoic acid,. A weaker positive association was observed for palmitoleic acid, ω6 docosatrienoic acid and arachidonic acid. A negative association was seen between systolic blood pressure and α linolenic acid.

Diastolic blood pressure, palmitic acid and ω6 docosatrienoic acid were found to be positively associated. A weaker positive association was found for arachidonic acid and ω6 docosatetraenoic acid and diastolic blood pressure. A weak negative relation between α linolenic acid and diastolic blood pressure was also noted.

Body mass index was positively related to palmitic acid, palmitoleic acid, oleic acid, arachidonic acid, ω6 docosatrienoic acid, ω6 docosatetraenoic acid, ω3 docosapentaenoic acid and total monounsaturates. Negative associations with body mass index were found for stearic acid and eicosaenoic acid. A weaker negative association was seen for α linolenic acid.

There were positive associations found between waist hip ratio and palmitic acid, palmitoleic acid, oleic acid, arachidonic acid, ω6 docosatrienoic acid, ω6 docosatetraenoic acid, ω3 docosapentaenoic acid and total monounsaturates. Negative associations were observed between waist hip ratio and stearic acid, linoleic acid, α linolenic acid, total polyunsaturates and the P:S ratio.

A weak positive association was found between total serum cholesterol and arachidonic acid, ω6 docosatetraenoic acid, ω3 docosapentaenoic acid and ω3 docosahexaenoic acid.

A positive association was found between serum HDL cholesterol and ω3 docosahexaenoic acid. A weak positive association was found for α linolenic acid and eicosaenoic acid and HDL cholesterol. A weak negative association between HDL cholesterol ,ω6 docosatrienoic acid and palmitic acid was also seen.

Non fasting triglyceride concentrations were positively correlated with palmitic acid, arachidonic acid, ω6 docosatrienoic acid and ω6 docosatetraenoic acid. A weaker positive association was observed for palmitoleic acid. Triglycerides were negatively associated with stearic acid, and weakly so with α linolenic acid.

The age of controls was positively associated with eicosaenoic acid, ω3 docosapentaenoic acid and ω3 docosahexaenoic acid.

Table 87. Pearson correlation coefficients for continuous control data

Fatty acid	Systolic BP	Diastolic BP	BMI	WHR	Total Cholesterol	HDL	TG	AGE
C14:0	-0.0700	0.0280	*-0.2172**	-0.1566	0.0051	0.0016	-0.1109	0.1452
C16:0	0.1275	*0.2115**	*0.2004**	*0.2300**	0.0961	-0.1829*	*0.2995**	0.0289
C18:0	-0.1510	-0.1430	*-0.4095**	*-0.3229**	-0.0913	0.1499	*-0.2860**	0.0183
C16:1	0.1909*	0.1526	*0.2695**	*0.2940**	0.1246	0.0120	0.1630*	0.0861
C18:1	0.0230	0.0275	*0.2233**	*0.2621**	0.0086	-0.0431	0.0104	-0.0754
C20:1	-0.0119	-0.0581	*-0.2098**	-0.1189	-0.0285	0.1687*	-0.1738*	*0.2432**
C18:2 ω6	-0.0939	-0.1323	-0.1504	*-0.2436**	-0.0914	0.0453	-0.1039	-0.0723
C18:3 ω3	*-0.2287**	-0.1991*	-0.2018*	*-0.2178**	0.0375	0.1997*	-0.2034*	-0.1304
C20:3 ω6	0.1866*	*0.2254**	*0.2895**	*0.2713**	0.1275	-0.1641*	*0.3896**	-0.0606
C20:3 ω9	-0.0550	-0.0712	-0.0813	-0.0333	-0.0588	0.0932	-0.0816	-0.0611
C20:4 ω6	0.1961*	0.1814*	*0.2984**	*0.2540**	0.1966*	0.0130	*0.2404**	0.0523
C20:5 ω3	-0.0413	0.0352	0.0255	0.1233	-0.0141	0.0032	0.0775	0.0144
C22:4 ω6	*0.2391**	0.1982*	*0.3835**	*0.3457**	0.1583*	-0.0921	*0.3791**	0.1262
C22:5 ω6	-0.0646	-0.0725	0.0502	-0.0090	0.1515	0.0603	-0.0482	-0.0242

Table 88. Pearson correlation coefficients for continuous control data (continued)

Fatty acid	Systolic BP	Diastolic BP	BMI	WHR	Total Cholesterol	HDL	TG	AGE
C22:5 ω3	0.1359	0.0869	*0.2206**	*0.2636**	0.1993*	0.1041	0.0992	*0.2240**
C22:6 ω3	0.0524	-0.0803	0.0130	0.1060	-0.1719*	*0.2275**	-0.0388	*0.2314**
TSAT	0.0230	0.0959	-0.0486	0.0169	0.0382	-0.0784	0.0893	0.0604
TMON	0.0957	0.0766	*0.2426**	*0.2912**	0.0560	0.0138	-0.0556	0.0152
TPOL	-0.0893	-0.1265	-0.1400	*-0.2285**	-0.0723	0.0590	-0.0954	-0.0644
P:S Ratio	-0.0737	-0.1243	-0.1145	*-0.2061**	-0.0675	0.0724	-0.1059	-0.0428

* $p < 0.05$, italicised items indicate $p < 0.01$

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Comparison of frequency of risk factors in cases and controls with and without adipose tissue samples

For treated hypertension

1. SCD cases

Including only those for whom there was a respondent, there was a history of treated hypertension more commonly found in the 6 cases that had not had a sample of adipose tissue taken at post mortem.

Table 89. Treated hypertension in sampled cases

Treated Hypertension	Sample	No sample
Yes	33	3
No	3	3

$\chi^2 = 7.29167$ p = 0.00693

With continuity correction $\chi^2 = 4.28588$ p = 0.03843

2. Controls

There was no difference in the frequency of reported treated hypertension in those controls who consented to have a sample of adipose tissue taken and those who did not.

Table 90. Treated hypertension in controls and sampling

Treated hypertension	Sample	No sample
Yes	200	8
No	31	54

$\chi^2 = 0.01131$ p = 0.91531

For diabetes

1. SCD cases

There was only one case of diabetes in the non sampled cases and none in the sampled cases.

2. Controls

There was no significant difference in the sampling frequency between the diabetics and non diabetics.

Table 91. Diabetes in sampled controls

Diabetes	Sample	No sample
Yes	6	5
No	225	57

With continuity correction $\chi^2 = 2.67189$ p = 0.10213

For a family history of heart disease in a first degree relative under the age of 60 years

1. SCD cases

There was no significant difference between the cases sampled and those not sampled with respect to the frequency of a positive family history.

Table 92. Family history and sampling of adipose tissue

Family history	Sample	No sample
Yes	13	2
No	23	4

$\chi^2 = 0.01728$ p = 0.89540

2. Controls

There was no difference between those sampled and those not with respect to the frequency of a family history of heart disease.

Table 93. Family history and sampling of controls

Family history	Sample	No sample
Yes	22	8
No	209	54

$$\chi^2 = 0.60740 \quad p = 0.43577$$

For cigarette smoking

1. SCD cases

There was no difference between those cases sampled and those not with respect to cigarette smoking.

Table 94. Smoking and cases sampled

Smoker	Sample	No sample
Yes	26	3
No	10	3

$$\chi^2 = 1.18833 \quad p = 0.27567$$

2. Controls

There was no difference between the controls who consented to have a sample of adipose tissue taken and those who did not with respect to the frequency of cigarette smoking.

Table 95. Smoking and control sampling

Smoking	Sample	No sample
Yes	57	13
No	174	49

$$\chi^2 = 0.36953 \quad p = 0.53426$$

Comparison of adipose tissue triglyceride fatty acid composition between cases and controls

Univariate analysis

All these analyses were performed on all adipose tissue data available from the 66 samples from the SCD cases and 229 samples from the controls.

1. Saturated fatty acids

Palmitic acid of the saturated fats was significantly lower in the cases compared to the controls by non parametric analysis. Stearic acid was significantly higher in the controls by non parametric analysis. There was no significant difference between cases and controls for myristic acid.

Table 96. Comparison of case and control saturated fatty acids

Fatty acid	Cases	Controls	p (t test)	p (Mann Whitney)
C14:0	3.36 (0.10)	3.30 (0.05)	0.544	0.8225
C16:0	21.45 (0.28)	22.00 (0.14)	0.074	0.0248
C18:0	4.65 (0.16)	4.94 (0.07)	0.064	0.0424

2. Monounsaturated fatty acids

Palmitoleic, oleic and eicosaenoic acid were significantly higher in the cases compared to the controls.

Table 97. Comparison of case and control monounsaturated fatty acids

Fatty acid	Cases	Controls	p (t test)	p (Mann Whitney)
C16:1	6.79 (0.24)	5.82 (0.10)	0.000	0.0002
C18:1	46.86 (0.30)	45.74 (0.16)	0.001	0.0003
C20:1	2.30 (0.08)	2.02 (0.03)	0.000	0.0011

3. Polyunsaturated fatty acids

Linoleic acid was significantly lower in the cases compared to the controls. ω6 Eicosatetraenoic acid, arachidonic acid and ω6 docosapentaenoic acid were significantly higher in the cases compared to controls. ω3 Eicosapentaenoic acid was significantly higher in the controls by non parametric analysis only.

Table 98. Comparison of case and control polyunsaturated fatty acids

Fatty acid	Cases	Controls	p (t test)	p (Mann Whitney)
C18:2	11.16 (0.41)	12.96 (0.27)	0.001	0.0009
C18:3 ω3	0.73 (0.03)	0.77 (0.02)	0.241	0.3162
C20:3 ω9	0.001 (0.00)	0.001 (0.00)	0.651	0.3410
C20:3 ω6	0.13 (0.01)	0.13 (0.07)	0.451	0.3625
C20:4 ω6	0.32 (0.01)	0.29 (0.09)	0.035	0.0445
C20:5 ω3	0.04 (0.01)	0.05 (0.00)	0.161	0.0390
C22:4 ω6	0.09 (0.01)	0.08 (0.00)	0.083	0.2509
C22:5 ω6	0.001 (0.0)	0.0	0.011	0.0019
C22:5 ω3	0.19 (0.01)	0.18 (0.0)	0.410	0.8815
C22:6 ω3	0.13 (0.01)	0.13 (0.01)	0.622	0.6667

4. Totals of each class of fatty acid and polyunsaturated: saturated ratio

Total saturates were significantly higher in the controls compared to the cases by non parametric testing. Total monounsaturates were higher in the cases compared to controls. Total polyunsaturates were significantly lower in the cases compared to the controls, but there was no significant difference between the cases and controls with respect to the P:S ratio.

Table 99. Comparison of fatty acid totals for case and controls

Class of fatty acid	Cases	Controls	p (t test)	p (Mann Whitney)
Total saturates	29.47 (0.45)	30.24 (0.20)	0.077	0.0034
Total monounsaturates	55.96 (0.48)	53.58 (0.22)	0.000	0.0000
Total polyunsaturates	13.07 (0.43)	14.83 (0.28)	0.002	0.0028
P:S Ratio	0.46 (0.02)	0.50 (0.01)	0.053	0.1039

Estimate of relative risk of sudden cardiac death due to coronary heart disease with respect to adipose tissue linoleic acid

Univariate estimate

An estimate of the univariate relative risk of having a low adipose tissue linoleic acid with respect to SCD case status was calculated. This was performed by using the distribution of adipose tissue linoleic acid percent in the controls. First, the adipose tissue linoleic acid percent from all 229 samples from the controls was ranked into quintiles. The percent linoleic acid for each case was then assigned to the corresponding control quintile according to its value. The distribution was then analysed by using χ^2 and by calculating the odds ratio for being a case of SCD in each quintile with respect to the quintile with the highest percent linoleic acid.

Table 100. Estimate of relative risk of sudden cardiac death with respect to linoleic acid

Quintile	C18:2 %	SCD cases	Controls	Odds ratio (95% confidence interval)
I	<9.522	23	45	5.75 (1.84 to 17.97)
II	9.522 < 11.496	16	47	3.83 (1.19 to 12.33)
III	11.496 < 13.057	11	45	2.75 (0.82 to 9.28)
IV	13.057 <15.706	12	47	2.87 (0.86 to 9.56)
V	≥ 15.706	4	45	1.00

For the distribution $\chi^2 = 11.54302$ p = 0.02109

There is an inverse relation between the estimated relative risk (odds ratio) of being a case of SCD due to CHD and the percent composition of adipose tissue fatty acids taken up by linoleic acid.

Using only the data from the 42 SCD cases who had data from a NSR resulted in the following analysis.

Table 101. Estimate of relative risk of sudden cardiac death with respect to linoleic acid

Quintile	C18:2 %	SCD cases	Controls	Odds ratio (95% confidence interval)
I	<9.522	12	45	3.00 (0.90 to 10.01)
II	9.522 < 11.496	8	47	1.92 (0.54 to 6.82)
III	11.496 < 13.057	6	45	1.50 (0.40 to 5.68)
IV	13.057 <15.706	6	47	1.44 (0.38 to 5.68)
V	≥15.706	4	45	1.00

For the distribution $\chi^2 = 4.35324$ p = 0.36031

A trend similar to the original analysis is seen.

Multivariate analysis

Using the multiple logistic regression analysis procedure available in SPSS/PC+ version 4.0 including data from all 229 controls and all 66 cases with adipose tissue data, the following results were obtained. This analysis was performed assuming that the 23 cases for whom smoking status was unknown were non-smokers.

The data is presented as independent odds ratio (with 95% confidence limits) for being a case of CHD taking into account quintile of adipose tissue linoleic acid percent (from the control distribution), confirmed smoking status (assuming those SCD cases who status was unknown were non-smokers), treated hypertension, diabetes and age.

Table 102. Multivariate analysis of estimate of relative risk of sudden cardiac death

Variable	Odds ratio (95% confidence interval)
Quintile I adipose tissue Linoleic acid	4.59 (1.41 to 14.91)
Quintile II adipose tissue Linoleic acid	3.49 (1.06 to 11.54)
Current smoking	2.63 (1.42 to 4.86)

On performing the same analysis with all SCD cases whose smoking status was unknown entered as being current smokers, the only variable remaining independently predictive of SCD case status was current smoking.

Table 103. Multivariate analysis of estimate of relative risk of sudden cardiac death

Variable	Odds ratio (95% confidence interval)
Current smoking	16.7 (7.6 to 36.7)

Using the data from the 36 SCD cases who had data available from NSR interview with the data from the 229 controls with data available from the controls spouse a multivariate analysis was performed. This involved forward stepwise multiple logistic regression. Entered into the regression were terms for the control quintile of adipose tissue linoleic acid percent, social class, current cigarette smoking, diabetes, treated hypertension, family history of heart disease in a first degree relative under the age of 60 years, and age.

The only variables in this analysis to make an independent and positive contribution to case status were current cigarette smoking and family history.

Table 104. Multivariate analysis of estimate of relative risk of sudden cardiac death

Variable	Odds ratio (95% confidence interval)
Current smoking	8.36 (3.66 to 19.13)
Family history	5.71 (2.89 to 14.25)

Using the conditional logistic regression procedure in STATA version 3.1,

entering linoleic acid as continuous variable, and stratifying the controls by their matched

cases and restricting the analysis to those 42 cases for whom the smoking data was

known for certain resulted in the following. This analysis included, age, linoleic acid,

treated blood pressure and diabetes.

Table 105. Multivariate analysis of estimate of relative risk of sudden cardiac death
(analysis by conditional logistic regression)

Variable	Odds ratio (95% Confidence Interval)	z score	p value
Linoleic acid	1.01 (0.90 to 1.14)	0.188	0.851
Current smoking	9.78 (2.69 to 35.45)	3.469	0.001
Treated BP	1.88 (0.35 to 10.07)	0.738	0.460

Smoking remained the only significant explanatory factor.

DISCUSSION

Main findings

This study has confirmed that sudden death as a result of coronary disease is a numerically important cause of death in the community. As a proportion of all deaths coming to post mortem it represents just over a quarter of the deaths, and as such is the biggest single category of cause of death. Together with the deaths out of hospital of men who had already been diagnosed as having CHD, coronary disease is responsible for over 40% of deaths. Suicide and trauma in this relatively young age group make up the next largest group of causes of death. The presence of left ventricular scars indicative of previous myocardial infarction in over a quarter of the SCD cases indicates that whilst these men had not been diagnosed as having CHD, it is possible, or even probable, that at least some had clinical syndromes which would have been compatible with this disease. The left ventricle was not separated for weighing in this study, which was not designed to look specifically at post mortem findings, but the high average weight of the intact hearts indicates that left ventricular hypertrophy was common. This is reflected in the high proportion of cases that were described as having left ventricular hypertrophy by the examining pathologist. From the Framingham echocardiographic study, the major determinants of left ventricular mass are systolic blood pressure and body mass index[135]. Just under a quarter of SCD cases were known to have hypertension, and it seems probable that elevated blood pressure would have been found in these cases prior to their death. The weight and dimensions of the bodies were not routinely recorded in the pathologists report so data is not available directly on body mass index of the deceased.

Information about the premorbid condition of the deceased and his social and medical condition in the months and years prior to death was gleaned from a number of sources. The Coroner's officer's information from their interview with the principal witnesses involved is the most complete, but not perhaps the most reliable source of information where tobacco consumption is concerned. The most accurate data was obtained from the deceased's wife, but this was not always available.

Approximately half of the deaths were witnessed but chest pain was known to have been present, at some point prior to death, in just under half of the SCD cases. Even

where there were symptoms presaging death, a third of the SCD cases with symptoms died within 60 minutes of these starting.

The significant difference in the distribution of the social class between the cases and controls reflects the higher mortality form CHD in those in social class IV and V. However, this could be due to a bias in the attendance of controls. The higher frequency of unmarried, or divorced men amongst the SCD cases is an interesting finding raising the possibility that men who live alone eat differently to those whose meals are more likely to be prepared by their wives.

Medical history and regular prescribed medication was not different between cases and controls. There was a significantly higher proportion of controls taking regular vitamin preparations raising the possibility that antioxidant consumption in the controls was higher as a result. This is possibly protective against CHD[136]. The absence of a difference in the frequency of diagnosed and treated hypertension in a study of this nature does not of course imply that there is a different frequency of hypertension. The frequency noted above of left ventricular hypertrophy indicates that there could have been more frequently undetected hypertension in the SCD cases. Chest pain reported by controls to the spouse would seem to be frequent. The presence of this symptom alone would not distinguish those who die from CHD. However, the question of the frequency and type of chest pains being a discriminatory feature has not been tested in this study.

Tobacco consumption in the form of cigarettes is associated strongly in both the univariate and multivariate analysis of this study. The frequency of current cigarette smoking being twice as frequent in the cases as in the controls, a finding that is not unexpected in this group of patients[137].

Alcohol consumption has been thought to be relatively protective against CHD[138], and there was no difference in the frequency of those drinkers in the general classes of drinking. However, there were more problem drinkers in the SCD cases. Heavy alcohol consumption in Finland and Sweden, and the United Kingdom has been shown to be associated with SCD[139-141]. There were no differences observed for fatty acids between the alcohol consumption groups in the cases, but a pattern emerged for linoleic acid in the control group. The group consuming a moderate amount of alcohol having the highest

percentage of linoleic acid. Those choosing to derive a lot of their food energy from food have been found, in common with smokers, to have certain patterns of nutrient consumption which may put them at risk of CHD[142]. It is known that cigarette and alcohol consumption are associated with the consumption, perhaps through taste, of foods with different nutrient content. These factors may act through a direct effect on fatty acid metabolism. This cannot be ruled out by this study. However, evidence from weighed dietary inventory supports the view that consumption is the main cause of the differences seen[143, 144].

The presence of a family history of a first degree relative under the age of 60 either dying or having definite CHD was three times as frequent in the SCD cases who had reliable witnesses for the family history. This was found for the univariate and the multivariate analysis with the SCD cases who had complete family history data. Familial hypercholesterolaemia could be the obvious connection but a common environment could also be a factor[145].

The analysis of the adipose tissue triglyceride fatty acids and the comparison of the findings for different conditions in the cases and controls reveals some consistencies between the two groups. Smokers in each of the two groups had lower linoleic acid percentages and higher oleic acid percentages. Palmitic acid and total monounsaturates were the only other consistent findings within and between the two groups being higher in the smokers. As linoleic acid is only fatty acid available from the diet this implies that smokers eat differently from non smokers. When the diet of smokers has been looked at systematically, the consumption of a several nutrients has been found to be lower in smokers[143, 144, 146].

Between the SCD cases and the controls a number of differences in fatty acid percent composition were found. Of the saturated fatty acids, palmitic acid and stearic acid were significantly higher in the controls compared to the cases. The monounsaturated fatty acids palmitoleic, oleic and eicosaenoic were all significantly higher in the SCD cases than the controls as were in total monounsaturates. Polyunsaturated fatty acids, apart from being higher in total in the controls compared to the SCD cases, were individually significantly higher for linoleic acid. Linoleic is the

most abundant PUFA. The SCD cases also had significantly higher percentages of arachidonic acid and ω6 docosapentaenoic acid. However, the coefficient of variation of the fatty acids present in relatively small proportions must lead to caution in interpreting these findings.

The relative risk associated with a low linoleic acid in adipose tissue triglyceride fatty acids for SCD was calculated in this study compared to the frequency distribution for linoleic acid in the CHD disease free controls. There was found to be a considerable, and significant elevation in SCD risk associated with being in the lowest quintile of adipose tissue linoleic acid.

On multivariate analysis, including known smoking habits, this finding remained significant and independent of the other risk factors that were known to be present in cases and controls. The consistent finding of current smoking, both in controls and cases, being associated with low linoleic acid percentages within groups is a significant finding. However, on repeating the analysis using conditional logistic regression, stratifying by matched controls, the relation of linoleic acid levels in adipose tissue to case status becomes attenuated and smoking remains the variable most strongly related to SCD.

I shall put these findings into perspective by reviewing the evidence for adipose tissue triglyceride linoleic acid's relation to CHD.

Linoleic acid and its relation to coronary heart disease from epidemiological studies
Cross cultural studies

An international comparison of the fatty acid composition of triglyceride fatty acids was performed by Riemersma and colleagues[53]. Healthy men aged 40 to 49 years of age in four regions were examined. The four areas were Edinburgh, Scotland, Sapri, in Italy and North Karelia, in Finland and South West Finland. All men invited and examined and finally included in the study were free of diagnosed CHD. They were randomly sampled from population registers held in each area. Adipose tissue samples were obtained from the subcutaneous layer of the abdomen in each area by the same open biopsy technique under local anaesthetic. All fatty acid analyses were performed in Finland by a similar method to that employed in the present study. The CHD mortality

rate in each area was known for men in the age group being considered. In Edinburgh this was stated as being (in 1979) 140 per 100,000 individuals, in North Karelia, 212 per 100,000, in South West Finland 146 per 100,000, and in Sapri 43 per 100,000 individuals. There were 131 samples from Edinburgh, 102 samples from North Karelia, 83 from South West Finland and 74 from Sapri. The percentage of linoleic acid was lowest in North Karelia with a level of 7.36%. The highest level was in Sapri in Italy with a level of 13.45%. Oleic acid was also highest in Sapri as well with a level of 54.30%. Palmitic, myristic, and palmitoleic acids were also highest in North Karelia and lowest in Sapri. Arachidonic acid in adipose tissue was also highest in Sapri. The total percentage of saturated fatty acids was lowest in Sapri and men from this area also had the highest total monounsaturated fatty acids and polyunsaturated fatty acids together with the highest P:S ratio. A high oleic acid would therefore not seem to be invariably associated with CHD incidence but palmitic and palmitoleic acids would. However, the main finding was that linoleic acid levels are consistently and inversely associated with CHD risk. The correlation coefficients between the classical risk factors also measured in this study and fatty acids shown to be different between the regions suggested that there was a negative relation of linoleic acid to smoking in Scotland only. There was no consistent correlation between the classical risk factors and linoleic acid. Total saturated fatty acids were negatively related to body mass index in Scotland, North Karelia and South West Finland but not in Italy. Correlation coefficients between total monounsaturates and other risk factors were not published in this paper. Because of the inconsistent relation of the fatty acids to the classical risk factors, they suggested that these factors are unlikely to explain the differences in the fatty acid profiles between the regions. Multivariate analysis was therefore performed with the fatty acids and other CHD risk factors to explore the strength of the association between linoleic acid and CHD risk. Linoleic acid remained a highly significant explanatory variable in the differences between the three countries with respect to risk of CHD even after taking into account the classical risk factors. These included age, smoking, blood pressure, total cholesterol, HDL cholesterol and body mass index. These analyses suggest that linoleic acid is associated with CHD

risk, and may be an important explanatory variable in addition to, and independent of, the classical risk factors.

Further evidence of adipose tissue triglyceride fatty acid linoleic acid levels in populations with different rates of CHD mortality can be found in the study by Logan and colleagues[52]. Healthy men aged 40 years were randomly selected from registers in Edinburgh and in Stockholm. It was known at that time that the CHD mortality for men in Scotland in the age group considered was 95 per 100,000 individuals and that in Sweden was 25 per 100,000. An adipose tissue biopsy was taken from the subcutaneous fat of the anterior abdominal wall and the triglyceride fatty acid analysis performed in Stockholm. From 107 men in Edinburgh and 82 men in Stockholm, the adipose tissue linoleic acid mean percent (standard error) was 7.3% (1.5) and 11.8% (2.1) respectively. This was also reflected in the cholesterol ester fatty acid percentages and that of the plasma triglycerides, which were all statistically significant. The adipose tissue P:S ratio was also significantly lower in the Edinburgh samples compared to the Stockholm samples. Correlation coefficients between linoleic acid percentages in adipose tissue, plasma cholesterol esters and plasma triglycerides were positive and highly significant but the correlations with other risk factors measured in this study were low. There was no difference for total cholesterol in this study, although triglycerides and HDL cholesterol were higher in the Edinburgh men. Although waist hip ratio was not measured in this study, insulin levels in response to an oral glucose load were. There was a higher level of insulin release in the Edinburgh men to attain the same glucose level throughout the glucose tolerance test. The inference from this is that the level of insulin resistance was higher in the Edinburgh men. The fatty acid composition of muscle membrane phospholipids has been shown to be related to differing patterns of insulin sensitivity[147].

Between population studies

Studies which have looked at the composition of adipose tissue triglyceride fatty acids between populations with differing rates of CHD mortality have confirmed the association between adipose tissue linoleic acid and CHD. Tavendale and colleagues analysed the fatty acid composition of 4,114 samples taken from 8,061 subjects from a total population of 10,359[54]. The subjects, men and women under the age of 60 years,

were randomly selected from 22 different health administration districts in Scotland. None were known to have been diagnosed as having CHD at the time of biopsy. The adipose tissue was taken from the subcutaneous layer of the skin on the upper arm by punch biopsy. They found there was a highly significant, and strong, inverse relation between the mean percent composition of adipose tissue linoleic acid and the standardised mortality ratio of each district. The age adjusted Spearman rank correlation coefficients were -0.60 for men and -0.62 for women. There was also a positive correlation between the standardised mortality ratio and oleic and palmitoleic acids for both men and women. This was 0.50 and 0.51 for palmitoleic acid, and 0.59 and 0.73 for oleic acid for men and women respectively. There was also a negative relation between the standardised mortality ratio for women with respect to myristic acid of -0.42 but this was not present for men. The P:S ratio was also negatively related to the mortality ratio for men, but not for women, with a correlation coefficient of -0.43. These strong relations of adipose tissue linoleic acid composition with routinely collected mortality data within Scotland are further evidence that dietary composition, as reflected by adipose tissue composition, is related to CHD.

Case control studies within populations

The odds ratio for SCD in the lowest quintile of adipose tissue linoleic acid in this study was nearly six fold compared with the highest quintile. This finding is consistent with the risk ratios found for adipose tissue linoleic acid in the studies of angina pectoris and first acute myocardial infarction performed in Edinburgh by Wood and colleagues[58].

They drew by stratified random sampling the names of 6,000 men aged between 35 and 64 from the central register of all men known to GPs in Edinburgh. These men were sent the Rose chest pain questionnaire and asked to complete and return it. The men who reported a positive history of having been told by a doctor that they had either angina pectoris or had been told that they had had a myocardial infarction were not studied further. Those who reported no history of a doctor's diagnosis of CHD and were negative for a history of chest pain interpreted as angina by the Rose questionnaire were used as a source of controls and a random sub sample of these men was drawn. All men who had no diagnosis of CHD but whose answers to the chest pain questionnaire were positive for

angina were invited to attend for examination as angina cases together with the controls. During approximately the same period, all men under the age of 55 years who were admitted to the coronary care units of two Edinburgh hospitals diagnosed as having had an acute myocardial infarction, but with no prior diagnosis of CHD, were identified. Adipose tissue samples were obtained by an open biopsy technique under local anaesthetic from the cases of angina pectoris and first acute myocardial infarction identified. A similar technique was used to obtain samples from the controls. Anthropometric measurements, blood pressure and serum lipid estimations were made from the cases and controls, although the measurements on the acute myocardial infarction patients for lipids and blood pressure were not included in the final analysis. The analysis of the adipose tissue triglyceride fatty acids was carried out by the same laboratory that analysed the samples for the present study. In addition, platelet membrane samples were prepared from whole blood and the fatty acid profile of the phospholipids of these platelet membranes analysed by gas liquid chromatography.

From 430 controls they obtained adipose tissue samples in 391. For the cases, 108 of the 125 angina pectoris cases consented to have samples of adipose tissue taken and 80 of 85 first acute myocardial infarction cases consented.

They found in this study that compared to controls, angina pectoris cases had significantly lower percentages of linoleic and stearic acids. They also had significantly higher percentages of palmitoleic and oleic acids. The P:S ratio was also lower in the angina pectoris cases. A similar pattern of differences was observed between the controls and the first acute myocardial infarction cases with the exceptions of stearic acid which was not significantly different and ω3 docosahexaenoic acid. ω3 Docosahexaenoic acid was significantly lower in the first acute myocardial infarction cases. Similar differences were observed for platelet membrane phospholipid fatty acid composition in both categories of CHD. Similar differences have been found in the present study between the cases of SCD and the live controls.

In contrast to their data, no significant relation was observed between linoleic acid and systolic or diastolic blood pressure for controls. The only significant correlation for linoleic acid was for waist hip ratio which was negative. This was not recorded in the

Edinburgh study. There was in this study, as in Edinburgh, no correlation between linoleic acid and total cholesterol, HDL cholesterol and non-fasting triglycerides.

Estimates of the relative risk associated with the lowest quintile of linoleic acid for angina pectoris and first acute myocardial infarction were performed in the same manner to the present study both in univariate and multivariate analyses. In the univariate analysis the odds ratio (95% Confidence limits) for being a case of angina pectoris for the lowest quintile of linoleic acid in comparison to the highest quintile was 3.2 (1.5 to 7.0) and that for first acute myocardial infarction was 3.0 (1.3 to 7.2). This is of a similar size to the univariate estimate of relative risk for SCD in the present study.

Multivariate analysis of the risk of being a case of angina pectoris or first acute myocardial infarction for the Edinburgh study was performed. This was done using forward stepwise logistic regression including quintiles of linoleic acid, age, smoking habit, blood pressure, total cholesterol, HDL cholesterol and non fasting triglycerides, height, weight and height/ weight index.. This analysis was based on 104 cases of angina pectoris and 380 controls with complete data. In this analysis linoleic acid made an independent contribution to the explanation of angina pectoris together with smoking habit and non fasting triglycerides. The estimated increase in risk for every 1% decrease in adipose tissue linoleic acid was 1.2 (1.1 to 1.3). For first acute myocardial infarction, a similar analysis, not including blood pressure and lipids, revealed that smoking habit and weight/ height index remained the only independent explanatory variables.

This result was similar to that found after forward stepwise multiple logistic regression analysis for the present study. Quintiles of linoleic acid remained independently related to SCD case status after including all cases for whom adipose tissue data was known and for whom smoking status was known or assumed. On assuming that the SCD cases whose smoking status was unknown were non smokers, linoleic acid remained independent of smoking, treated hypertension, diabetes and age. Recoding the assumed non smokers to being active smokers prior to death resulted in cigarette smoking being the only variable independently associated with SCD.

The mean level (standard error of the mean) of linoleic acid in the Edinburgh study by Wood and colleagues for controls was 9.81% (0.14). This is considerably lower

than the mean (standard error of the mean) for the controls in the present study. This may reflect the change in dietary habits over a period of time or regional differences in diet due to the availability of foods, or both. The absolute rate of CHD mortality in the two areas is consistent with this difference in linoleic acid between the control populations. However, the relative risk for being in the lowest quintile for the cases is similar.

In 1980, Wood and colleagues studied the adipose tissue composition of men aged 45 to 54 years of age drawn at random from the population registered with GPs in the Edinburgh area[57]. From the 448 men who attended for examination, 371 provided adipose tissue samples. From these men, 28 were identified as having newly diagnosed CHD either by electrocardiographic criteria for old definite Q wave myocardial infarction or by chest pain questionnaire. They found in this cross sectional survey that there were significantly lower percent levels of linoleic acid in adipose tissue triglyceride fatty acids in the newly identified cases of CHD compared to men with normal electrocardiograms and negative chest pain questionnaires. They also found higher palmitoleic acid and lower dihomo-γ-linolenic acid (ω6 linolenic acid) in the new cases. There was no difference in the levels of oleic acid between the two groups in this study. The mean level of adipose tissue linoleic acid (standard error of the mean) in the controls in this study was 8.94% (0.13). In this study cigarette smoking was associated with a lower level of linoleic acid There was a weak negative relation between linoleic acid and both systolic and diastolic blood pressure but no relation to lipids or anthropometric indices. In this study, samples were analysed from men who had already been diagnosed as having CHD. The linoleic acid level was higher in this group than in the controls, indicating that those that had been told of their diagnosis may have altered their diet and thereby increased the percent of linoleic acid consumed. A 7 day weighed dietary record in this study confirmed a positive and highly significant correlation between the linoleic acid consumed in the habitual diet and adipose tissue triglyceride fatty acid levels. Multivariate analysis including total cholesterol, HDL cholesterol, age, current cigarette smoking, systolic and diastolic blood pressure, triglycerides, weight/ height index and glucose was performed using case status as the dependent variable. This was based on the 26 cases and 319 controls with complete data. Linoleic acid, age, total cholesterol, and

weight/ height index all were independently and significantly related to case status. Including all the other fatty acids in the multiple logistic regression equation resulted in dihomo-γ-linolenic acid being the only fatty acid to be independently related to risk together with age, weight/ height index and glucose. No data was presented in this paper for the estimated relative risks of being a case of CHD with respect to linoleic acid control quintiles.

A similar pattern of adipose tissue linoleic acid composition in a case control study of acute myocardial infarction was found by Kirkeby and colleagues in Oslo[40]. In 1970 they obtained adipose tissue samples from the buttocks of 79 men between the ages of 40 to 70 years of age admitted to their hospital with a diagnosis of acute myocardial infarction. Forty three of these men had no prior diagnosis of CHD and the rest had either had a previous acute myocardial infarction or angina pectoris before the admission at which the sample was taken. They obtained samples from 25 men admitted to the surgical wards to act as controls. They found a significantly lower level of linoleic acid in the newly diagnosed cases of acute myocardial infarction compared to the controls. Those with a previous diagnosis of CHD had higher levels of linoleic acid than the controls. Palmitoleic acid was higher in the newly diagnosed cases compared to the controls as was oleic acid. Stearic acid was also significantly lower in the new cases compared to the controls. The mean (standard deviation) percent of linoleic acid in the controls in this study was 9.5% (2.9). No multivariate analysis data was presented in this paper.

Again there seems to be a consistent pattern of differences even in this population taken from a Nordic country with respect to the cases freshly diagnosed, in that linoleic acid is lower, as is stearic acid whereas palmitoleic and oleic acid are higher.

Another case control study of adipose tissue and CHD was performed in the United Kingdom by Thomas and colleagues[148]. They obtained adipose tissue from 59 cases of CHD taken at post mortem in the South Wales area and compared them to the adipose tissue composition of 61 controls also taken at post mortem. It is uncertain whether they included those who were already known to have CHD prior to their death. They reported the mean level of control fatty acids and the differences (positive or negative) between the cases and controls. The mean level of linoleic acid in the controls

was 6.85% for *cis, cis* linoleic acid. The mean for the cases they obtained was 0.37% higher, a difference not significant by analysis of variance. They also present data in this survey on the presence of *trans* fatty acids suggesting that the percentage of *trans* forms of linoleic acid is significantly lower in the controls.

From a study in New Orleans the adipose tissue composition of men aged between 25 and 44 years who came to post mortem during the 10 year period 1969 to 1978 was analysed. There were a total of 66 cases who were diagnosed as having died of CHD, 78 cases who were found to have significant CHD at post mortem who had died of other causes and 988 other samples taken from post mortems where the cause of death was unrelated to CHD. Adipose tissue triglyceride fatty acid composition was determined by thin layer chromatography on samples taken from two sites: the peri-renal fat and from the subcutaneous layer of the buttock. The data is presented for myristic, palmitic, palmitoleic, stearic, oleic and linoleic acid by several different categories. They are split into two age bands; 25 to 34 years, and 35 to 44 years; into Black or White racial origins, and whether the fatty acids were from peri-renal fat or buttock fat. There are no statistics presented in the paper for the differences for the fatty acids between the groups studied, but linoleic acid was higher in the CHD cases compared to the deaths from other causes in both peri-renal and buttock adipose tissue.

From the description in this paper of the collection of material it is clear that no distinction was made between those who had a clinical diagnosis of CHD prior to their death, and had possibly changed their diet and raised their level of linoleic acid in adipose tissue. This is clearly what happens in the Scottish and Norwegian populations as outlined from the studies cited above.

Other means of assessing the dietary intake of linoleic acid and the subsequent risk of death or acute myocardial infarction using the fatty acid composition of the serum lipids have been investigated. Kirkeby and colleagues in addition to obtaining samples of adipose tissue also analysed serum lipids in their case control study of acute myocardial infarction[39]. They found there was a consistently lower percentage of linoleic acid in the newly diagnosed cases, whichever compartment of serum lipids were studied. There was a lower percentage of linoleic acid in cholesterol esters, phospholipids and serum

triglycerides in newly diagnosed cases compared to the controls. There was a significantly higher percentage in cholesterol esters for palmitoleic acid but not phospholipids and serum triglycerides. Oleic acid was significantly higher in cholesterol ester but not in serum triglycerides and phospholipids. Palmitic acid was higher in the cases in cholesterol esters but not in phospholipids and triglycerides. Eicosapentaenoic acid was higher in the cholesterol esters of the cases and the phospholipids but not in the triglycerides. The long chain fatty acids of docosapentaenoic acid and docosahexaenoic acid (the position of the first double bond not specified) were higher in the phospholipids of the newly diagnosed cases compared to the controls but not in the cholesterol esters or the triglycerides. A more complicated and varied picture would therefore seem to be present in the more dynamic composition of serum lipids.

A 5 year follow up study of patients with peripheral vascular disease by Kingsbury and colleagues, who had the fatty acid composition of their plasma cholesterol esters determined, confirms the importance of linoleic acid[60]. Compared to those 40 subjects who remained alive and well, the level of linoleic acid in the cholesterol esters of subjects who died of vascular causes (all CHD related deaths) was significantly lower. The arachidonic acid level in those who died was also significantly lower compared to those who remained well. The subjects who developed acute myocardial infarction had lower linoleic acid levels compared to those who were well, but those who developed angina pectoris had a slightly higher linoleic acid level. There were no other significant fatty acid differences. Dietary linoleic acid would again seem to be important in determining outcome in those who have already got disease. The relevance of this finding in this population with respect to the other standard risk factors is not available from the data and analysis given in this paper.

A formal prospective study of blood lipid fatty acid composition, particularly linoleic acid, was performed by Miettinen and colleagues[61]. They followed up 1,222 middle aged men who were initially free of clinical CHD for 7 years. From this cohort 33 men experienced a fatal or non fatal myocardial infarction or SCD during the period of study. Controls were selected from the remaining 1,189 men and were matched for age, blood pressure, cholesterol and triglyceride concentrations, smoking habit and glucose

tolerance at the start of follow up. The fatty acid composition of phospholipids, cholesterol esters and triglycerides was determined from a fasting serum sample taken on entry to the study. A significantly lower linoleic acid level was found in the phospholipid fraction of the cases compared to the controls. As all the controls were matched for other risk factors, this can be considered to be an independent finding. In the previously described Edinburgh Stockholm study there was a positive correlation between serum phospholipid linoleic acid and adipose tissue triglyceride linoleic acid. This result indicates that linoleic acid could be a determinant of the development of CHD.

A similar study was carried out in Prague by Valek and colleagues[62]. They followed up 107 survivors of acute myocardial infarction and measured the fatty acid composition of serum phospholipids and triglycerides at the start of follow up. After 5 years there had been 23 vascular deaths. Compared to the survivors the percent composition of linoleic acid in those who had died was low. This seemed to be independent of the other major risk factors in relation to vascular death.

What emerges from the review of the data available on adipose tissue and blood fatty acid composition in relation to CHD is that linoleic acid is consistently, although not invariably, found in lower proportions in those who present with clinical CHD. When it has been found that linoleic acid is not lower than in controls, the possibility has always been present that prevalent cases of CHD have been sampled. These findings in themselves do not prove an aetiological association between the presence of low levels of adipose tissue linoleic acid and CHD. I shall now consider the relation that linoleic acid may have to other risk factors for CHD.

Relation of linoleic acid to other coronary heart disease risk factors

As outlined above, a consistent relation of adipose tissue linoleic acid to the classical risk factors of age, blood pressure, total and HDL cholesterol and cigarette smoking, have not been consistently found within study populations. From the four areas studied by Riemersma and colleagues there was no consistent relation between any of the fatty acids in adipose tissue and any of the classical risk factors[53]. The only relation to be found was between cigarette smoking and linoleic acid in the Scottish population.

Cigarette smoking is recognised as being a major risk factor in the development of CHD[137]. Precisely what it is about cigarette smoking that leads to CHD is not known apart from the influence of smoking on fibrinogen levels[149, 150]. In both SCD cases and controls in the present study smokers consistently have lower adipose tissue linoleic acid percentages. However, what has not found to be present in other European populations would now seem to be so for at least two population areas within the United Kingdom.

Analysis of the correlations between the continuous variables of systolic and diastolic blood pressure, total cholesterol, HDL cholesterol and triglycerides in this study, and in the others cited above, have not identified any consistent association between the other major risk factors and adipose tissue linoleic acid or any other fatty acid. The significant relations, in positive and negative directions, seem to be more often found with the saturated and monounsaturated fatty acids. However, the Pearson correlation coefficients are less than 0.5. The significant relations could be as a consequence of multiple statistical associations due to chance. Trials of fish oil feeding to lower blood pressure have shown that high intakes of these fatty acids can influence blood pressure[151]. However, because of the very low percentages of the $\omega 3$ fatty acids in adipose tissue reliable estimates of the relation to dietary intake cannot be obtained and the absence of statistical relations in this study does not exclude a real effect.

Epidemiological studies of sudden cardiac death

This present survey has confirmed that SCD due to CHD, is the single most common form of death in the population of men studied at post mortem in the Southampton and South West Hants health district. This confirms the public health aspect of this problem demonstrated by Kuller and colleagues over twenty years ago in Baltimore[78, 121]. By taking a subsample of all deaths in the Baltimore area between 1964 and 1965 and classifying them into those that were probably and possibly "sudden" they identified those definitely due to CHD and those due to other causes, and identified 489 sudden deaths due to CHD. Relatives of the next of kin were contacted and the authors state they were "92% successful" in interviewing the next of kin. It is unclear whether this percentage refers to the all the sudden death cases or to those who had relatives that were

known, or to those relatives who were known and responded. In this survey, SCD due to CHD accounted for 31.5% of all deaths in Baltimore in the period studied, but the actual number of SCD established as being the first manifestation of the disease was 55 of the 489 (11.3%) definitely identified as SCD. In 28.4% of these deaths the cause was established at post mortem . The presence or absence of CHD prior to death in the cases identified is not given in this paper. They looked at a number of factors but in particular they did not identify any specific "level of activity" undertaken by those who died suddenly prior to death. It is clear the survey described here cannot be as complete as Kuller's original survey as no attempt, apart from ensuring that all hospital deaths were catalogued, was made to collect all deaths possibly interpretable as being SCD. That would require a retrospective review of all the death certificates issued for residents of the area in question for the period of the survey.

A similar study to Kuller's was performed in Belfast in 1966[79] where all deaths certified as being due to arteriosclerotic heart disease, or disease involving the coronary arteries, were identified and then information was gathered on the individual's circumstances of death from several different sources. These included the patient's general practitioner, hospital and coroner's records, ambulance service records and relatives of the deceased. They identified 998 such instances of death during this period and they could reliably establish the time between the onset of symptoms and death in 871 (87.2%). Two thirds, 644 of 998 (66.5%), were male. Information from this survey is given in the paper on those men whose "first attack" was fatal; it is not clear whether this refers to those who had no prior symptoms of CHD or whether it also includes those who had angina. Of those in whom the attack was their "first", 107 of 365 (29.3%) died within 15 minutes of symptoms starting and 241 of 365 (66%) were dead within 12 hours. They also found that men who were older and whose attack was their second or subsequent attack tended to survive longer.

Bekker & Grunfeld[91] reviewed all the death certificates from the whole of Denmark for September 1972. They established the time from the onset of symptoms to death from the death certificate itself, supplemented by information from the patient's doctor and from post mortem if available. Of the 3,971 persons that died in Denmark

during that month, they established that 299 men had died within 24 hours of the onset of symptoms. All of these were reported to have been well prior to their last illness. In this group there were 215 of 299 (71.9%) who had died from "Acute Heart Disease". It is not specified in the paper whether this means CHD alone. The proportion of those dying of acute heart disease who were known to have had a previous diagnosis of angina, acute myocardial infarction or "ischaemic heart disease" was 129 of 215 (60%). Hypertension or diabetes was known to have been present in 46 others, and only 77 (30.3%) had none of these illnesses diagnosed prior to death. The overall post mortem rate is stated to be "low" but is not specified for the male SCD. Danish law may require an inquest into death but not necessarily a post mortem examination. During the same period, 99 of 152 (65.1%) women died of SCD; 17 of 99 (17.2%) being under 65 compared to 86 of 215 (40%) of the men who died suddenly. Approximately the same proportion of women 30 of 99 (30.3%) had no previously diagnosed illness. The proportion of all SCD, male and female, that were known to be dead within 1 hour of symptoms starting was 57%.

The Minnesota Heart Survey published its findings in 1983[103] on the review of all residents 30 to 74 years old living in the Minneapolis-St Paul metropolitan area who died between 1970 and 1980. The place of death was recorded and they defined those persons who died outside hospital or in hospital "emergency rooms" as SCD. Whilst there is no information from this survey on the presence or absence of pre-existing disease, there is information on the International Classification of Diseases (ICD) classes of death rates from 1970 and 1978. For the ICD code 410-413 for men, which includes all forms of CHD, the rate of death per 100,000 population fell from 311 for death out of hospital or in the emergency room in 1970 to 244 in 1978. The overall death rate for men for all the aforementioned ICD classes declined from 508 per 100,000 in 1970 to 366 in 1978. There were two to three times the number of deaths in the emergency room in 1978 than there were in 1970, but the overall rate of CHD death was lower, as was their defined SCD rate. From the above figures it can be seen the proportion of deaths that were sudden in 1970 and 1978 remained approximately two thirds (61.2% and 66.7% respectively). This does indicate that although CHD rates have fallen SCD is still a major public health problem.

Sudden death in younger males aged 35 to 44 was studied by Kuller and colleagues[106] for the deaths occurring in Allegheny County in Pennsylvania between 1970 and 1981. They identified 793 records from the coroner and from the other death certificate records in this age group as having been due to cardiovascular causes. They then obtained further information on these deaths from various sources. Post mortem examinations had been performed in 379 (47.8%), in 408 (51.4%) coroner's records were available, 248 (31.2%) had hospital records, in 125 (15.8%) information from relatives was available but in 24 (3.0%) of cases no further information was available. Based on this information, a review of the original death certificate diagnoses was performed by a single physician who assigned causes of death. Of the 603 cases originally classified as being due to CHD, 60 (10%) were apportioned to other causes of death by the reviewing physician. Of the 190 deaths due to causes other than CHD, 13 (7%) were reclassified as being due to CHD. The proportion of the eventual total of 556 SCD due to CHD, who had no recorded history of CHD prior to death was 57.2%. Compared to those with a prior history of CHD, their place of death was more frequently out of hospital: 76.4% compared to 58.5% for those with a prior history. The absolute rates for SCD out of hospital without a prior history of CHD in this survey fell from 36.9 per 100,000 for this age group in 1970-72 to 15.3 in 1979-81. The corresponding rates for in hospital death in the same area and age group were 11.7 in 1970-72 and 6.3 in 1979-81. They argue that the decline in death rates from CHD, and SCD in particular, reflects an overall decrease in the incidence of CHD which has been reflected in the changing mortality due to CHD in the United States during the same period. What they cannot confirm, is what proportion of all deaths during the periods in question were made up of SCD.

All death certificates were reviewed for deaths occurring in the city of Belfast over a period of one year[108, 152]. The deaths attributed to ICD numbers 410-414 were provisionally included in this survey. Other death certificates where the death was considered to have been possibly or probably due to CHD were collected and recorded for further enquiries to be made. Verification of all these collected deaths was then undertaken by the investigators using data from post mortem reports, medical records from hospitals and general practitioners, and by interview with relatives of the deceased.

A panel of cardiologists then assessed the data from all the cases where there was doubt as to the final coding of the cases as being due to CHD or not. Of the total of the 1,654 deaths that were coded as being due to CHD, or included as being possible or probable CHD, 1,288 deaths were coded as ICD 410-414 but 108 (8.4%) of these deaths had to be excluded because of doubt about the final cause of death. From the other 366 deaths coded under rubrics other than ICD 410-414, 223 (60.9%) were finally excluded by having no data to support the final diagnosis as being due to CHD. They found that the final number of those actually attributed to CHD was 2.8% higher than the original total of 1323. This data was compared and correlated to the information on all deaths where a coronary care ambulance was called to in the same area during the same period. Of the 1323 deaths, the death was unwitnessed in 287 (21.7%). The time from onset of symptoms to death was known in 562 of the remainder. For the men under the age of 70 years, of whom there were 332, the time from the onset of symptoms to death was known in 206 (62%). In just under half of these case, death was known to have occurred within 1 hour of symptoms starting (102/206). A third of these men were deemed to be dead within 4 minutes of symptom onset. Analysis is given of the deaths occurring out of hospital in men and women under the age of 70 years. There were 128 such deaths, 97 of whom were male. In this group of deaths, 61 (49%) were known to have had a clinical diagnosis of CHD prior to the final illness. In a further 14 (11%) there was a history suggestive of CHD of recent onset or for some time prior to the final illness. The presentation of the final illness in 80 (62%) of these cases was by sudden collapse and loss of consciousness. The median survival time was 8.25 minutes for this group.

The death certificate review method could be criticised for its reliance on the diagnosis being made without confirmatory evidence if it was not for the painstaking methods outlined above. The Minnesota Heart Survey has also published data on the validation of death certificate data in cases of suspected SCD[113]. In this study they took a random sample of the out of hospital deaths occurring in residents of their area between January and December 1979. This constituted a third of all such deaths for residents aged between 30 and 74. They then collected information on the death from as many sources as possible. This information was then reviewed by one physician using a computer-

assisted decision program. The deaths were then assigned to five categories: (i) definite fatal myocardial infarction; (ii) definite sudden death due to coronary heart disease; (iii) definite fatal coronary heart disease; (iv) possible fatal coronary heart disease; and (iv) non-coronary heart disease death. They remark, almost in passing, that the analyses were "hampered by missing data"! They also calculated the sensitivity, specificity and positive and negative predictive values for the original death certificate data. The sample yielded 413 cases, 285 (69%) of whom were men. Almost 50% of these men were over 65. They were unable to contact an "informant" for the death in 33% of cases and the post mortem rate was 24.9%. Approximately one third of the deaths were unwitnessed. The cause of death was CHD, assigned by the study physician, in 72.9% of cases. Even the study physician was uncertain as to the actual cause of death in 3.2% of these deaths. Excluding the uncertain cases the sensitivity of death certificate data for CHD was 90.3%, the specificity 82.7%, the positive predictive value 94.1% and the negative predictive value 73.6%. Of the eventual deaths that were assigned to CHD, 114 of 247 (46.2%) were in the category of definite SCD. Six of these had been given an alternative, non-CHD, diagnosis on the original death certificate. There was a positive CHD history in the SCD cases, from at least one source, in 54 of 114 (47.7%).

One of this group, Gillum, has gone on to review the death certificate data for out of hospital and emergency room death rates from 48 of the 50 states of the United States of America (U.S.A.), and interpreting them on the basis that they are most likely to reflect SCD[116, 117]. On analysis of this data he has found an approximate 20% reduction in the rate of SCD in the U.S.A. in all age groups between 1980 and 1985. He has also observed a geographic variation throughout the U.S.A. in the proportion of all deaths from CHD being SCD in the male 55 to 64 year age group in the period 1984 to 1986. There is a wide variation also in the standardised mortality ratio for SCD, from 0.513 in New Mexico to 1.223 in New York (the standard mortality being 1.00). No satisfactory explanation for this geographic variation exists.

The register method of obtaining information on cases of a disease as it arises in a community was pursued in several populations in the late 1960's supported by the WHO.

Publications from individual data collection sites involved in this project have yielded much information on the public health aspects of SCD.

From July 1968 to June 1969 in Stockholm and the surrounding counties there were 1,740 deaths from CHD[81] in men of all ages, of whom 434 (24.9%) were under 65. Wikland, in this study, defined deaths as sudden if they were "medically unattended". In the 30 to 64 year age group 236 (54.4%) fitted into this category. A post mortem was performed in 193 of these cases (81.7%). Of the "medically unattended" deaths under 70, death occurred within 15 minutes of the onset of symptoms in 145 of 338 (42.9%). For 102 (30.1%) of these deaths, no time from the onset of symptoms to death could be attributed. When considering the prior medical history of those men under 70, only 75 of 338 (22.2%) had no known, or "suspected" medical history, i.e. either myocardial infarction, angina, hypertension or diabetes. The post mortem findings are difficult to interpret by age group from the tables printed, but undoubtedly in all age groups there were old infarcts found at post mortem examination in those who did not have a prior history of myocardial infarction. In all age groups 47 of 147 (32.0%) with no prior history of any sort had an old infarct at post mortem. Of these 47, 16 (36%) had a ruptured left ventricle and 17 (37%) had an old myocardial infarction.

In Nashville, Tennessee between July 1967 and June 1968, all acute myocardial infarctions and SCD's under the age of 75 were gathered by monitoring and recording physician and hospital activity[83]. During this period 258 SCD cases were collected. A large proportion of these (55.3%) were known to have died within 2 hours of the onset of symptoms. A positive history of previous myocardial infarction was obtained in 93 of the 255 (36.4%) in whom information was available. The presence or absence of angina pectoris is not commented upon. Previous myocardial infarction was known to have occurred in 58 of the 173 males (33.5%) compared to 13 of 66 women (19.7%).

A similar register was maintained in Edinburgh over a 14 month period[85], and whilst SCD was not named as a definite diagnostic category, the time from the onset of symptoms was determined as far as possible for each registered fatal case. The age limit for entry into this study was 70. The total number of male deaths under 70 was 397 during the period of this study, 248 (62.5%) of which were (medically) unattended. Of

these unattended deaths, the time from the onset of symptoms to death was unknown in 67 (27%) but of the rest, 142 of 181 (78.5%) were dead within 1 hour. The proportion of first attacks of myocardial infarction presenting as unattended death was 23.1% for men and 28% for women but the frequency in these groups of a prior history of angina pectoris alone is not stated. The post mortem rate in this survey was 6.4%.

Romo[87] conducted a register in the city of Helsinki area between January and December 1970 during which time he recorded 184 SCD in males under the age of 65. The WHO criterion of "definite acute myocardial infarction", that is unequivocal ECG, biochemical or post mortem evidence of acute myocardial infarction, was fulfilled in 50 of this 184 (27.2%). Post mortem examination was performed in 143 of these cases (77.7%). A prior history of ischaemic heart disease was present in 133 (72.3%), 69 of whom had a prior myocardial infarction. Hypertension was said to have been present in 30.5% prior to death and diabetes in 9.4%. Current smokers made up 103 of the 184 SCD cases (56%). There were 192 post mortems from 239 SCD cases, of whom 159 (82.8%) were males.

There was a register kept of all incident cases in the East End of London between April 1970 and December 1972[89]. A total of 1,039 events were recorded in that time in men and women under the age of 65. Rates of attack are given and no other figures are presented in the paper but it is stated that "a quarter of the men and a third of the women were dead or in irreversible cardiac arrest when first seen by a doctor in the attack (half of them with no previous history of angina or infarction)". There were 340 deaths out of the total number of attacks occurring outside hospital or in the casualty department, that is 32.7% of all attacks. Of the deaths occurring within 28 days of the attack, 70% occurred within 4 hours of the attack starting.

A register method survey was also used in Framingham, Massachusetts for the period from June 1970 to June 1971[92]. During this time there were 13 male SCD, 5 of whom (38.5%) had a prior history of CHD.

Kaunas, in the Baltic republic of Lithuania had a register of SCD from 1970 to 1975[94] during which time there were 197 male SCD cases. Post mortem examinations

were performed in 165 (83.8%). In the paper it is stated that "further enquiries" were

made in 127 cases. Of those 127 cases 75% were said to have had a prior history of CHD.

The findings of a register of SCD between 1971 and 1973 in the region of

Bohemia in Czechoslovakia were published in 1977[95]. They found 150 cases of sudden

death due to natural causes, 130 of which (86.7%) were attributed to CHD. There were 30

women in this group, 9 of whom were older than 70 years of age. Of the remaining 100

men there were 15 over the age of 70. They state that "one half" of the total number of

deaths from SCD had a prior history of CHD, 37 of whom had a history of myocardial

infarction. From the table in this paper on post mortem findings there was said to be

evidence of old myocardial infarction in 62 of the cases. Over half of the SCD died

within 1 hour of symptoms starting.

Data on SCD from Gothenburg and Boden in Sweden and Helsinki and Tampere

in Finland were pooled from 1971 for a publication in 1977[96]. The findings from the

Helsinki population have been noted above[87]. The recorded number of definite SCD in

the total population of males aged 20 to 64 in these four cities was 394 in 1971. This

represents 23.9% of the total number of "coronary attacks" during 1971. The proportion

of these SCD that were dead within 1 hour of symptoms starting was 64.9%.

Symptomatic CHD prior to death was reported to be present in 74% of the males dying

suddenly. In addition, 30% of the males had been diagnosed as having hypertension.

Only 13% had no reported cardiovascular disease prior to SCD. Whilst they do not

provide the figures they report that while smoking was as common in the SCD group of

attacks as it was in the other forms of incident CHD during this period ,"heavy smoking"

was "much more common" in the SCD group. They also report that hypertension was

more common in the SCD group compared to the other manifestations of CHD. Relative

weight, physical activity, social class or marital status did not seem to be associated with

SCD.

The Rochester Epidemiologic Project in Rochester, Minnesota has available all

the medical, hospital and post mortem examination records of the residents of this area

from a period of many years. Records have been kept since early in the century and

records of first diagnoses of CHD, angina pectoris, myocardial infarction and SCD are

available[99, 100, 107]. They have published figures from their records from 1950 for SCD with and without prior CHD. Of the 1,054 cases of all SCD, 704 were male and 347 (49.3%) of these had no prior history of CHD. The proportion of cases of SCD under the age of 70 from 1950 to 1975 was 275 of 544 (50.4%); the proportion of males is not given. From 1950 to 1982, there had been a total of 694 cases of SCD with no prior history of CHD; the proportion being male was 60.2%. The annual age adjusted rates of SCD with no prior history are given and there has been a decline from 2.17 per thousand males in 1950 to 1954 to 1.03 per thousand males in 1978 to 1982; a 52.5% fall. The corresponding female rates are 0.62 and 0.49; a drop of 21%. The proportional rate (adjusted for age) of presenting in this manner would seem to have declined from 1950-54 to 1978-82. In 1950-54 the overall rate of presentation of all manifestations in males of CHD was 8.99 per thousand and in 1978-82 was 7.58 per thousand. The proportion of CHD presenting as SCD, with no prior history of CHD, was 24.1% of the overall rate in 1950-54 and 13.5% in 1978-82. The corresponding figures for women were 17% and 13%.

Fredericksborg County in Denmark was used as a population base for a SCD survey in 1982[105]. The whole population of 332,000 was monitored from May 1982 to October 1982. During this period there were 56 male deaths under the age of 70 and 18 female deaths. Of the total number of SCD under 70, male and female, 41 of 74 (55.4%) had a prior history of CHD and hypertension, and of the remaining 33, 7 (21.2%) had a known history of hypertension. The total number of SCD in all ages and both sexes during this period was 166 and of these, 100 (60.2%) were dead within 1 hour of symptoms starting. SCD accounted for 12.7% of all deaths in this survey. Prodromata of chest pain prior to SCD was more often present in those who had a history of CHD (54% compared to 26%). They also calculated rates per thousand population for SCD with and without a CHD history. The rate for men less than 50 years of age without a history of CHD was 0.095 per thousand per year whereas that for men from 50 to 69 was 1.8 per thousand per year. The corresponding rates of SCD for those with a prior history of CHD were 7.5 and 19.3; that is those males under 50 with a history of CHD were 79 times

more likely to die suddenly than those without; men from 50 to 69 were 11 times more likely.

In the town of Tecumseh in Michigan State, between 1959 and 1960, 8,641 people (88% of the total population) were examined and the 3,643 persons older than 30 years of age were followed up[80]. During the period 1959 to 1965, 98 persons died of CHD; 45 of these (45.9%) were deemed to have been SCD. There were 29 men and 16 women in this group (64.4% male). Those who were free of CHD before they died of SCD made up 27 of the 45 (60%). However, of these 27 cases of SCD, "hypertensive heart disease" had been diagnosed prior to death in 5 and diabetes was present in 6. Only 17 of the 45 (37.8%) were free of CHD, "hypertensive heart disease" or diabetes prior to death. The presence of "codable" abnormalities on the ECG was found in 38 of the 45 SCD victims. Only one person of the original 3,643 persons who had no evidence of CHD, "hypertensive heart disease", diabetes, elevated blood pressure, high relative weight, raised serum cholesterol, diabetes or any ECG abnormality at initial examination suffered SCD.

Eighty six per cent of the population, older than 15 years, of three counties in Bohemia, Czechoslovakia were studied between 1959 and 1969[82]. The prevalence and incidence data on 1,793 persons older than 30 were then collected. It is not clear from the description of the methods in this paper whether these people were examined and then followed up or the examinations were conducted over a longer time interval. There were a total of 77 deaths in males under the age of 70 in this period, 32 (41.6%) were due to CHD. The proportion of these deaths that were SCD's was 15 of 32 (46.9%). It is not possible to ascertain from this paper what fraction of the men who experienced SCD had any manifestation of CHD prior to death.

Employees in a factory in North Carolina were examined in 1959[86] and followed up until 1970. Out of 1,224 male workers originally studied, 118 were known to have died. Death certificates were available in 116. Sixty of the deaths were attributable to CHD (51.7%); 38 of these were SCD (63.3%). The time from symptom onset to death was known in 50 of the 60 deaths from CHD (83.3%). In the SCD group, 27 of the 38 (71.1%) died within 1 hour of symptoms starting. It is not possible to calculate from this

paper the number of men who had a diagnosis of CHD prior to death but the presence of ventricular premature beats on the initial ECG alone was not associated with an significantly increased relative risk of SCD (0.9) or CHD death (1.3). The presence of "other" ECG abnormalities alone was associated with a relative risk of SCD of 2.5. The combined presence of ventricular premature beats and "other" ECG abnormalities did seem to be associated with a significantly increased relative risk for SCD (2.1). No significance levels or confidence intervals are given in this paper however. The rate of development of abnormalities in the succeeding years in the ECG, or of CHD, was not given in this paper.

Male employees of the Chicago People's Gas Company were examined in 1958[88]. A total of 1,465 were followed up over the next 15 years. At initial examination they were classified into three groups: (1) those who were free of CHD; (2) those suspected of having CHD; and (3) those who definitely had CHD. The rates of SCD after 10 years of follow up per thousand population were for group (1) 19; group (2) 29; and for group (3) 126. In group (1) SCD accounted for 19% of all CHD deaths whilst in group (3) SCD accounted for 40% of all deaths. Analysis of the rates associated with various risk factors for CHD were also presented: the presence of serum cholesterol $\geq$ 250mg/dl; diastolic blood pressure $\geq$ 90mmHg; and the consumption of $\geq$ 10 cigarettes per day. For group (1), those who had none of these factors present at initial examination had a SCD rate, adjusted for age, of 13.0 per thousand after 15 years of follow up. The rate of SCD in this group for those who had one or more of the above factors present at the initial examination was 39.5 per thousand after 15 years. The corresponding rates for groups (2) and (3), combined, for those with no risk factor present was 38.6, with any one risk factor present it was 48.6 and for those with two or more present it was 91.7. An interesting further observation was that within group (1) and also within combined groups (2) and (3) the mean heart rate increased successively with the presence of one risk factor to two or more risk factors.

Tunstall-Pedoe[97] recorded the ECG in 8,228 employed men who were classified as having no symptoms suggestive of CHD at initial examination. They were followed up for a period of 4 years. During that time there were 160 deaths, 64 (40%) of which were

due to CHD. Of the deaths from CHD, 51 (79.7%) were classified as SCD. He found that the absence of Minnesota code abnormalities on the ECG was associated with 0.6 deaths due to SCD per hundred individuals, the presence of any one abnormality was associated with a rate of 0.4, any two abnormalities with a rate of 1.8 and the presence of three or more with a SCD rate of 2.2. Of the total number of men examined 1,760 (21.4%) had no Minnesota code items on their recording and 13 of 51 SCD (25.5%) came from this group. The rates of SCD for post infarct patients in this study varied with the frequency of ECG abnormalities, but it was at least five times the rate of symptomless men even if no abnormalities from the Minnesota code were present on the ECG.

In the MRFIT study, 12,866 men were randomised into a trial of multifactorial intervention, of whom 6,428 were randomised to receive special intervention and 6,438 to the usual care group[102]. After six years of follow up there had been 265 deaths in the special intervention group, 115 of whom (43.4%) were due to CHD; 75 of these (62.6%) were SCD. In the usual care group there were 260 deaths after six years, 124 of whom (47.7%) were due to CHD; 81 of these (65.3%) were SCD. Further analysis was performed using the total number of CHD deaths and SCD figures were not given in this paper for comparison by level of risk factor. The proportion of SCD in each group dying within 1 hour of symptoms starting was 54 of 75 (72%) in the special intervention group and 58 of 81 (71.6%) in the usual care group.

The Manitoba Study has followed up 3,983 men who were initially examined in 1948 aged 15 to 64 years of age[101]. Electrocardiograms were analysed using the Minnesota code and the follow up data on these findings after thirty years were given in this paper. The rates available from this study exclude those who developed CHD, including silent myocardial infarctions, during the follow up period. During this period there were 70 SCD's. They found that 20 of 70 (28.6%) had no coded abnormalities. The age adjusted incidence of SCD in this group is not given. The incidence rate for various codes is given but the combination of ECG findings and analysis of their independent contribution to SCD was not given in this paper.

Hinkle and colleagues have published data on a relatively small cohort of employed men followed up for a period of twenty years[153]. This study looked principally

at the mode of death of a group of 356 middle aged men, 301 of whom entered the study. They specifically classified deaths as being due to arrhythmia, even if the medical condition leading to death was not due to heart disease. The majority of deaths in this category were due to CHD, but not all. They then analysed data for univariate and multivariate associations with the mode of death. The men were aged between 54 and 62 years of age at entry into the study in 1962. They were drawn from telephone company employees selected by random numbers corresponding to their social security number. Examinations were performed and they were asked to complete various questionnaires. Regular examinations were then conducted every two years to detect specific conditions. Data was obtained in detail for all deaths. The deaths were classified by likely mechanism into those that were due to cardiac arrhythmia and those due to primary circulatory failure. During the twenty year follow up there were 148 deaths; 65 deaths were classified as being due to arrhythmia. Of the arrhythmic deaths, 36 occurred within 1 hour of symptoms starting. Just under three quarters of the deaths occurring outside hospital were classified as being arrhythmic. In the univariate analysis, the electrocardiographic diagnosis of chronic myocardial ischaemia, left bundle branch block, and early cycle ventricular ectopics were associated with arrhythmic death. The other characteristics most strongly associated in the univariate analysis were systolic blood pressure and the number of cigarettes smoked per day. Other associated factors were lack of exercise, low education, high uric acid, high alcohol intake, chronic airways disease and age. There was a significant negative association between arrhythmic death and the number of years spent in full time education. In the multivariate analysis left bundle branch block and other intraventricular conduction delays were independently and positively associated with arrhythmic death as were systolic blood pressure and the number of cigarettes smoked. It is interesting to note that none of the terms coding for the presence of ventricular arrhythmias did not appear in the final statistical analysis. All but three of the arrhythmic deaths had heart disease diagnosed prior to the final event.

Prospective studies of sudden cardiac death and its relation to classical risk factors

Prospective studies which have published detailed information by multivariate analysis on factors related to SCD due to CHD are The Framingham Study[84, 90, 93, 104,]

[110, 123-126] and The Honolulu Heart Program [111, 115]. A recent publication on the Italian cohort from the Seven Countries Study has also reported on CHD presenting as SCD using multivariate analysis[118]. Factors have thus been identified which can, mathematically, be linked to SCD as an initial presentation of CHD. The independent contribution of the various factors can be calculated from multiple regression equations. These studies by virtue of their length of follow up and consistency of classification are the gold standards by which factors implicated by other types of study can be weighted. This can help in the identification and selection of modes of intervention to be tested by trials or to be included in public health initiatives.

The Framingham study recruited 5,209 subjects, 2,336 men and 2,873 women, in 1948 and has regularly published the morbidity and mortality rates in relation to the bi-annual examination findings, biochemical and ECG information on these individuals. During the first fourteen years of follow up there were 120 deaths from CHD before the age of 65[84]. The total number of deaths due to all causes during that period was 156. The number of CHD deaths that were SCD was 66 of 120 (55%). From this group of 66, 42 of these (63.6%) had occurred in people who had no known history of CHD. Of the 37 men who were in this group, 14 had ECG evidence of left ventricular hypertrophy prior to death, and a further 11 had "definite" hypertension (a systolic blood pressure reading greater than 140 mmHg and a diastolic blood pressure reading greater than 90 mmHg, or both) at one of the examinations carried out before death.

The Framingham study and a study of male civil servants in Albany, New York pooled their information on SCD in 1975[90, 93]. This provided information on 4,120 individuals, in whom, after 16 years of follow up there had been 109 SCD's. This was a population of men aged between 45 and 74 years of age. Of the SCD group, 62 (56.9%) had no prior history of CHD. The annual rate of SCD for those free of CHD in the 45 to 54 year age group was 0.7 per thousand, and 2.4 per thousand in the 55 to 64 year age group.

Subsequent publications from this study and from other cohort studies have produced multivariate regression coefficients in relation to several factors. These are listed in Table 107.

Age is a consistently significant factor in all the prospective studies that have been published on SCD due to CHD; it can be seen from the table that it has a strong influence on the incidence of SCD. Relative weight or obesity, however defined, would also seem to be independently related, as would blood pressure and ECG evidence of left ventricular hypertrophy. Resting heart rate is also related independently. Those who have already been diagnosed as having congestive cardiac failure are also liable to SCD.

Cigarette smoking is consistently related to SCD but cholesterol level is not so. One analysis from the Framingham study looked at this in a slightly different manner by using the term cholesterol multiplied by age and this even became inversely related to SCD, albeit very weakly.

For the men in the Honolulu Heart Program, relative risks for SCD due to CHD for the presence of several risk factors at initial examination, and subsequent development during follow up, have been published (Table 108.). All the classical risk factors would seem to be significantly related to SCD presentation. One of the index terms for obesity in this survey, the sum of skin folds would not seem to have a strong relationship and body mass index was not apparently related. Cholesterol, systolic blood pressure, smoking and fasting glucose would all seem to have a similar degree of independent risk elevation associated with being in the top quartile. The presence of hypertension elevates risk by a factor of 2.71, but by far and away the largest influence would appear to be the presence of ECG left ventricular hypertrophy at 4.87. Most interesting from this analysis, with respect to its known effects on blood pressure and the myocardium, is alcohol consumption. Those who consume alcohol would seem to have an independent decrease in risk of SCD in this survey .

The above review would indicate that although much has been published on SCD due to CHD, there is much that remains to be explained in terms of the risk profile of those destined to present in this manner. They do not seem at present to be distinguishable from their peers who present with an acute myocardial infarction or with angina pectoris although angina pectoris itself, once present, is a significant risk predictor for SCD. There is a need for better estimates of how men, and women, at risk can be

identified, and once identified effective measures taken to avoid an often tragic and untimely death.

From the summary of the data presented above, apart from left ventricular hypertrophy, the level of the classical risk factors do not help to distinguish between the SCD victim and those who die by other means. Adipose tissue linoleic acid is known to be inconsistently related to the classical risk factors, apart from the case of cigarette smoking, and that has only been demonstrated in the United Kingdom. Where it may fit in into the picture of CHD and those that present suddenly is not known at present. I shall explore some of the possible mechanisms in a subsequent section.

Table 106. Summary of studies on sudden cardiac death in coronary heart disease

Reference	Type of study	Population size	Duration of study	Number of SCD cases	Post mortem rate
77	I	73,573	6 years	336	Not available
78	DC	263,601	1 year	1077	Approximately 40%
79	DC	Belfast	1 year	348	Not available
80	CO	3,643	6 years	45	Not available
81	R	640,781	1 year	1,023	75.8%
82	CO	2,277	10 years	28	57.1%
83	R	166,811	1 year	258	Not available
84	CO	5,209	14 years	66	Not available
85	R	500,000 (approximate)	1 year	226	6.4% (overall all deaths)
86	CO	1,224	11 year	38	Not available
87	R	522,929	1 year	239	80.3%
88	CO	4,120	16 years	109	Not available

Table 106. Summary of studies on sudden cardiac death in coronary heart disease

Reference	Type of study	Population size	Duration of study	Number of SCD cases	Post mortem rate
89	R	82,200	1 year	245	Not available
91	DC	5,100,000	1 month	314	27.5%
92	R	64,048	1 year	19	Not available
94	R	Not available	5 years	193	85.5%
95	R	120,000	3 years	131	100%
96	R	725,600	1 year	486	82.9%
97	CO	17,705	4 years	178	Not available
98	I	120,000	6 years	197	Not available
99	R	60,000	25 years	544	Not available
100	R	60,000	25 years	1,054	Not available

Table 106. Summary of studies on sudden cardiac death in coronary heart disease

Reference	Type of study	Population size	Duration of study	Number of SCD cases	Post mortem rate
101	CO	3,983	30 years	70	35.7%
102	CO	12,866	7 years	153	Not available
103	DC	Not available	10 years	Rates only	Not available
104	CO	5,209	26 years	196	Not available
105	R	332,000	6 months	166	50% (approximately)
106	DC	Not available	10 years	556	Not available
152108	DC/R	355,980	1 year	No definition of SCD	25% (approximately)
107	R	60,000	32 years	694	Not available

Table 106. Summary of studies on sudden cardiac death in coronary heart disease

Reference	Type of study	Population size	Duration of study	Number of SCD cases	Post mortem rate
109	R	Not available	14 years	169	10% (approximately)
110	CO	5,209	38 years	264 (+165 possible)	Not available
111	CO	11,148	7 years	58	100%
112	CO	15,481	7.5 years	41	Not available
113	DC	Not available	1 year	114	23.7%
114	PM	Not available	3 years	189	100%
153	CO	301	20 years	36	Not available
115	CO	7,591	16.5 years	96	Not available
116	DC	Not available	5 years	Rate only	Not available
117	DC	Not available	2 years	Rate only	Not available
118	CO	Not available	23 years	67	Not available

DC Death certificate, CO Cohort study, I Insured persons, R Register of incident cases, PM Post mortem study

Table 107. Multiple regression analysis results from cohort studies for SCD in men

Factor	Reference	Beta	se Beta	t value	p value
AGE	104	0.186			0.0003
	125	0.5382			≤ 0.001
	126	0.0627			≤ 0.001
	118	0.0749	0.0236	3.17	
RELATIVE WEIGHT	88	0.0227	0.0058	3.95	
	124	0.263			
	104	0.0196			0.0268

Table 107. Multiple regression analysis results from cohort studies for SCD in men

Factor	Reference	Beta	se Beta	t value	p value
SYSTOLIC BLOOD PRESSURE	88	0.0125	0.0044	2.82	
	124	0.399			
	104	0.0105			0.0411
	126	0.0107			≤ 0.05
MEAN BLOOD PRESSURE	118	0.0191	0.0092	2.07	
CIGARETTES/DAY	88	0.0376	0.068	3.52	
CIGARETTE SMOKING (YES/NO)	124	0.347			
	104	0.0189			0.0197
	126	0.0216			≤ 0.01

Table 107. Multiple regression analysis results from cohort studies for SCD in men

Factor	Reference	Beta	se Beta	t value	p value
CHOLESTEROL	88	0.0025	0.0023	1.09	
	124	0.303			
	104	0.0340			0.0032
CHOLESTEROL × AGE	126	0.0062			≤0.01
ECG-LVH	88	1.3298	0.3652	3.64	
	124	0.432			
	104	1.84			0.0000
	125	0.2453			≤ 0.001
	126	0.7453			≤ 0.001
QRS SUM	118	0.0073	0.0037	2.00	

Table 107. Multiple regression analysis results from cohort studies for SCD in men

Factor	Reference	Beta	se Beta	t value	p value
HEART RATE	125	0.3867			≤0.001
	118	0.0185	0.0084	2.21	
PREVIOUS CHF	126	1.0903			≤0.05

CHF Congestive heart failure, ECG-LVH Electrocardiographic left ventricular hypertrophy

QRS SUM Sum of voltage of QRS complex in all 12 electrocardiographic leads

Table 108. Multivariate analysis of variables and relative risk of sudden cardiac death in Hawaiian Japanese men

The Honolulu Heart Program 1965-83[115]

Variable	Relative risk	95% Confidence interval
Systolic blood pressure	2.71	1.72 to 4.26
Cholesterol	1.81	1.15 to 2.84
Pack years of smoking cigarettes	1.67	1.02 to 2.75
Glucose	1.85	1.31 to 2.62
Sum of skinfolds	1.20	0.69 to 2.11
Alcohol consumption	0.54	0.30 to 0.96
Left ventricular hypertrophy or strain	4.87	2.75 to 8.61

Post mortem series

Post mortem series of SCD have been frequently published (see Table 109.). Interpreting the findings from such surveys is often very difficult even allowing for the fact that they are necessarily highly selected cases. Definitions of what constitutes a SCD are sometimes not clearly explained and often the relation of post mortem findings such as heart weight and old scars of myocardial infarction are not related to known prior histories of CHD or hypertension. What is clear from these surveys is that SCD due to CHD makes up the larger proportion of diagnoses in the category of sudden unexpected death. This would even appear to hold true in the younger age groups[154] although diagnoses other than CHD then begin to make up a larger fraction of the total. There are a number of these publications but as generally there is no data published to be able to interpret the findings in the wider epidemiological context, I shall not review them further here.

Table 109. Summary of post mortem series published on sudden cardiac death

Reference	Site	Method of case selection	Year	Number
Spain et al[155]	Westchester County, NY, USA	Coroner	1949-59	584
Crawford et al[156]	London, UK	Not stated	Not stated	75
Adelson & Hoffman[157]	Cleveland, OH, USA	Coroner	Not stated	500
Myerburg & Davis[158]	Dade County, Miami, FA, USA	Coroner	1956-62	1348
Luke & Helpern[154]	Manhattan, NY, USA	Coroner	1965-67	275
Spain et al[159]	Westchester County & Brooklyn, NY, USA	Coroner	Not stated ("3 year period")	102
Spain & Bradess[160]	Westchester County & Brooklyn, NY, USA	Coroner	Not stated	189
Scott & Briggs[161]	Albany, NY, USA	Hospital Records	1966-70	183

Table 109. Summary of post mortem series published on sudden cardiac death

Reference	Site	Method of case selection	Year	Number
Roberts & Buja[162]	Bethesda, MD, USA	Not stated	Not stated	107
Friedman et al[163]	San Francisco, CA, USA	Coroner	Not stated	64
Liberthson et al[164]	Dade County, Miami, FA, USA	Coroner	1970-73	150
Myers & Dewar[165]	Newcastle Upon Tyne, UK	Coroner	1971-72	100
Reichenbach et al[166]	Seattle, WA, USA	From Emergency Squad callouts	1972-75	130
Lovegrove[167]	Perth, Western Australia	Coroner	Not stated	500
Rissanen et al[168]	Helsinki, Finland	Register & Forensic cases	1970-71	151
Baroldi et al[169]	Milan, Italy	Forensic Institute Cases	1967	208

Table 109. Summary of post mortem series published on sudden cardiac death

Reference	Site	Method of case selection	Year	Number
Gwynne[170]	Otago, NZ	Coroner	1971-79	408
Davies & Thomas[129]	London, UK	Not stated	Not stated	100
Dienstl et al[171]	Innsbruck, Austria	Forensic Institute	Not stated ("Five years")	126
van Dantzig & Becker[172]	Amsterdam, Holland	Selected from heart registry	Not stated	16
Phillips et al[173]	US Air Force	All deaths in recruits	1965-85	21
Zaija[174]	China	Register & coroner	1974-80	Unclear
Raymond et al[175]	Durham, NC, USA	Retrospective survey of hospital records	1980-86	83
Drory et al 1991[119]	Israel	Forensic Institute Records	1975-86	162

Abbreviations are standard for the States in USA, and for other countries

Limitations of the present study

Definition of sudden cardiac death

The definition utilised in this study of SCD is broad. This was deliberate to try to ascertain as many potential true "instantaneous" or arrhythmic deaths as possible. The result being that the manner of death, whilst being representative of the population as a whole, is heterogeneous. Mechanisms of death, either arrhythmic or ischaemic cannot be properly identified in this study as there is no electrophysiological data available. There are practical limitations in a population survey of this nature and although insisting on documentary evidence of ventricular fibrillation would have been desirable, identification of such cases would been practically impossible. The collection of cases fulfilling the required criteria would also have been extremely slow.

The use of post mortem material

There will be a few cases during the period of the study that will have collapsed suddenly outside hospital and been resuscitated to survive more than 24 h and died or survived long enough to have been discharged home. The loss of these cases could have biased the results, but I believe that these cases are few. As evidence for this I can only cite my personal experience on the Southampton Hospital wards.

Adipose tissue will tend to decompose after death although the proportion of fatty acids in adipose tissue remains stable at 37°C in animals for up to 36 h after death (R. A. Riemersma Personal Communication). However, it was specifically decided to exclude those cases that were severely decomposed. The loss of these cases and the samples that were not taken due to administrative errors is a serious source of bias. The proportion of cases that were identified and sampled was satisfactory in being over 70% of all cases identified. However, the misunderstanding with the pathologists that a prior diagnosis of heart disease did not include hypertension diagnosed as being present before death meant that there were significantly more cases with missed samples diagnosed as having high blood pressure. Although the differences in the fatty acids between the controls diagnosed

as having high blood pressure did not include linoleic acid it cannot be excluded that a bias will exist.

The use of live controls

It could have been possible to obtain adipose tissue samples from post mortem specimens of trauma victims. However, the age structure of this group is quite different from that of the CHD group. This could have introduced bias although on the analysis of the control fatty acid data there does not seem to be a great influence of age on fatty acid composition. More serious would have been the low numbers of post mortem victims available for sampling as SCD due to CHD was the largest single group and obtaining two controls for every case would have been difficult.

The invitation of controls unfortunately leads to the more affluent, health conscious members of the population attending for examination. This is reflected in the social class structure of the controls. This does introduce a serious source of bias as there seems to be a significant difference in the fatty acid composition of the adipose tissue with respect to social class which is a reflection of the diet consumed. The selection of controls only in the same social class as the cases may have led, however, to over matching and no difference in diets being found.

Dietary assessment by biochemical means

Dietary data cannot be obtained from the SCD victim himself and the use of proxy interviewees to obtain such data has not been validated. It was decided at the outset of the study not to collect such data. The correlation between adipose tissue composition and the dietary fatty acid composition only seems to hold well for linoleic acid. In long term feeding experiments it has been used to document adherence to the regime. However, the possibility exists that acute events may alter the proportion of linoleic acid in adipose tissue triglycerides. There does not seem to be a differential release of linoleic acid from triglycerides(R. A. Riemersma Personal Communication).

There are other limitations in the use of adipose tissue triglyceride information in the testing for exposure in the diet to other fatty acids which may be important in other

compartments. For example, the fatty acids that make up the largest proportion of triglyceride fatty acids are oleic acid, palmitic acid and linoleic acid. The smaller proportions of the other fatty acids, with their necessarily larger coefficient of variation in the measurement of these fatty acids, make assessment of their contribution to the differentiation of case and control difficult. ω3 Docosahexaenoic acid ω3 eicosapentaenoic acid may have a role to play in the influence of diet on arrhythmias but their level in adipose tissue does not reflect this[176]. Measuring the fatty acid composition of different compartments, such as membrane phospholipid, may be of more relevance in this regard[177-180].

The use of proxy interviews to obtain data

As can be seen from the comparison of control spouse responses and the control responses, use of a proxy interviewee is, for some categories of information, unreliable. The robustness of the data for the cases has to be interpreted in the light of this knowledge. It is reassuring, however, that smoking and treated hypertension are fairly accurately recorded by the spouses of the controls. However, care in the interpretation of the data is required as recall bias in, for example, the reporting of a family history of CHD is quite likely. Further validation would be necessary to test these assumptions.

The use of proxy interview data was used to investigate the hypothesis that oral contraceptive use was associated with an increased rate of myocardial infarction and sudden death at the end of the 1970's[181]. In this study the investigators collected information on all deaths from all women between the ages of 15 to 44 who died of an acute myocardial infarction over an eighteen month period in the five largest Standard Metropolitan Statistical Areas in the United States from January 1974. They required a face to face interview with the next of kin, or closest relative or friend available. A proxy respondent for the controls was used to attempt to validate this information. Of the 358 potential cases that they identified for this study, 91 were not included because it was thought that myocardial infarction was not the cause of death or the cause of death was unknown, 20 others were considered ineligible because of lack of data concerning the

death, in 19 cases no informant (i.e. a next of kin or close living relative or friend) could be identified and in 65 cases the informant refused to participate in the study. Therefore, just over thirty percent of those eligible for this study were dropped from further analysis because of inability to interview a proxy for the deceased. The approach used in the analysis was to drop information from these cases altogether. This obviously could bias the conclusions of any survey considerably.

The use of the case control method to investigate sudden cardiac death

As is apparent from the tabulation of the response rates of the NSR to approaches from the GP and myself, there is a lot of information that has been lost in this survey. This has, as has been pointed out, a common problem in the collection of retrospective information whenever sudden cardiac death has been investigated in the past. The use of case control studies as a valid means of testing hypotheses of association and causation must be called into question. Techniques for avoiding the problem of non response and investigating exposure to confounding variables by other means may have to be developed.

Similar problems have been encountered with this method in the survey alluded to above[181]. Attempting to approach the NSR almost immediately after the morbid event may be associated with a higher refusal rate than waiting for at least six months to elapse. The grieving process may take a considerable time and contact during this time may not be appropriate. This approach has been used in the "verbal autopsy" method used in surveys of causes of death in the developing world where very few deaths come to post mortem[182]. The optimum time over which accurate information can be obtained about the final illnesses from relatives that causes least distress is yet to be precisely defined.

Alternatively, biochemical markers for exposure to confounding variables may be used in order to reduce the bias introduced by non response. Measuring cotinine levels in samples of urine taken at post mortem would be a possible method to establish whether the deceased had been a current smoker before death. However, this would not help to differentiate between the ex-smokers and life-long non-smokers.

There are considerable difficulties in the use of the case control method to investigate sudden death, and as a consequence it would be important to corroborate evidence from such studies with data from long term follow up of large cohorts.

Possible mechanisms for the role of linoleic acid in the development of coronary heart disease

Dietary linoleic acid as measured by adipose tissue triglyceride fatty acid percentage is consistently and strongly and inversely related to CHD when this diagnosis is being made for the first time. Recognition of the diagnosis is associated with higher percentage levels of linoleic acid in both adipose tissue and blood. As the diet is the only source for this fatty acid it is likely there is a change in the proportion of this fatty acid in the habitual diet of those suffering from CHD. This could be due to either an increase in the absolute amount of linoleic acid consumed or to a reduction in the amount of saturated fat consumed thereby altering the relative proportion of linoleic acid in the diet. There would also seem to be, in Britain at least, an inverse relation between linoleic acid consumption and cigarette smoking. This has been shown to be due to a true effect of the food choice of smokers[144].

In considering what physiological pathways might be responsible for the observations outlined above there are several possibilities. However, it cannot at present be categorically stated which one is the true mechanism and, indeed, all may be playing a role in linoleic acid's reduction in CHD risk.

Keys attributes the recognition of the connection between cholesterol levels in the blood and dietary habits to original observations by a Dutch ex-patriate in Java[183]. Keys' own studies in the 1950's together with others[184-188] showed that the serum cholesterol level in man could be reduced by suitable alteration of consumed dietary fat. The fact that the principle fat related to cholesterol reduction was linoleic acid, being the main polyunsaturated fat in the diet, was thought to be doubly significant by Hugh Sinclair[189]. He proposed that as linoleic acid was an essential fatty acid it was a relative deficiency of this species that was responsible for atherosclerotic disease. This hypothesis was

vigorously refuted by Keys himself[190]. However, the fact remains that increasing the proportion of linoleic acid in the diet will reduce the total cholesterol concentration in man[191]. The mechanism of lowering serum cholesterol in man is still being debated[192]. It was initially suggested that the principle effect was the reduction in amount of cholesterol carried in each LDL particle as a result of the esterification of cholesterol with linoleic acid. However, this seems unlikely as the secretion of apolipoprotein B decreases in parallel with the decrease in LDL. The rate of cholesterol synthesis does not seem to be altered by linoleic acid but there is an increase in the output via the faeces of neutral sterol compounds and bile acids. There is also an effect on the hepatic production of VLDL. As this species is also the precursor of a proportion of the circulating LDL it is possible that this is responsible for some of the reduction in cholesterol brought about by linoleic acid. There is also a reduction in HDL production as measured by the production of apolipoprotein AI. How polyunsaturated fats actually bring about these changes is not yet understood[26, 193, 194]. There are strong genetic influences, as demonstrated by twin studies, but with any given genotype there are definite responses to changes in the diet[195]. There is also a concomitant reduction in HDL, but the overall effect of the reduction in total cholesterol overrides any particular concerns that may arise as a result of this. Animal studies on the regression of atheroma in primates fed linoleic acid after diets which have been shown to be atherogenic have given weight to the cholesterol reducing hypothesis for linoleic acid's action[196].

The prostaglandins were being named and investigated by von Euler and Goldblatt [197]at around the same time as Burr and his wife were performing their dietary exclusion work on rats. In 1962 Bergström demonstrated that prostaglandins were naturally occurring fatty acids. Subsequently it was shown that arachidonic acid and dihomo-γ-linolenic acid were metabolised to the prostaglandin series. The metabolism of linoleic acid to dihomo-γ-linolenic acid and arachidonic acid was also shown during the 1960's. The natural inference from this work was that essential fatty acid deficiency led to a deficiency of prostaglandins and it was this that was responsible for the syndrome of

essential fatty acid deficiency. Infusions of prostaglandin E into essential fatty acid deficient animals did not alleviate the features of fat deficiency however. The discovery by Vane and colleagues of a substance that caused strips of rabbit aorta to contract, and the subsequent discovery of the inhibition of its production by aspirin led to an explosion of biochemical investigation in cardiovascular disease[198, 199]. Vane and his colleagues in 1976 discovered a novel prostaglandin, subsequently named prostacyclin[200]. Its properties as being the most active vasorelaxant and platelet activation inhibitor, gave new importance to the essential fatty acids, their products, and cardiovascular disease. The discovery of the inhibition of platelet activation by aspirin in 1971 has helped to turn attention back to the role of thrombosis in arterial disease. Trials carried out in the late 1970's demonstrated that a reduction in the incidence of subsequent events in those who had already suffered from CHD could be achieved by treatment with aspirin. Primary prevention trials involving aspirin in men in the 1980's have shown benefit in terms of coronary heart disease but at a cost of an increased frequency of haemorrhagic cerebral events[201]. In addition to this evidence, trials in the 1980's have subsequently shown that aspirin is synergistic with thrombolytic agents in the treatment of acute myocardial infarction[202]. Attention is now focused on the interaction between blood components and the vessel wall. The discovery that naturally produced fatty acid derivatives could influence the vascular endothelial layer[203] and platelet activity provides another potential pathway for dietary fatty acids to modify disease activity. The metabolic pathways to the species of the prostaglandin series and other vasoactive lipids have become clear over the past thirty years[204, 205]. At the start of this pathway are the essential fatty acids. The finding of the importance of the prostaglandins in platelet and vessel wall biology opened up a new avenue for the investigation of dietary influence on the atherosclerotic and thrombotic process. Animal and human studies have suggested that manipulation of the diet can influence the balance of molecules which are physiologically and pharmacologically active, not only in the cardiovascular system, but as mediators of physiological and pathological activity in every system[206-208]. This would seem to be true

not only in terms of the platelet and vessel wall interaction, but also in the electrical and other properties of membranes[147, 178, 179, 209-224].

Essential fatty acid deficient rats have been noted to have an alteration of the morphology of their electrocardiographic complexes[225]. Induction of arrhythmias due to cardiac ischaemia in rats fed diets with differing fatty acid composition has demonstrated that rats fed on diets with high levels of linoleic acid have a reduction in the incidence of such arrhythmias[177, 179, 180, 226]. Precisely what the mechanism of protection from arrhythmias is in these animals is not yet known. Incubation of single myocytes from rats with ω3 eicosapentaenoic acid has demonstrated an antiarrhythmic effect[178] but similar investigation of linoleic acid has not been performed. Studies on the basic electrophysiology of whole rat hearts do indicate that there are changes with the alteration of the proportion of linoleic acid in myocardial membranes[227]. This study indicated that the effective refractory period is shortened by increasing the linoleic acid percentage in membranes, an effect that would not conventionally be expected to be antiarrhythmic. However, the relevance in terms of the cellular electrophysiology is not at present certain. No formal electrophysiology has been performed on single myocardial cells from animals fed on diets containing differing fatty acid compositions.

Chemical modification of the lipoprotein structures in vivo has also been shown to be associated with atheroma development[5]. This modification is possibly the result of low antioxidant levels within the lipoprotein particles. Chemical reactivity of the unsaturated carbon to carbon bonds can lead to changes in the larger structures causing them to become agents for pathological change within an artery wall[228]. Not only may this be important in the development of atheroma but in vitro and animal studies have suggested that chemically modified LDL may influence normal vessel wall responses[229-231]. A case control study based in Edinburgh has indicated that consumption of antioxidants may be associated with protection from coronary heart disease[232]. Vitamin E, or one of its components, α-tocopherol, is known to have antioxidant properties and is a lipid soluble

compound present in abundance in vegetable seed oils. So it may not only be linoleic acid alone that may be the protective factor but its natural accompanying antioxidants.

Adipose tissue composition as a measure of dietary fat consumption

Adipose tissue studies investigating the influence of the dietary fatty acid pattern on the percent composition of adipose tissue triglyceride fatty acids have been performed[50, 51]. Adipose tissue composition is a good surrogate for dietary assessment in groups of individuals[26, 233]. The correlation for polyunsaturated fatty acids is good with a Pearson correlation coefficient of 0.8. The corresponding values for saturated fat and monounsaturated fat in the diet are both of the order of 0.5.

Dietary surveys using the weighed inventory method have shown that there is a good correlation between the linoleic acid percent of fatty acids in the diet and that found in adipose tissue triglyceride fatty acids[146, 234].

It has also been established that because of the variation of dietary intake from day to day in the free living population, several days of monitoring are required to establish with accuracy the habitual intake of many dietary constituents[25]. This could be one the reasons for the negative relations of CHD to dietary nutrients observed in surveys that have utilised a 24 h recall method on one occasion to characterise a habitual diet. This was the method of dietary assessment in the Framingham study[235]. Similarly analysis of the diet by recall methods and correlating death rates in the Seven Countries study did not show a relation between CHD mortality and polyunsaturated fat in the diet[236]. However, there was a positive relation to saturated fat in the diet and a negative relation to monounsaturated fat. It is known that dietary recall methods are not particularly accurate[237] when assessing some dietary constituents and linoleic acid was not identified as a specific food constituent in the Seven Countries study.

There are difficulties in establishing with accuracy the actual nutrient intake of human individuals. For linoleic acid consumption in the diet as a *proportion* of the fatty acids in the diet, adipose tissue triglyceride fatty acid analysis is a very useful tool. The

limitation is that it cannot quantify the amount an individual is consuming in the diet, nor the actual source of the fatty acid. Ascertainment of this data can only be gained by weighed inventory or duplicate meal composition analysis.

Clinical trials of polyunsaturated fatty acid consumption and coronary heart disease

Dietary trials to test the converse of the aetiological association between linoleic acid and CHD, namely that increasing linoleic acid in the diet reduces the risk of CHD, have not been performed. However, in primary and secondary prevention trials diets low in saturates and high in polyunsaturated fatty acids have been used. The effect of varying one kind of fat at a time has not been tested in a large intervention trial with clinical endpoints. The logistical problems associated with doing such trials are enormous and problems with palatability of diets are frequently encountered.

A trial of secondary prevention of CHD events was performed on men who had suffered myocardial infarctions in the New Jersey area[238]. They observed that increasing the proportion of polyunsaturated fats in the diet reduced the cholesterol level and there was a reduction in CHD events in the group in whom this diet was introduced.

Secondary prevention of further CHD events following an initial myocardial infarction by modifying the diet was attempted in Australia[239]. Men who had suffered a myocardial infarction, or who had angina pectoris of recent onset (possibly unstable angina), were randomised to receive dietary intervention or not. Dietary intervention consisted of nutritional counselling and monitoring of dietary change by regular interview by standardised questionnaire. Two hundred and thirty seven men were randomised to no modification of their diet, unless they were overweight. The other two hundred and twenty one men were tutored individually to reduce saturated fat intake and to reduce their intake of cholesterol. They were encouraged to use foods containing polyunsaturated fatty acids. They found that modification of the diet did not seem to prevent CHD death as there were 28 deaths in the control group compared to 39 deaths in the dietary intervention group. However, the numbers entered into the trial were relatively small and there was no attempt

to balance the degree of severity of disease in the trial intervention groups. The severity of angina, however, was a strong influence on survival. For example, it is possible that patients with low ejection fractions, or severe unstable angina, were put more frequently into the dietary intervention group. The poor prognosis associated with a low ejection fraction following myocardial infarction is now well understood[240] and this factor could probably override any mechanism that may be operating through dietary modification. Furthermore, no data is presented on the occurrence of further CHD events other than death.

In an institution in Los Angeles for elderly men, vegetable oils were substituted for two thirds of animal fat in an experimental diet fed to a random half of the men[241]. Linoleic acid accounted for 38% of all the fatty acids in the meals regularly prepared for this diet compared to 12% of the control diet. Within two years, the adipose tissue level of linoleic acid had risen to a mean of 24% in the men exposed to the experimental diet compared to 9% in the control group. After 8 years of follow up, there was a reduction in the number of atherosclerotic events in the experimental group. This was especially so in the men in the younger age groups. However, the overall death rate was not lower in the dietary intervention group compared to the control group. In the men under 65, of which there were just over 200 in each group, there were 28 deaths due to an "acute atherosclerotic event" (possibly SCD) in the control group and 7 such deaths in the experimental group. However, there was a total of 45 deaths in the control group and 47 deaths in the experimental groups in this age category. This probably reflects the underlying chronic disease states that required these relatively young men to be in a long term health care institution.

Two mental hospitals in Finland were used to try to establish whether changing the dietary fat composition altered the frequency of CHD events[242]. One hospital initially changed its habitual diet for the patients by replacing dairy fats almost totally by vegetable oils. The other hospital acted as the control. After five years the hospitals switched over so that the experimental diet was being used in the former control hospital and vice versa.

Whilst total fat intake remained the same overall, there was a considerable reduction in saturated fat, no change in monounsaturated fat but an increase in total polyunsaturates. The P:S ratio in the experimental diet became 1.48 compared to 0.25 in the control diet. The adipose tissue linoleic acid level in samples taken from those consuming the experimental diet was a mean of 27% compared to 10.3% in the controls after 5 years. Just over 670 patients were present in either hospital for the entire period of the study and 72 patients experienced at least one CHD event. The incidence of CHD events was lower in each of the experimental groups in both periods. The incidence of CHD events during the experimental diet period was 13.5 per 1000 man-years compared to 24.3 in the control diet period. Total mortality in the intervention groups was not reduced which is perhaps to be expected given the total number of participants.

In contrast to the two trials above the Oslo diet heart study performed an intervention on 1232 middle aged men with hypercholesterolaemia and high coronary risk scores[243]. Dietary modification in these men considered to be at high risk was accompanied by attempts to stop cigarette smoking. Their dietary intervention was mainly aimed at a major reduction in the consumption of saturated fats with only a minor increase in polyunsaturated fat. The men were randomly allocated to intervention or no intervention and followed up for 5 years. The dietary intervention had the effect of increasing the P:S ratio in the intervention group: it was 0.39 in the control group compared to 1.01 in the intervention group. No direct measurement of linoleic acid was made in this study. There was a reduction in the incidence of CHD in the intervention group but again total mortality was not affected. There were 3 SCD cases in the intervention group and 12 SCD cases in the control group; the event rate for SCD was significantly lower in the intervention group. However, although the total number of deaths for all causes was lower in the dietary intervention group, 16 deaths as opposed to 24, this was not statistically significant.

Although all these trials aimed to generally increase the amount of polyunsaturated fat in the diet, the principle polyunsaturated fat in these diets was linoleic acid. The potential reason for the effects observed in these trials could possibly have been due to the

linoleic acid per se in the diet, or as alluded to above, the antioxidant intake associated with this. The use of commercially hardened vegetable oils in some of these trials and the presence of *trans* isomers of linoleic acid being introduced in these diets may have introduced another complicating factor, however, as there is some evidence to suggest that consumption of this type of polyunsaturate is associated with an increase in risk of CHD[244].

Conclusions

This population case control study has shown that there is an inverse relation between adipose tissue triglyceride linoleic acid and the risk of SCD due to coronary artery disease. Furthermore, this relation is independent of treated hypertension, diabetes and age and is possibly also independent of smoking habit although multivariate analysis excludes this possibility when confirmed smoking is used in the equation. There is a strong relation between adipose tissue linoleic acid and smoking, at least in the British population, from weighed dietary inventory studies and adipose tissue analysis. It is also known that adipose tissue triglyceride levels of linoleic acid are directly related to diet although an independent metabolic effect of smoking cannot be excluded. The confounding effect of smoking is difficult to interpret confidently in this study because of the extent of non response of the nearest surviving relative. The true contribution of smoking may have been under (or over) estimated but from the analysis performed limited to those for whom smoking status is confirmed, smoking is by far the strongest risk factor for being a case. However, as outlined in the discussion, smoking itself may determine food choices and therefore the pattern of fatty acid consumption. The exact mechanism through which smoking exerts its effect is not known, but linoleic acid would not seem to consistently displace it from the equation.

Notwithstanding the influence of smoking, dietary habits leading to the low proportionate consumption of linoleic acid would seem to place individuals at high risk of developing coronary disease and succumbing to sudden death. The order of magnitude of this risk is as great if not greater than that demonstrated for the classically defined risk factors in univariate analysis. The mechanism is not clear although there are a number of plausible biological pathways. Low linoleic acid consumption is probably not a specific risk factor for dying suddenly of coronary disease, but animal evidence would suggest that low levels of polyunsaturated fats in the diet may enhance susceptibility to this mode of death.

These data are in keeping with physiological changes and mechanisms known to be operating in biological models of atheroma development and ischaemic arrhythmias and confirms the relation found between adipose tissue linoleic acid in other population studies.

Recommendations

Further work would be necessary, in animal and single cell models, to elucidate the precise mechanism, or mechanisms, by which linoleic acid seems to confer benefit in terms of CHD morbidity and mortality. It is also possible that linoleic acid itself is a marker of the consumption of some other beneficent species of molecule, or molecules, the antioxidants being one example. To test the hypothesis that linoleic acid per se is the active principle would require a large scale randomised controlled trial. Unfortunately, because of the unstable nature of linoleic acid itself, providing it in the diet without antioxidants may be impossible.

Concerning the possibility that an absolute increase in the amount of linoleic acid consumed may confer benefit, one may only speculate without the data from large trials. Concern has been expressed that altering the balance of $\omega 3$ and $\omega 6$ fatty acids in the diet may have implications for other disease processes such as cancer[245]. One must be cautious about extending these observations to wholesale endorsement of absolute increases in linoleic acid consumption. However, as the linoleic acid in adipose tissue triglycerides is an expression not only of the absolute amount in the diet, but the proportion of this fatty acid as a fraction of all fat in the diet, recommendations about the general reduction of the calories derived from fat in the diet would be upheld by the data from this study.

work myself. I therefore gratefully acknowledge the time and effort put into the preparation and analysis of adipose tissue and serum samples by The Cardiovascular Research Laboratories in Edinburgh University. Dr Rudolf Riemersma gave thoughtful advice and encouragement throughout the study and supervised the laboratory. The time consuming preparation and analysis of samples was performed by Karin Lyall, Jacqui Lawrence and May Walker and I am glad that my working relationship with them has been so good. I really appreciate now how much effort goes into the production of the numbers that epidemiologists like to play with. They also helped me to understand biochemical principles that I should have remembered, or perhaps never really knew!

The General Practitioners in the district willingly reviewed the lists of controls that we regularly sent them and kindly took the trouble to check them. I also gave them extra work by finding several more hypertensive and hypercholesterolaemic men and women about whom they would have been otherwise ignorant. They also kindly allowed me to interview their patients relatives. The staff of the Hampshire Family Practitioner Committee were very helpful in identifying controls and the identity of the cases. I am grateful for the help of these people who, although being busy with routine work, found the time to help us.

Many controls and their wives willingly took time out from their work, often without pay, to attend a clinics that may not have always run to time. They had to put up with the appalling car parking arrangements at the hospital (so they must be superhuman and not really representative of the population as a whole!).

The purpose of medical research is to find new ways of alleviating human suffering. I have met many young widows during the course of this study. They have given me insight into the emotional strength of women. It is sad to have met them in such circumstances. I hope that my work will help to reduce the frequency with which such tragedies occur in the future. I am indebted to their kindness in allowing me to interview them.

Throughout the time that I have spent reading about this project, collecting the data, interviewing subjects, and in the preparation and analysis of the data and now the writing up of the findings, I have been supported, in more ways than one, by Professor David Wood. I must thank him, not only for having faith in me to be able to carry through a project that was his entirely in terms of the original idea and protocol, but also for the way in which he has made it a real pleasure to come to work. I have thoroughly enjoyed carrying the task I was set. I hope I have done it justice.

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