A. Stewart Truswell

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The Research on Diet and Coronary Heart Disease 1900–2000

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Foreword

Only once in a great while does a book come along that really does the job in addressing a major medical issue. When this happens, all can be joyful – and also grateful. Stewart Truswell has provided just such a volume about a large, labyrinthine subject: the causal inter-relations of diet, blood lipids, atherosclerosis, and coronary heart disease, the "diet-heart hypothesis." Opening with an accurate and expeditious tour of the early history – the Russian experimental feeding of human diets to rabbits and the crucial recognition of clinical coronary syndromes – the bulk of the book is devoted to description and analysis of the complex permutations of the diet-lipid-disease relationships. Readers will find all their favorite dietary puzzlements dealt with, from longchain fatty acids in oily fishes to cholesterol-elevating cafestol in Norwegian boiled coffee. As for alcohol, the absence of even a mention of resveratrol suggests that Truswell must agree with the quotation he cites from Archie Cochrane: "If wine is ever found to contain a constituent protective against CHD then we consider it almost a sacrilege that this constituent should be isolated. The medicine is already in highly palatable form . . .."

With consummate scholarship, clarity, and brevity, Truswell sifts out the chaff and identifies the critical questions, the responsible investigators, and the key studies. He shows that while the trail of "diet-heart" may be a tortuous one, the actual research has been particularly collaborative, multi-disciplinary, and international. In providing "a series of short accounts of the different parts of this remarkable section of the history of science and medicine," he avoids lengthy, glowing biographies of the key figures, and is thereby able to offer more coverage to the contributions of many others. He gives due credit, of course, to main characters – to Ancel Keys and John Gofman, for example, for the idea that "normal" cholesterol levels in affluent society are not physiologically optimal, and to Joseph Groen for the earliest distinction of animal and vegetable fat effects – and he also notes that it was a less renowned practitioner, Weldon J. Walker, who in 1974 first called attention to the dramatic decline in US coronary disease mortality rates, a trend largely unrecognized by the experts.

Truswell brings a wealth of experience as a pioneer participant, himself, in dietheart research dating from the 1950s, which included among his important findings the low serum cholesterol levels of hunter-gatherers and of children. He sees

research on diet and coronary disease as an "integral part of the larger efforts toward the causes, treatment, and prevention of coronary disease." He also maintains, and he should know, that "it was the most exciting part of nutrition research in the second half of the century." Truswell consummates a thoroughgoing analysis of each issue with a reasoned conclusion. In addressing the reduction in coronary mortality rates in many industrial countries, for example, he rounds out the discussion of plausible mechanisms with the broader judgment that the trend of polyunsaturated fatty acids replacing saturates in the habitual diets of these cultures was not only consistent with, but probably the cause of the decline.

For comparable ease of understanding and engagement by the reader in such a vast complex subject, I am put in mind of Jacques Barzun's review of 500 years of Western civilization: From ***Dawn*** *to Decadence.* Like that impressive historical overview by a wise observer of the larger scene, Truswell provides an intelligent, pellucid summary of diet-heart science. In it he resolves much conflict and provides us a rich perspective.

Minneapolis, MN Henry Blackburn

Preface

The twentieth century was a golden age for research in human nutrition, especially on vitamins and coronary heart disease (CHD). Yet in 1900 there were no departments, research groups or other professionals devoted to its study and practice. Since then, one of the most rewarding areas of research in nutrition has been the discovery that what we eat influences risk of coronary heart disease (CHD) and, by various approaches, working out different components of our food and drink that add to, or reduce the risk. This research has been done by workers in cardiology, epidemiology, pathology, animal experimentation, clinical science, food chemistry, biochemistry, haematology, the pharmaceutical industry and the emerging nutrition scientists. The discoveries depended on development of scientific methods including the ECG, cholesterol measurement, food analysis, the ultracentrifuge, gas chromatography, radio-isotope labelled studies, prospective cohort studies and methods in epidemiology and statistics.

The history of science and medicine is not like classical history, where year dates stand out because of wars, government changes and treaties. The person who first suggests a scientific idea or does a new experiment may not be noticed in the general noise, or dismissed by majority opinion. Sometimes a new concept has been tried for a time, appears to work but is then abandoned and resurrected somewhere else. In the diet-heart history several ideas were actually developing at any particular time, with different momentum and in different places, and there were usually opponents to each new idea. We can't use reigns of kings or terms of presidents to subdivide the time because this is a very international story. To follow the parallel development of different scientific ideas it seems unhelpful to try and produce a series of condensed annual reviews. I have instead described the stories of different topics in short chapters which are placed about where the development started to have serious impact.

This is, to my knowledge, the first broad account of the history of research on diet and coronary heart disease from 1900 to 2000. Sometimes the story here is not directly about nutrition but it is relevant. As the first chapter shows, we can't look at diet and CHD until the disease was being diagnosed and recorded. I have not provided biographies of the main characters. They would be interesting to read, but picking out lead players understates how much was done and contributed by so many of us minor players. Each of my short chapters could be expanded. The

intention here is to provide a series of short accounts of the different parts of this remarkable section of the history of science and medicine.

I know of ten major participants in CHD research who have been fascinated by its history and wrote important essays on different parts of it:

- Leary T (1935) Atherosclerosis, the important form of arteriosclerosis, a metabolic disease. JAMA, *105*: 475–481 (*Pathology*).
- Dock G (1939) Historical notes on coronary occlusion: from Heberden to Osler. JAMA, *113*: 563–568 (*much on 19th century*).
- Snapper I (1953) Diet and atherosclerosis: trust and fiction. Am J Cardiol, *11*: 283–289 (*quotes de Langen; brings in unsaturated fatty acids*).
- Dock W (1958) Research in atherosclerosis – the first fifty years. Ann Internal Med, *49*: 699–705 (*in praise of early Russian work*).
- Oliver MF (1987) Dietary fat and coronary heart disease. Br Heart J, *58*: 423–428 (*British view*).
- Gordon T (1988) The diet-heart era. Outline of a history. Am J Epidemiol, *127*: 220–225 (*Keys vs. Gofman; slow to believe HDL*).
- Goodman DWS (1989) Cholesterol revisited. Molecule, medicine and media. Arteriosclerosis, *9*: 430–438 (*The cholesterol saga illustrates well the important links between cholesterol and medicine*).
- Steinberg D (1989) The cholesterol controversy is over. Why did it take so long? Circulation, *80*: 1070–1078.
- Epstein FH (1996) Cardiovascular disease epidemiology. A journey from the past into the future. Circulation, *93*: 1755–1764. (*History of development of this leading branch of epidemiology*).
- Steinberg D & Gotto AM (1999) Preventing coronary artery disease by lowering cholesterol levels. Fifty years from bench to bedside. JAMA, *282*: 2043–2050 (*The cholesterol story 1948–1998*).
- Connor WE (1999) Diet-heart research in the first part of the 20th century. Acta Cardiol, *54*: 135–139 (*Connor selects 14 key experiments: Ignatowski to Kinsell*).

Sydney, Australia A. Stewart Truswell

Acknowledgments

In 1963 and 1964 I was a full time researcher on coronary heart disease (CHD) at the MRC Atheroma Research Unit in Glasgow, directed by Dr Brian Bronte-Stewart. The unit closed when he died (of cancer) aged 39. After that I was an academic general physician in Cape Town, then professor of human nutrition first in London University, then at Sydney University.

Throughout these careers diet and CHD has been my continuing research interest. At least 25 colleagues co-authored 100 publications with me and there were others on major committees I was privileged to work with. All these people have shared ideas with me, which must be behind what appears in this book. I first lectured on diet and CHD in 1963 and have done so regularly ever since. The contents changed every year and a single lecture is no longer enough.

I was fortunate to be trained in writing by the apprenticeship system as junior author with the late Dr Reg Passmore of Edinburgh. Together we wrote two editions of the major European textbook "Human Nutrition and Dietetics". I took the place of Sir Stanley Davidson who had retired. These were not multi-author books; the two of us wrote all 756 and 641 pages on every aspect of the subject.

I want also to record my thanks to the late Professor Jack Brock of Cape Town who gave me my first opportunity to do research and supervised my doctoral thesis. He was the best head of a large Department of Medicine I have even seen.

This book was typed by Marianne Alexander. She worked through two or more drafts and sometimes improved the wording. I received a grant to help with preparing it from the Australian Nutrition Trust (Chairman Professor Paul Nestel). Flavia Fayet checked the chapters as they were written. Jennifer McAthur did fine work scanning the figures. Theodora Sideratou and Kamrul Zaman helped at proof stage.

When the first draft was finished, five specialist colleagues read it and provided comments:

Professor Jim Mann, University of Otago, New Zealand
Dr Masoud Mirzaei, Health Policy Unit, University of Sydney
Professor Paul Nestel, Baker Medical Research Institute, Melbourne
Associate Professor Samir Samman, Human Nutrition Unit, University of Sydney
and Dr Christopher Sempos, Office of Dietary Supplements, National Institutes of Health, Bethesda, MD.

I am very grateful for their support and advice. Each suggested one or two additions that have gone in, and I realised that Salt deserves a place – it has become the longest chapter of the book.

Sydney, Australia A. Stewart Truswell
October 2009

Contents

Chapter 1
Emergence of Coronary Heart Disease as a Diagnosis

Angina pectoris had been described in the eighteenth century. In 1900 there were still different opinions among authorities about its cause, though Osler thought it was usually associated with coronary artery disease.

"Coronary thrombosis" was only diagnosed at post mortem until individual living cases were described in 1910 and 1912. The new electrocardiogram was used to support the diagnosis in 1919. The first 100 cases of "cardiac infarction and coronary thrombosis" was published in the Lancet in 1928; "disease of the coronary arteries" appeared in international mortality statistics soon after. Years 1900–1928.

Most of the important diseases have been recognised for centuries, at least since the nineteenth century but "coronary disease" and "coronary thrombosis" were not diagnoses that any clinical doctor made in the first 10 years of the twentieth century – and most not for the first 20 years. Thrombosis and coronary narrowing and myocardial degeneration were known to pathologists and angina pectoris was known to clinicians, but clinical and pathological correlation was missing. None of the modern objective tests of cardiac function were available: electrocardiograms, biochemical changes, echo cardiographs, coronary angiograms. Diagnosis depended solely on the physician's experience and judgement. Different schools of thought attributed angina to cramp of the heart muscle or coronary artery narrowing or (according to Sir Clifford Allbutt) aortic pathology or to neuralgia of cardiac nerves or to a psychoneurosis.

1.1 In Osler's Textbook (1904)

The leading textbook of medicine on both sides of the Atlantic in the first 15 years of the century was written by Dr William Osler [1], who was head of the Department of Medicine at Johns Hopkins Hospital Medical School and later became Regius Professor of Medicine at Oxford University as Sir William Osler. He wrote about what we would now call coronary heart disease in the 1904 edition of his textbook, in three places.

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Angina Pectoris (p. 761 in Osler) is described in the section Neuroses of the Heart: a rare disease occurring almost exclusively in men "characterized by paroxysms of agonizing pain in the region of the heart, extending into the arms and neck . . ." Gout, diabetes and syphilis are important factors. Attacks come on during exertion most frequently. "Mental emotion is the second very potent cause . . . The paroxysms last from several seconds to a minute or two during which, in severe attacks, the patient feels as if death were imminent". Death may occur suddenly with an attack, or in angina subjects without pain . . .. "Almost without exception the subjects of true angina have arterio-sclerosis . . . with changes in the coronary arteries". Elsewhere (p. 763) "Extreme sclerosis of the coronary arteries is common, and a large majority of the cases present no symptoms of angina. Even in the cases of sudden death due to blocking of . . . the anterior branch of the coronary artery, there is usually no great pain either before or during the attack". There was also *Pseudo-Angina Pectoris*, most common in women, associated with nervous symptoms, pain lasts one to two hours, never fatal (p. 764).

Osler also wrote a section on Affections of the Myocardium [1] Lesions due to Disease of the Coronary Arteries (pp. 746–747). The account of anaemic necrosis or *white infarct* describes the pathology and is not clinical. "Sudden death not infrequently follows the blocking of one of the branches of the coronary artery . . .. In medico-legal cases it is a point of primary importance to remember that this is one of the common cases of sudden death".

In another section, Osler dealt with *arteriosclerosis* under Diseases of the Arteries (p. 770). Among the circumstances listed as bad use of good vessels is Overeating. "I am more and more impressed with the part played by overeating in inducing arteriosclerosis. There are many cases in which there is no other factor". People over the age of 50 should reduce their aliment, and reduce it further gradually and sensibly every 7 years (one of George Cheyne's aphorisms).

1.2 First Reports of Coronary Thrombosis with Survival

It was not until 1912 that the first two cases of acute coronary artery obstruction with survival (52 h; 7 days) were reported in the English language by JB Herrick in Chicago [2]. One case was confirmed by necropsy which showed a recent thrombus completely obstructing the left coronary artery, with infarcted areas in the left ventricle and overlying pericarditis. Two years earlier Obrastzow and Straschesko (in Kiev) had reported cases in a German medical journal [3] and Osler in his 1910 Lumleian lecture [4] appears to have described such a case. There was very little reaction in the literature to Herrick's first paper – the First World War was priority.

Herrick published again in 1919. In the meantime the electrocardiogram (ECG) had been invented by Einthoven in 1901 and FM Smith (Herrick's colleague) investigated changes in dogs' ECGs after coronary artery ligation in 1918 [5]. One of the 3 cases in Herrick's 1919 paper [6] lived 5 months after his first attack and his ECG (the first taken after a myocardial infarction Fig. 1.1) showed striking

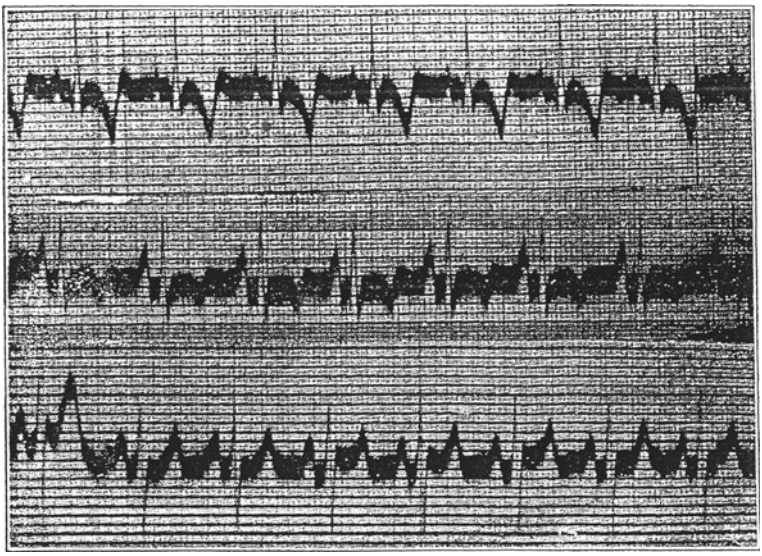

Fig. 1.1 The first electrocardiogram (ECG) of a patient in 1919 who survived thrombosis of the left coronary artery (later demonstrated at autopsy). Top tracing is lead 1, which shows steeply inverted T waves. On the same page of the journal an ECG was reproduced from a dog whose coronary artery was ligated, showing remarkably similar ECG changes. From Herrick [6]

downward sloping ST and sharply inverted T waves, very similar to a pattern seen in dogs' ECGs by Dr Smith. Herrick concluded: "This work needs confirmation as to the regularity of the results obtained and especially as to the interpretations and conclusions. Confirmation from necropsies is particularly desired. Patients with this condition do not present themselves very often. A large proportion with coronary obstruction die a sudden death or are too ill to come to the office or a hospital where they can have electrocardiographic tracings taken . . .."

1.3 Papers Appear in British Journals

The first papers on coronary thrombosis in British journals appeared in 1925, reporting a few cases. The classic paper by Parkinson and Bedford in 1928 described 100 cases seen in London hospitals [7]. It has a memorable opening: "When a man of advancing years is seized while at rest with severe pain across the sternum which continues for hours and which is accompanied by shock, collapse and dyspnoea he has an anginal attack of no ordinary kind. It is only reasonable to suppose that something definite and material has happened in the heart, and investigation is actually proving that such attacks are the results of an acute infarction of the heart muscle from coronary occlusion".

Delays in recognizing coronary heart disease clinically were later discussed by Dr Bedford in his 1968 Harveian lecture [8]. In the early twentieth century Sir Clifford Albutt, professor of medicine at Cambridge University had strongly opposed the coronary theory of anginal pain. Bedford recalls "As a student I had never heard of coronary thrombosis and did not make my first clinical diagnosis until 1924 in a case admitted to the surgical ward at the Middlesex Hospital as an acute abdomen, as often happened in those days. Working at the London Hospital with Parkinson in 1926, I watched the epidemic spreading round the neighbourhood as local practitioners began to recognize coronary thrombosis, and by 1930 it had become a common and familiar illness".

1.4 Added to International List of Causes of Death (1930)

In 1930 "Disease of the coronary arteries" was added to angina pectoris in the 4th revision of the International List of Causes of Death (predecessor of the International Classification of Disease) [9]. From then on epidemiologists could follow CHD mortality statistics over time and compare differences between countries. These changes and differences in coronary death rates have provided important clues and hypotheses in research on diet and CHD.

Chapter 2
Experimental Pathology in St. Petersburg

In Russia, at the Royal Military Academy in St. Petersburg mixed animal foods fed to rabbits produced aortic lesions (1908). Stuckey (1910, 1912) found egg yolk and brain produced the strongest aortic changes. Anitschkow had the idea that the cholesterol in these feeds might be responsible. He demonstrated (from 1913) that high doses of cholesterol in sunflower oil produced arterial lesions containing lipid droplets in the suboptimal layer that in polarised light appeared to be cholesterol ester. Anitschkow described their experiments in more detail in English, after the Russian revolution (in 1933). Coronary arteries also showed these lesions, but they could not be produced in other animals such as rats. Though several other pathologists conformed these findings, they were largely ignored and not thought applicable to humans.
Years 1908–1913 (1933).

2.1 Atherosclerosis Introduced as a Pathological Term

Early in the century developments took place in pathology and experimental pathology. In 1904, at a congress in Leipzig, Marchand [10] first proposed the term *atherosclerosis* which combined atheromatous changes under the intima with degenerative changes in the media of arteries. Windaus in 1910 undertook analyses for the pathologist L. Aschoff and found that atheromatous aortas contained 6 times more free cholesterol than normal arteries and over 20 times more cholesterol ester [11].

2.2 Pioneering Experimental Work 1908–1913 Feeding Cholesterol to Rabbits in St. Petersburg

Meanwhile in Russia, at the Pathology Institute of the Royal Military Medical Academy in St. Petersburg, Ignatowski [12] fed animal foods, meat, milk and eggs to rabbits in long-term experiments. The animals developed nodular lesions of the aorta. His investigations were initiated by order of the Tzar [13]. Ignatowski thought the lesions were due to the toxic influence of foreign proteins in the diet.

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Stuckey [14] did similar experiments but could not produce lesions with a diet very rich in animal protein, egg white and meat juice. He examined different foodstuffs and found that egg yolk and brain caused the strongest aortic changes [15], but pure neutral fats, animal or vegetable did not produce these changes.

Nicolai Anitschkow [16] in the same pathology institute at St. Petersburg's Royal Military Medical Academy had the idea that the substance in those feeds that produced lesions in the rabbit's aortas might be cholesterol. Cholesterol had been demonstrated in human atherosclerosis by Aschoff and Windaus; egg yolk and brain are rich in cholesterol. Pure cholesterol was available (from Merck) for feeding experiments. With Chalatow he fed rabbits cholesterol dissolved in sunflower seed oil by stomach tube daily for 10, 25, 26, 79, 81 and 139 days. The total cumulative dose of cholesterol administered ranged, according to duration, from 3 to 82 g.

Fatty changes were produced in the subintimal layer of the aorta. They increased with longer duration of cholesterol feeding, i.e., there was a dose response. They resembled the earlier changes in rabbits fed egg yolk and brain. Microscopically there were small fat-like droplets in macrophages. The droplets were confined to the luminal side of the internal elastic lamina. They stained yellowish-red with Sudan III. In polarised light the droplets were doubly refractive and displayed glittering cross figures (typical for cholesterol esters). The lipid droplets appeared first, before there were cellular changes, i.e., the process seemed to be infiltrative rather than degenerative. The anisotropic fatty material also accumulated in other parts of the rabbits' bodies – liver and kidneys.

Anitschkow then fed white rats with egg yolk in milk for different periods, up to 5 months but no macroscopic or microscopic change could be found in the aortas. So to produce the "dietetic atherosclerosis" the kind of animal was important, as well as the chemical constitution of the feed.

2.3 Anitschkow Described His Work Further in 1933 American Monograph

Anitschkow continued working through the Russian revolution, publishing in German, though there was a gap in his publications between 1916 and 1921. In 1933 he wrote a major 50 page chapter (in fluent English) on Experimental Arteriosclerosis in Animals in Cowdry's book on Arteriosclerosis [17]. He was by this stage Professor of Histology at the University of Leningrad. This chapter records his continued experiments and the development of his ideas. It has valuable pictures of the rabbits' aortas (naked eye and histological) (Fig. 2.1) even of their narrowed coronary arteries.

Despite the title, the chapter is all about atherosclerosis. Anitschkow asserted that the only way to achieve an animal model of atherosclerosis is with cholesterol feeding of some species: rabbits and also guinea pigs. The cholesterol doesn't have to be given by stomach tube. Stuckey first showed in 1910 that the same lipoid changes were seen after rabbits were given egg yolk in milk along with their usual food. Coronary arteries are also affected [18]: one picture in the chapter shows an early

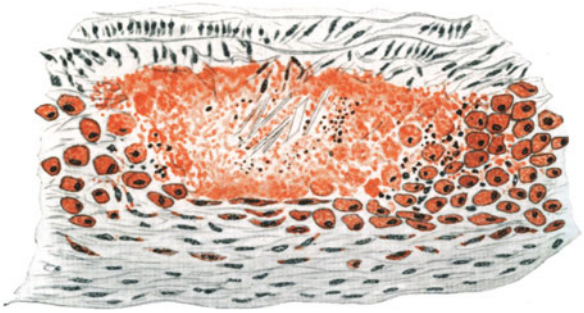

Fig. 2.1 Portion of an atherosclerotic plaque in the aortic arch of a rabbit fed with cholesterol for 124 days and then kept alive 101 days longer without further cholesterol feeding. The centre shows an atheromatous focus with lipoid masses, cholesterol crystals and scattered calcium granules. Groups of large lipoidal cells are to be seen on either side. The *upper* part shows a broad fibrous layer between the atheromatous focus and the lumen. (Stained with Sudan III, highly magnified). From Anitschkow [17]

stage of involvement, another shows severe narrowing of a rabbit's left coronary artery by a fibrous atheromatous plaque after 122 days cholesterol feeding followed by 1,000 more days without cholesterol [18]. Regression occurs if animals are kept alive after cholesterol feeding; lipids slowly disappear, fibrous tissue remains. The lipid goes faster in internal organs than in the arteries.

2.4 Anitschkow's Early Rabbit Work Confirmed in Other Countries

Anitschkow's experimental atherosclerosis was repeated and confirmed, by Wacker and Heuck [19], Kon (1913), Aschoff (1914), Waristscheff (1914), Bailey (1915), Bailey [20], McMeans and Klotz (1916), Schönheimer (1924) and others later [all referenced in Anitschkow's Chapter [17]].

2.5 Rabbits' Arterial Lesions Compared with Human Atherosclerosis

Anitschkow reasoned that cholesterol in the arterial walls must come from the blood in the lumen. These rabbits have greatly raised blood cholesterols. There are no histological features of cellular abnormality in the early stages. This experimental atherosclerosis differs from the usual human form in that there is more lipid in the rabbits' arteries, more lipoid changes in other organs (yet the cerebral arteries are not affected) and the plasma cholesterol is higher. But if the rabbits are fed with smaller amounts of cholesterol for, say, 2 1/2 years the arterial lesions look more like human atherosclerosis and lipid deposits are less in internal organs. He suggested that in human atherosclerosis there may be a primary disturbance of cholesterol

metabolism. “Atherosclerosis belongs among the metabolic diseases and cholesterol is the ‘materia peccans’”.

Box 2.1 Leary repeat’s Anitschkow’s work in Boston (1934)

T. Leary, a Boston pathologist (who had read Cowdry’s Survey [17]) repeated Anitschkow’s experiments, feeding cholesterol in oil to rabbits and chickens [21]. His interest in atherosclerosis arose from post mortem observations that people with alcoholism showed fewer arterial lesions than expected for age. He wrote a major paper in JAMA in 1935 [22], in which he went further than the experimental pathologists and proposed the rabbits’ arterial changes are a model for human atherosclerosis, which he described in detail. The rabbit is normally only exposed to plant sterols (which are poorly absorbed) and has weak mechanisms to respond to cholesterol, but in humans something similar was seen in diabetics on very high fat diets in the 1920–1930 period who had premature atherosclerosis. “Atherosclerosis is a metabolic disease” he wrote, but he was about 20 years ahead of most experts.

Chapter 3
Is Plasma Cholesterol Raised with Human Atherosclerosis?

Atherosclerosis was first described by Marchand in 1904. Windaus and Aschoff found increased cholesterol ester in these lesions (1910), confirmed by Schoenheimer (1926). Comparisons of plasma cholesterol levels in people with atherosclerosis and controls yielded conflicting results until 1948. After this, larger numbers of subjects and statistical methods showed average total cholesterol was higher in CHD patients. In 1950–1955 the association was narrowed to low density or β-lipoprotein cholesterol.

Keys et al. (from 1954) found in Mediterranean countries, Japanese and Africans that serum cholesterols were lower and there was less CHD than in Western countries. It emerged that *average* serum cholesterols in affluent countries might not be *normal* cholesterol levels. Years 1904–1955.

3.1 Cholesterol Characteristic in Human Atherosclerosis

Windaus and Aschoff's finding of increased cholesterol ester in human atherosclerosis (1910) was confirmed by Schönheimer [23] in 1926. Total cholesterol content of the aorta increased with severity of atheroma and the ratio of cholesterol ester to free cholesterol (about 3:1) was the same as in plasma. Schönheimer thought these lipids in the wall came by direct infiltration from blood in the arterial lumen, and it was most improbable that such large amounts could be synthesized inside the aorta. The lipids in aortas have since been chemically analysed in increasing detail by Weinhouse and Hirsch [24], Buck and Rossiter [25], Mead and Gouze [26] and Böttcher and Woodford [27]. The lipid which characteristically accumulates with increasing severity of atheroma is cholesterol ester and its most abundant fatty acid is linoleic, as in cholesterol esters in the plasma.

3.2 At First Not Clear If Plasma Cholesterol Raised with Human Atherosclerosis

In the first half of the century measurements of plasma cholesterol in people with atherosclerosis and controls yielded conflicting results (for a number of

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references see Kritchevsky [28] and Davis et al. [29]). There are several reasons for this. All were case-control studies; numbers of subjects were small and not age-standardised; plasma cholesterol falls for a time after a myocardial infarct; diagnosis of atherosclerosis in some studies was based on palpation of peripheral vessels; in one study blood lipids were obtained post mortem. Familial hypercholesterolaemia has, however, been recognised to be associated with CHD from 1938 [30, 31].

So at the end of the 1940s Paul White, in his textbook Heart Disease [32], wrote on the cause of atheroma "faulty cholesterol metabolism, local arterial strain or overwork, hypertension, infection, allergy, endocrinopathy and heredity are among the many factors suggested, but none has been proved or consistently found". Neither Paul White nor Paul Wood (in the British textbook) [33], though they mentioned lipid substances in the arterial lesions, made the connection between blood cholesterol and CHD, let alone between dietary fat and blood cholesterol or dietary fat and CHD [34].

3.3 Improved Statistical Design Shows Average Plasma Cholesterol Higher with CHD

Between 1948 and 1953 four larger case-control studies were published, all showing clearly higher average serum cholesterols in people with CHD than controls. Ages were now matched in all these studies. Morrison et al. [35] had 200 cases and 66 controls; Gertler et al. [36] had 97 cases and 97 controls; Steiner et al. [37] had 82 cases and 112 controls; Oliver and Boyd [38] in Edinburgh, Scotland had 200 consecutive admissions (men and women) with CHD and 200 miscellaneous convalescent in-patient controls. However, all these reports noted that there was an overlap in plasma cholesterols between CHD cases and controls.

3.4 It Is Low-Density Lipoproteins that Are Associated with CHD

Meanwhile Gofman et al. [39] used the high technology ultracentrifuge to see if they could narrow down a specific lipoprotein class that is particularly associated with CHD. They found in 104 patients with proven myocardial infarction that almost all the men had cholesterol-bearing low-density lipoproteins of the Sf 10–20 class, while only half the controls showed this. Gofman suggested that the controls who carried Sf 10–20 were developing atherosclerosis that would be clinically evident later in life. Oliver and Boyd [40] used the simpler technique of paper electrophoresis to quantify α and β lipoprotein cholesterols in 50 cases and 50 controls. β-lipoprotein (which corresponds to low density lipoprotein) cholesterol was increased in CHD cases and α lipoprotein cholesterol (corresponding to HDL-cholesterol) was lower. Differences between cases and controls were striking, probably because they had deliberately chosen young cases (average age 41 years).

3.5 "Normal" Cholesterol Level in Affluent Countries May Not Be Healthy Cholesterol (See Box 17.1, p. 74)

The third development at about this time was the realisation that levels that had been taken as "normal" plasma cholesterols in affluent western countries may not be optimal or healthy for the arteries of older people.

3.6 Serum Cholesterols Lower in Naples and Madrid . . . and Hunter Gatherers

The leader of enquiries into plasma cholesterols in different countries was Ancel Keys, Director of the Laboratory of Physiological Hygiene at the University of Minnesota. He had earlier completed a large study of the effects on physiology of partial starvation in conscientious objectors [41]. It started in 1944, commissioned by the US government because starvation was anticipated during and after World War II, and it was published in 1950 as a two volume book [42]. At the end of the 1940s his interest turned to the question of coronary heart disease. In 1951–1952 he had a sabbatical year as Senior Fullbright Scholar associated with Dr Hugh Sinclair at Magdalen College, Oxford and he spent time in Naples with Professor Flaminio Fidanza. In his 1952 paper in Voeding [43] he already reported that serum (total) cholesterols in men over 35 years were about the same in England as in Minnesota but they were lower in Naples and even lower in poor workers in Madrid. The bloods were collected by, or in collaboration with Italian and Spanish colleagues and analysed by a well standardised method in Minneapolis. The Naples [44] and Madrid [45] cholesterols were fully published in 1954. Keys brought in collaborators in Cape Town who found big differences in cholesterols between black, Coloured and white people in that part of Africa [46]. He then found other collaborators with whom he measured serum cholesterols in Japan [47] and Croatia [48]. By this stage other researchers and groups had begun to measure serum cholesterols in different communities all over the world. Keys and his collaborators and followers were soon collecting statistics on CHD to compare with serum cholesterol numbers in the different groups and it appeared that some countries had much lower rates than northern Europe and the USA, associated with lower serum cholesterols. Keys maintained that serum total cholesterol was as significant as "the various lipoprotein complexes and giant molecules" measured in the ultracentrifuge [43, 49] (i.e., Gofman's Sf 10–20). It is certainly much more practical [50]; it was and is used to assess large and ever increasing numbers of people all over the world.

A later perspective on how we assess "normal" is that !Kung hunter-gatherers were found to have serum cholesterols around 3.0 mmol/l [51]. Newborn human babies and all other mammals (unless fed cholesterol) have similar or lower levels [52].

Chapter 4
Diet *Can* Have Worthwhile Effects on Human Plasma Cholesterol

The first well designed experiments, feeding cholesterol to humans, by Keys (reported around 1952) showed surprisingly little effect on serum cholesterol, but total dietary fat did raise serum cholesterol. Next, from around 1956 it was found that effects differed with the predominant fatty acids in the dietary fat. When saturated fatty acids dominated, serum cholesterol was increased, while polyunsaturated fats tended to lower serum cholesterol. It was LDL- or β-lipoprotein cholesterol that was increased or decreased by the fatty acid pattern. Further work showed the different effects of individual saturated and unsaturated fatty acids. Cholesterol balance experiments are complicated because of plant sterols in the diet but it appeared that different fatty acids do not change serum cholesterol by altering cholesterol excretion. They probably affect activity of the LDL-receptor in cell membranes (discovered by Brown and Goldstein [89]). Years 1952–1965.

4.1 Dietary Cholesterol

Up to 1950 surprisingly few researchers had examined the effect of changing dietary cholesterol on people's plasma cholesterol. There was little motivation to investigate the effect of diet on human plasma cholesterol until its role as a risk factor for CHD became established. Mjassnikow (1930) found no effect on feeding 8 eggs to 3 healthy subjects for 4 days [53]. Gardner and Gainsborough (1928) in London mostly looked at the effects of single meals containing cholesterol (which were variable). One subject, recovering from colitis and kept in bed was given three diets. Plasma cholesterol was lower after 7 days on a very low cholesterol, also very low fat diet and a little higher than at the start on an ordinary full diet plus calves brain (4 g cholesterol/day) [54]. Okey and Stewart [55] gave three diets, each for a month to four nutrition students. Mean whole blood cholesterols were consistently slightly higher with 4 eggs or liver (3 g/day cholesterol) and probably higher with an equivalent amount of crystalline cholesterol added to butter in 3 of the subjects [55]. The authors also noted that the higher cholesterol intake had very little, if any effect on the subjects' menstrual variation in blood cholesterol.

Ancel Keys looked into diet cholesterol and plasma cholesterol in a more statistical way. From 1947 he and colleagues measured the cholesterol intakes of over 400

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men. There was no difference in the serum cholesterols between those with high and those with low cholesterol intakes [56]. A group of 41 middle-aged men lowered their dietary cholesterol by 50%: their mean serum cholesterol did not change [56]. Later Keys varied the diets of 21 men with schizophrenia in their metabolic ward. Dietary cholesterol had no significant effect on serum cholesterol, but changes in (total) fat intakes had a substantial effect [43].

4.2 Dietary Fat Has More Effect than Dietary Cholesterol

So Keys reached the counter-intuitive conclusion "there can be little doubt that, other things being equal, the serum cholesterol level is markedly influenced by the proportion of calories supplied by fats in the diet, that vegetable as well as animal fats have this effect, and that the dietary cholesterol itself is unimportant at all levels of intake practicable with natural foods [43]". This effect of fat intake on serum cholesterol had been noticed on Kempner's rice-fruit diet for treatment of hypertension (very low in fat) [57, 58]. It was confirmed by Mellinkoff et al. [59], by Hildreth et al. [60] and by Mayer et al. [61].

4.3 And the Type of Dietary Fat

A third important effect of diet on plasma cholesterol was discovered soon after. Groen et al. in the Netherlands in a feeding trial with 60 men found a decrease in plasma cholesterol when a diet high in vegetable fat was substituted for one high in animal fat [62]. Ahrens et al. [63] found the same. Kinsell and Michaels [64] noted the same effect. They used soy, corn and peanut oils but added an addendum graph that shows in one subject an increase of plasma cholesterol when the fat was coconut oil (with a low iodine value[1]). Ahrens et al. fed six different fats or oils and found that the serum cholesterol was lower with corn oil than on the same intake of olive oil, coconut oil or lard [65].

4.3.1 Saturated and Polyunsaturated Fats, Rather than Animal or Vegetable Fats

Bronte-Stewart et al. in Cape Town tested the effect of some 13 different fats and oils in a small number of volunteer subjects in a metabolic ward [66]. Each served

[1] A measure of the average net unsaturation of the fatty acids, used before gas chromatography became generally available. Unsaturated bonds take up iodine; a high iodine value indicates more unsaturated fatty acids.

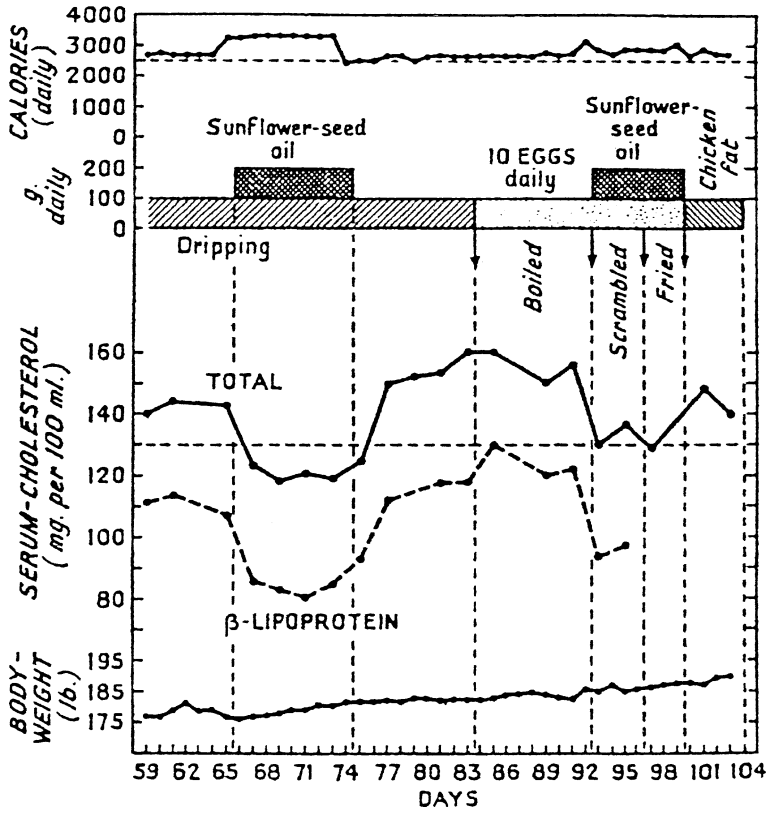

Fig. 4.1 One of the first published individual subject's sequence of serum cholesterols as he was fed different fats. Serum cholesterol fell (due to fall of β-lipoprotein cholesterol) when (polyunsaturated) sunflower seed oil was added to the diet. From Bronte-Stewart et al. [66].

as his own control over many weeks while different fats were given for about a week, with brief rest periods between. Total cholesterol, β-lipoprotein cholesterol and body weight were monitored repeatedly. Serum cholesterol went up on butter, down with sunflower seed oil (Fig. 4.1). Olive oil had an intermediate effect. Pilchard and seal oil, though animal, did not raise the serum cholesterol but hydrogenated ground-nut fat did raise it. The authors suggested that "a possible common difference between animal fats and hydrogenated vegetable fat on the one hand and natural vegetable and marine oils on the other is not the cholesterol, sitosterol, vitamin or protein content but is in some way connected with the proportion of highly unsaturated and saturated fatty acids in the fat concerned [66]". The eight graphs in the paper all show the β-lipoprotein cholesterols going up and down in parallel with the total cholesterol, i.e., the effect of type of fat/oil on total cholesterol was because of changes in β-lipoprotein cholesterol (LDL-cholesterol).

4.3.2 Repeated Tests by Keys' Group with Different Fats and Oils

These findings were promptly confirmed by a small British study with sunflower seed oil [67], and a comprehensive study with 15 different animal and vegetable fats/oils by Ahrens et al. [68] in New York and by Malmros and Wigand in Sweden [69]. In 1957 came the first reports of the trials of different fats by Keys' group in the Metabolic Research Unit of the Hastings State Hospital, Minnesota. The subjects were men with chronic schizophrenia, assessed to be physically and metabolically normal and selected for the trials as being co-operative and relatively stable emotionally. They were under 24 h surveillance and all their meals were prepared in the special diet kitchen. The first paper [70] reports trials with butterfat, lard, olive, cottonseed, corn, sunflower seed, coconut and sardine oils. There were 12–27 men in each of four series of experiments, C, F, H & J. In experiment C the diets, in random order were low fat, olive oil, cottonseed oil; in F they were low fat, cottonseed oil and butterfat; in H low fat, cottonseed oil, corn oil, hydrogenated coconut oil; in J they were low fat, butter, olive, corn and sunflower oils and sardine oils. Volunteer aides from a neighbouring church lived with the men and supported the professional staff during the 4 years 1952–1956. Based on these extensive experiments Keys proposed a first version of his classic equation:

$$\Delta \text{ cholesterol} = 2.74\Delta\text{sat} - 1.31\Delta\text{poly}$$

where the change (Δ) in serum cholesterol is in mg/100 ml and changes in fatty acid classes are expressed as changes in percent of total calories [71].

Box 4.1 De Langen in Dutch East Indies 1916–1922

CD De Langen was a Dutch doctor, working in the Public Health Service of the Dutch East Indies. He was probably the first (1916) to report a relationship between usual diet, serum cholesterol and CHD. He observed that native Javanese did not experience the CHD and atherosclerosis that he saw in the Dutch immigrants. He also noticed their very different diets. The Javanese ate a largely vegetarian diet, based on rice. De Langen measured serum cholesterols; they were much lower in the Javanese, but Javanese stewards who worked on Dutch passenger ships had serum cholesterols nearer to the European levels [72].

De Langen went on to do apparently the first human feeding experiment of diet and serum cholesterol with a clear result. He fed 5 Javanese subjects a diet rich in eggs, butter and meat for 3 months. Their mean serum cholesterol rose from 128 to 168 mg/100 ml, increasing in all 5 subjects [73]. Connor, who later drew attention to this work, made a table of this with statistical analysis [74].

De Langen incidentally noted that gallstones were rare in Javanese. Their bile had a low cholesterol content [73], again different from the Europeans.

His papers in Dutch and in a tropical medicine journal were not noticed by the mainstream till the 1960s [75].

4.3.3 Not All Saturated Fatty Acids Have the Same Effect on Serum Cholesterol

The second large series of human metabolic studies of the effects of different fats was carried out by Hegsted et al., at Harvard [76], also in subjects with chronic schizophrenia, housed in a special isolation ward with its own metabolic kitchen. Ten men participated and each test period lasted 4 weeks. The test fats were similar to those used by Keys' group, with the addition of safflower oil and cocoa butter. From the regression equations it emerged that myristic acid (14:0), though never in high proportion, was the most potent cholesterol-elevator. This was later confirmed by Zock et al. [77].

Meanwhile Keys' group analysed their own and other researchers' human experiment results and concluded that stearic acid (18:0), as well as saturated fatty acids containing fewer than 12 carbon atoms have little or no effect on serum cholesterol [78]. This was later confirmed by Bonanome and Grundy [79]. Stearic acid can be converted to oleic (18:1) in the body [79].

Thus the effects of fats or oils on human serum cholesterol in 1955 appeared to be related to the iodine number, or its square root. Two years later, the equation 2 sat – 1 poly seemed to fit better. By 1965 it was becoming clear that only or mostly lauric + myristic + palmitic were the saturated fatty acids involved in raising serum cholesterol. Hugh Sinclair [80] suggested that the cholesterol-lowering effect of polyunsaturated fatty acids is correction of essential fatty acid (EFA) deficiency. But the vast majority of people whose cholesterol falls on polyunsaturated fat diets show no signs of EFA deficiency and highly unsaturated fish oil, low in EFA lowers serum cholesterol as effectively as polyunsaturated plant seed oils [81, 82].

Box 4.2 Contribution of Better Methods

In food fats the method of gas-liquid chromatography, invented around 1950 [83] and soon widely used [84] was an improvement over the iodine number, which did not indicate individual fatty acids. The older chemical methods of analysing fatty acids were slow and cumbersome and could not easily be

used to check the fatty acid pattern of fats used in experiments or sampled in epidemiological studies.

For the thousands of serum cholesterols measured in the 1950s the introduction of the "Abell Kendall" method [85] was simpler and quicker than previous methods and it was reliable. All the epidemiological studies co-ordinated by Ancel Keys used this method.

4.4 How Does It Work? Could It Be by Changing Cholesterol Excretion?

By what mechanism(s) do saturated fatty acids raise and (poly)unsaturated fatty acids lower plasma LDL cholesterol concentration? From 1957 papers appeared from many laboratories now interested in cholesterol, reporting sterol balances when their subjects changed between e.g., butter and safflower oil as their main dietary fat. At first more of the papers reported increased faecal bile acids and/or neutral sterols when dietary fat was polyunsaturated and serum cholesterol lower [86]. Methods were relatively simple. It was realised later that phytosterols eaten in plant oils (which pass to the faeces as neutral steroids) confuse the picture and that individual faecal bile acids and sterols have to be separated chromatographically and then quantified. The most thorough work was published by Spritz et al. [87]. The methods take 4 pages of fine print in their paper. Only 5 subjects were studied in the metabolic unit at Rockefeller University for 3–6 months. They showed no correlation between changes in serum cholesterol and excretion of bile acids and sterols in the faeces.

Increased cholesterol excretion was thus, at best inconsistent. Decreased cholesterol absorption was ruled out by all who measured it. This leaves altered cholesterol biosynthesis or a shift of cholesterol from plasma to other tissues. Animal experiments support the latter.

Spritz and Mishkel [88] suggested the hypothesis that since unsaturated fatty acids with their bent molecules occupy a greater area than saturated acids they alter the spatial configuration of the lipids so fewer lipid molecules can be accommodated by the apoprotein of LDL.

4.5 Discovery of the Low Density Lipoprotein Receptor

The probable answer came after, when Brown and Goldstein discovered the receptor for plasma LDL in cell membranes [89] (for which they were awarded the 1985 Nobel prize for Medicine). If triglycerides containing myristate or palmitate are fed, LDL-receptor activity is suppressed and less plasma LDL-cholesterol is taken up by

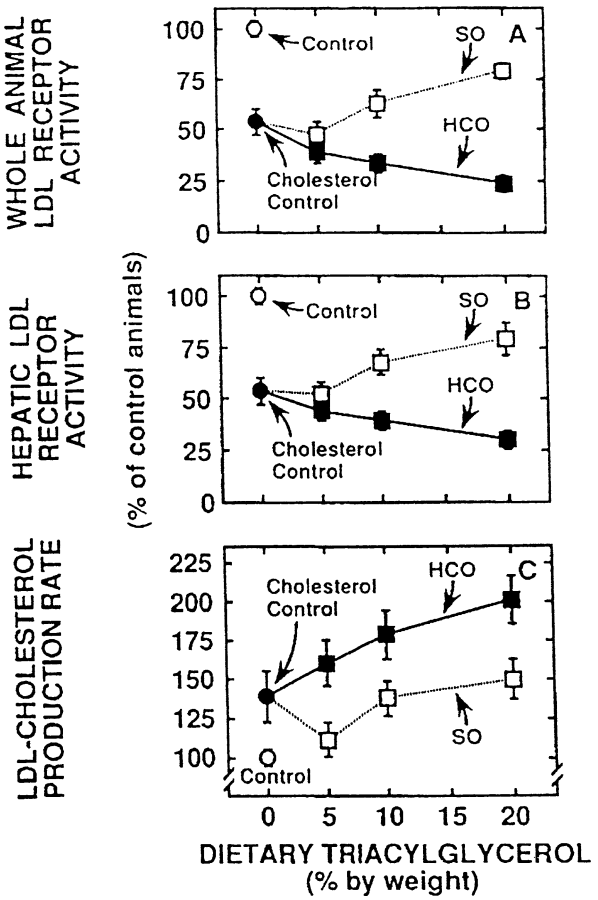

Fig. 4.2 LDL-receptor activity in hamsters (at constant cholesterol intake) with increasing intakes of saturated triglyceride (hydrogenated coconut oil, HCO) or polyunsaturated triglyceride (safflower oil, SO). Whole animal activity (*top*) and hepatic activity (*middle*) are suppressed by saturated triglycerides and (independently) stimulated by polyunsaturated fat in the diet. From Woollett LA, Spady DK & Dietschy JM (1992) Saturated and unsaturated fatty acids independently regulate low density lipoprotein receptor activity and production rate. J Lipid Res, *33*: 77–88

the cells [90]. Triglycerides with predominantly unsaturated fatty acids have the opposite effect. Saturated fatty acids reduce the size of the intra-cellular cholesterol ester pool; unsaturated fats increase it. The size of this pool appears to regulate activity of the hepatic LDL receptor [90] (Fig. 4.2).

Chapter 5
A New Type of Observational Epidemiology

Around 1950 a new type of epidemiological study was invented, the *prospective* or cohort study, in which people at moderate risk of a disease are examined and answer questions about lifestyle. They are then observed for 5, 10 or more years. Incidence of disease can then be related back to aspects of lifestyle (including diet) and to findings at the initial medical examination. Those who develop the disease of interest had been questioned and examined in exactly the same way as those who do not. The first prospective study of CHD was conducted in the town of Framingham, Massachusetts, USA. Here, and in other prospective studies that followed, the big three factors predicting risk of CHD have been raised serum cholesterol, blood pressure and cigarette smoking. Years 1949–1968.

5.1 Invention of Prospective, Cohort Studies

Doll and Bradford Hill [91] wrote "Further retrospective studies of that same kind would seem to us unlikely to advance our knowledge materially or to throw any new light upon the nature of the association. If, too, there were any undetected flaw in the evidence that such studies have produced, it would be exposed only by some entirely new approach. That approach we consider should be *prospective* (according to the Oxford English Dictionary: Characterized by looking forward into the future. 'He was a retrospective rather than a prospective man'). It should determine the frequency with which the disease appeared, in the future, among groups of persons whose smoking habits were already known". This appears in the introduction to one of the two first big prospective studies. This one, on the relation of smoking to the mortality of British doctors started in 1951.

5.1.1 The Framingham Study, Started 1949

The other pioneer prospective study, investigating antecedents of CHD was started in 1949, in the town of Framingham, Massachusetts, about 30 km west of Boston but a separate community with a population then of 28,000 [92]. TR Dawber was the

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first director. Randomly selected men and women aged 30–60 years were carefully examined and those 5,127 then free of CHD were regularly followed up at the Study Centre maintained in the town and kept under close medical surveillance. Despite occasional threats to the federal funding the subjects were followed up for over 30 years and the study included risk factors for stroke and even extended to offspring of the original Framingham cohort and their spouses [93].

Prospective studies are more expensive than case-control studies and they take at least 5 years, preferably longer before they produce results. They have to be set up in communities that have a relatively high incidence of the disease in question. Large numbers of subjects are needed for the cohort. The community should be stable and, of course, cooperative and supportive. The US National Heart Institute thought that such a long time study would be easier to run in a single community and that cooperation and coverage would be better in a small town than a large city. Massachusetts State Health offered cooperation and Framingham had been the site of the first community study of tuberculosis, which ran successfully from 1917 to 1923 [92].

5.2 Results Emerge from Framingham and Subsequent Cohort Studies

The term "risk factor" first appeared in a publication of early Framingham results [94, 95]. The big three risk factors for CHD emerged early and have since been confirmed in many subsequent prospective studies (Table 5.1): a high serum (total) cholesterol, a high blood pressure and cigarette smoking (the smoking risk was already reported in Doll and Bradford Hill's preliminary report [91]).

5.2.1 Big Three Risk Factors: Serum Cholesterol, Smoking and Blood Pressure

After 10 years of follow up the Framingham investigators [96] could see that higher cholesterol, higher blood pressure and smoking each compounded the risk. If one was abnormal the risk doubled, with two abnormal it was increased 3.5 times. If all three were abnormal risk was increased about tenfold.

This demonstration that a raised serum cholesterol could significantly predict a CHD event in Framingham and almost all the subsequent prospective studies (Table 5.1) gave strong support to the cholesterol hypothesis.

The largest cohort study that reported in 1986 serum (total) cholesterol was the 6 year mortality data from the 361,662 men screened for the Multiple Risk Factor [97]. Examinations were made over 1973–1975. There were seventy times more subjects here than in the Framingham cohort. Mortality increased very gradually at first between serum cholesterols 140 and around 200 mg/dl and then more steeply (Fig. 5.1).

Table 5.1 Early Prospective Studies for CHD started in 1950s and 1960s (or before)

N	Name	Start year	Lead authors	C B S	Other
3,983	Manitoba	1948	Mathewson		BMI
281	Minnesota businessmen	1948	A Keys	C B	
5,127	Framingham, MA	1949–1950	Kannel	C B S	
41,000	British doctors	1951	Doll & Bradford Hill	S	
687	London busmen	1956–1960	JN Morris	C B S	
5,397	Western Electric (Chicago)	1957	O Paul, RB Shekelle	C B S	
1,465	People's Gas, Chicago	1958	Stamler	C B S	
1,858	Tecumseh	1959–1960	Ostrander, Epstein		
11,579	Seven Countries Study	1958–1964	A. Keys et al.	C B	
485	North Bohemia	1959–1964	Reinis	B	
1,712	Italian rural	1960	Menotti	C B S	
3,751	Oslo, Norway	1960	Westlund & Nicolaysen	C B S	
3,154	Western Collaborative	1960	Rosenman & Friedman	C B S	Type A personality
3,486	Stockholm Prospective	1961	Carlson + Bottiger	B S	Triglycerides
10,000	Israeli IHD	1963	Goldbourt	C B S	
1,227	Belgian Bank study	1964/1965	Kornitzer	C B S	
1,648	Finnish Insurance Co	1965–1966	Pelkonen	C	
6,217	Japanese in Honolulu/Hawaii	1965–1968	T. Gordon	C	HDL-c
7,993	Paris Prospective	1967–1972	Richard	C B S	
17,718	Whitehall (London)	1968–1970	Grose & Shipley	C B S	
1,935	Domolo-Tel Aviv study	1969	Brunner	C	

C = raised serum (total cholesterol) a risk factor; B = raised blood pressure a risk factor; S = cigarette smoking a risk factor.

5.3 Tentative Recommendations to High Risk Patients Start in 1960s

During the 1960s heart associations and individual specialists in a few countries, USA, Australia and UK started to suggest low saturated fat, increased polyunsaturated fat diets for people with, or at risk of CHD [98–103].

From 1968 the new findings, epidemiological and dietetic appeared in dietary guidelines for the general population, first with the Nordic Medicinska synpunkter på folkkosten i de Nordiska länderna [104], which included:-

2. Total fat consumption should be reduced from the present around 40% to between 25 and 35% of total calories.

3. The use of saturated fat should be reduced and consumption of polyunsaturated fat should be increased simultaneously . . .

Of the serum lipids measured in the prospective studies: (cholesterol, phospholipids and fractions of lipoproteins by ultracentrifuge), cholesterol appeared to make the most significant independent contribution to CHD risk. At this stage the Framingham authors considered there was "little advantage in obtaining batteries of lipids, or indexes derived from lipids" [96]. No critical or "safe" level of serum cholesterol could be identified, below which there was no risk. There appeared to be a gradient of CHD risk from the lowest to the highest serum cholesterols found in the cohort. Kannel et al. [96] did not however think they could demonstrate cholesterol to be a necessary or sufficient cause of CHD. "The disease must be looked upon as resulting from the interplay of multiple inter-related factors". The Framingham team did not measure serum triglycerides in their early examinations.

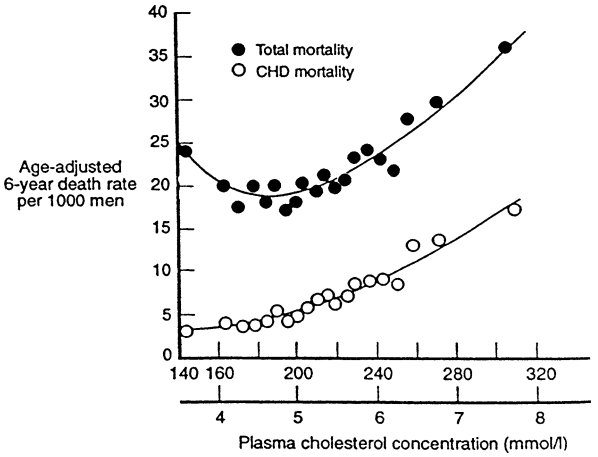

Fig. 5.1 Cohort study in 361,662 US men, followed for 6 years, showing relation between plasma cholesterol and subsequent CHD and total mortality. The increased total mortality (only) at the lowest cholesterol concentration is thought to reflect acute and chronic illnesses (which often lower plasma cholesterol). From Martin et al. [97]

Chapter 6
Serum Triglycerides? Another Risk Factor

In addition to serum cholesterol, plasma triglycerides (TAG), measured in fasting morning blood, were claimed first by Albrink et al. [106] from a case-control (not prospective) study to be a better risk factor for CHD. Carlson's Stockholm prospective (1972, 1980) also reported that serum fasting triglycerides showed as a risk factor. (They had not been measured in the original Framingham study.) Serum triglycerides were however, strongly correlated with serum cholesterol. As data accumulated from the numerous prospective studies it became clear that fasting triglycerides are only in the second row of risk factors. Plasma triglycerides correlate with blood glucose and inversely with exercise. They may be an independent risk factor in women. Years 1961–1980.

6.1 Endogenous and Exogenous Serum Triglycerides

Time of day has little effect on serum cholesterol concentration but the serum triglycerides (triacylglycerol, TAG) that were next proposed, in the 1960s, as a risk factor for CHD have to be measured in blood taken before breakfast after an overnight fast. These are endogenous triglycerides, or VLDL, typically induced by excess carbohydrate intake [105].

[There have also been a smaller number of studies investigating whether raised triglycerides after a meal, i.e. chylomicrons, or exogenous triglycerides might be related to CHD.]

6.2 Early Claims that Serum Triglycerides Predict Risk of CHD

Albrink et al. [106] in the USA in a retrospective study of 115 patients who had had a myocardial infarction reported that an increased serum (fasting) triglyceride was more characteristic than increased cholesterol. Carlson, in Sweden [107] found that increased serum triglyceride associated better than cholesterol in patients with myocardial infarction if they were under 50 years of age. But Tibblin and Cramer [108] made serial measurements after a myocardial infarct and observed that while

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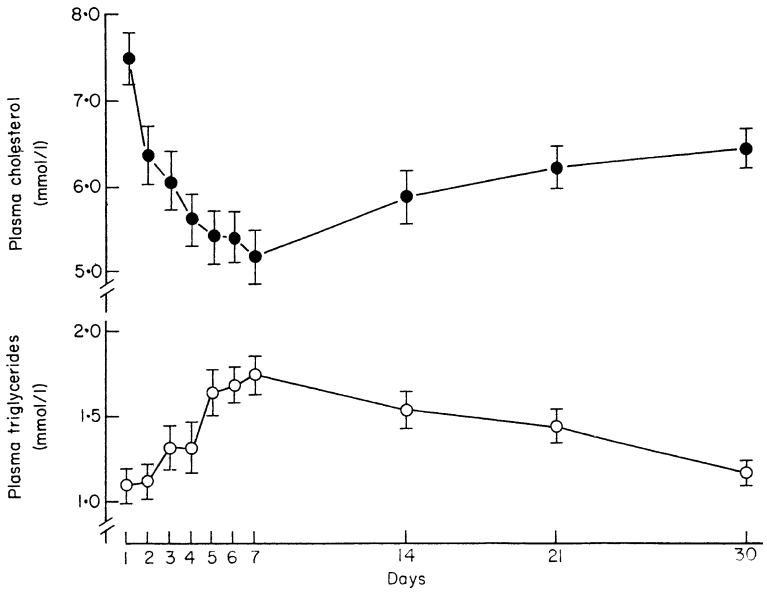

Fig. 6.1 Changes in plasma cholesterol and (fasting) triglycerides over 30 days in 15 patients after a myocardial infarct. Serum cholesterol falls, triglycerides rise. From Avogaro P et al. (1978). Variations in apolipoproteins B & A during the course of myocardial infarction. Eur J Clin Invest, *8*: 121–129

serum cholesterols go down in the first week, triglycerides go up and stay high for at least 3 months. Others have confirmed this phenomenon (Fig. 6.1). So the question was whether serum triglycerides are high prospectively and secondly whether any such association with CHD is independent of other risk factors. The first prospective study to measure and report triglycerides was in Stockholm. Here, raised triglyceride appeared to be a risk factor for CHD events along with, and independent of, raised cholesterol at the 9 year follow up [109]. At the 14.5 year follow up, serum triglycerides were more significantly associated with deaths than cholesterol was [110]. There was strong correlation, however, between cholesterol and triglyceride. These were controversial findings.

6.3 Fasting Serum Triglycerides Not a Strong Independent Risk Factor

Blood was not collected after overnight fast at the start of the first prospective studies (so unsuitable for triglycerides), but in most of the prospective studies reviewed by Hulley et al. in 1980 [111] triglyceride did not hold up as an independent risk factor when there was multivariate adjustment for a full set of major risk variables, including total cholesterol, obesity and high density lipoprotein cholesterol (see Chapter 10). Hulley et al. [111] recommended "that widespread screening and treatment of healthy persons (for raised triglycerides) should be abandoned until more

persuasive evidence becomes available". Since then triglycerides have not been discarded but they are in the second row of CHD risk factors today. They show greater variation between and within individuals than cholesterol, and are reduced by exercise [112]. In Framingham they appeared to be a significant independent CHD risk factor in women [113]. They correlate with blood glucose [114] – and diabetes increases the risk of CHD. They correlate inversely with HDL-cholesterol, and the triad of raised triglycerides, small dense low density lipoproteins and reduced HDL-cholesterol is a common associate of myocardial infarction [115]. As the chapter in Harrison's textbook puts it succinctly: "Elevated cholesterol levels, diabetes and lack of physical activity are important risk factors for CHD in both men and women. Triglyceride levels are an independent risk factor for CHD in women but not in men [116]".

Chapter 7
Fredrickson's Classification of the Hyperlipoproteinaemias

Serum lipids can be raised in association with several diseases, such as hypothyroidism and nephrotic syndrome (secondary hyperlipidaemia). Hypercholesterolaemia or hypertriglyceridaemia may also be primary, probably genetic. Fredrickson (1967) classified and described hyperlipidaemias, based on which classes of lipoprotein were elevated, so hyperlipoprotinaemias. Paper electrophoresis of lipoprotein was added to measurement of serum cholesterol and triglycerides. He described five types of hyperlipoprotinaemias, Types I to V. They require different management. Subsequently (1970) type II (hypercholesterolaemia) was subdivided into IIA or IIB – the latter accompanied by raised triglycerides. In 1972 familial combined hyperlipidaemia was defined. Years 1967–1973.

Donald Fredrickson was Head of the Section on Lipoproteins at the Laboratory of Molecular Diseases of the National Heart Institute, Bethesda, Maryland. He had written many of the chapters in the Lipid section of the great classic "Metabolic Basis of Inherited Disease". In 1967 he re-worked his chapter Familial hyperlipoproteinemia in the 2nd edition of the book [117], joining secondary hyperlipidaemias to the genetic ones and structuring the writing for clinicians [118].

7.1 Five Types of Hyperlipidaemia, and Three Types of Low Serum Lipoproteins

No longer were there only people with increased serum cholesterol, or increased triglycerides, or neither. From now on people with a raised plasma lipid concentration belonged to one of Fredrickson's 5 types. The classification was based on which *lipoprotein class* was increased, as visualised on paper electrophoresis and supported by chemical quantitation of plasma triglycerides and cholesterol. The 5 types were:-

Type 1: increased chylomicrons in fasting serum (triglycerides); rare.
Type II: increased β-lipoprotein, or LDL (cholesterol); common.
Type III: broad β-band (cholesterol and triglycerides); rare.

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Table 7.1 Summarised diet prescriptions for the different types of hyperlipoproteinemia *based* on Fredrickson et al. (1973)

Diet prescription	Type I	Type IIa	Type IIb and III	Type IV	Type V
	LOW FAT 25–35 g/day	Low cholesterol Increased PUF Decreased sat fat	Low cholesterol 40en% fat 40en% CH_2O 20en% protein	Controlled CH_2O Approx 45en% Mod restrict cholesterol	Restrict fat 30en% Control CH_2O 50% Mod restrict cholesterol
Calories	–	–	Reduce weight Priority if above "ideal" weight	Reduce weight Priority if above "ideal" weight	Reduce weight Priority if above "ideal" weight
Fat	Restrict to 25–35 g/day	Limit sat fat Increase PUF	Controlled to 40% PUF substituted for sat fat	Eat considerably less sat fat and more unsaturated fat	Restrict to 30en% PUF preferred
Cholesterol	–	As low as possible Some meat allowed	<300 mg/day Some meat allowed	Moderately restrict 300–500 mg	Moderately restrict 300–500 mg
Carbohydrate	–	–	Controlled Restrict concentrated sweets	Controlled Restrict concentrated sweets	Controlled Restrict concentrated sweets
Protein	–	–	High protein	–	High protein
Alcohol	Not recommended	May be used with discretion	Limit to 2 servings	Limit to 2 servings	Not recommended

Fredrickson DS, Levy RI, Bonnell M & Ernst N (1973) Dietary management of hyperlipoproteinemia. A handbook for physicians and dietitians. National Heart & Lung Institute, Bethesda, MD. DHEW Publication No (NIH) 73-110.

Type IV: increased pre-β, or VLDL (triglycerides); common.
Type V: increased pre-β, (VLDL) and chylomicrons (very high triglycerides); rare.

The review also included (rare) deficiencies of serum LDL or HDL;

Abetalipoproteinaemia: no β-lipoprotein (LDL),
Hypobeta-lipoproteinaemia: abnormally low β-lipoprotein,
Alpha-lipoprotein (HDL) deficiency (first discovered in a family on Tangier Island).

These different plasma lipoprotein syndromes have multiple causes, genetic or secondary, and different dietetic and pharmacological treatments (Table 7.1). The 44 page article [118] which appeared in five successive issues of the New England Journal of Medicine, quickly became the manual for a new clinical specialty of LIPIDOLOGY, and stimulated doctors to set up Lipid (or Cholesterol) clinics.

7.2 Further Developments of Fredrickson's Types

It was soon found that some of the people with type II (increased β or LDL) also had increased pre-β-lipoproteins (triglycerides) so type II was subdivided into IIa (hyper β-lipoproteinaemia) and IIb (with increased β and pre-β lipoproteins) by a WHO Committee [119]. Goldstein et al. [120] carried out extensive family studies of 176 survivors of myocardial infarction in Seattle. They recognised families with familial hypercholesterolaemia, which had been well known for over 20 years [31]. In affected members the hyperlipoproteinaemia is of type IIA and there is diminished expression of the LDL receptor [89]. But another familial hyperlipidaemia emerged in which some affected family members had increased LDL-(cholesterol), some had increased VLDL-(triglyceride) and others had both, i.e. Fredrickson types IIa, IIb or IV. When the family data were extensive enough and lipid levels were measured in children, this disorder could be distinguished from familial hypercholesterolaemia and from familial hypertriglyceridaemia. Goldstein et al. gave it the name Familial Combined Hyperlipidemia (FCHL) [120]. There is over-production of apolipoprotein B_{100} in FCHL [121] and it carries an increased risk of CHD. It is about as common as familial hypercholesterolaemia [122].

Chapter 8
The Seven Countries Study (7CS)

Ancel Keys and collaborators ran synchronous prospective studies with standardised methods in 16 contrasting communities in 7 countries (Italy, Greece, Yugoslavia, Netherlands, Finland, Japan and USA). CHD incidence after 5 and 10 year follow-up confirmed the predictive value of serum cholesterol (mean initial values of the cohorts ranged from 160 to 260 mg/100 ml), and blood pressure (not as strongly). But smoking was not related in Mediterranean countries and Japan. For the first time saturated fat intake (% calories) was found to correlate significantly with CHD incidence. The lowest incidence of CHD was in Crete, highest in East Finland.

Cohorts started 1985–1964; 5 year results published 1970; 10 year results in 1980.

8.1 Setting Up

Around 1954 Ancel Keys with collaborators in Italy, Spain, Yugoslavia (as it then was) and South Africa was reporting cross sectional differences in serum cholesterol between different groups of people beyond the USA (see Section 3.6). Usual diets appeared to be different and incidence of CHD lower in groups with serum cholesterols that were low by American standards at that time. To subject these impressions to scientific scrutiny Keys developed, with the support of Dr Paul White (America's leading cardiologist) and collaborators across the world, a study that was prospective and broad as well. Broad in that subjects were in seven contrasting countries and quantitation of the most relevant aspects of their diets was built into the design. It is at first puzzling to see many more than seven data points on graphs of results from the 7CS (Fig. 8.1). There were actually 16 different communities. In most of the countries two small areas were selected with contrasting lifestyles. The design was that they should be stable communities eating traditional diets, hence they were mostly rural (Table 8.1).

In Croatia one group was on the coast in Dalmatia (more fish and olive oil), the other in the interior in rural Slavonia (more animal fat). In Japan Ushibuka was a fishing area and Tanushimaru a farming town. In Finland, East Finland was known

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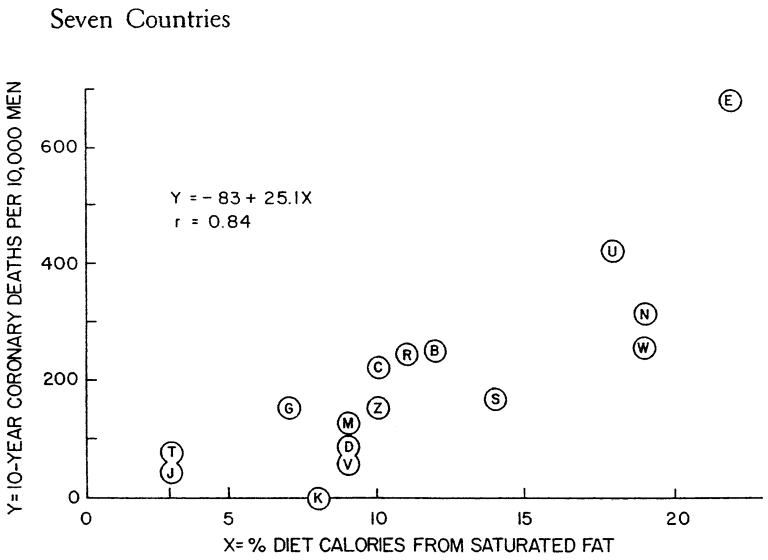

Fig. 8.1 Seven countries study. Ten year coronary death rates plotted against mean saturated fat intake in each of 16 cohorts. T & J in Japan, K = Crete, E = East Finland. There was a strong correlation. From Keys et al. [123].

Table 8.1 The seven countries study: 10-year incidence (hard CHD = CHD death or definite myocardial infarction)

Start year	Cohort	N	All-causes deaths	Hard CHD
1959	US railroad[a]	2,571	294	–
1958	Dalmatia	671	61	14
1958	Slavonia	696	124	22
1958	Tanushimaru	508	58	9
1959	East Finland	817	147	115
1959	West Finland	860	127	61
1960	Ushibuka	502	70	13
1960	Crevalcore	993	136	51
1960	Montegiorgio	719	74	27
1960	Zutphen	878	109	59
1960	Crete	686	42	4
1961	Corfu	529	43	19
1962	Rome railroad[b]	768	77	31
1962	Velika Krsna	511	63	8
1963	Zrenjanin	516	60	14
1964	Belgrade	538	27	19
	Total	12,763	1,512	466

[a]No 10-year examinations.
[b]Ten-year examinations incomplete.
Source: From Table 1.2 in Keys et al. [123].

to have a very high mortality from CHD; for comparison a second group was studied in South West Finland.

It took years of negotiations, fund raising, planning and trial runs before the first cohorts started in 1958. Central organization came from Minneapolis in the USA but local funding, enthusiastic professionals and facilities were provided in the countries. The project was one of the fruits of the USA's political mood of liberal internationalism after World War 2. The countries in the Study had been badly affected by the war and were in the process of recovering.

8.2 Methods

The subjects were individual men, initially 40–59 years old. Serum cholesterols were standardised. Serum was put on filter paper with a calibrated micro-syringe, dried in room air and flown to Minneapolis for analysis by the Abel-Kendall method [124]. For electrocardiograms the Minnesota code was developed [125]. The 5 year follow up of the cohorts was reported in 1970 [126] and the 10 year results in 1980 [123].

As well as cardiac and lipid data, respiratory function was measured, and in some countries, e.g., Finland much additional research was stimulated. The very large differences of CHD mortality in these cohorts, from East Finland (highest) to Japan (lowest) was confirmed. Mean serum cholesterols of the cohorts ranged correspondingly, from 260 down to 160 mg/100 ml (6.74–4.15 mmol/l).

8.2.1 Results

The predictive value of serum total cholesterol was confirmed. At 10 years the correlation coefficient of CHD mortality with median cholesterol at entry between the 16 cohorts was 0.80. This risk factor was estimated to account for 64% of the cohorts' variance in CHD death rate [123]. Blood pressure had the next most influence but its relationship was stronger for the Finnish than the Mediterranean cohorts [123]. The results for smoking habits were surprising. When percent smoking 20+ cigarettes/day was plotted by cohort against 10 year CHD the correlation coefficient was –0.03. However, within the cohorts different slopes could be calculated for cigarette dose against CHD mortality: steep in N. Europe, flatter progressively through Yugoslavia, then Italy and Greece, and in Japan the slope was horizontal. The importance of smoking thus appeared to be related to the frequency of CHD in the community. The risk of a smoker having a heart attack is greatly increased if his serum cholesterol is high. Keys suggested that smoking may trigger a heart attack by increasing irritability of the myocardium if its blood supply is already compromised by severe coronary atherosclerosis [123]. For overweight, the overall conclusion from the 7CS was that differences in CHD mortality were not significantly related to difference in average relative weight or skinfolds. Keys criticised the Life insurance companies who have loaded policies of overweight people on the basis of risk of death from CHD [123].

8.2.2 Dietary Results

Dietary data from the 7CS were only represented in the primary reports by % calories from total fat, saturated, monoene and polyene fats. These were reliable estimates from fatty acid analysis in a central laboratory of replicate meals collected from the subjects in the different areas. Correlation of 10 year CHD incidence rate with mean total fat per cohort was r + 0.39 (not significant) but with saturated fat r was +0.84 (highly significant) (Fig. 8.1) at 5 years and with monoene fatty acids it was –0.42 (a non-significant trend). Polyunsaturated fatty acids did not correlate significantly but their range of cohort means in the 7CS (1.9–6.9% Calories) was much smaller than for saturated (2.9–23.7% Cals) or monoene fatty acids (2.9–25.8% Cals). A potentially controversial result was significant correlation of sucrose intake and CHD incidence [123]. However, this was explained by correlation of sucrose with saturated fat intake. Partial correlation analysis showed that, with dietary saturated fat held constant the correlation between sucrose and CHD was not significant [127].

Data for food and nutrient intake is much more cumbersome than single numbers per subject for cigarettes smoked or serum cholesterol, and dietary data was not generally published until Kromhout, who took over the Zutphen cohort, re-calculated and for Crete re-estimated the original food groups and proximate nutrient intakes [128, 129]. This is now a historical record of what men in these poor rural communities were eating as they were recovering from the war. The food pattern in Crete, 1960 has been taken as an ideal, healthy Mediterranean diet because the Cretan cohort had the lowest CHD incidence in the 7CS [130]. On the other hand, Italian nutritionists advocate the 1960 food pattern in Southern Italy as an ideal Mediterranean diet [131, 132]. Both these diets have since changed greatly and Greece no longer has such a good life expectancy at age 60 [133].

Box 8.1 Mediterranean Diets

Eighteen countries have coasts on the Mediterranean Sea, 8 in Europe, 3 in the Middle East, 5 in Africa and 2 island countries, all with different national diets. The ideal for today's nutritionists seems to be what Italians OR Cretans were eating as their countries started to recover from being World War 2 battlefields (when the Seven Country Study was operating). The food intake side of the 7CS concentrated on fatty acid analysis of samples from the diet. Food group intakes, g/day in men (in the following table) were not published until 1989 by Kromhout et al. [128, 129].

	Nicotera 1960 South Italy	Montegiorgio 1960 Central Italy	Crete 1960	Dalmatia/Croatia 1960
Total cereals, including bread	488	529	410	499
Potatoes		56	190	214
Legumes	49	21	30	7
Vegetables	344	194	191	200
Fruit	101	28	464	6
Meat	53	85	35	117
Fish	42	35	18	96
Milk	33	8	235	434
Cheese	15	9	13	4
Olive oil	46	24	95	72

Alcohol was estimated to provide 8% of energy overall in the 7CS. There were considerable differences between these four Mediterranean communities in 1960. Well before the end of twentieth century these dietary patterns had changed, with e.g., increased cereals, increased meat, cheese and sugar. The Italian diet was accurately measured in the Euratom Study in the 1960s. The Cretan diet had been carefully measured by Allbaugh in 1948 as a case study of an underdeveloped area.

For Ferro-Luzzi and Sette [132] "describing the Mediterranean diet which was supposed to be a quite easy task, has turned out to be a demanding and almost impossible enterprise since data are lacking, incomplete or too aggregated. It appears that currently there is insufficient material to give a proper definition of what the Mediterranean diet is or was either in terms of well defined chemical compounds or even in terms of foods". Ferro-Luzzi and Sette were too modest. Their review, which also drew on FAO food balance sheets makes it clear that there is no single Mediterranean food pattern, so one ought to state the Mediterranean country (even part of the country) whose diet is being discussed and at what year in history.

References

Euratom Study (1969) cited by Ferro-Luzzi & Sette (1989).
Allbaugh LG (1953) Crete: a case study in an underdeveloped area. Princeton University Press, Princeton, NJ.

8.3 Implications from the 7CS

The 7CS showed that the average serum cholesterol of north Americans and NW Europeans of the 1960s, though seen in many apparently healthy people may be above the healthy level for the long term. As the WHO Expert Committee (1982)

noted “The Expert Committee knew of no population in whom CHD is common that does not also have a relatively high mean level of total cholesterol i.e., greater than 5.17 mmol/l (200 mg/dl) in adults” [134]. The 7CS also showed for the first time a prospective correlation between saturated fat intake and CHD, which strengthened the case for the dietary-fat-cholesterol hypothesis. It would be wrong, however, to dismiss a role for polyunsaturated fat from the 7CS’s failure to find a relation of linoleic acid with CHD incidence. The communities varied little in polyunsaturated fatty acid intake [129] and the importance of long chain omega-3 (especially in the Japanese cohorts) was discovered later.

8.4 Longer Follow Up

Longer follow up of 7CS cohorts to 25, even 30 years [135] was affected by the law of diminishing returns. Lifestyles and diets changed, so did risk factors; people put on weight, effective medication became available for moderate hypertension.

Late benefits from the 7CS included a community programme in Finland, starting in North Karelia (i.e. East Finland) that achieved big reductions in CHD mortality [136, 137] (see Section 31.3). In Zutphen, Kromhout later set up a new cohort and reported from 1985 on CHD incidence and intake of fish (see Chapter 18), and flavonoids. As Blackburn puts it “The Seven Country Study provided new and strong evidence for a policy of risk reduction at the population level. And it infected with the epidemiological method and mystique the medical and public health ‘virgin forest’ in each of its seven countries” [138].

Chapter 9
Sucrose – An Alternative Dietary Hypothesis

Added (extrinsic) sugar (sucrose) consumption increased greatly from 1900 to 1960 in Britain and similar countries. John Yudkin suggested this was responsible for the appearance and increase of CHD. Sucrose could act by increasing plasma triglycerides. This hypothesis was presented in a popular best seller "Pure, White and Deadly".

On further examination the evidence incriminating sugar was inadequate, but official policy in Britain did not embrace the lipid-cholesterol hypothesis until 1984. Years 1964–1984.

9.1 Yudkin's Proposal

In the 1960s John Yudkin, then one of the two professors of nutrition in London University, pointed out that national consumption of sugar (i.e., added sugar, then nearly all sucrose) had increased in Britain more than any other commodity in the preceding 100 years and also that, between (developed) communities, sugar consumption correlated at least as closely with CHD mortality as total fat consumption [139]. So it could be the sugar, not the fat that was responsible for CHD. Yudkin supported his observations with a very small case-control study [140]. Dietary histories for sugar in 20 men (45–65 years) soon after a myocardial infarct (MI) were compared with 25 miscellaneous middle-aged men as controls. MI (median) 113 g; controls 56 g sugar/day (an additional 25 men with peripheral arterial disease also scored high on sugar intakes – 128 g/day). This was a full paper in the Lancet!

Yudkin did feeding experiments with rats. High sugar diets increased serum triglycerides; less consistently cholesterol. At about this time serum triglycerides were being suggested as good a risk factor as cholesterol for CHD [107, 109] and Ian Macdonald was reporting that high sugar intakes raised fasting serum triglycerides in men [141].

Yudkin's idea was warmly supported by the dairy, egg and meat industries who were beginning to feel the impact of "reduce saturated fats". He popularised his sugar theory through the media and a best-seller paperback with a striking title (but no references) [142]. He had a previous successful Penguin book "This Slimming Business" (1958).

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Yudkin's ideas were popular in Britain in the 1960s and early 1970s. They weakened the first official COMA (Committee on Medical Aspects of Food Policy) report on Diet and CHD from the government's Department of Health and Social Security [143]. It contained a minority report by Yudkin.

9.2 Reactions

Few other scientists were impressed by the sugar hypothesis. Keys refuted it [127, 144]. For Stamler et al. [145] "several sets of evidence, animal-experiments, clinical and epidemiological render the hypothesis untenable that sucrose is a prime and decisive factor influencing atherogenesis".

By the 1980s these sets of evidence included:-

- Sucrose does not ordinarily raise plasma cholesterol.
- It does not raise plasma triglycerides if energy balance is maintained and sucrose compared with starch at usual intakes [146].
- There is no plausible mechanism from animal experiments whereby sucrose could lead to CHD.
- Experimentally induced atheroma in primates REGRESSES on very low fat, high sucrose diets [147].
- Several countries with high sugar intakes had low rates of CHD, e.g., Cuba, Venezuela, Costa Rica [144].
- Sugar intake has not been found an independent risk factor in prospective studies.
- No prevention trial of CHD with sugar restriction has been completed, started, planned or even contemplated.

A much needed report by the joint working party of the Royal College of Physicians and the British Cardiac Society (RCP/BCS) (1976) [148] (with Gerry Shaper, Michael Oliver and Geoffrey Rose among its members) considered there was no firm evidence linking intake of dietary sugar and CHD. They did not mention sugar in their recommendations, except as a common source of excess energy.

Despite this, the sugar hypothesis held up establishment advice on CHD prevention in the UK until the Department of Health's COMA produced another report in 1984 [149], which shared the opinions of the RCP/BCS committee, and on sugar recommended only that the public should not increase their intake. CHD mortality started to come down from 1966 in USA, Finland and Australia. It took 10 years before this happened in the UK (see Section 31.4).

Chapter 10
HDL-Cholesterol Is Protective

Cholesterol (being insoluble in water) all has to be carried in plasma in lipoproteins. Most is in low density lipoprotein (LDL), whose concentration is higher in CHD cases. But one-quarter to one-third of plasma total cholesterol is in high-density lipoprotein (HDL). There were some observations in the 1950s that HDL-cholesterol levels (HLD-c) were *lower* with CHD. The concept that HDL-c might be PROTECTIVE started with the Millers' 1975 discovery that is was inversely related to body total cholesterol pools. Supportive epidemiology reports followed. Higher HDL-c levels help to explain the lower (age-standardised) incidence of CHD in women. Dietary influences on HDL-c are quite different from those that affect plasma LDL-cholesterol and total cholesterol (since LDL-predominates in plasma). Years 1953–1979.

10.1 Early Observations

Oliver and Boyd [40] had noticed in their large retrospective study with paper electrophoresis that serum β-lipoprotein cholesterol was higher in CHD cases than controls but α-lipoprotein (i.e., HDL) cholesterol was lower. They noted that this pattern had been first reported by Barr et al. in 1951; who used Cohn's protein fractionation method [150]. Nikkilä had also noticed, in Helsinki, lower alpha lipoprotein cholesterols in patients with CHD in 1953 [151]. But researchers were preoccupied with a possible pathogenic influence of β-lipoprotein cholesterol – with its increased cholesterol/phospholipid ratio – on CHD. Keys and others focused on the association of total cholesterol with CHD, and β-lipoprotein cholesterol makes up the majority of total cholesterol. It is straightforward thinking to envisage that accumulation of cholesterol in arteries is related to an increase of cholesterol-carrying lipoproteins in the plasma.

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10.2 The Millers' Hypothesis

Miller et al. [152] in 8 patients with hypercholesterolaemia measured body cholesterol pools and turnover by 2-pool analysis of the plasma cholesterol specific activity-time curve after intravenous infusion of radiolabelled cholesterol. "Possibly the most interesting information to emerge from this study", they wrote, was that . . . "the sizes of both the rapidly and slowly exchanging pools of body cholesterol were unrelated to plasma total, VLDL or LDL cholesterol concentration, but showed strong negative correlations with HDL cholesterol concentration. As only the liver can catabolize or excrete cholesterol in important amounts, the elimination of cholesterol from extrahepatic tissues is assumed to be performed by plasma lipoproteins, and there is evidence that this may be a function of HDL", both from in vitro studies with cultured cells and from the model of Tangier disease, in which there is defective synthesis of HDL apoprotein and cholesterol accumulates in a variety of tissues. GJ Miller and NE Miller formally proposed the HDL hypothesis in the Lancet in 1975: that reduction of plasma HDL concentration may accelerate the development of atherosclerosis, and hence CHD by impairing the clearance of cholesterol from the arterial wall [153]. HDL-cholesterol does not simply go down reciprocally if LDL-cholesterol is high [154]. In the Millers' review the correlation coefficient between HDL and LDL was –0.67 in men and only –0.41 in women [153].

From around 1970 simpler chemical methods were introduced for serum HDL-cholesterol that were suitable for large numbers of subjects in epidemiological or routine clinical work. VLDL (and chylomicrons) and LDL are precipitated by heparin (or dextran) and manganese chloride. Cholesterol is measured before and after this precipitation [155].

10.2.1 It Ran Against the Grain

In an essay about this revolutionary HDL idea, Tavia Gordon wrote [156] "My guess is that the idea of a negative relation simply ran against the grain. It was easy to believe that too much cholesterol in the blood could 'overload' the system and hence increase the risk of disease, as Anitschkow had argued; but how could 'too much' of one part of the cholesterol reduce the risk of disease? To admit that fact challenged the whole way of thinking about the problem. Who wants to rethink any idea?"

10.3 Epidemiological Support

The Millers' paper was quickly followed between 1976 and 1979 by a wave of epidemiological reports from developed countries of significant inverse association of serum HDL-cholesterol and CHD, in large case-control studies [157–159] and prospective studies [160–162]. Some of the later large prospective studies reported the same [163]. HDL-cholesterol or apolipoproteins A-I or A-II had not been

measured at the start of several of the early prospective studies. HDL-cholesterol now emerged as "the good (serum) cholesterol" in journalists' parlance.

10.4 HDL_2 or HDL_3?

High density lipoprotein(s) can be subdivided in the ultracentrifuge into two major subclasses: HDL_2 (density 1.063–1.125 g/ml) and HDL_3 (density 1.125–1.21 g/ml). Gofman first reported that HDL_2 was more reduced than HDL_3 in 38 men who subsequently developed clinical CHD [164]. Miller [165] and several others subsequently have mostly (but not always) noted the same phenomenon – in case-control studies. This has potential dietetic implications. For example, in moderate alcohol drinkers HDL_3 was increased, not HDL_2 [166]. However, Stampfer et al. [163] went to the trouble of measuring HDL_2 and HDL_3 by differential precipitation and apolipoproteins in 500 physicians in their prospective study. They did not confirm that HDL_2 was a better predictor of myocardial infarction. For them the HDL subfraction did not add significantly to prediction from total/HDL cholesterol.

10.5 In Developing Countries?

But in countries where total and LDL-cholesterols are low, HDL-cholesterols also tend to be lower than in affluent communities. The Wageningen group investigated this. HDL-cholesterols average lower in boys (7–9 years) in Nigeria, Ivory Coast, Pakistan, Ghana and Philippines – all countries with low rates of CHD – than in boys in the USA and European countries. This difference can still be seen in men, but is not as striking [167]. Influences like alcohol intake (which increases HDL-cholesterol) and obesity (which reduces it) complicate the picture in adults. Hautvast et al. gave student volunteers different diets. High fat diets increased serum HDL-cholesterol, whether the fat was saturated or high in polyunsaturated fat, though the first raised and the second lowered serum total cholesterol [168]. So they suggested in affluent communities a high HDL-cholesterol indicates a better capacity to handle the dietary saturated fat. On the other hand in developing countries, with low intakes of fat there are low levels of HDL and total cholesterol and CHD risk is low [169].

10.6 In Women

Plasma HDL-cholesterols are higher in women. This is one obvious explanation for their lower (age-standardised) incidence of CHD. HDL goes up with ovulation in the menstrual cycle [170] when oestrogens peak, and makes a substantial contribution to the usual increase of total cholesterol concentration in pregnancy [171].

10.7 Diet Affects HDL-Cholesterol Quite Differently

Diet affects HDL-cholesterol (HDL-c) quite differently from LDL-cholesterol. HDL-c tends to be low with obesity, increased by weight reduction and by alcohol intake. It is low when the dietary carbohydrate to fat ratio is high and higher when fat is a larger proportion of total energy. It tends to go down if polyunsaturated fat intake is very high, with P/S ratio (polyunsaturates/saturates) >1.5 [172]. It is increased by regular exercise, such as regular brisk walking [173].

Chapter 11
Critics and Sceptics

With every new discovery and concept about diet and CHD there have been critics and sceptics. They were naturally supported by dairy, egg and meat industries and by journalists who look for a debate with two sides. Most of the critics have faded into the past and industries have adapted. Two strong critics of the fat-cholesterol hypotheses in 1977–1979 (along with Yudkin, in Chapter 9) are recalled here: Sir John McMichael in Britain and George V Mann in USA. Years 1977–1979.

A review in 1981 had the ambitious title "A survey of 246 suggested coronary risk factors" [174]. More have probably been added since. Kritchevsky, author of the first (English language) monograph on Cholesterol, wrote "One thing must be made clear at the outset of this discussion: the literature relating to atherosclerosis is so extensive that it is possible for find conflicting views on practically every aspect of the disease" [175] – and that was 50 years ago!

In the early years of research on atherosclerosis and CHD most people knew little about it and were not affected, but by the 1970s major food industries started to feel under threat and "Cholesterol" entered ordinary people's vocabulary. "It is a common lament that almost everything that one enjoys in life is either illegal, immoral or fattening. To these we must add – and hazardous to the heart" [176]. But people need to be convinced before they will give up things they enjoy.

With all creative ideas there have been critics and sceptics. Growth of our information on diet and CHD did not roll on smoothly either. Two of the most prominent multifactorial critics were *Sir John McMichael*, who had been Professor and head of the Department of Medicine at the Royal Postgraduate Medical School in London [177, 178] and *Dr George V. Mann*, a career investigator funded by the US National Heart, Lung and Blood Institute.

11.1 Sir John McMichael, London

Two important points that McMichael made in his earlier papers were about the pathology of atherosclerosis. The lipid-laden lesions that Anitschkow produced are only occasionally seen in human cases of xanthomatosis. [But Constantinides

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[179] could produce lesions similar to ordinary human atherosclerosis in rabbits by feeding smaller doses of cholesterol over longer periods of time.] McMichael also thought the dietary fat hypothesis did not take into account the proposed role of thrombosis in atherogenesis (see Chapter 12).

Several of McMichael's other criticisms were also made in more detail by George Mann (see below).

McMichael in his later two-page article [178] raced through over 20 pieces of criticism. Several were general, quoting other people's opinion, or travellers' tales (like two orders of monasteries or workers on the Indian railways) or factually wrong or about drugs that didn't work. Most of these points in his critique were rebutted by Jim Mann [180]. McMichael ignored positive evidence, e.g., three primary prevention trials and used the recent decline of CHD deaths in the USA to argue *against* the fat-cholesterol hypothesis.

11.2 Dr George Mann, USA

George Mann's critique [181] contained more scientific material than Sir John's. In his long introduction he accused colleagues of voting with the majority and some segments of the food industry of making money! He appeared to be angry that the predictive role of α-lipoproteins ("several times more predictive for CHD than LDL") was not established earlier. He then offered a set of points against diet-heart:

- *In Framingham [182] and Tecumseh [183] there was no relation between dietary habits and cholesteremia*. This phenomenon was a frustrating problem for the fat-cholesterol-CHD hypothesis. It has been the usual result in other studies in homogenous communities. Apart from the difficulties with accurate quantification of individual's diets, down to different fatty acids, there is considerable between-individual variation of serum cholesterol in an affluent community. Kannel and Gordon [182] stressed that their results do not mean that differences between Framingham and some other population (e.g., Japan) in serum cholesterol is not related to differences in their diet. Still less does it mean that serum cholesterol levels cannot be changed by changes in dietary intake. It was interesting that haemoglobin levels (separately in men and women) did not correlate with estimated iron intake either.
- *Mortality trends since 1950 do not support the argument that extensive dietary propaganda has had an effect in clinical events.* Mann looked at crude overall death rates. If he had looked at age-standardised CHD mortality he would have seen that it peaked in 1966 in USA and has been coming down steadily since then.
- *No diet therapy has been shown effective for the prevention or treatment of coronary heart disease.* Dietary trials are discussed in later sections (Chapters 14 and Chapters 23). There have been a few successes but the difficulties are formidable. The 1982 WHO Expert Committee [134] considered the total cholesterol-CHD relationship to be causal without waiting for the perfect randomised, controlled

trial. Numbers of participants and cost would be huge (and by now, after we have seen what statins can do, it would probably be unethical as well as impractical to try and keep a large number of middle-aged people (controls) on a serum cholesterol-maintaining diet).

- *There is no safe efficacious drug known for the management of cholesterolemia*. The statins were discovered [184] about the time Mann was writing!
- *Risk factors for coronary disease do not count after the age of 55*. This example of ageism has been proved incorrect in the randomised controlled statin trials.
- *Some of the corn oil and spreads are hydrogenated, so they contain a percentage of the unnatural trans unsaturated fatty acids, which can have a hypercholesterolemic effect*. True in some cases. It took about 15 years for nutrition scientists to attend to this, (see Chapter 21) and then the edible fat industry started to act.
- *What about the Masai*? They serve as an example of various small remote groups that seemed to be exceptions to the dietary fat theory. Mann had published about the Masai in East Africa [185], reported to have a diet largely consisting of (fermented) milk and blood, but no CHD. But the Masai men have considerable seasonal variation of food supply and milk, with periods of shortage. Their diet includes grain. They were lean and very active [186].
- Lastly, "No systemic trials of the effectiveness of exercise in preventing CHD have been done – a commentary on the lost generation of diet-heart enthusiasts". True. No one has managed to achieve a large randomised controlled trial where half do prescribed exercise for years and half do not. But there are several instructive observations of groups who exercise because their work requires it or because they enjoy it in leisure time [187].

George Mann's paper stimulated a crop of correspondence in following issues of the New England journal, in general saying that he was dogmatic, and omitted large areas of animal experimental and human metabolic ward investigations. The president and past presidents of the Chicago Heart Association ended their long letter: "In view of recent positive progress and the large tasks ahead, it is particularly unfortunate that Dr Mann chose this time to present such a poorly documented paper" [188].

That recent progress in the USA was lower consumption of dairy fat, lard and eggs, fall of cigarette use, more people being treated for hypertension, apparently lower serum cholesterols and decreasing CHD mortality since 1966.

Chapter 12
Thrombosis on and in Atheroma

Research efforts and findings concentrated at first on plasma cholesterol and atherosclerosis. Coronary artery THROMBOSIS was more difficult to research because it occurs rapidly and tests for liability to thrombosis in glass outside the body may not reproduce what happens in living blood vessels.

As well as thrombosis that can occur on top of atherosclerotic narrowing in a coronary artery, Duguid (1954) produced evidence that mural thrombi can contribute to the build-up of atherosclerotic plaques. With specific immunofluorescent staining pathologists demonstrated fibrin and also platelets in plaques (1967). Thus lipid infiltration and mural thrombi can both be involved and individual atherosclerotic lesions can differ in structure and composition. Years 1954–1967.

12.1 Thrombosis a Separate, Acute Process

A myocardial infarct comes on in minutes and thrombosis is usually found in the adjacent coronary artery over an atheromatous plaque. This thrombosis is a separate process from the much slower development of atheroma. It was largely neglected between 1950 and 1985 while research effort and findings focussed on lipids and arterial wall pathology. But for Sir George Pickering (Regius Professor of Medicine at Oxford) the lipid hypothesis saw this major event of thrombosis "as almost an irrelevant accident happening because alterations in lipid metabolism also predispose to thrombosis; or by supposing that the thrombosis is a by-product, as it were of the nodule (plaque) which sets up eddies and so leads to thrombosis. Neither is very satisfying to a clinician since it assigns a minor role – in fact almost an afterthought – to the event that determines life or death" [189].

12.2 Tests for Liability to Thrombosis Are Indirect

Association of higher serum cholesterols with CHD gave impetus to international research and prevention trials testing the lipid hypothesis. Plasma lipids are presumably the same in a clinical biochemistry lab as inside the body and methods

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can be readily standardised. But to assess liability to thrombosis multiple tests are needed because numerous reactions are involved. These tests in glass may not be the same as what occurs inside living blood vessels, and some of the methods are very difficult to standardise.

Merskey and collaborators (Cape Town) in 1960 carried out 12 tests in 28 cases who had recently recovered from a myocardial infarct or had stable angina; controls were 50 office workers in a large insurance company [190]. The tests were coagulation time, prothrombin level by 4 different methods, prothrombin consumption, factor V, factor VII, antihaemophilic globulin (factor VIII), thromboplastin generation, fibrinogen and fibrinolysis. The only difference between cases and controls was higher fibrinogen levels in CHD cases over 50 years of age. There were more differences in several tests between white subjects – cases or controls – and healthy Bantu (Black) migrant workers. Coagulation and fibrinolysis tests are done in vitro to represent complex enzymatic processes that occur in vivo inside living blood vessels. There was no easy way of searching for environmental influences on thrombosis.

12.3 Or Can Mural Thrombosis Become Atheroma?

As well as thrombosis superimposed on existing atheroma there is the question whether mural thrombosis is the basis of atheroma, rather than lipoprotein infiltration. The thrombogenic hypothesis was first proposed by Rokitansky in 1841, before Virchow postulated the lipogenic theory in 1856. Virchow's theory was dominant in pathology teaching until it was challenged with careful histological studies by Duguid (professor of pathology in Durham University, UK) in 1946 and further developed in 1954 [191]. He claimed that mural thrombi in time become covered with endothelium. Their interior can undergo fibrous and fatty change. Duguid found that in cholesterol-fed rabbits the aorta becomes dilated not narrower. He suggested that an artery's lumen can only be narrowed if there is thrombosis superimposed on the inner lining.

Duguid's contribution was supported when histopathologists demonstrated fibrin, the basic material of mural thrombi in human atherosclerotic material by immunofluorescent staining. This could have originated by infiltration from the plasma or from incorporated mural thrombosis. Stronger evidence of thrombosis material was demonstration of platelets in plaques by using highly specific antiserum [192].

Pathologists were moving toward the present concept of atherosclerosis, that lipid infiltration and mural thrombosis can both be involved and that individual atherosclerotic lesions can differ in structure and composition [193].

Chapter 13
Dietary Cholesterol *May* Affect Plasma Cholesterol

Keys in the 1950s discovered that dietary triglycerides increase plasma cholesterol more reliably than dietary cholesterol (Chapter 4). At first it seemed to him that any effect of dietary cholesterol was unimportant. In 1965 after more carefully controlled experiments he suggested that plasma cholesterol's rise was related to the square root of increased dietary cholesterol. Isotope labelled sterol human balance studies in the 1970s showed that as more cholesterol is absorbed, its excretion is increased, and endogenous synthesis decreased by suppression of HMG CoA reductase. In the 1980s research focussed on individual differences in response of plasma cholesterol to dietary cholesterol, but when cholesterol feeds were repeated later in the same subjects, the responses were only partly consistent. One genetic basis for cholesterol responders is the 4/4 genotype of apolipoprotein E.

There has been no clear epidemiological evidence that addition of one egg to a daily diet increases risk of CHD. Years 1965, 1971, 1986, 1999.

13.1 Keys Had Asserted Dietary Cholesterol Does Not Raise Plasma Cholesterol

In the 1950s Keys and his collaborators communicated their important discovery that in humans dietary cholesterol does not increase serum cholesterol, contrary to animal (rabbit) experiments (see Section 4.1). Instead serum cholesterol (and possibly even CHD) can be reduced by changing dietary fatty acids, with less saturated and more polyunsaturated fats. This new and unexpected prescription might not have been taken up so promptly round the world if it had a second label attached to it: "and probably cholesterol intake as well".

13.2 Other Early Studies Inconclusive

The effect of dietary cholesterol has been difficult to clarify. Keys et al., could not see an effect in cross-sectional studies or in free-living people who intended to change their cholesterol intake – but perhaps made other dietary changes at the

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same time [43, 194]. Kinsell too saw no change in serum cholesterol when a very large amount of cholesterol was added to patients' diet [195]. However, a few other researchers in the early 1960s found it hard to believe that dietary cholesterol could have no effect, and did their own human experiments. Beveridge et al. [196], Connor et al. [197], Steiner et al. [198] and Erikson et al. [199] all reported some increase of serum cholesterol with extra dietary cholesterol. Their methods would be criticised today – periods too short [196], formula diets [197], very large dose of cholesterol, use of prisoners [199] but they were published in major journals.

13.3 Keys' Group and Hegsted's Group Re-examine

In 1965 Grande et al. [200] (i.e. Keys' group in Minneapolis) reported results of three sets of strictly controlled experiments in the Metabolic Unit of the Hastings State Hospital with 22 stable schizophrenic in-patients. They added 500 or 1,500 mg of crystalline cholesterol per day to a low cholesterol diet and also 500 mg as egg. Diets were 40% fat, high in polyunsaturated fat or saturated fat, or low fat. Serum cholesterols were higher with added cholesterol. Incorporating other recent experiments [196–199] into the data Keys [201] calculated that change of serum cholesterol *is* related, but to the *square root* of the change in cholesterol intake. This meant that on the usual range of cholesterol intakes differences in serum cholesterol are quite small, about 7.5 mg/100 ml and easily masked by inter- and intra-individual variance in serum cholesterol and the effect of other dietary changes.

Hegsted et al. [202] at Harvard around the same time (1965) were also testing men with chronic schizophrenia at Danvers State Hospital, who were given a low fat adequate diet to which 36 different oils or fats were added to groups of 10 men. In some of these, extra cholesterol (as egg yolk) was given. From all the data it appeared that across intakes up to 600 mg of dietary cholesterol the response of serum cholesterol was linear; it increased about 5 mg/100 ml for each added 100 mg of cholesterol. Mattson et al. [203] gave formula diets containing 0, 106, 212 or 317 mg of cholesterol for 6 weeks to groups of at least 10 prisoners in Holmsburg, PA. At these intakes (below a US average intake at the time) the response of serum cholesterol was better described by a linear than a square root relationship.

13.4 Metabolism of Labelled Cholesterol

When rabbits are fed large amounts of cholesterol, it accumulates in many parts of the body [204], not only in the plasma. On the metabolic ward at Rockefeller University Hospital, Quintão et al. [205] used ^{14}C and ^{3}H labelled sterol balances to measure absorption, synthesis and excretion of cholesterol, and hence accumulation in 8 patients with CHD and/or hypercholesterolaemia. Each study lasted 6–19 weeks. They showed that with increased cholesterol intake (500–4,000 mg/day) its absorption increases, though percentage absorption goes down from around 50 to

25–30%. When the amount of absorbed cholesterol increases, there is compensatory increased re-excretion as faecal neutral steroids, and reduced cholesterol synthesis. But in three of their patients this compensation fell short and it appeared there was accumulation of body cholesterol (in the short term) though this was not reflected in change of plasma cholesterol. "Indeed plasma cholesterol concentrations reflect the total body content of cholesterol imperfectly and may even misrepresent the effects of high cholesterol diets on tissue concentrations of cholesterol" [205].

In Canberra, Nestel and Poyser [206] subsequently carried out similar isotopic sterol balances in 9 subjects. They tested more moderate cholesterol intakes: 250 and 750 mg/day and confirmed compensatory faecal re-excretion of cholesterol and suppressed cholesterol synthesis when cholesterol absorption was increased. Interestingly at 750 mg cholesterol/day intake in those subjects whose compensation was mostly cholesterol re-excretion, plasma cholesterol increased (+64, +67, +142 mg/100 ml); if compensation was mostly by suppressed synthesis, plasma cholesterol changed little (+3 to +26 mg/100 ml).

Box 13.1 Species Differences in Cholesterol Metabolism

Dietschy and Wilson [207] explained why animals have different responses to cholesterol feeding. In all species on very low cholesterol intake (e.g., human vegans) the body's cholesterol comes from endogenous synthesis. In rabbits fed cholesterol (not part of their normal diet) more cholesterol is absorbed than is normally synthesised and excretion as bile acids cannot increase. In humans, cholesterol absorption is limited; the amount absorbed can be compensated by inhibited synthesis alone, so the body's cholesterol pool is only slightly enlarged. In dogs and rats, inhibited synthesis and increased bile acid formation compensate for a large increase of cholesterol absorption: there is only a modest expansion of the body's cholesterol pool.

13.5 Many Human Experiments with Dietary Cholesterol 1960s and 1970s

Many human experiments of the effect of cholesterol intake were done in the 1960s and 1970s, often with small numbers or short periods or other design weakness. McGill [208] wrote a thorough review of all these reports in 1979. He concluded that independent association of dietary cholesterol and serum cholesterol has not been found in cross-sectional studies. But in controlled experiments, increases up to 600 mg cholesterol/day have been accompanied by average serum cholesterol increases of 3–12 mg/100 ml/250 mg cholesterol. Increases above 600 mg/day dietary cholesterol have usually produced no additional rise of serum cholesterol. "Many investigators and reviewers have commented on the marked variability

among individuals in response to dietary cholesterol". McGill thought the frequency distribution of serum cholesterol responses may be as important as the average response. "If an average change of, say, 10 mg/100 ml is made up of a few persons who respond dramatically, and a large number who respond slightly or not at all, the strategy to identify the extreme responders and recommend dietary modification only for them might be advisable" [208].

13.6 Are Some Individuals Responsive to Dietary Cholesterol, Not Others?

Individual variation of response of plasma cholesterol to cholesterol intake became a focus of research in the 1980s. The first article to have this in the title was by Mistry et al. [209]. With 6 egg yolks/day (1,500 mg cholesterol) added to the usual diet of 37 healthy medical students, mean plasma total cholesterol rose 29 mg/100 ml but the individual range was –6 to +75 mg/100 ml. Addition of half this amount of dietary cholesterol produced nearly the same mean plasma cholesterol increase (+24 mg/100 ml), confirming earlier reports that the response curve has a flat top. The increased plasma cholesterol was mostly in low density lipoprotein, with a smaller amount in HDL. They also showed that extra dietary cholesterol suppressed activity of HMG CoA reductase (the rate-limiting enzyme in cholesterol biosynthesis) in freshly isolated blood mononuclear cells.

The important question about individual responsiveness of serum cholesterol is whether it is reproducible. Katan and Beynen did a series of repeat experiments to find out. First, they re-tested 34 subjects who had participated in a trial by colleagues 6 years earlier [210], comparing at least one egg a day, with none, each for 3 weeks. In the first experiment serum cholesterol decreased 0.16 (range –1.27 to +0.51) mmol/l; in the later experiment it decreased 0.31 (range –0.94 to +0.48) mmol/l. Correlation between the two individual responses was $r = 0.32$ ($P < 0.05$) [211]. Responses were thus only partly consistent but the subjects had been free-living. The Wageningen investigators next provided all the diets and ran three sets of experiments [212]: low cholesterol (around 120 mg/day), followed by extra egg yolk (625, 673, 989 mg cholesterol/day). Experiment 1 with 94 subjects served to find hyper- and hypo-responders. They were tested again (23 and 18 in each group) and then a third time (for twice as long). Hyper-responders as a group in the first experiment had significantly higher serum cholesterol responses in the second and third experiments but there was a great deal of overlap. It seemed that the wide scatter of responses in single experiments is largely due to within-person fluctuations. Overall mean changes were intermediate between predictions by Keys et al. [201] and by Hegsted et al. [202].

One reason for consistent individual variation in response of serum cholesterol to cholesterol intake is genetic. People with the 4/4 genotype of apo E had a significantly higher rise of serum total cholesterol after 300 mg added dietary cholesterol than people with genotypes 3/4 and 3/3 [213].

McNamara et al. [214] carried out 75-twelve week sterol balance studies at Rockefeller University, measuring the responses to adding 3 eggs/day to the diet of healthy men (dietary cholesterol increased from 190 to 820 mg/day). 52/75 (69%) men were "compensators": their plasma cholesterol did not change. They compensated by decreasing cholesterol synthesis. In "noncompensators" plasma cholesterols rose and their mononuclear cells showed no significant change in sterol synthesis rate.

13.7 Effect of Basal Cholesterol Intake

It was fairly clear, from the time of Keys' square root equation that adding larger amounts of dietary cholesterol have proportionally smaller effects on serum cholesterol. Hopkins [215] confirmed this in mathematical analyses of published data from studies with controlled diets supplied by a metabolic kitchen (Fig. 13.1).

His new finding was that cholesterol has a greater effect on serum cholesterol if it is added to a low cholesterol diet; at moderate (basal) cholesterol intake serum cholesterol changes little. Reading from his graph, 500 mg added dietary cholesterol would increase serum cholesterol by 0.60 mmol/l (20 mg/100 ml) if baseline cholesterol intake was zero; the corresponding increase would only be 0.08 mmol/l (3 mg/100 ml) when baseline was 500 mg cholesterol per day.

In the last meta-analysis of the century, Howell et al. [216] collected 9 different published prediction equations for serum cholesterol responses to changes in cholesterol intake. The most recent predictions for 100 mg/day increase in dietary

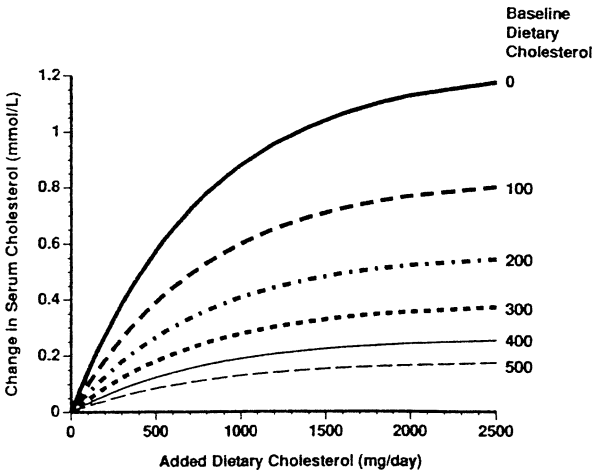

Fig. 13.1 Effects of added dietary cholesterol on serum total cholesterol. Different estimated *curves* for baseline dietary cholesterols. At moderate baseline cholesterol intakes little increase in serum cholesterol expected. From meta-analysis by Hopkins, 1992 [215]

cholesterol had come down from about +0.1 mmol/l (+4 mg/100 ml) to about +0.06 mmol (+2 mg/100 ml).

There has been no clear epidemiological evidence that addition of one egg to the daily diet increases the risk of CHD [217].

13.8 Different Recommendations by Advisory Committees

Government advisory committees have taken somewhat different positions about dietary cholesterol on the two sides of the Atlantic. In the USA, committee after committee have recommended that people's dietary cholesterol should be reduced to around 300 mg/day [218–220]. Yet McNamara in "Present Knowledge in Nutrition" [221] calculated that a population reduction of 150 mg (from 450 to 300 mg) cholesterol intake would only lead to a 1.4% reduction of plasma cholesterol.

British committees have taken neutral positions. In the 1984 COMA report [222] "There are no specific recommendations about the dietary intake of cholesterol. Cholesterol intake is approximately 450–450 mg/day in adults and is likely to fall if the recommendation regarding intake of saturated fatty acids (decrease) is implemented. We believe the current intake is not excessive and that evidence for an influence of this level of intake on blood cholesterol is inconclusive". Ten years later the same British committee [223] said "The influence of dietary cholesterol varies between individuals. The average intake . . . is now about 245 mg/day. At this level of intake, an increase in dietary cholesterol can have a significant effect on plasma cholesterol, though quantitatively less than that of saturates. . .. We recommend that the average dietary intake of cholesterol should not rise".

The uncertain disadvantage of eating moderate amounts of egg has to be balanced against the valuable nutrients in this inexpensive and convenient food, such as the best utilized of all dietary proteins.

Chapter 14
First Controlled Trials

By the late 1950s serum cholesterol was emerging as a major risk factor for CHD. Changing a (western) diet, with lower saturated fat and more polyunsaturated fat could reduce serum cholesterol. Controlled dietary trials were started. Between 1965 and 1972 six had reached reporting stage, three small trials from Britain and (one each) from USA, Norway and Finland (see Table 23.1). Four were secondary prevention trials, in subjects who had recovered from a myocardial infarct; two were primary prevention trials, in middle aged people. The first three trials had disappointing results but the next three, in Los Angeles, Oslo and Helsinki were larger, lasted longer and had encouraging results. Years 1965–1972.

Dietary intervention trials for prevention of CHD were started in the late 1950s. They were needed because the contributions from epidemiology and from clinical experiments could not *prove* a causal relationship. Even if we accept that nutritional elevation of serum cholesterol *is* a causative factor in atherosclerosis and CHD we are left with uncertainty whether change of diet in older people can undo the accumulated damage. And are cholesterol-lowering diets, long term, free of undesirable effects? [224].

The largest of the early trials, the New York Anti-Coronary Club started in 1957 [225]. Members who followed the "prudent diet" actively for 10 years had only half the incidence of new coronary events seen in the controls, a different group of age-matched men who had presented at a cancer detection clinic. (Club members may have also decreased smoking and increased exercise.) Randomisation of subjects to intervention or control was going to be needed to prove the hypothesis.

14.1 First Two Randomised Dietary Trials, 1965

The first two randomised controlled trials, in different London hospitals, were reported in 1965. Both were secondary prevention trials – participants had recently recovered from a myocardial infarct. In the *low-fat diet trial* ($n = 264$) there was no significant difference in CHD recurrence rate, although serum cholesterols

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were lower on low fat than on "normal" diet [226]. In the *corn oil trial* (only) 28 patients were asked to take 80 g corn oil/day (which many didn't like) and reduce foods high in saturated fat for 2 years; 26 controls continued their ordinary diet [227]. The corn oil group had lower serum cholesterols but twice as many CHD recurrences.

Several questions followed these disappointing results [228]. A trial with oestrogens *in men* had reduced serum cholesterols but did not prevent CHD recurrences [229]. Perhaps after a myocardial infarct it was too late to expect benefit from reducing cholesterol. Perhaps triglycerides are involved, perhaps dietary sugar. Perhaps linole*n*ic (not linoleic) acid would be more helpful, by reducing platelet aggregation [230]. And the sceptics arguments were strengthened.

14.2 Two More Dietary Trials

Around 1968 two more diet trials reported. The *soya bean oil trial* [231] at the same four London hospitals as the low fat trial [226], and the *Oslo trial* [232] (that also used soy bean oil) were both also secondary prevention trials. Numbers were about the same (393 and 412 participants). Serum cholesterols came down in the diet groups. In the London trial there was no difference in CHD recurrences; the average time on diet was around $3\frac{1}{2}$ years. In Oslo the trial lasted 5 years; there were significantly fewer re-infarctions in the diet group but the same number of sudden deaths. Patients started in the London trial around 5 weeks after their myocardial infarct; in Oslo the earliest entry was at 13 months. Medical records on the Oslo participants were reviewed at 11 years [233]. The benefit in re-infarctions was maintained, e.g., fatal myocardial infarctions 32 (diet), 57 (control), with no difference in sudden deaths. Total mortality was 101 (diet), 108 (control).

At this stage (1968) the Lancet [234] was beginning to think that an increased polyunsaturated, reduced saturated fat diet could only have a small, if any benefit for secondary prevention of CHD. "Larger investigations will be required to uncover a possible small reduction in myocardial reinfarction. Whether sufficient enthusiasm and resources exist for this next stage is another matter... Perhaps it was too optimistic to hope for a dramatic change in the course of a chronic arterial disease once it has impaired myocardial function. The efficacy of a polyunsaturated-fat diet as a means of preventing the onset (primary prevention) of CHD is only in the early stages of examination.... It has recently been stated: 'There are two extremes: diet is nonsense; and diet is crucial'. No one has irrefutable proof of either. Thus, human beings currently are presented with the choice of following a daily special pattern of living, which is far from easy, or forgetting the whole thing. A great majority of people follow the latter choice. Except for reduction of excess weight, the facts at present indicate that they may well be right".

The next two dietary trials to report were larger, with more subjects and longer duration and based in residential institutions. They were the first *primary* prevention trials, although a minority (7%) in Los Angeles had ECG evidence of a previous myocardial infarction.

14.3 The Los Angeles VA Trial, Reported 1969

The Los Angeles trial was perhaps the best designed of all the dietary trials [235]. It was planned in 1957–1958 and recruitment started in 1959. There were 846 subjects, men age around 65.5 years at entry who were Veterans (ex-servicemen) in a large Domicile. They volunteered for the study, were randomised and then for up to 8 years half ate the experimental diet and half ate the control diet in separate dining rooms. Both groups were told their diet had been changed (but not how). The aim was for the iodine value of the mixed fats to average 100 in the experimental diet and 55 in the control diet. Each week's diets were collected, homogenised and analysed. Serum cholesterols were measured every 4 months. Fatty acids were analysed in diet, serum lipids and adipose tissue biopsies. The experimental group had a large increase in linoleic acid intake and decrease in palmitic. Serum cholesterols averaged 13% lower in the experimental group. There was little change in body weight. The men's health and illnesses were carefully followed (physicians double blind) including standardised ECG reports. Any who needed to go to hospital went to the VA hospital adjacent to the Domicile. Autopsies were obtained on most of the men who died. The five authors thanked about 50 medical, dietetic and laboratory colleagues for their work in this trial [235]. The experimental group had fewer myocardial infarcts *and* sudden cardiac deaths, there were also fewer cerebral infarcts in the experimental group. When these are added to cardiac events the difference for combined cardiovascular events was significant ($P = 0.01$). Atherosclerotic events were more reduced in the younger experimental subjects.

Subjects ate most but not all their meals in the dining room. Careful records with meal tickets showed that experimental group subjects ate an average 78% of the Centre's meals and the controls averaged 80% while they were resident. There was some turnover of the participants. Some were discharged, most during the first year. A few withdrew from the trial in the first 2 years. When these movements are taken into account the meal adherences were 49% experimental and 56% control [235]. The Los Angeles trial was supportive of the diet fat/cholesterol/CHD hypothesis but one of its results set off a new question. There were more non-cardiac deaths in the experimental group, notably 7 from cancer (most bronchogenic) against 2 from cancer in the controls. Could this be a long-term adverse effect of increased intake of polyunsaturated oils?

14.4 Helsinki Mental Hospitals

The next dietary trial to report was planned in 1958 and started in 1959 in Finland. The incidence of CHD was compared in two large mental hospitals near Helsinki. In Nikkilä Hospital (N) the diet was changed by replacing milk fat with soybean oil and replacing butter with soft polyunsaturated margarine, also less meat, fewer eggs, more root vegetables. At Kellokoski Hospital (K) the usual hospital diet continued; this was the control hospital. At this time many people with chronic psychosis spent

years in institutions; only some early anti-psychotic drugs were available and experience of how to use them had still to build up. Patients accepted the new diet in hospital N. Adipose tissue biopsies there showed increased 18:2 and decreased 14:0 fatty acids and serum cholesterols fell 18% in male and 12% in female patients. At the end of 6 years, in the men CHD mortality (age adjusted) was significantly lower in hospital N (5.7/1,000 person years) than in hospital K (15/1,000 person year) and all cause mortality was lower [236]. Women too had lower CHD and total mortality, though neither were statistically significant.

These results were presented at the 8th International Nutrition Congress in Hamburg [237] where members of the audience urged the investigators to cross over diets between the hospitals and carry on another 6 years. This they did, and the results were reversed but the differences were now less striking. In hospital K (the new diet hospital) patients were younger and the average stay was shorter during the second 6 years. (The revolution in management of mental illnesses was starting.) Because of this, Miettinen et al., presented results age-standardised and per person year(s) [236]. The trial was criticised by writers from NIH who complained that subjects were not randomised, some came in and some left and the house physicians were not blinded [238]. The Finnish authors in reply [239], explained that new subjects were added only once a year. Anyone admitted after the annual recruitment date and discharged before the next failed to enter the trial. Their study design may be "far from ideal", but ideal designs "it seems are so elaborate and costly that trials according to them may perhaps never be conducted" (prophetic words) [239].

Further analysis of the Finnish Mental Hospitals trial, now adding young adults to the middle-aged in the earlier results were published later. The total number of subjects was 1,900 men and 2,836 women. Fewer ECG abnormalities in the diet groups were significant in the men, not in the women. Examination of a number of potentially confounding variables indicated, the investigators wrote, that these had only small effects and could not account for the considerable reduction in incidence of CHD events on the serum cholesterol-lowering diets [240, 241].

At the stage when these six diet-only prevention trials had been reported, Mann and Marr plotted percentage difference in CHD rates (and confidence limits) against % serum cholesterol reduction [242]. The last four trials, the longer ones – the soya bean oil trial [231], Leren in Oslo [232], Dayton in LA [235] and Finnish mental hospitals men [236] fit near the regression line. The two small (or very small) 1965 trials don't.

For a time the sceptics (and the dairy industry) emphasised the possible cancer risk from long term increased polyunsaturated fatty acids in the diet. But the trend of more cancer cases in the Los Angeles trial was not confirmed in the other dietary trials [243].

While results of these dietary trials were not wholly convincing, trials with serum cholesterol-lowering drugs were also going on. Being less expensive and easier to carry out they might demonstrate a clearer prevention of CHD than the dietary trials. But the effect depends on the drug.

14.5 Drugs Trials

In the 1960s results of placebo-controlled clinical trials with drugs that can lower serum cholesterol were reported. Oestrogens (in men), D-thyroxine (not the natural L-isomer), neomycin (by mouth) and triparanol all proved unsuitable because of serious side effects. Nicotinic acid and cholestyramine resin had longer trials: they were promising but caused some side effects. Clofibrate ("Atromid") had a large trial, reporting in 1978: excess mortality in the clofibrate group was a concern. Years 1967 onwards.

14.5.1 Early Trials with Cholesterol-Lowering Drugs

Oestrogens in men can lower serum (total) cholesterol but did not reduce CHD incidents; there were side effects, feminisation and more thrombo-embolic episodes [229].

D-thyroxine usually lowers serum cholesterol, apparently by accelerating cholesterol oxidation or excretion but the effect wears off and higher doses can provoke angina [244].

The ability of oral *neomycin* to lower cholesterol was discovered serendipitously when a patient with salmonella gastroenteritis was treated with neomycin. It lowers serum cholesterol by increasing faecal bile acids but it can also cause general malabsorption and induce antibiotic resistant Gram negative bacteria; it is partly absorbed and can be ototoxic, so after some interest in 1959–1962 [245] it was not thought desirable for long-term cholesterol-lowering.

Triparanol lowered serum cholesterol by inhibiting the very last step in its biosynthesis, from desmosterol to cholesterol. It was withdrawn when it was found that desmosterol accumulates in plasma, in arterial lesions [246] and led to cataract and hair loss.

14.5.2 More Promising Drugs Followed

Nicotinic acid was found to lower serum cholesterol by Altschul et al. (in Saskatchewan) in 1955 [247]. They discovered its action while trying to see if a substance that could raise levels of NAD might increase oxidation of cholesterol. The dose used was around 1 g thrice daily, about 150 times the nutritional RDA, and nicotin*amide* is ineffective here. The major mechanism of action was later found to be blocking of free fatty acid release from adipose tissue [248]. It was effective in lowering serum cholesterol over 1–2 years [249]. Flushing and several other side effects were frequent and some patients stopped taking the drug. It was most effective in lowering lipids where triglycerides were elevated, with or without hypercholesterolaemia [250]. Nicotinic acid (niacin) was suitable for prevention trials. In the Coronary Drug Project, niacin for 5 years reduced serum cholesterol 10% and

reduced definite non-fatal myocardial infarct by 26% but total mortality was the same as in control subjects [251].

14.5.3 "Atromid" or Clofibrate

A new drug, "Atromid" which became *clofibrate* (ethyl chlorophenoxyisobutyrate) was first developed by Thorp and Waring (1962) at ICI in England [252]. It was found to lower serum triglycerides more than cholesterol. Several possible modes of action have been demonstrated, including stimulation of lipoprotein lipase and increased biliary excretion of cholesterol [253]. Primary CHD prevention trials were started with clofibrate in 1964. The WHO Cooperative Trial had 10,627 men, 30–60 years with moderately high serum cholesterols, half on clofibrate, half controls [254]. After 4–8 years serum cholesterols were reduced 9%, triglycerides more. Non-fatal CHD occurred in significantly fewer men, but fatal CHD occurred in 36 (clofibrate) and 34 controls. Non-coronary deaths were 108 (clofibrate) and 79 (controls). Some of the excess deaths were from cancer, some from complications of gallstones.

The Lancet [255] thought "this trial should spell the end of the use of clofibrate, except maybe in the desperate problem of hypercholesterolaemia". The BMJ speculated further [256]: "the other lipid-lowering regimes recommended for general use may possibly share the same problems: and regimens of this kind – including, perhaps, dietary modification with polyunsaturated fats – will require equally careful consideration in the future if we are to achieve benefit without at the same time causing more harm than had individuals been left untreated".

Some time later Davey Smith et al. [257] added up data from CHD prevention trials and noted that raised mortality from causes other than CHD was seen in some of the drug trials but not overall in the dietary trials.

14.5.4 Cholestyramine Resin

Cholestyramine, a quaternary ammonium resin was first used in medicine around 1960 to relieve the pruritus in patients with obstructive jaundice because it binds bile acids in the intestine and carries them out in the faeces. Soon it was realised that preventing the entero-hepatic cycle of bile acids might achieve a negative cholesterol balance. Bile acids are one of the two main forms in which cholesterol gets excreted. Lowering of high serum cholesterol by cholestyramine in small series of cases were reported from 1965 [258]. It was also given to children with familial hypercholesterolaemia, though gastro-intestinal side effects reduced long-term compliance to 50% [259].

Further dietary trials reported in 1978–1990 (see Chapter 23 and Table 23.1). Trials were completed with cholestyramine and new drugs were developed.

Chapter 15
Dietary Fibre

The dietary fibre hypothesis attracted popular and research attention in the 1970s. Trowell expected that wheat fibre (wholemeal bread) would lower plasma cholesterol. In controlled human experiments it did not, but subsequently viscous types of dietary fibre did lower plasma cholesterol – pectin, guar gum, oat bran and psyllium. The mechanism appears to be entrapment of bile acids in the terminal ileum, preventing their reabsorption, hence a negative sterol balance.

There is also more recent epidemiological evidence that high intakes of whole grain cereals are negatively associated with CHD (mechanism unclear) and so are high intakes of vegetables and fruits. Years 1976 onwards.

The term dietary fibre, formerly called "roughage" or "residue" or "unavailable carbohydrate" came into general use in 1972 [260], introduced by a number of British physicians interested in nutrition, including M Eastwood, NS Painter, D Burkitt, H Trowell and K Heaton. Hugh Trowell proposed a definition that it is remnants of plant cell walls that are not hydrolysed by the alimentary enzymes of man [261]. He subsequently redefined dietary fibre as plant polysaccharides and lignin which are resistant to hydrolysis by the digestive enzymes of man [262] which extended the definition to storage polysaccharides like guar gum.

The basis of the fibre hypothesis was that refined carbohydrate foods are unnatural, from the evolutionary point of view and several of the common degenerative diseases in industrial countries might be due to lack of dietary fibre. Trowell and Burkitt had both worked very productively in Uganda, as physician and surgeon respectively: Trowell wrote the classic book on kwashiorkor [263] and Burkitt discovered Burkitt's lymphoma. In Africans, Western diseases [264] were seldom seen [265]. Trowell noted how the mortality from diverticulitis (of the colon) shadowed CHD between countries and over time. The effect of dietary fibre on colon function and diverticulitis is quite direct. In the UK, CHD (and diverticulitis) mortality stopped rising during World War 2 when all the bread had to be 85% extraction (i.e., containing more wheat fibre). These diseases increased again when white bread was allowed again after the War.

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There was a large output of publications on dietary fibre from 1972 [260]. Trowell had expected that wheat fibre – brown bread or bran – would lower serum cholesterol, but in controlled human experiments, it did not [266]. However, viscous or "soluble" forms of dietary fibre: pectin [267] guar gum [268], oatmeal [269] and psyllium [270] did lower cholesterol in most of the published controlled human experiments. The mechanism of their action appears to be entrapment of bile acids in the viscous contents of the lower small intestine so that their usual reabsorption does not take place, faecal bile acids are increased so there is a negative sterol balance [271]. There are also experiments showing small reduction of serum cholesterol in people eating large amounts of legumes [272] although the design was weak in some of the experiments [273].

Box 15.1 Improved Cholesterol Lowering by Adding High-Fibre to Fat-Modified Diet

Perhaps the best reduction of serum cholesterol by diet was achieved by adding high fibre foods (cereals, vegetables, legumes, fruits) to a fat-modified diet in 12 monks, volunteers in a closed abbey in the Netherlands. Four diets, each taken for 5 weeks, were compared in random order. All the foods were prepared in a separate area in the monastery kitchen.

Compared with the subjects' reference "Western" diet, mean serum cholesterol was 21.6% lower on the fat-modified diet (P/S 1.0). Adding high fibre to this, (total) cholesterol was 29% lower and LDL-cholesterol 34.5% lower (fibre 55 g/day, including pectin 6 g/day).
Lewis B et al. [274].

Despite the absence of effect of wheat fibre on serum cholesterol, several large prospective studies in different countries have found, fairly consistently that high intakes of whole grain cereals are negatively associated with CHD incidence [275]. The mechanism can only be speculated. It is unlikely to be via lowering cholesterol. Confounding remains a possibility.

Vegetables and fruit, another group source of dietary fibre may be protective against CHD [276–278]. Foods – plant foods – that provide dietary fibre contain other components that could protect against CHD, or hypertension (a risk factor for CHD), e.g., folate, potassium, flavonoids (see Box 22.2).

Chapter 16
Obesity

Assessment of relations between overweight/obesity and CHD is affected by the cut-offs used for desirable weight. The (NY) Metropolitan Life Insurance Company's 1959 desirable weights were widely used in the 1960s and 1970s. They were based on actuarial statistics (but to have life insurance requires a secure income). In the 1980s countries changed to use much simpler Body Mass Index (BMI) numbers – usually 25 kg/m^2 for the start of overweight and 30 kg/m^2 for obesity.

Epidemiologists have, however, used many different criteria for obesity. Its relation to CHD largely disappears if high blood pressure, high serum cholesterol and diabetes are excluded. In 1983 workers in Gothenburg found that waist circumference or intra-abdominal adiposity was more closely related to cardiovascular disease than BMI. Years 1959, 1981, 1983.

In the first half of the century obesity got little medical attention. It occupies only two pages in Osler's 1904 textbook [1], with no mention of complications. However, the life insurance companies were soon loading premiums of people who were overweight because their actuarial data in 1912, 1929 and 1959 reports showed it increased mortality. In The Society of Actuaries 1959 study the risk of cardiovascular-renal disease with overweight was 1.49 in men and 1.77 in women [279]. People in this follow-up had policies issued in the USA between 1925 and 1934. They had been prosperous enough to afford life insurance and their mortality rate was lower than in the general American population [280].

16.1 Desirable Weight for Height

For diagnosis of overweight/obesity four references or standards have been used successively in the twentieth century (Table 16.1). The first three were based on Americans who took out life insurance. Numbers were very large but they were not random samples of the USA, let alone the world population. Heights were measured in shoes (with flat heels) and in indoor clothes. (This is how weights and heights had been usually measured by the medical examiners for Life insurance.) Small, medium and large frame were incorporated in the standard in 1959 and 1983 but

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Table 16.1 Desirable weight standards

Date	Source	For example: Weight, lbs[a] for man 5 ft, 8 in. (1.725 cm)
1912	Association of Life Insurance Medical Directors and Actuarial Society of America [281]	150 (20–24 years) to 168 (>55 years)
1959	Metropolitan Life Insurance Co, New York. Desirable weights [282]	136–145; small frame; 142–156; medium frame; 151–170 large frame
1983	New desirable weights [283]	142–151; small frame; 148–160; medium frame; 155–176 large frame
From 1982	Body Mass Index, kg/m^2 [286]	135–168 lbs (BMI 20–25)

[a]To convert to kg multiply by 0.4536.

they were never validated. With the first three standards there were separate tables for women (for whom weights for height were a little lower). So there were tables with 180 numbers.

For example, desirable weight(s) in pounds (lbs) for height 5 ft 8 in. (1.725 m):

	Small frame	Medium frame	Large frame
Men	136–145	142–156	151–170
Women	130–140	136–151	145–165

The 1959 set of desirable weights were widely used and accepted in other developed countries beyond the USA for about 25 years. It was replaced in 1983. The new set of desirable weights for height was based on the Society of Actuaries 1979 Build and Blood Pressure Study (4 million US men and women insured from 1954). Desirable weights increased by around 5%, and more for short people. This reflected the improved mortality of moderately overweight people who had life insurance in the USA. Fewer were smoking, treatments had improved for hypertension and CHD mortality had started to come down. There were two sorts of reaction to the new desirable weights. A headline in the Washington Post read "The Weighting List: Suddenly it's Okay to put on the Pounds". Others pointed out that less privileged Americans were not represented in the cohort and that the follow up was probably too short (average 6.6 years) to be sure of the long-term effects of overweight [284].

16.2 Body Mass Index

Before the 1983 weight tables were widely used a revolution took place in weight assessment. The cumbersome tables of desirable weights were quietly replaced over

about 10 years by the simple "body mass index" (kg ÷ metres squared) originally proposed by the Belgian astronomer Quetelet in 1871 [285]. This BMI was recommended to assess overweight in Britain in 1981 [286], then by the US Surgeon General (1988) [287], then by WHO in 1990 [288] and is now used universally as the main reference. Of all the indices of weight divided by height to the power of N, W/H^2, weight/height squared (i.e., BMI) is the most independent of height (W/H makes the tall people appear too fat and W/H^3 makes short people appear too fat) [285]. Back in 1972 Keys et al., had [289] concluded that, in the absence of skinfold measurements, BMI is the most satisfactory index of obesity based on weight and height. The general reference BMI range of 20–25 corresponds to the desirable weights at all the heights in the 1959 Metropolitan Life tables [290]. For women the same numbers are used as for men, and the healthy reference range can be adjusted for particular populations.

In the first half of the century weight reduction for obesity was the major non-drug treatment for angina: "Obesity is an added burden on the heart and should be reduced whenever possible" [291]. "A large proportion of patients are of the overweight, thick-set type, and in these reduction in weight is probably the most potent therapeutic measure" [292].

When the new epidemiological studies relating to CHD got under way some measurement of overweight or adiposity was naturally among the factors examined. The early studies 1963–1972 are summarised in Table 16.2.

The Pooling Project [304] (1978) combined the results of five US longitudinal studies (Albany, Chicago People's Gas, Chicago Western Electric, Framingham and Tecumseh): "Relative weight is less strongly and consistently related (than serum cholesterol, hypertension and smoking) to non-fatal myocardial infarction and coronary mortality in the Pooling Project studies and the relationship is statistically significant only for the 40–49 age group, not at older ages. Since it has been shown that overweight and weight gain are associated with a worsening of atherogenic traits (e.g., hypertension), the limited role of overweight as a risk factor in middle age remains an enigma needing further investigation" [304].

The Joint Working Party of the Royal College of Physicians and the British Cardiac Society [148] put it clearly in 1976: (i) "Obesity is associated with an increased mortality in general and may be associated with an increased risk of some forms of CHD". (ii) Obesity is commonly associated with hypertension and glucose intolerance (diabetes mellitus) and in the presence of these factors and/or of raised plasma cholesterol levels, obesity becomes a risk factor for CHD. Where obesity is completely unassociated with any other risk factors (which is probably unusual) it is apparently not an important risk factor for CHD. (iii) "Correction of obesity will beneficially affect glucose intolerance, blood pressure and plasma lipid levels, particularly of triglycerides. For these reasons alone it is desirable, despite the lack of direct evidence that weight reduction by itself will lower the risk of CHD" [148].

Overweight people tend to have elevated serum total and LDL-cholesterol [305, 306].

Table 16.2 Overweight and obesity and risk of CHD in major epidemiological studies 1963–1972

Study	Authors	References	Findings
NY City	Spain et al.	[293]	Small increase in CHD in overweight men, disappeared if those with high BP or diabetes excluded
Western Electric	Paul et al.	[294]	Men who developed CHD had not weighed heavier, but had somewhat fatter skinfolds
Du Pont Co	Pell and D'Alonzo	[295]	Overweight small increased risk of MI only in men under 45 years
Tecumseh	Epstein et al.	[296]	Overweight increased risk of CHD in men, not in women, and partly dependent on serum cholesterol
Albany	Doyle 1966	[297]	Increased risk of CHD only in grossly obese persons
London busmen	Morris et al.	[298]	High sum of 3 skinfolds small increase of CHD (less than high BP or serum cholesterol)
Framingham at 12 years	Kannel et al.	[299]	Obesity increased risk only of angina or sudden death only in middle-aged men. Effect in women negligible unless also high BP & serum cholesterol
Israel	Medalie et al.	[300]	CHD was not related to ponderal index (height/cube root of weight)
Western Collaborative	Rosenman et al.	[301]	Ponderal index not a significant predictor of CHD if cholesterol or BP controlled
Los Angeles	Chapman et al.	[302]	Weight index (ht/cube root wt) risk factor for MI only in men 30–39 years
Evans County, GA	Heyden et al.	[303]	Very low or absent relationship of overweight with subsequent MI
Seven Countries Study	Keys et al.	[126, 123]	Neither relative weight nor sum of 2 skinfolds helped to explain difference in CHD between populations. Life insurance companies have exaggerated heart risks of overweight/obesity
Stockholm	Carlson and Bottiger	[109]	Rate of CHD did not increase with increasing weight/height index

MI = myocardial infarct.

Nestel et al. [307], in their measurements of cholesterol pools and turnover with cholesterol-4-^{14}C found that cholesterol production rate was increased in subjects with excess body weight, possibly this is synthesised in adipose tissue. Serum HDL-cholesterols, on the other hand, tend to be lower in obese people [306] and increase if they reduce body weight (see Chapter 10).

16.3 Waist Circumference – Visceral Adiposity

In 1983 [308] and 1984 [309, 310] the Gothenburg prospective study report brought a new dimension to the question of an independent association of obesity and cardiovascular disease. In 792 men neither BMI nor sum of 3 skinfolds were correlated significantly with CHD or stroke or death (any cause), but waist/hip circumference was. In 1,400 women waist/hip was a stronger predictor of all three outcomes than BMI. The idea of fat distribution and disease stimulated further research. It was found that adipose tissue within the abdomen, i.e. visceral (e.g., omental) is much more sensitive to the lipolytic action of catecholamines than fat elsewhere in the body [309]. Free fatty acids from inside the abdomen pass directly to the liver where they contribute to increase synthesis of triglycerides and insulin. It later appeared that either waist circumference alone [311] or waist/height [312] correlate better with intra-abdominal fat mass, estimated by computerised tomography than waist/hip, and this avoids the hip measurement for which it is difficult to choose the right level.

Waist circumference, or intra-abdominal fat by imaging, correlate with serum triglyceride (positively), HDL-cholesterol (inversely) and with glucose and insulin areas in glucose tolerance tests [310]. In the Manitoba Heart Survey metabolic variables and blood pressure were compared between people with central obesity, or with high BMI but low waist/hip ratio or (thirdly) without obesity. The metabolic variables in over 2,000 people were more abnormal with central obesity but they were still not normal in those with non-central obesity [313].

Waist circumferences are larger in men and tend to increase with age [314]. Mean values differed between the 19 populations that participated in the WHO MONICA project [315], 75% of this variation was related to BMI. (MONICA stands for Monitoring Trends and Determinants in Cardiovascular Disease; a major international collaboration, see Section 19.4.)

Except in Gothenburg, waist circumferences were not measured at the start of the many prospective studies of CHD, and these studies are not being set up any more. However, in case-control studies visceral adiposity by computed tomography has been found to be associated with coronary artery narrowing by angiography [316] and with impaired left ventricular function [317]. Waist circumference is now the second tool for assessment of obesity in the WHO report “Obesity: Preventing and Managing the Global Epidemic” (2000) [318]. Disease risks at a particular circumference are not the same in all populations, and abdominal fatness is a higher proportion of total fat in South Asian people.

16.4 At the End of the Century the Cardiac Risk of Overweight Remained Variable for Moderate Overweight

The American Heart Association's Nutrition Committee in its 1997 statement [319] noted that "until recently the relation between obesity and CHD was viewed as indirect, i.e. through covariates . . . including hypertension, dyslipidaemia (particularly reduction of HLD-c) and impaired glucose tolerance or non-insulin dependent diabetes mellitus. . . Long-term longitudinal studies, however, indicate that obesity . . . independently predicts coronary atherosclerosis". The three supporting references are cohort studies of US nurses, from Framingham, and from the Manitoba study. In these the index of obesity must have been BMI, though the statement does mention waist/hip. The Committee were less clear whether an obesity/CHD relation operates in Hispanics, Pima Indians and African-American women. They do not seem to have looked beyond North America.

In 1998 a 14 year follow-up of over 6,000 obese patients in Germany [320] as in a 25 year follow-up of Seven Country Study cohorts [321] – BMIs of 25–30 (i.e., overweight, not obese) were not significantly related to increased subsequent mortality.

There are confounding factors in examining a relationship between overweight/obesity and CHD: smoking; illness that causes loss of weight; whether adjustment is (or is not)made for hypertension, dyslipidaemia, diabetes; lack of exercise; socio-economic status and the fact that BMI indicates body muscle as well as fat [322].

Chapter 17
Thrombosis Treated Early

Around 1980 meticulous serial sections of injected coronary arteries from early fatal cases found that there were occlusive thrombi in most cases. About the same time procedural cardiologists started doing coronary angiography within the first 24 h after onset of symptoms of myocardial infarct. They were sometimes able to relieve spasm and they injected streptokinase or tissue plasminogen activator to try and achieve reperfusion of ischemic, yet not necrotic myocardium. This new treatment, in specialised cardiac units, reduced mortality and extent of myocardial damage.

Also in the early 1980s the Northwick Park (London) prospective measured a set of initial haemostatic functions. They found that raised factor VIIc and fibrinogen predicted subsequent CHD. Factor VIIc is increased by dietary fat or high plasma triglycerides. Fibrinogen is increased by smoking, not diet.

Aspirin was introduced to reduce platelet aggregation by inhibiting COX 1 and stopping synthesis of thromboxane A2. Dietary polyunsaturated oils appear to reduce platelet aggregation. Years: early 1980s.

By the mid 1980s evidence had further accumulated that Herrick (1919) was right to call myocardial infarction "Thrombosis of the coronary arteries" [2], although fresh thrombus could often not be found in the responsible coronary artery at routine necropsy [323]. Davis et al., made meticulous examinations of coronary arteries from early fatal cases, taking serial sections every 3 mm after injecting barium/gelatine suspension at 100 mmHg and fixation in formalin [324, 325]. They were able to find occlusive thrombi in the majority of cases. Thrombi were adjacent to fissures or rupture of atheromatous plaque, as first described by Constantinides [326].

17.1 Acute Phase Coronary Angiography

Brave developments were happening in the Coronary Care Unit. It had been (reasonably) assumed that injection of contrast medium into the coronary circulation during the acute stage of a myocardial infarction could only aggravate the condition. But in the late 1970s some procedural cardiologists did coronary angiography during

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the first 24 h after onset of symptoms in the hope that they might be able to treat the diseased artery and limit the area of infarction. They were sometimes able to do this by relieving spasm with local nitroglycerine. DeWood et al. [327] reported on early coronary angiography in 322 patients with acute myocardial infarction. (Only 2 patients did not survive the procedure.) In the first 4 h after onset of symptoms 87% had total occlusion. This proportion decreased after 4 h, presumably because of natural fibrinolysis. In some patients DeWood et al., were able to remove the thrombus with a special catheter. On microscopical examination the recovered thrombi had acute inflammatory cells at the leading edge; the consistent feature of the distal part of every thrombus was a thickened layer of fibrin and platelets. In the middle portion red cells were interspersed with fibrin.

Dealing with thrombosis now became the main focus of early management of acute myocardial infarction. Thrombolytic treatment to achieve reperfusion of ischaemic, not yet necrotic myocardium was found to be possible with intravenous tissue plasminogen activator (TPA) [328] or streptokinase, with or without oral aspirin [329]. The reduction of vascular and all cause mortality was highly significant for patients treated in the first few hours after onset of symptoms. Thrombolysis, often combined with angioplasty has revolutionised management of myocardial infarction in patients who can be brought to a specialised cardiac unit early enough.

17.2 Clotting Factors in a Prospective Study

Also in the early 1980s, the first prospective study that had made initial measures of haemostatic function, at Northwick Park Hospital, London reported that of all the clotting factors measured only plasma levels of factor VIIc and of fibrinogen were (both) significantly associated with increased risk of subsequent CHD [330, 331]. The association with fibrinogen was soon confirmed in other cohort studies [332, 333]. Meade thought “There is increasing reason to consider the prevention of thrombosis as an effective approach to the prevention of IHD. The case for doing so is strengthened by the possibility that the biochemical disturbance in IHD may lie as much in the coagulation system as in the metabolism of cholesterol” [331].

Plasma fibrinogen is an acute phase protein; its concentration rises in response to a number of stimuli (inflammation, injury, etc.). In the prospective studies fibrinogen and smoking were strongly related to one another. Plasma fibrinogen is a major determinant of blood viscosity as well as a coagulation factor. No clear dietary influence on fibrinogen has been found [334].

Factor VIIc, however is affected directly by the fat intake; the response is prompt [334]. Plasma factor VIIc increases after a fatty meal, reaching its peak 2–3 h after the triglyceride peak [335]. Changes in fat intake were followed within 12 h by change in factor VIIc of the same sign. With prolonged high fat intake factor VIIc reached almost double its level on a low fat diet [334]. Factor VIIc antigen (factor VII ag) does not change, so the increase of the VIIc (clotting activity) is due

to activation, perhaps by free saturated fatty acids [335]. Treatment of hypertriglyceridaemias results in reduction of factor VII and also of factors VIII and X [336].

Part of the effect of dietary fats on CHD may thus be via increasing factor VIIc, as well as the better known increase of LDL-cholesterol by saturated fatty acids, and one mechanism for the role of smoking may be by increasing fibrinogen concentration, though high fibrinogens were associated with CHD in non-smokers too [331].

17.3 Platelet Function

Platelet aggregation is the other complex process involved in thrombosis. Inhibition of platelet aggregation with lower dose aspirin (classified as an anti-platelet drug since around 1980) works by irreversibly inhibiting cyclo-oxygenase I (COX I) in platelets, hence stopping their synthesis of thromboxane A_2 (TX A_2) [337]. Aspirin is given as standard part of the management of acute coronary syndromes, whether the approach is invasive or conservative [338, 339]. As with the demonstration that high levels of some coagulation factors precede clinical CHD, the question was naturally asked whether they had hyperactive platelets [340]. In a case-control study, Meade et al., at Northwick Park Hospital, measured platelet aggregation by the in vitro light transmission method invented by Born in 1952. Aggregability to ADP was greater in men than in women, in white people than in blacks, higher with high plasma fibrinogen and lower in those who consumed more alcohol. But between people with a history or ECGs indicating CHD, and those without, aggregability was only slightly greater and not significantly [341]. No prospective study has demonstrated hyperactive platelets as a risk factor for CHD [342].

Ingenious methods have been used to see if different diets might make platelets less able to aggregate. Hornstra devised a siliconised polythene loop filled with heparin and inserted (under anaesthesia) in the abdominal aorta of rats [343]. When they were fed high intakes of sunflower oil the obturation time before flow ceased in the loop, was lengthened from 94 to 200 h. He combined with others [344] to use a special microfilter through which blood was drawn at slow constant rate from an arm vein in human subjects. The pore size allowed passage of red and white cells but would be occluded by platelet aggregates. The aggregation time was significantly longer in subjects on a long term diet with high P/S ratio than in those on the control diet (The subjects were participating in the Finnish Mental Hospitals Trial) [236].

Platelet aggregation in vitro was also found to be reduced (in response to collagen) in bloods from people on diets rich in polyunsaturated vegetable oil, i.e., linoleic acid (18:2) [345] compared to their usual diet. Platelet TXB_2 formation, the metabolite of (very short half life) thromboxane A_2 was reduced. Platelets do not convert linoleic to arachidonic acid (AA) and the increased platelet linoleic acid [346] appears to interfere with conversion of arachidonic acid to thromboxane A_2. All these platelet function studies are difficult to interpret. Behaviour of platelets in

glass is likely to be different from behaviour in a living artery and must be affected by drawing blood through a cannula, presence of an anticoagulant and centrifugation [340]. Change to more ω-6 PUFA in the diet did not affect bleeding time [344].

Box 17.1 What's a Normal Plasma Cholesterol?

Plasma cholesterol concentrations can differ remarkably in healthy people. In the author's 1970s collection of 118 published plasma cholesterols in groups of 40 year old men round the world the *means* ranged from 99 [347] to 286 [348] mg/dl (2.56–7.41 mmol/l).

Health professionals use laboratory reference values when advising whether action is called for by a particular blood biochemical value, like the cholesterol.

Davidson's textbook of medicine 1974–1981 editions [349] gave 140–300 mg/dl as reference values for plasma cholesterol, later 140–260 mg/dl in 1984–1991 editions [350]. Harrison's textbook 1987 gave 158–276 mg/dl as "5th–95th percentiles (not ideal)" for 40 year old men [351]. Annals of Internal Medicine's 1987 list of reference values in clinical chemistry (for implementation of SI units) gave "less than 245 mg/dl (<6.35 mmol/l)" at age 40 years [352].

A change came at the end of the 1980s. The next (1991) edition of Harrison's textbook gave 5.2 mmol/l (200 mg/dl) [353] as the *desirable* plasma cholesterol and above 6.21 mmol/l (240 mg/dl) as a *high* cholesterol. (These numbers continue to the 2008 edition.) Instead of using a laboratory's or a survey's range (or 5–95% percentiles) the numbers now were based on risk from prospective studies.

The American Health Foundation held a meeting in New York in 1979 "Plasma lipids: optimal levels for health" [354]. In the report, Henry Blackburn, writing up the Epidemiology workshop presented three bell curves:

- Present US mean 210 mg/dl (it had been round 235 mg/dl in the 1950s)
- Ideal mean 160 mg/dl
- Feasible mean 190 mg/dl.

160 mg/dl had predicted the lowest risk of CHD in the Seven Country Study.

The same three bell curves next appeared in the WHO's 1982 expert committee "Prevention of Coronary Heart Disease" [355] (Blackburn was the rapporteur). The committee chose under 5.17 mmol/l as the optimum

population mean total cholesterol (i.e. the old – and memorable – 200 mg/dl). This was adopted as the population target at the NIH Consensus Development Conference in 1985 [356]. Then the Expert Panel of the National Cholesterol Education Program (1988) [357] set 200 mg/dl as desirable, 240 mg/dl as high, with levels between as "borderline-high" for individuals. "Serum total cholesterol should be measured in all adults 20 years of age and over, at least every 5 years". The European Atherosclerosis Society's Study Group focussed on hyperlipidaemias, rather than population, but their treatment group A (least severe) had a target cholesterol of 200 mg/dl (5.2 mmol/l) [358].

Levels of LDL-c and HDL-c should both be taken into account in assessing CHD risk. Total cholesterol/HDL-c is the best single figure way to express this. In most laboratories HDL-c is measured directly, while LDL-c is derived by difference (and can be confused by VLDL or lipaemia). Some epidemiologists found that total/HDL-cholesterol is a better predictor of CHD risk than total cholesterol [359].

For doctors' advice to individuals, including whether treatment with statins is worth the cost, tables and charts were introduced in the late 1990s which also include age, sex, blood pressure, smoking, diabetes (and perhaps family history) to estimate absolute CHD risk: the Sheffield table [360], the Joint British recommendations [361] and the New Zealand cardiovascular risk assessment charts [362]. They were mostly based on Framingham data and tests of their applicability to different populations and eras continued into the next century [363].

Chapter 18
Fish Oil

In Eskimos living their traditional lifestyle the diet is high in (marine) fat yet CHD appeared uncommon. Bang and Dyerberg made successive summer visits to a community in Greenland. They confirmed that in these Eskimos CHD was rare, plasma cholesterols were relatively low, triglycerides very low. Seal, whale and fish predominated in the diet. The Eskimo plasma lipids had high saturated fatty acids, very low linoleic and linolenic and exceptionally high eicosapentaenoic acid (EPA; 20:5 ω-3). EPA reduces platelet aggregation.

In other populations Kromhout first reported that fish consumption appears to protect against CHD in a Dutch cohort (1985). Then in Adelaide (Australia) animal experiments (1985–1993) showed that prior feeding fish oil prevented ventricular fibrillation that often follows coronary ischaemia. A secondary prevention trial in Italy (1999) found that on EPA + DHA (22:6) there was significantly lower mortality (notably fewer sudden deaths). Fatty fish or fish oil (or EPA produced microbiologically) were added to the list of recommended foods. Years 1971, 1985, 1999.

18.1 Traditional Eskimos: High Fat Diet but CHD Uncommon

In a 1953 symposium "Unusual diets for humans" Hugh Sinclair told how the traditional Eskimos[1] had a high fat and protein diet – much of it from marine animals – yet rarely appeared to suffer CHD [364]. Serum cholesterols were low in some reports from far north Canada [365, 366], not in others (from Alaska) [367]. Nutrition scientists were also interested in Eskimos' low intakes of vitamin C, carbohydrate and fibre. Their hunter-gatherer lifestyle at the top of North America was being displaced by modern manufactured food flown in from further south in the continent [368].

Over in Greenland, Eskimos continued hunting and gathering in the 1970s. Bang and Dyerberg from Denmark made repeated summer visits to the Umanak community, who live on a coastal strip below the ice plateau and nearby islands at latitude

[1] In the twenty-first century "Inuit" is preferred.

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70.5° on the west coast. They confirmed the rarity of CHD in the area. Plasma cholesterols in 130 men and women were lower than in Danes and in Greenlanders living in Denmark [369]. The Eskimos' triglycerides and pre-β-lipoproteins were strikingly lower than in Denmark. Bang and Dyerberg thought their plasma lipids were low because of the Eskimos' environment. Seal and whale meat and fish predominated in these Eskimo's diet [370]. The Danish investigators now measured 30 fatty acids in plasma cholesterol esters, triglycerides and phospholipids (separately) in 129 Greenland Eskimos, 32 Greenlanders in Denmark and 30 Danish controls [371]. In Greenland, Eskimos averaged plasma lipids had higher percentages of saturated fatty acids and lower (total) polyunsaturated fatty acids; the P/S ratio was lower in the Eskimos than in Denmark [371]. Linoleic acid (18:2) was much lower in the Eskimos' plasma lipids but eicosapentaenoic (EPA) (or timnodonic) acid (20:5 ω-3) was 7 times higher than in Denmark. Dyerberg et al. suggested that quantitative differences in the polyunsaturated fatty acids might turn out to be important. In their next major paper they were joined by Moncada and Vane from the Welcome Research Laboratories who had recently (with Samuelsson) discovered new active derivatives [372], thromboxanes and prostacyclins, the eicosanoids, which came from three polyunsaturated 20 carbon fatty acids (Box 18.2).

18.2 Eicosapentaenoic Acid Reduces Platelet Aggregation

Box 18.1 Eicosanoids, Derived from C20 Polyunsaturated Fatty Acids [372]

Pro-aggregating	Platelets	Precursor acid	Vessel wall	Anti-aggregating
No	TXA_1	Dihomo-γ-linolenic ← C20:3 →	No PG1 type compound	No
Yes	TXA_2	← Arachidonic C20:4 →	$PG1_2$	Yes
No	TXA_3	← Eicosapentaenoic C20:5 →	$PG1_3$	Yes

Arachidonic acid (20:4 ω-6) in platelets produces thromboxane A_2 which is pro-aggregatory; in blood vessel walls it can produce prostacyclin (PGI_2) which is a vasodilator [373]. But the other 20 carbon polyunsaturated fatty acid, EPA (20:5 ω-3) in platelets produces thromboxane A_3, which does not induce aggregation. Dyerberg et al. demonstrated with aggregometers that EPA does not induce platelet aggregation [372]. Since EPA is the major C20 polyunsaturated fatty acid in Eskimos' plasma lipids, here was another special explanation for their low rate

of CHD. The EPA must come directly from the Eskimos' diet since its content of linolenic acid (18:3 ω-3) (which might be chain-elongated to EPA) is low. In their next expedition to Greenland Dyerberg & Bang measured the fatty acid profile of platelets in Eskimos to compare with Danish controls. The Eskimos had significantly longer bleeding times than Danes and 16 times higher platelet EPA's [374].

Other researchers confirmed the striking lowering of plasma triglycerides with fish oil feeding [375] (due to reduced production from the liver [376]), and reduced platelet aggregation [377, 378] with lower formation of TXB_2, the stable hydrolysis product of thromboxane A_2. Sinclair put himself on a diet strictly limited to marine animal food for 100 days. His bleeding time became very high, with spontaneous haemorrhages and his platelet count fell dramatically [379]. He suspected the thrombocytopenia might have been caused by too much of another fatty acid, cetoleic (22:1, ω-9) also present in fish. Meanwhile in Japan, Hirai et al. [380] compared people in a fishing village with a farming village. The fishing people ate three times more fish, had higher plasma lipid EPA and EPA/arachidonic acid ratio than the farmers and less aggregation of platelets.

18.3 Epidemiological Studies: Fish Consumption and CHD

The fish oil story moved next to epidemiology. The Dutch cohort of the Seven Country Study was in Zutphen. Kromhout, who took over its management from van Buchen and den Hartog, looked into the relation between fish consumption and death from CHD [381]. The surprise finding was that those who in 1960 were eating >45 g fish/day had a risk ratio of 0.42 and there was an inverse dose response relation across the lower categories of fish intake. Only about one third of this fish was fatty fish, and two thirds lean fish. Kromhout et al. could see no relation between fish intake and the major risk factors for CHD; the inverse association with fish intake appeared to be independent. Dutch fish intake was lower than Japanese and much lower than traditional Eskimos' consumption. They wondered whether small amounts of EPA might have a preventive effect against CHD. The Zutphen finding was quickly supported in a letter to the New England Journal from the larger Western Electric prospective study. Shekelle et al. [382] reported a significant inverse correlation between fish consumption and 25 year CHD mortality in Chicago. This also appeared to be independent of other risk factors (The 30-year follow up of this cohort, with the same overall result was reported in detail in 1997) [383]. Eleven cohort studies had reported by the end of the century. They were critically reviewed by Marckmann and Gronbaek [384], who concluded: "Of 4 studies judged to be of high quality, the 2 largest were performed in populations at low risk of CHD. They found no protective effect of fish consumption. The other two high-quality studies were relatively small and included individuals at higher risk. They found an inverse relationship between fish consumption and CHD death, suggesting that 40–60 g fish/day is optimal and associated with a risk reduction of

40–60%. Results of 4 studies of intermediate quality support that fish consumption is inversely associated with CHD mortality in high-risk populations only" [384].

18.4 Polyunsaturated Oils Prevent Dangerous Arrhythmias in Rats

Editorials in the 1980s, reviewing the possible health benefits of oily fish described the reduction of plasma triglycerides and of platelet aggregation. Another line of animal research developed in 1985 in Adelaide, South Australia but was not picked up by mainline clinical commentators until the end of the century. Work at Unilever Research Laboratories in the late 1970s had suggested that different dietary lipids could affect the function of isolated rat heart preparations [385]. Murhaghan (in Dublin) reported effects of sodium salts of different fatty acids bound to albumin in the perfusion solution on the ventricular arrhythmia threshold in isolated rabbits' hearts. Saturated and monounsaturated fatty acids lowered the current required during hypoxia but polyunsaturated fatty acids raised the threshold current [386]. Around 1983 Charnock and McLennan (in Adelaide) found that papillary muscles isolated from rats fed a diet supplemented with polyunsaturated vegetable oil were less susceptible to develop fast arrhythmias when treated with catecholamine than rats fed a saturated animal fat diet [387]. They then used an in vivo model, in which under anaesthesia they opened the rat's chest and threaded a fine ligature round the left coronary artery. The ECG was recorded as the coronary artery was occluded. Age and a saturated (sheep) fat diet increased the risk of ventricular fibrillation – and if this was prolonged – sudden cardiac death [388]. In their next series of experiments the duration and type of ventricular arrhythmia (tachycardia or fibrillation) were recorded in rats after the left coronary artery was ligatated for 15 min, then released so that the effect of reperfusion could be monitored. Before the experiment the animals had been fed a standard diet or this supplemented with 12% sunflower seed oil (SS oil) or with sheep perirenal fat or with tuna fish oil [389]. Both SS oil and fish oil prevented ventricular fibrillation during occlusion about equally but during reperfusion fish oil was much more effective in reducing severe arrhythmias. Moreover the ω-3 PUFA content of the fish oil diet was about half the ω-6 PUFA content of the SS oil diet. In further experiments the Adelaide group showed in small primates, marmosets that the ventricular fibrillation threshold dose of isoproterenol was significantly higher after either SS oil or fish oil diets [390]. Olive oil was not protective [391].

The cellular mechanism was investigated by Alexander Leaf and colleagues at Harvard Medical School with an in vitro microscopical preparation of neonatal rat cardiac myocytes. Their spontaneous beating rate was recorded with a video camera. Addition to the perfusion medium of agents known to cause arrhythmia in patients – ouabain or isoproterenol, or external imposition of a low voltage [392] initiated fast arrhythmias but these could be prevented or abolished by low concentrations of free PUFAs: EPA, DHA, also linolenic and linoleic, but not arachidonic acid.

They showed that free PUFAs in the medium inhibited the voltage dependent Na^+ channels [393] and also the Ca^{++} channels [394].

18.5 Human Prevention Trials with Fish Oil

The relevance for human cardiovascular disease of these animal experiments was strongly supported by two randomised controlled secondary prevention trials. Burr et al. [395] ran a diet and re-infarction trial (DART) in Cardiff, south Wales. Over 2,000 men who had recovered from myocardial infarction were allocated randomly to receive or not receive advice on each of three dietary factors: lower total fat with increased P/S ratio, increase in fatty fish, or increase in cereal fibre intake. In the 1,015 subjects in the fish group those who could not tolerate fish were given 3 Maxepa (fish oil) capsules, daily. Compliance was reasonably good, checked with plasma fatty acid measurements. There was a 29% reduction in 2 year total and CHD mortality in the fish group. The intake of fatty fish averaged 300 g/week, supplying 2.5 g of EPA. The effect of the fish diet appeared early in the trial. Burr et al. suggested that prevention of ventricular fibrillation during myocardial ischaemia might be an explanation. The "fat change" and "increased fibre" groups had no significant benefits [395].

Ten years after DART, came GISSI [396], the Gruppo Italiano per lo Studio della Sopravivenza nell Infarto Miocardico, a network with hundreds of collaborators across Italy. They enrolled over 11,000 men and women who had survived a myocardial infarct in the previous 3 months. They were randomly assigned supplements of EPA + DHA ethyl esters (1 g/day) or vitamin E, synthetic α-tocopherol (300 mg/day) or both or neither. Follow-up was for 3.5 years. The relative risk of cardiac or coronary death was 0.65 (highly significant) in the EPA/DHA group and their total mortality was 0.80 (0.67–0.94). Vitamin E disappointed; it had no significant benefit. The pure EFA and DHA in GISSI would correspond to about 100 g of fatty fish/day and here it was the pure long chain ω-3 PUFAs that were tested, not fish. The GISSI authors evidently thought that the anti-arrhythmic effect of the ω-3 PUFAs is one of the most likely explanations for their results [397]. In both GISSI and DART the benefits happened without change in the plasma cholesterol.

Leaf and Weber [398] wondered "How can a naturally-occurring dietary factor (ω-3 PUFAs) possess so many different effects in our bodies?" They suggested that from around 1800 A.D. total fat in human diets has increased with more saturated and ω-6 PUFAs while ω-3 fatty acid intake of modern humans is lower than it was in paleolithic times, the long period of human evolution.

By 1994 recommendations to eat more fatty fish started to appear as part of general dietary policy to reduce CHD. That year the British COMA recommendations included "R 3.1 We recommend that people eat at least two portions of fish, of which one should be oily fish, weekly" [399]. The consequential questions were going to be: "Where is the whole world going to find all the oily fish recommended" and "can EPA or DHA be produced from micro-algae (e.g. Schizochytrium) or would linolenic acid in plant oils be able to substitute adequately?"

Chapter 19
Alcohol

Pathologists had often noted relative absence of atheroma at post mortem examination of high alcohol consumers. Klatsky (1974) reported the first epidemiological study suggesting alcohol protects against CHD. In the years 1977–1999 many prospective studies reported on estimated alcohol intake (Table 19.1 has examples): moderate consumption, 1–4 drinks per day have been generally found protective against CHD. Public health authorities cannot, however, recommend alcohol because its responsibility for violence and accidents in young people removes more life-years than are saved by the CHD benefit in older people in affluent communities.

The best established mechanism for alcohol's protective effect is that it raises HDL-cholesterol. Although red wine contains more antioxidants, no specific disease benefit has been associated with one type of beverage. Years 1974–1997.

19.1 Impressions at Post-mortem Examination

In the first half of the century experienced pathologists taught that they saw little atheroma at post mortem in people who died of alcoholic cirrhosis of the liver [400]. The explanation was not settled. A quick suggestion that alcohol in the blood helped to dissolve lipids was very unlikely. Blood alcohol seldom exceeds 0.2% and the blood lipids are attached to proteins. Perhaps undernutrition and low fat diets in chronic alcoholics could slow atheroma formation. People tend to die younger with cirrhosis than from atherosclerotic diseases. Wilens [401] found little difference in degree of atherosclerosis in a large necropsy series at Bellevue Hospital, New York, when alcoholic and non-alcoholic cases were age-matched. Ruebner et al. in another large postmortem series at Johns Hopkins [402] even with age-matching, still found myocardial infarction significantly less common in those with portal cirrhosis. But they quoted Cornfield [403] who pointed out the statistical principle that even if two lethal diseases have no relationship in a living population, there will nevertheless be a negative association between them in a postmortem study. Moore and Pearson, in their major review [404] list 15 necropsy studies of alcohol history or cirrhosis and coronary atherosclerosis or myocardial infarction. Though some showed less

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atherosclerosis with alcoholism or cirrhosis and some did not, none found a *positive* association of alcohol and CHD.

19.2 Negative Association with CHD in 1974 Case-Control Study

When the epidemiological studies of CHD got started in the 1960s one question you would expect at the initial history and examination – as in all thorough medical interviews – is "Do you drink alcohol? How many drinks per day or week?"[1] In the early results of prospective studies alcohol intake was not related to CHD events [294, 405] unlike cigarette smoking, which came out strongly positive from the start. In 1974 Klatsky et al., at the Kaiser-Permanente Medical Center published a case control study in which there was a larger portion of teetotallers among 464 patients with a first myocardial infarction than in controls [406]. This was well controlled for smoking and other major risk factors, with controls from the enormous records of the Kaiser-Permanente Health Plan.

As Klatsky et al., wrote in their introduction [406], in the previous decade the medical literature about alcohol consumption and heart disease had been dominated by interest in alcoholic cardiomyopathy. Most reports did not discuss any relation, positive or negative, of alcohol consumption to CHD, although many studies required absence of evidence of CHD as a criterion for the diagnosis of alcoholic heart disease. Klatsky et al., carefully looked for bias, an effect of ethnic differences or reduced alcohol intake because of CHD symptoms. Could alcohol affect known risk factor(s), reflect an unknown risk factor or be due to a statistical artefact? They suggested that while large intakes of alcohol can damage myocardial cells, moderate amounts might improve coronary blood flow. "It thus seems fair to say that there is some clinical and experimental evidence for a beneficial effect of alcohol on the coronary circulation. But the case for this is far from convincing. . .. Although the finding of a statistically significant negative association between use of alcohol and a first myocardial infarction cannot at this time be interpreted as proof of a protective effect of alcohol on the coronary vessels, this happy possibility does exist" [406].

19.3 Epidemiological Studies Build Up the Case

In 1977 Yano, Rhoads and Kagan [407] reported from their cohort of Japanese men in Honolulu a strong negative association between moderate alcohol consumption (up to 60 ml/day), mainly from beer, and the risk of nonfatal myocardial infarction and death from coronary heart disease [407]. This association remained significant in multivariate analyses, taking into account smoking and other risk factors. Yano

[1] Surprisingly, there seems to be no "alcohol" question in the Initial Data forms for the Seven Country Study [126] though there was a large section about smoking. Perhaps Ancel Keys had already made up his mind.

et al. found a decreasing gradient in CHD incidence with four increasing steps of daily alcohol consumption. They also noted that in a probability sample taken from their study cohort, alcohol consumption correlated *positively* with alpha (high density) lipoprotein cholesterol, which this Honolulu team were among the first to report (in 1970) as having an inverse relationship with CHD [157].

In 1979 St. Leger, Archie Cochrane[2] and Moore [408] from the MRC Epidemiology Unit in Cardiff published a correlation study of CHD mortality in 18 developed countries (1970 data) with a number of environmental factors. They warned that results of an ecological study like this must be interpreted cautiously. They found relatively little association with indices of health services, e.g. doctor and nurse density. Cigarette consumption and saturated fat were positively associated, polyunsaturated fat and gross national product negatively associated with CHD mortality. There was also a strong negative correlation with *wine*. They concede that Yano et al. [407] had evidence in favour of beer but thought that if wine has a protective effect it is more likely due to aromatic components and other trace components which give wine its distinctive character, rather than its alcohol content. They called for research on the effect of wine on blood-lipids, platelet aggregation, etc. "If wine is ever found to contain a constituent protective against CHD then we consider it almost a sacrilege that this constituent should be isolated. The medicine is already in highly palatable form (as every connoisseur will confirm). We can only regret that we are as yet unable to give information to our friends about the relative advantages of red, white or rosé wine".

Between 1976 and 1985 seven case-control studies and then some 15 prospective studies were reported. The majority showed a higher incidence of CHD in teetotallers and/or less CHD in people taking on average a few alcoholic drinks per day. Framingham, at the 24 year follow-up [409] now showed the incidence of CHD was inversely related to the amount of alcohol regularly consumed. These relationships, for men and women were weak, but statistically significant.

As time wore on there were probably more epidemiological studies for CHD reporting estimated alcohol intake than any other dietary constituent. Alcohol intake is easier to ascertain and its estimation more standardised. In the years 1985–1999 the major prospective studies reported [410–417]. They all found reduced CHD events in people who took 1–4 alcohol drinks per day, exemplified in Table 19.1. With over a million subjects in the USA and Britain they abundantly confirmed the early reports of an apparent protective effect of moderate alcohol intake against CHD. Shaper's suggestion [411] that this could be explained by drinkers who become ill moving to be non-drinkers was excluded by having a category of ex drinkers, who were found to have the same CHD risk as lifelong abstainers [418]. While some degree of confounding is possible – people who manage to take only a few alcohol drinks per day may be more in control of their lives – the consistency of results is striking and the question arose of public health policy.

[2]Inspiration for the Cochrane collaboration.

Table 19.1 Alcohol and CHD – major prospective studies 1988–1998

Author(s) (References)	Name of prospective study	Number of subjects	Result
Stampfer et al. [410]	Nurses Health Study	87,526 females (34+ years)	Few drinks/day ↓ ↓ RR 0.6–0.4
Shaper et al. [411]	British men	7,735 (40 year +)	Mortality U-shaped curve
Boffeta and Garfinkel [412]	American Cancer Society prospective	27,802 men (40+ years)	Mortality CHD RR reduced for 1–6 drinks TOTAL mortality up from 3 drinks
Rimm et al. [413]	Health Professionals FU study	51,529 men (40+ years)	CHD RR down to 0.52 for 3 drinks
Doll et al. [414]	British male doctors	12,321 men (48–78 years) 13 years FU	Mortality U-shape curve (steeper on left)
Tunstall-Pedoe et al. [415]	Scotland population study	11,629 men and women (40+ years)	Alcohol significant protective effect against CHD: men and women Shallow U-curve
Thun et al. [416]	Large prospective study of US adults	490,000 men and women (ages 30+ years)	Mortality CVD reduced but total mortality only sl reduced
Muntwyler et al. [417]	Physicians Health Study, 5 year FU	90,150 men (mean age 63–65 years)	After MI, total & CVD mortality reduced for 2–6 drinks/day

19.4 Alcohol Only Benefits Health of Older People in Developed Countries

A health benefit of reduced CHD only improves mortality rates in older people in affluent countries where CHD is common. In younger people in all countries alcohol at all levels increases disease and death from violence, accidents and other complications of alcohol abuse. In over 49,000 young Swedish men (conscripts) followed up for 25 years, death rate was lowest for abstainers and increased stepwise with daily alcohol consumption [419]. In countries where CHD is uncommon alcohol drinking increases overall morbidity and mortality from its association with injuries and accidents, cirrhosis and several types of cancer.

Scragg [420] estimated the *life-years* affected by alcohol in New Zealand. Alcohol was estimated to have caused 3% of deaths among 0–14 year olds, 20% of deaths among 15–34 year olds, mostly from road injuries and to have prevented 0.5% of all deaths among 35–64 year olds and 3.4% of deaths among ≥65 year olds due to alcohol's protective effect against CHD. Young people have longer life expectancy, hence more life years were lost than saved by alcohol across the whole country. Murray and Lopez [421] published an instructive figure (Fig. 19.1)

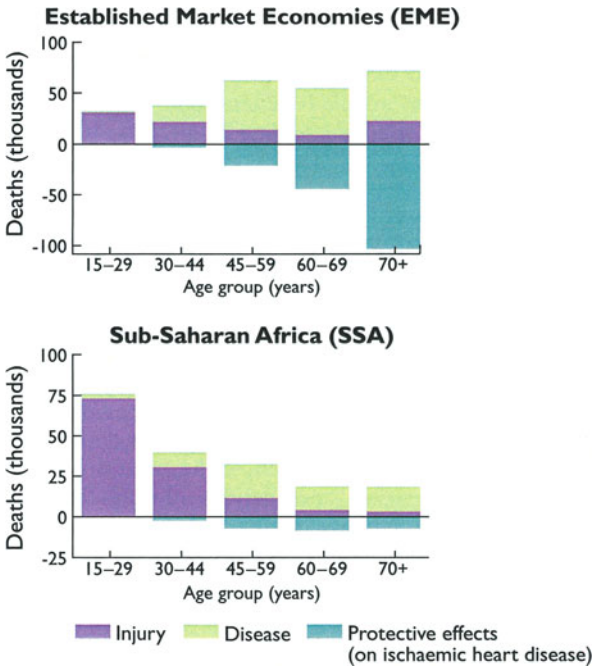

Fig. 19.1 Male deaths, attributable to and averted by alcohol use by age in Established Market Economies and Sub-Saharan Africa. Disease and injury exceed protective effects except in the oldest age group in rich countries. From Murray and Lopez [421]

showing male deaths attributable to, or averted by alcohol use: protective effects are only equal or dominant in people over 60 years in Established Market Economies. By contrast, in Sub-Saharan Africa nearly all the deaths are caused, not saved by alcohol.

19.5 Which Type of Alcoholic Drink?

Although St. Leger et al. [408] found wine consumption was negatively related to CHD incidence, they did mention that Yano et al. [407] had found it was mostly beer that had the same effect in Hawaii. In Finland it was spirits that were associated with reduced risk of CHD [422]. Klatsky and Armstrong looked at their Kaiser-Permanente data [423]. They suggested that wine drinkers were at an advantage over beer or spirits drinkers because wine is often taken with food, slowly and with regularity (in California). Rimm et al. [424] reviewed 12 ecological, 3 case-control and 10 separate prospective cohort studies of alcohol and CHD in which type of alcohol was reported. Of the 10 prospective studies equal numbers found inverse associations with CHD for wine, for beer and for spirits, so a substantial proportion of the benefits of all three types of beverage are attributable primarily to the alcohol

content rather than to other components of each drink. Doll reached the same conclusion in his masterly 1997 paper "One for the Heart" in the Christmas issue of the BMJ [425]: "There is no specific benefit associated with one type of beverage, but the benefit derives from the content of ethanol and the extra benefit associated with wine in some studies can be accounted for by differences in the pattern of drinking". The effects of ethanol on blood constituents that affect the risk of thrombosis last less than 24 h so it would seem to be more effective when comparatively small amounts are taken regularly, which is the way wine is drunk in many countries, than when the same total is taken 1 or 2 days a week, in the way that beer and spirits often tend to be drunk.

19.6 How Does Alcohol Reduce the Risk of CHD?

Three mechanisms have been proposed: increased HDL-cholesterol and/or haemostatic effects and/or antioxidants.

19.6.1 Serum HDL-Cholesterol (HDL-c)

Johansson and Laurell (in Malmo) in 1969 noticed increased serum α-lipoprotein (HDL) in alcoholics [426]. Later Johansson and Medhurst (1974) studied this in heavy drinkers at different stages. On admission to hospital intoxicated all had raised α-lipoprotein, but if they had not been drinking for a week α-lipoproteins were in the normal range. When people who were admitted with acute alcoholism stopped drinking, α-lipoproteins came down to normal in 7–18 days [427]. Berg and Johansson also measured the effect of moderate alcohol intake (50 g/day) in healthy volunteers (1973). Alpha-lipoprotein rose progressively over 5 weeks in all 8 men, by about 20% and came down when the alcohol was stopped [428]. Mishkell (McMaster, Canada) reported in 1974 a single case: "I wish to report a finding that I have not seen described before: an elevation of alpha-lipoprotein cholesterol that appeared to be related to heavy alcohol ingestion" [429]. Then, following the Millers' major paper on CHD and HDL in 1975 [153], five reports of positive relations of alcohol consumption and HDL-cholesterol appeared in 1977 from Hawaii [407], from Rome, from Sweden and from two groups in the USA. One of the latter [430] reported 7,700 subjects from five prospective studies and a "dose-response" of HDL-c to alcohol could be seen in each of the studies. This quantitative relationship was also found in two other large studies, from the BUPA Health Fund in Britain [431] and from nine lipid research clinics in the USA [432].

Increased HDL-cholesterol has been accepted as the most likely main explanation for less CHD with moderate alcohol consumption [425]. A randomised controlled trial hardly seems possible. There was confusion for a time with reports that the subfraction HDL_2 is inversely associated with CHD but moderate alcohol intake only increases HDL_3, while heavy drinking is needed to increase HDL_2 [433]. Others have found that HDL_3 also appears to protect from CHD [163] and

that moderate alcohol intake in cohort studies (where the intake is long term) increased HDL_2 about as much as HDL_3 [434] as it did in a 6 week controlled experiment with 46 g alcohol/day from beer [435]. Alcohol increases the secretion of apolipoprotein A_1 from human liver cells (Hep-G2 line) in culture [436].

19.6.2 Haemostatic Effects

Alcohol consumption has been repeatedly shown to reduce platelet responsiveness in vitro, first by Haut and Cowan [437] whose subjects were taking a quart of whiskey per day. Then with more ordinary intakes by Mikhailidis et al. [438], by Renaud in several different communities from 1984 [439] and by Meade et al. [440]. Particularly, secondary aggregation to ADP is reduced, even with moderate intake. Reduction of serum fibrinogen [441] and changes in fibrinolytic factors were also reported [442].

19.6.3 Polyphenolic Antioxidants in the Beverage

The substances that St. Leger and Cochrane [408] suspected of having a favourable influence in wines are polyphenols, especially flavonoids, which are antioxidants. There are more of these in red wine than white wines and other alcoholic beverages. Multiple compounds occur in wine and some are in polymeric form. Frankel et al. (at UC Davis) [443] in 1995 reported these compounds in different wines, others have continued these analyses. Individual flavonoids were separated by HPLC and a standard method was used for total phenol activity. Red wines had around 10x the phenol activity of white wines. The most abundant phenolic compounds are a group of catechins. In vitro, red wines reduced Cu^{++} oxidation of human low-density lipoprotein. The catechins in red wine are quite well absorbed by human subjects, better absorbed than the flavonoids in vegetables and fruits because they are more soluble in this medium. They appear in plasma, nearly all as metabolites, and conjugated [444]. If these polyphenols are protective against CHD, people regularly drinking red wine should develop less CHD prospectively than white wine, beer and spirit drinkers. But this was not found in a 7 year follow up of 129,000 persons insured with the Kaiser Permanente health fund [445].

19.7 The French Paradox

The French have appeared to eat a diet relatively high in saturated fat (pâté de foie gras, etc.) yet have a low mortality from CHD. Bronte-Stewart [446] was perhaps the first to be puzzled by this in 1958. There have been several suggestions to explain this French paradox. One recurrent suggestion is influence of the unusually high wine intake in France [447, 448]. Serum HDL does not seem higher in France, though platelet reactivity may be lower. Another suggestion is more vegetables,

vegetable oils, dietary antioxidants and folate [449]. McMichael earlier wrote that France's leading cardiologist told him French doctors put "heart failure" on a death certificate when in other countries "chronic CHD" would be the diagnosis [450].

The WHO's MONICA project [451] looked inside national health statistics at cardiovascular disease and major risk factors in 74 regions in 25 countries with standardised methods. Three of the MONICA centres were in France (Bas-Rhin, Haute-Garonne and Lille). Their 1984–1986 mortalities from CHD *and* from cardiovascular diseases were lower than in all the other countries except Japan and China. However, with all causes mortality, the three French centres had about the average of the centres (ranks 40, 41 and 42 out of 74). Though they have a partly inexplicable low mortality from CHD, French people have higher mortality from chronic liver disease, cirrhosis, etc., than in similar developed countries [452].

Chapter 20
Coffee

Until 1990 medical interest in coffee focussed on the (fairly mild) pharmacological properties of its caffeine content. Epidemiological studies produced different results on whether coffee increased CHD or serum cholesterol. Two meta-analyses (misleadingly) demonstrated no relationship. Between 1985 and 1989 Nordic and Dutch workers found that boiled coffee raises serum cholesterol but not if it is filtered (caffeine passes through the filter). Katan's group concentrated the thin lipid supernatant of boiled coffee in large scale food equipment. When fed to volunteers it raised serum cholesterol. In 1994 Katan and a group in Germany showed independently it is two diterpenes in coffee lipid, cafestol and kahweol that raise LDL – and total cholesterol in human subjects. These diterpenes are removed when coffee is filtered and in the manufacture of instant coffee. Tea does not contribute to CHD risk. Years 1985, 1990, 1994.

Until the last decade of the century medical interest in coffee focussed on *caffeine*, the most widely used psychoactive substance in the world and the main reasons people drink (and grow) coffee. Caffeine, a methylxanthine was discovered in coffee in 1821 and characterised chemically before the twentieth century.

20.1 Coffee and Caffeine

Concerns about coffee between 1900 and 1960 were seldom about serious diseases and mostly not about its cardiac effects. Caffeine is distributed in the total body water, crosses the blood-brain barrier and antagonises the action of adenosine on A_1/A_2 receptors. Caffeine was known as a gentle cerebral stimulant, not really addictive, though when regular users stop coffee they may have headaches for a few days. Taken in the evening coffee can delay sleep. Excess use can cause an anxiety state, "caffeinism". Coffee stimulates gastric secretion, so there was interest in its relation to digestive diseases. It increases urine flow. Multiple cups of coffee many increase blood pressure a little, though this effect seems to fade with regular usage. Coffee was sometimes found useful for asthma (theophylline is closely related) or for migraine. "Many ills have been ascribed to caffeine but few have been

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substantiated" [453]. The physiological effects of the caffeine in ordinary consumption of coffee are benign. On the heart, caffeine can have a mild dilating effect on the coronary arteries and, at the same time a positive inotropic effect on the muscle, increasing the cardiac output. The increased coronary blood flow to the myocardium is likely to balance the increased oxygen demand [454]. Coffee avoidance has been part of the standard advice for people with paroxysmal tachycardia [455].

20.2 Coffee and CHD (Direct) Epidemiology

When the epidemiological studies of coronary heart disease got under way in the second half of the century, the questions "Do you drink coffee"? and "how many cups a day"? must have been asked in many of them. But unlike the alcohol question, to which the prospective studies showed a negative association, coffee in most of them showed no significant association (positive or negative) with CHD, provided adjustment was made for cigarette smoking, which tends to go with drinking coffee.

The major CHD prospectives (Tables 5.1 and 19.1) reported no significant association of coffee with CHD. This was the result at Kaiser-Permanente (1973), Framingham [456], in Hawaiian Japanese (1977), in Evans County, GA (1978) in the "Health Professionals Follow Up Study" [457] and in Willett's Nurses cohort (1996) and also in the less well known Lutheran Brotherhood Study (1981) a 12 year follow-up of nearly 17,000 men in northern USA by a life insurance company [458].

Four exceptions did find a significant association of CHD with coffee. The Boston Collaborative Drug Surveillance Program (BCDSP) reported a case-control study in 1972 [459]. This was a time of growing interest in the new speciality of clinical pharmacology. Eight hospitals participated (in USA, Canada, Israel and New Zealand), relating use of a broad range of drugs to various diseases in people admitted. One question was about coffee or tea drinking. Patients with myocardial infarction were matched with multiple controls who had a variety of other diagnoses. People drinking more than 5 cups of coffee/day had twice the risk of acute myocardial infarction of those drinking no coffee. In their second paper [460] the group reported on 440 myocardial infarction patients and over 12,000 controls who had other diagnoses in hospitals in the Boston area. Results were about the same: risk of myocardial infarction 1.5 for 1–5 cups of coffee/day. Sugar preferences did not explain this.

La Croix et al. (1986) reported [461] a 19–35 year follow up of 1,130 former medical students at Johns Hopkins Medical School. CHD here included myocardial infarction, angina and sudden cardiac death. Coffee consumption was recorded several times. Relative risk for 5 or more cups, compared to non-drinkers (adjusted for smoking) was 2.49 (for most recent coffee intake measure). La Croix wrote "Although most previous prospective studies have not supported a positive association between coffee drinking and coronary disease, these . . . differed in that they employed only a single measure of coffee consumption, . . . distant in time from the first manifestations of coronary events".

The Chicago Western Electric Company prospective, one of the studies in Table 5.1, reported a significant association of coffee with CHD mortality in smokers and non-smokers. The authors tried, but could not explain why their results were different from those of the other CHD prospective studies [462].

In Norway a prospective study was based on attendance at screening in three counties, one in the far north. 38,500 men and women were followed for 6 years [463]. Here the subgroups of coffee intake included a top one of 9 or more cups/day! The overall risk of CHD mortality with coffee was pulled up by the very high risk in the northern county, Oppland, where an earlier survey had found very high use of "boiled coffee".

The evidence about a direct association of coffee drinking and CHD was clearly inconsistent [464]. This was a situation that might be helped by meta-analysis, which was developing in the 1990s. Greenland's (1993) meta-analysis [465] found for 5 cups of coffee versus none a risk of 0.92 for the five early cohorts studies and of 1.27 for the subsequent nine. The meta-analysis by Kawachi et al. (at Harvard) [466] found the relative risk for all 15 cohort studies was 1.05. Both meta-analyses found risks of around 1.5 for the smaller number of case-control studies. But "the experience of having had a myocardial infarct may have affected the patients' rating of coffee consumption" [467]. The possibility still remained that high coffee consumption really did increase CHD risk in some communities, not in others.

20.2.1 Coffee and Serum Cholesterol: Human Experiments

On this aspect researchers were soon able to do good experiments. Little et al. [468] were perhaps first to report a correlation between daily cups of coffee and serum cholesterol, in a small case-control study in Toronto. Dag Thelle et al., in Tromsø [469], in the north of Norway, reported in 1983 that in 14,580 men and women coffee consumption was strongly related to serum total cholesterol, with a clear dose-response. This held up after multiple adjustments. "Why is this strong association not well established in the literature?" Thelle asked; it was not found in Framingham. Subsequent correspondence in the journal [470] from the Netherlands, NHANES 2, Western Electric and Heidelberg, Germany all confirmed Thelle's impression: there was no significant effect of coffee on serum cholesterol in these different large studies.

In 1985, Kark et al. (in Jerusalem) [471] reported that they did find correlations of coffee cups per day and serum cholesterol in men and women. Mathias et al. (at La Jolla Lipid Research Clinic) [472] could only find a correlation in women, not in men. They provided a table reviewing, by then, 14 publications. At least half had found no significant effect of coffee. The same year Thelle's group moved to an experiment [473]. Thirty-three men with hypercholesterolaemia were divided into four parallel groups. One continued their usual coffee intake, one abstained from coffee for 10 weeks, one abstained for 5 weeks then went back to boiled coffee, the fourth group abstained for 5 weeks then drank filter coffee. Serum cholesterol fell when coffee was removed, then rose again with boiled coffee, not with filter coffee.

The trial was too small for conclusive answers but the brewing method needed further study. Aro et al. (in Finland) [474] had larger numbers – 42 subjects also with serum cholesterol around 8 mmol/l. In random order they each drank for 4 weeks boiled coffee, filter coffee and tea. Serum cholesterols went up with boiled coffee, down with tea and slightly down on filter coffee. Traditionally Scandinavians have boiled coffee grounds in water and then poured the fluid into a cup without filtering it. Aro et al., inferred that caffeine cannot be responsible for the cholesterol-raising effect, since it passes through filter paper [474].

A third experiment was done by Bak et al. (in Rotterdam) [475]. Young subjects, with normal cholesterol levels were randomly assigned (in groups of 33) to take 4–6 cups/day of boiled coffee or filtered coffee or no coffee for 9 weeks. Boiled coffee increased serum cholesterol by almost 10%. (Incidentally prolonged boiling was not used here. Boiling water was poured on coffee grounds and kept at a temperature about 93° in thermos flasks for 10 min.)

The question: "is there a cholesterol-raising substance in boiled coffee?" was tackled in Katan's laboratory at Wageningen University. They wondered if the substance might be in ground particles floating in the brew and centrifuged some boiled coffee in a blood centrifuge. Surprisingly, the centrifuge tubes contained a thin floating layer of oil-like material [476] – boiled coffee contains 1–2 g of lipid/l. Using large scale food technology equipment Zock et al. [477] prepared a lipid-rich supernatant from boiled coffee with the lipid concentrated 10-fold. Ten volunteers took 80 g of this per day with custard for 6 weeks. Serum total cholesterol rose from 4.66 to 5.72 mmol/l, LDL-c from 2.92 to 3.78. Triglycerides also rose but HDL-c was unaffected. All returned to start levels when the coffee lipid concentrate was stopped. It provided 1.3 g of coffee lipids. Since 1 g/day of triglycerides could not increase serum cholesterol, Zock et al., reasoned it was the 120 mg/day unsaponifiable part of this lipid that raised the cholesterol.

In 1991, van Dusseldorp et al. [478] had three groups each of 21 healthy volunteers take 0.9 l boiled coffee or the same boiled coffee that had been passed through a filter paper or no coffee for 97 days. LDL and total cholesterol went up with the boiled coffee, but not significantly with the boiled filtered coffee. Caffeine intakes were the same on the two coffee preparations. The filter paper was found to retain 88% of the lipid present in the boiled-type coffee. Ahola et al., in Helsinki found the same [479].

20.2.2 It's Cafestol and Kahweol in Coffee that Raise Serum Cholesterol

A collaboration with Martijn Katan [480], with specialised chemistry and food technology searched for the identity of the cholesterol-raising factor. Three volunteers (two of the authors) took pure cafestol (73 mg/day) plus kahweol (58 mg/day) both as palmitate for 5 weeks. [Cafestol and kahweol were known to be major constituents of the unsaponifiable fractions of coffee oil]. Serum cholesterol went from 200 to 265 mg/100 ml and alanine aminotransaminase (ALT) rose to 50 U/l. In 150 Norwegians habitually drinking 5 or more cups of boiled coffee/day serum

cholesterols were higher than in 150 drinking filter coffee but serum ALTs were almost the same. It was reassuring that in Norwegians who were used to boiled coffee serum ALTs were not elevated. No other biochemical indicators of liver function were changed in these experiments. Cafestol/kahweol is clearly more potent in its effect on LDL-c than any other known dietary component. Each 2 mg of this increased serum cholesterol by 1 mg/100 ml.

The same year Heckers et al. (in Giessen, Germany) [481] reported independently that they extracted a large quantity of coffee beans with diethyl ether in a Soxhlet apparatus and removed the ester lipids by alkaline hydrolysis. In the unsaponifiable lipid fraction, by 4-fold crystallisation they obtained crystals which they demonstrated by thin layer chromatography to be cafestol and kahweol (diterpenes), confirmed by other chemical tests. Five volunteers were given 148 mg/day of the isolated coffee diterpenes for 30 days. Serum LDL- and total cholesterol, and triglycerides rose 50, 32 and 135% respectively. They found instant coffee to be free of cafestol.

After this, Urgert and others with Katan investigated different methods of preparing coffee. They found that cafestol and kahweol are present in floating coffee grounds [482]; the elevated ALT was still high after 6 months of cafetière (French press) coffee though it had partly subsided [483]. Both pure cafestol and pure kahweol raise ALT; cafestol has a stronger cholesterol-raising action [484]. Cafestol is present in all cultivated species of coffee, and wild Coffea species in Africa [485]. No animal model has yet been found, in which coffee diterpenes raise LDL-cholesterol as much as in humans [486].

Cafestol appears to act on the liver but by 2000 the biochemical details of its action still awaited elucidation. The experiments with coffee and serum lipids yielded a surprise finding, that the ignored tiny lipid one-thousandth of a coffee drink contains a potent cholesterol-raising substance. This explained why in some communities, coffee drinkers tended to experience more CHD. It was nothing to do with caffeine.

The practical conclusion from this research is that coffee brews could be classified [487]:

Low diterpenes: filtered, instant and percolated;
Moderate levels of diterpenes: espresso;
High levels of diterpenes: cafetiere (French press), Turkish and boiled coffee

People at increased risk of heart disease who drink much coffee should be advised to select coffee brews low in diterpenes.

20.3 And Tea?

Tea served as the control drink in some of the research on coffee and CHD. Scientific research on tea, relevant to CHD risk was done towards the end of the century. In general it confirmed the general belief that tea is a healthy drink. The water has been boiled (so has no pathogenic bacteria); the infusion provides potassium and

useful amounts of fluoride and manganese and the caffeine content is about half that in coffee. Few epidemiological studies have focused on tea. More often they claimed an inverse association with CHD but there have been exceptions [488]. Confounding depends on the other things tea drinkers do and take. Tea contains antioxidant flavonoids – about a third of dry weight of the leaf [489]. Brewed green tea inhibits copper oxidation of LDL *in vitro*. In black tea, the usual drink in Western countries, the flavonoids are polymerised and less active as antioxidants. Drinking tea, green or black does not appear to prolong the lag time of LDL oxidation ex vivo [490]. In human experiments 6 cups of black tea did not affect the plasma cholesterol of healthy subjects, either with a parallel design [490] or crossover [491], compared with a placebo drink (of mineral water, or caffeine in warm water).

Chapter 21
Trans-Fatty Acids

By pumping hydrogen into edible oils (with heat and nickel catalyst) their fatty acids become more saturated and the oil solidifies. Hydrogenation was used from around 1912 to make the original margarines, which became the poor persons' butter. If hydrogenation is not taken to completion and some double bonds remain, some of them now have their hydrogen atoms on opposite sides i.e., in trans form (changed from natural cis form, with both hydrogens on the same side of a double bond). The molecule changes shape and the oil's melting point goes up. The major industrial trans fatty acid is 18:1 trans, called elaidic.

Early human experiments with trans fatty acids (TFAs) had inconsistent effects on serum cholesterol, but from 1990, with more precise composition of test fats comparing 18:1 trans (elaidic) with 18:1 (oleic), TFAs raised serum LDL-c and at very high intakes (8–11% energy %) also lowered HDL-c. As to epidemiology, cohort studies with food frequency questionnaires suggest TFAs increase risk of CHD; case-control studies based on adipose tissue TFA did not.

Trans fatty acids are also made by microbial biohydrogenation in the rumen of cows and sheep so a small percentage of the fatty acids in beef, lamb and dairy foods is natural trans 18:1, vaccenic acid. Its double bonds are in different position from elaidic. There were no human experiments in the twentieth century to test the biological effects of vaccenic acid, i.e., non-industrial TFA. Years (1975), 1990–1995.

21.1 Hydrogenation of Liquid Oils to Make Solid Margarines

Unsaturated fatty acids can potentially have the hydrogens at each double bond in one of two configurations: both on the same side, in the natural or *cis*-form, or opposite in the *trans*-form. Human consumption of *trans*-unsaturated fatty acids was small until Wilhelm Norman (in Germany) invented hydrogenation of edible oils in 1903. By feeding pure hydrogen into the oil, with a fine nickel catalyst in a closed apparatus with heat some of the unsaturated fatty acids are converted to the corresponding saturated acids. This raises the melting point, making vegetable and marine oils more solid and more stable against oxidation. *Trans*-fatty acids are also produced as a by-product (Fig. 21.1).

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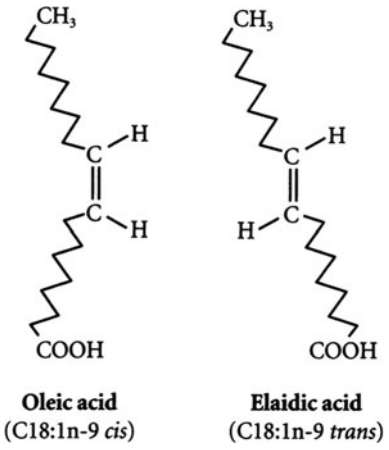

Fig. 21.1 Structure of a *cis* and a *trans* monounsaturated fatty acid (18:1). Note that *cis* 18:1 is bent at the double bond; *trans* 18:1 is straight (resembling saturated fatty acids)

From around 1912 these hydrogenated oils were used to make margarines which became the poor man's butter and, in the 1914–1918 and 1939–1945 wars largely replaced butter. But from around 1960 margarines with increased polyunsaturated fatty acids were developed, they were not as solid, so kept in tubs in a home refrigerator.

21.2 Human Experiments: Trans Fatty Acids and Serum Cholesterol

Trans fatty acids have been considered in CHD research since investigators started measuring the effects of different fats, oils, hence fatty acids on human serum cholesterol from 1956. Bronte-Stewart et al. [66] found that hydrogenated groundnut oil (iodine value 55) raised total and LDL-cholesterol while natural ground nut oil (iodine value 89) did not. Ahrens et al. [68] reported two subjects whose serum cholesterol rose on hydrogenated corn oil. Fatty acid analyses showed increased saturated and *trans* fatty acids, decreased linoleic. Any or all of these three changes in composition might have produced the serum cholesterol response.

In this work with *trans*-fatty acids the medical researchers have relied on fats and oil companies for special variants of the usual commercial product and on specialised laboratories for fatty acid analyses. Before gas-liquid chromatography was invented this involved iodine values, spectrophotometric analysis after alkali isomerization and, for *trans* fatty acids infra-red analysis [68]. Some of the technical language of fats and oils chemistry came into medical vocabulary.

The first experiment designed to test an effect of *trans* fatty acids was done by Anderson, Grande and Keys (1961). Twenty-three men took (for 3 weeks, in random order) a corn/olive oil mix, and the same mix that had been selectively hydrogenated, and butterfat. The selectively hydrogenated oil contained over 40% *trans*, mostly *trans* 18:1; its saturated and polyunsaturated fatty acids were almost the same as the

natural corn/olive. Serum cholesterols were 188, 204 and 233 mg/dl on the natural oil, oil with *trans* and butterfat [492].

In 1972–1975 came two reports on *trans* fatty acids and serum cholesterol, both from research laboratories with major fats and oils companies. Vergroesen [493, 494] (with Unilever, the Netherlands) gave liquid formula diets with different known fatty acid composition to Trappist nuns in a monastery. It appeared that elaidic acid (18:1 *trans*) at 14% raised serum cholesterol moderately, compared with oleic (18:1 *cis*) provided one egg/day was in the diet. But numbers were small and serum cholesterols fluctuated considerably in the parallel control group [494]. Mattson et al. [495] (with Procter & Gamble, USA) had prisoners in Philadelphia as subjects. Half of them took a blend of vegetable oils (all *cis*); half took the same oil with 40% of the unsaturated fatty acids in *trans* form (15 en% as elaidic). There was no significant difference in serum cholesterol.

By 1987 the British Nutrition Foundation's Task Force [496] concluded "Studies of the effect of diets containing different amounts of *trans* fatty acids in man do not suggest any consistent effects of *trans* monounsaturated fatty acids on plasma cholesterol levels that is attributable to the *trans* geometry per se". There was no general concern about trans fatty acids.

Then between 1990 and 1994 six carefully conducted human experiments were reported. Mensink and Katan [497] compared 3 diets, each taken for 3 weeks. Their fatty acids and the rest of the diets were identical and natural except that against 10% of daily energy from oleic acid in the first, the second provided this as *trans* isomers of 18:1 specially made by Unilever research and in the third they were saturated fatty acids. They found that the *trans* diet raised total and LDL-cholesterol (not as much as the saturated fat diet) and – a new finding – it lowered HDL-cholesterol significantly. Reasons could be suggested why these results were different from the earlier experiments: (i) all the *trans* fatty acids were 18:1; there were no *trans* versions of linoleic acid. (ii) Earlier experiments had only measured total cholesterol: increased LDL-c plus decreased HDL-c could give little change in total cholesterol. (iii) The *trans* fatty intake was higher than people would normally eat, even in industrialised countries.

Nestel et al. [498] tested a more moderate *trans* intake of 4% of daily energy with four different special margarines (formulated by Meadow Lea Foods, Australia). On the elaidic acid-rich diet plasma total and LDL-cholesterols were higher than on the corresponding oleic margarine, but HDL-cholesterol was unchanged.

The same year Nestel et al. [499] presented results for three margarine blends, one providing 4 energy percent as *trans*, the other two had 7 energy percent as *trans,* mainly elaidic, and more polyunsaturated fatty acids. "Provided the overall ratio of linoleic to palmitic acid in commercial edible oil blends exceeds that in the prevailing national diet, partial hydrogenation will not negate the LDL-lowering potential". Again, no fall of serum HDL-cholesterol was seen [446].

Zock and Katan [500] compared the effects of linoleic acid and its hydrogenation products, stearic acid and elaidic acid in 56 men and women, each at 7.7% of total energy. Serum LDL-cholesterol increased and HDL-cholesterol fell but to smaller extent in this trial than in their earlier trial at 11 energy percent *trans* [497].

Lichtenstein et al. (at Tufts University) [501] compared corn oil with a corn oil "stick" margarine. These were ordinary commercially available foods. On the stick margarine dietary *trans* fatty acid intake was 10 times higher (4 en%). Total and LDL-cholesterols were 11% higher but HDL-cholesterol was unchanged.

Judd et al. [502] (Beltsville Human Nutrition Research Center) in 58 men and women compared the effects on plasma lipids of 4 diets: high oleic, moderate *trans* (3.8% of energy), high *trans* (6.6% of energy) and saturated. (Member companies of the Institute of Shortening and Edible Oils supplied the special diets.) Over 98% of the *trans* fatty acids were isomers of 18:1; the most abundant was 18:1 *n*–8. Serum LDL-cholesterol increased 6% on moderate *trans* and 7.8% on high *trans*. HDL-cholesterol was unchanged on moderate *trans* but slightly lower, – 2.8% on high *trans*.

The *trans* fatty acids tested have been isomers of 18:1 (elaidic acid). There were no experimental results on effects of diene trans fatty acids or the trans acids in dairy foods (vaccenic acid) before 2000.

Box 21.1 Trans Fatty Acids

Oleic, palmitic and stearic acids were discovered in 1815 and 1816; linoleic in 1844. Natural vegetable oils contain these, and other fatty acids only in the *cis*-form. *Trans*-fatty acids in vegetable oils are a by-product of industrial (partial) hydrogenation.[1] The first *trans*-fatty acid was discovered by Moore in 1919 [503]. This was ELAIDIC acid, 18:1 *trans n*–9. Smaller amounts of other *trans*-fatty acids also occur in hydrogenated oils. From *cis* linoleic acid, 18:2 are derived 9 *cis*-, 12 *trans*-, 18:2- and 9 *trans*-, 12 *cis* 18:2 and 9 *trans*-, 12 *trans* 18:2 – the *trans* diene fatty acids. If fish oils are hydrogenated, containing unsaturated 20 and 22 carbon acid other trans fatty acids are produced. Evidence on the biological effects of *trans* fatty acids is nearly all based on *trans*-18:1.

With advances of gas chromatography not only could *cis*- and *trans*- isomers of individual fatty acids be separated, but even the positional isomers. In hydrogenated oils the double bond in *trans* 18:1 can be anywhere from Δ6 (*n*–12) to Δ13 (*n*–5), though it is usually maximal at the Δ9(*n*–9) position [504, 505]. These different positional isomers may be metabolised at varying rates [505].

Trans-fatty acids are also made by microbial biohydrogenation in the rumen of cows and sheep, so that a small percentage of the fatty acids in beef, lamb meat, milk, butter and dairy foods is natural *trans* 18:1, VACCENIC acid. It was discovered in cow's milk by Bertram in 1928 [506]. Its double bonds are nearly all at the Δ11 (*n*–7) position, unlike the *trans*-18:1 in hydrogenated oils [505]. Dairy fat also contains small amounts of *trans* 16:1 and *trans* 18:2. There were no experiments in twentieth century to test in humans the biological effects of vaccenic acid but in vitro Δ11 18:1 and

Δ9 18:1 behave differently with desaturase enzymes [507]. In experiments with vegetable oil *trans*-fatty acids, meat and dairy intake was held constant. About 1/4–1/2 of industrial countries' *trans* fatty acid intakes comes from meat and dairy foods. This proportion has been estimated from the ratio of 18:1 *trans* Δ11 (*n*–7) to *trans* Δ9 (*n*–9) in serum lipids and in adipose tissue biopsies.

[1] If hydrogenation should be complete, all the fatty acids would be saturated and there would be no unsaturated fatty acids, either *trans* or *cis*. In practice "hydrogenated fats" are usually, in fact, partially hydrogenated.

21.3 Epidemiological Studies

In 1993, Willett et al. [508] reported from their large US nurses cohort a study of CHD and *trans* fatty acid (TFA) intake. 85,000 subjects were involved and they had developed a detailed questionnaire for type of fat for their 1980 semi-quantitative food frequency questionnaire (FFQ). Risk of CHD was significantly higher in the top quintile of TFA intake. This was all associated with vegetable, i.e. hydrogenated fats. The risk number with different adjustments was between 1.35 and 1.67 and statistically significant. There was, however, no convincing dose response across quintiles.

Other opinions questioned the reliability of the TFA intakes. As well as the subjectivity of FFQs, data for TFA content of foods was incomplete and it changed between 1980 and 1993 [509, 510]. Two comparisons by Willett's group, of TFA intake and the objective measure of adipose tissue TFA reported correlation coefficients of 0.51 in over 100 women [511] and only 0.24 in over 100 men [512].

Ascherio et al. [513], in Willett's group, reported on a similar cohort study of TFA intake and myocardial infarction on over 43,000 male health professionals. Results were somewhat similar to the nurses but, adjusted for fibre intake the risk was not significant and the TFA results did not appear in the Abstract. In Finland, Pietinen et al. [514] had obtained intake data in men in the Alpha-Tocopherol, Beta Carotene Cancer Prevention Study. The multivariate risk of CHD death in the fifth quintile of TFA intake was 1.39 but no dose response could be seen.

Because of the difficulty of accurately quantifying TFA intake and because TFAs in the body all come from the diet [515], the other more objective approach would be a case-control study of definite CHD, e.g. myocardial infarction with adipose tissue biopsy to estimate TFA intake. Two substantial studies of this type were reported in 1995, in the same issue of the Lancet. EURAMIC [516] stood for European Acute Myocardial Infarction and Cancer Study. Needle aspiration biopsy specimens were taken from 671 men admitted with a first acute myocardial infarction and from 717 carefully matched controls in the same community. The centres were in 9 European countries. Adipose tissue was analysed in Finland by gas chromatography for 18:1 *trans*. Mean for all centres was from over 2.0% total fatty acids in

Israel, Netherlands, Norway and Scotland down to 0.41% in Spain. Overall there was no significant difference in adipose tissue trans fatty acids between cases and controls.

In the other study [517] adipose tissue was obtained at necropsy from 66 cases of sudden cardiac death and in life from 286 healthy controls, drawn from lists of the general practitioners with whom the cases had been registered. Cases and controls were in the Southampton area, UK. Fatty acid analysis by gas chromatography was done in Edinburgh. It gave 18:1 *trans* and 18:2 *trans*. The former averaged a little *lowe*r in the sudden death cases; the latter was the same in cases and controls. In Britain there is a standard way of grading social class in research and Roberts et al. noted the concentration of TFAs were lower in the upper social classes I and II in their controls.

21.4 Conclusion

To sum up, research on TFAs and CHD required very careful food science and difficult analytical chromatography and the amounts in diets are smaller than the other dietary lipid classes. It was shown experimentally that TFAs increase LDL-cholesterol (but rather less than myristic and palmitic acids) and, at unusually high intakes may reduce HDL-cholesterol. As to epidemiology, cohort studies with food frequency questionnaires suggested TFAs increase risk of CHD; case control studies based on adipose tissue analysis did not.

On one thing there was now international agreement: “It is unacceptable to use marketing claims such as ‘low in saturates’ when the product is high in *trans* (unsaturated) isomers” [518]. And there were calls for mandatory labelling of the *trans* fatty acid content of foods [519, 520] or to add trans and saturated fatty acids to make a single number [521]. The big fats and oils companies started to take active steps to reduce or even remove TFAs from margarines and spreads [522]. The Trans story was an excellent example of cooperation between a part of the food industry and medical scientists.

Chapter 22
Antioxidants

Experimental pathology, reviewed by Steinberg (1989) indicates that the LDL-receptor of macrophages cannot take up enough LDL to become foam cells (central cells in atherosclerosis) unless the LDL has been oxidised so that it can be recognised by the cells' scavenger receptor. Although it is unlikely the LDL is modified in the plasma, high intakes of vitamin E appeared to protect against CHD in two prospective studies (1993). Five large prevention trials were set up, some with cancer as well as CHD as outcome (reporting 1994–1999). Neither vitamin E (2 trials) nor β-carotene (4 trials) prevented CHD (or cancer). There has been much speculation why these antioxidant nutrients, carried in LDL were ineffective.

However, several epidemiological studies indicate that increased intake of fruits + vegetables are associated with lower incidence of cardiovascular diseases. Antioxidants are only one of several possible mechanisms. Years 1989–1999.

22.1 LDL Oxidation and Atherosclerosis – Hypothesis

In 1989 Steinberg et al., started a new approach to CHD prevention with their Mechanisms of Disease review "Beyond cholesterol. Modifications of low-density lipoproteins that increase its atherogenicity" [523]. Their hypothesis was an extension of Brown and Goldstein's discovery and elucidation of the cells' LDL receptor [89]. Lipid laden macrophages, "foam cells" are a central part of atherosclerosis but normal monocytes and macrophages incubated with high concentrations of native LDL cannot take up enough LDL to become "foam cells". To get much more LDL into these cells it has to go in by another route. Goldstein et al. [524] were the first to describe how LDL modified by acetylation is taken up by a "scavenger receptor" or "acetyl-LDL receptor" at rates much higher than native LDL can go into the cell. The most likely modification of the LDL is oxidation: peroxidation of part of the polyunsaturated fatty acid, linoleic acid, forming aldehydes that attach to ε amino groups of lysine on the apolipoprotein B so that the modified LDL is now recognised by the scavenger receptor and not the LDL-receptor [524]. The hypothesis was

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based on cell culture work and supported by experiments with transgenic rabbits that lack the LDL receptor. It appears that the LDL must be modified within the intima of arteries, not in the plasma. "If oxidised LDL were generated in the plasma it would be swept up within minutes by the liver, and oxidation is inhibited by plasma so would require the favourable conditions of a sequestered microenvironment" [523]. Despite the presumed location Steinberg [523] and Witztum [525] thought that intervention trials with natural lipid-soluble antioxidants such as vitamin E or β-carotene should be encouraged, and so did a 1992 workshop [526].

22.2 α-Tocopherol the Major Antioxidant Inside LDL

The most abundant antioxidant inside LDL is α-tocopherol (the principal form of vitamin E in the body); each LDL particle normally contains about 6 molecules of it (Box 22.1). It is the principal chain-breaking antioxidant in plasma. With very high supplement dosage α-tocopherol concentration in LDL can be increased 3-fold. From 1991 to 1993 several laboratories showed that if healthy subjects are given high doses of vitamin E for several weeks, then their blood is taken, its LDL separated and exposed to an oxidant, usually copper, the chemical signs of oxidation of the LDL (appearance of lipid peroxides) were delayed [527]. Extra vitamin E went into the LDL in vivo but the oxidation was in vitro. The dose of vitamin E to achieve this effect had to be at least 400 IU. (As this was given as synthetic [racemic] α-tocopherol it is equivalent to over 200 mg of natural (RRR) α-tocopherol which is about 13 times the RDA of 15 mg/day for adults.)

Box 22.1 Antioxidants in LDL

One molecule of LDL (on average) contains 2,420 mols total fatty acids, of which half are polyunsaturated, mostly linoleic (18:2), and 6 mols of α-tocopherol, 0.5 mols of γ-tocopherol and 0.33 mols of β-carotene [528].

22.3 Vitamin E and CHD in Cohort Studies

Some cross-sectional and case-control epidemiology in the early 1990s suggested higher intakes or serum levels of vitamin E protect against CHD [529], others did not [530]. Then two large cohort studies reported from Harvard in May 1993, in 87,000 female nurses [531] and nearly 40,000 male health professionals [532] that high intakes of vitamin E were associated with a CHD risk of about 0.65. Reduction of risk was nearly all in the top quintile; here the mean vitamin E intake jumped into amounts that could only come from supplements. From foods the range of vitamin E intakes is quite small, the 5th–95th percentile range in men is about 4–18 mg/day

[533] but in the top quintiles in these cohorts intakes were 208 [531] and 419 IU/day [532].[1] So, even if ordinary diet cannot contribute enough it looked as if generous supplements of vitamin E could protect against CHD. Randomised controlled intervention trials were needed.

22.4 Randomised Controlled Prevention Trials with Vitamin E

The first of these, the Cambridge Heart Anti Oxidant Study [534], had the acronym, CHAOS. Its results were equivocal. In this secondary prevention trial there were fewer (non fatal) CHD events in those taking vitamin E (400 IU or 800 IU) but slightly more cardiovascular deaths. Correspondence in the journal expressed the disappointment and search for explanations that these results provoked [535].

There were only 2,000 subjects in the Cambridge trial, but a very large trial in Finland, ATBC (standing for alpha tocopherol, beta carotene) started to recruit in 1985. It had 29,133 middle-aged male smokers. It was funded principally by the US National Cancer Institute and its main focus was on lung cancer. In 1981 Peto and Sir Richard Doll had asked, in a reasoned paper in Nature "Can dietary beta-carotene materially reduce human cancer rates?" [536]. The Finnish trial had 4 treatments: alpha-tocopherol 50 mg/day or beta-carotene 20 mg/day, or both or neither. The first main publication [537] reported the disappointing news: no prevention of lung cancer by either supplement – possibly some increase in the β-carotene group. A bar chart also showed 602 ischaemic heart disease deaths with α-tocopherol and 637 deaths without (? not significant). On CHD, a paper appeared in 1997 [538] reporting the 1,862 men who came into ATBC with a history of previous myocardial infarction, i.e. they constituted a secondary prevention trial. They actually had more CHD deaths with α-tocopherol and especially with β-carotene. The authors could only wonder whether α-tocopherol limits "preconditioning" of the myocardium.

Two years later a big Italian secondary prevention trial, GISSI (with 11,300 subjects) reported that, fish oil capsules reduced recurrent CHD events significantly, but high dose Vitamin E (300 mg/day) had no benefit [396]. (The hope that vitamin E supplements could help to prevent CHD or reduce recurrences had almost gone by the end of the century, and in the first five years of the twenty-first century another five large trials all reported no benefit.)

22.5 Trials with Beta-Carotene

Beta-carotene is the other antioxidant lipid carried in LDL and its concentration can be increased 5 or more times with dietary supplements. Because the earlier hypothesis for antioxidants was that β-carotene might help prevent cancer, trials

[1] To convert these international unit (IU) doses of synthetic α-tocopherol to mg of natural (RRR) α-tocopherol multiply by 0.67 [532].

with β-carotene had started before the oxidised LDL hypothesis, and four of these reported results before 2000:

> ATBC (see above) [538], CARET (with 18,314 subjects) [539],
> the Physicians Health Study (with 22,071 subjects) [540] and
> the Women's Health Study (with 39,071 subjects) [541].

They gave the β-carotene group 20 mg/day or 50 mg alternate days or 30 mg/day. (The usual dietary intake is around 2 mg β-carotene/day.) None of these reduced cancer incidence. For CHD or cardiovascular disease there were also no significant benefits (or harm) from β-carotene in any of these 4 trials in over 100,000 subjects.

These were big disappointments. Leading scientists had reasoned for or directed the trials. Roche provided all the α-tocopherol and β-carotene for the 8 year long ATBC trial (and a few years later gave up its vitamin business). The discussion "How did we get it wrong" has to be different for vitamin E and for β-carotene. From the viewpoint of CHD the idea for trials was started by the oxidised LDL hypothesis and encouraged by in vitro LDL-oxidation studies and the two Harvard prospectives. Increasing alpha-tocopherol was more likely to protect polyunsaturated fatty acids in LDL [542] than β-carotene.

22.6 Why Didn't Alpha Tocopherol or Beta Carotene Work?

Differences in vitamin E intake from diet are not large. The clues for trialling vitamin E came from supplement intakes, not dietary variation. In the end it is difficult to see any other explanation for the failure of vitamin E except that LDL must get oxidised inside the arterial intima in ways that can't be inhibited by trebling vitamin E concentration in the LDL particles. Whatever dietary antioxidants or supplements may do in the body, it is clear they don't prevent CHD. The debate about β-carotene was broader. It concerned mainly cancer, other carotenoids and misinterpretation of epidemiological data [543].

Box 22.2 Fruit and Vegetables – Part of Favourable Dietary Patterns

Generous intakes of fruit and vegetables have been part of dietary patterns associated with low rates of CHD and cardiovascular diseases:

- Traditional Mediterranean diets (see Chapter 8)
- Traditional Japanese diets (see Chapter 8)
- Some hunter-gatherers' diets [544]
- and Vegetarian diets.

Key et al. [545] reported a combined prospective study of 5 groups of vegetarians with socially matched omnivore controls (76,000 men and women in all) in four countries. The vegetarians had significantly lower mortality from CHD (corrected for smoking), but not from cancer. The authors of one of these cohort studies pointed out that it might be several other features of vegetarian diets rather than avoidance of meat that reduce the risk [546].

Ness and Powles reviewed the literature on fruit and vegetable intake and cardiovascular disease [276]. For CHD, 9/10 ecological studies, 2/3 case-control studies and 6/16 cohort studies found a significant protective association with consumption of fruit and vegetables or surrogate nutrients. In another review, Law and Morris report that higher vitamin C intakes predicted less CHD in 6/7 cohort studies [277]. Vitamin C was used here as a biomarker of fruit and vegetable intake. Pure vitamin C supplements have failed to prevent CHD in three randomised controlled trials [547]. Fruits and vegetables in general provide (or lack) a number of constituents that may help reduce CHD. They are low in calories, total fat, saturated fat [548] and sodium; they donate potassium, folate and flavonoids [549] to the diet and they contain no cholesterol. Vegetarians tend to have lower plasma cholesterols than omnivores [545, 550]. In the DASH trial, blood pressures were lower with extra fruits and vegetables [551]. In a cohort of over 39,000 female health professionals in the USA, higher fruit and vegetable intake was associated with lower risk of myocardial infarction [552].

Chapter 23
More Controlled Dietary Trials

Another four controlled prevention trials with serum cholesterol-lowering diets reported between 1979 and 1988, in Finland, Norway, UK and USA. They are combined in Table 23.1. The bottom line of the 10 trials in the table was significantly fewer CHD events and lower mortality (not significant) in the diet groups. The trials with largest product of % serum cholesterol reduction x years on diet showed much better results.

Two large multi-factorial trials, reported in the late 1980s. Both were ambitious and involved multiple centres, MRFIT in USA and the WHO Collaborative trial in factories in four European countries. Their main results were disappointing for a variety of reasons but there were a few encouraging aspects.

Four small dietary trials were assessed by coronary angiography. Serum cholesterols fell 10–20% in all of them and coronary narrowing was significantly less (measured "blinded") in the diet group.

A dietary trial in Lyon, France had exceptional lower CHD results compared with controls, without change in serum cholesterols. One interpretation is an effect of linolenic acid (18:3, ω-3) in the canola oil supplied. Years 1979–1999.

After the long dietary trial in two Helsinki mental hospitals (Chapter 14) additional controlled trials with change(s) of dietary fat for the prevention of CHD were reported between 1978 and 1992. Three of these and part of another can be added to the earlier six dietary trials reviewed in the first section on dietary trials (Chapter 14). They are summarised in Table 23.1. These 10 trials in the table, though they have their flaws, may be seen as the best experimental answer to the question whether changes of dietary fats to lower plasma cholesterol reduce the risk of CHD.

23.1 Further Dietary Trials in Table 23.1

Kallio et al. (1979) in Finland [553] divided 375 men and women, who had been treated in hospital for acute myocardial infarction in Turku or Helsinki randomly into intervention or control groups. The intervention groups were given dietary

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Table 23.1 Randomised controlled trials of dietary fat changes to lower serum cholesterol: effects on mortality and CHD

Trial		Type	*n*	Percentage of reduction of serum cholesterol × years (product)	Total deaths I/C	CHD fatal & non-fatal I/C	References
1. Research committee low fat (1965)		SD	264	–8 × 3 year (–24)	20/24	31/34	226
2. Rose corn oil (1965)		SD	52	–9 × 2 year (–18)	5/1	12/6	227
3. MRC soy-bean oil (1968)		SD	393	–15 × 3.4 year (–51)	28/31	45/51	231
4. Dayton VA (1969)		PD	846	–13 × 7 year (–91)	174/177	60/78	235
5. Leren (1970) 11 years		SD	412	–14 × 5 year (–70)	101/108	79/94	232, 233
6. Helsinki Mental Hospitals (1972)	Men	PD	1,900	–15 × 12 year (–180)	188/217	34/76	236, 237,
	Women		2,836	–13 × 12 year (–156)	415/456	73/129	239, 240, 241
7. Kallio (1979)		SM	375	–11 × 3 year (–33)	41/56	69/76	553
8. Hjermann (1986)		PM	1,232	–13 × 5 year (–65)	19/31 × 0.6 = 11/19	24/45 × 0.6[a] = 15/27	554, 555
9. DART fat advice (1989)		SD	2,033	–3.5 × 2 year (–7)	111/113	132/144	395
10. Frantz Minnesota (1989)	Men	PD	4,393	–14 × 1 year (–14)	158/153	69/74	556
	Women		4,664	–14 × 1 year (–14)	111/95	62/47	
				Totals	1365/1459 = 0.936	681/836 = 0.814[b]	

I = intervention group; C = controls; S = secondary prevention trial; P = primary prevention trial; D = diet change only; M = multifactorial, including diet.

[a]Hjermann's correction, to allow for smoking effect.

[b] $P < 0.02$.

advice and encouraged to stop smoking. The authors give no details of the diet in their Lancet paper but serum cholesterols were 11% lower in the intervention group. Both groups reduced their smoking by about the same 50% "thus smoking habits do not account for the differences in mortality observed in this study" [553]. There were more sudden deaths in the control group.

Hjermann et al. (1981) in Oslo, Norway [554, 555] selected 1,232 healthy men (40–49 years of age) who were found at screening to have high serum cholesterol; most were smokers. There were several exclusions (abnormal resting ECG, clinical diabetes, disabilities, psychiatric disease, alcoholism) and those already on a lipid-lowering diet. They were randomised into intervention and control groups. The former were instructed in a reduced saturated, increased polyunsaturated diet and given anti-smoking advice; if they were overweight or had raised fasting triglycerides reduced energy intake was advised. After 5 years serum cholesterols in the intervention group averaged 13% lower, triglycerides were lower and so were cigarettes/day (5.5 cf 10 in controls). There were fewer fatal and non-fatal CHD events in the intervention group. The authors estimated that the cholesterol reduction was responsible for 60% of the better outcomes, smoking reduction for 25%. A factor of 0.6 is therefore applied to the outcomes in Table 23.1.

The next dietary trial to report was the *Diet and Reinfarction Trial (DART)* (1989) run by the British Medical Research Council Epidemiology Unit in south Wales [395]. It was a secondary prevention trial of three dietary factors. 2,033 men who had recovered from myocardial infarction were allocated to receive or not to receive advice in reducing fat intake and increasing the polyunsaturated to saturated fat ratio OR increasing cereal fibre OR increasing fatty fish, all in a factorial design, with 2 years follow-up. The fatty fish arm was described in 18.5. The change in the dietary fat arm (shown in Table 23.1) achieved only a 4% reduction of serum cholesterol and small reductions in non-fatal CHD, no reduction in CHD.

Also in 1989 *Frantz et al.* [556] reported a primary prevention trial among the residents in six Minnesota state mental hospitals. This was a similar idea to the Helsinki mental hospitals trial but the two diets – usual institutional or P/S 1.6 and low cholesterol were served in a single line. The food servers issued the diet indicated on a computer code that the subject was carrying. The trial was randomised and double-blind. Though the serum cholesterols were 14% lower in the treatment group there was no difference in the outcomes. But the mean time on the diets was only just over a year. The authors wrote that "By the end of the initial 3-year pre-treatment observation period, the practice of vigorous drug treatment and early discharge to the community was in full swing". They expected that a fair length of time would have been required for the diet to exert an effect. Table 7 in their paper deserves more prominence: myocardial infarction and all deaths in men and women 35–55 years who were on diets for more than 2 years were only about half the numbers in the control group – and this was for 1,568 subjects.

Six of the trials were secondary, four were primary prevention trials. They tested a reduced saturated fat, increased polyunsaturated fat diet against an approximately equal number of randomised subjects on usual diet. The results clearly showed no increase in total mortality on the serum cholesterol-lowering diet. This is in contrast

to the earlier drug trials, some of which had increased non-CHD mortality [557]. Secondly, the total CHD events in the diet groups were 0.814 that in the controls ($P < 0.05$ by a simple Wilcoxon test). Then, if the trials with the largest product of % serum cholesterol reduction x years on diet, trials 3, 4, 5, 6, 7 and 8 in Table 7, are looked at separately the outcome numbers for the combined diet groups were only 0.89 of the controls for total mortality and 312/531 or 0.59 of the controls for CHD events.

Two reported trials are not included in Table 23.1. Woodhill et al. [558], published in a little known journal, did not give numbers for CHD between diet and control groups. The authors felt that any small effect of diet was overshadowed by changes in smoking, physical activity weight loss, etc. "This study has brought home to us the almost insuperable difficulties of conducting intervention studies of this nature in CHD". This Australian trial was not included in a 20 page review by the Australian National Heart Foundation's Nutrition Committee [559]. The other omitted trial was by RB Singh of Mooradabad, who has been discredited [560].

23.2 Two Large Multi-Factorial Trials

In the mid to late 1980s two much larger controlled trials reported. In order to have large numbers they had to be multi-centric and rely much less on one-to-one professional advice and motivation of the subjects. But by aiming to reduce several risk factors these were demonstrations of what can and should be achieved in practice and it was hoped that significant disease reduction might be achieved.

The Multiple Risk Factor Intervention Trial, MRFIT, run by the National Heart, Lung and Blood Institute in the USA started in 1973–1976. 12,866 healthy middle-aged men were selected in centres in 18 different cities. They were randomised to usual care (UC) or SI (special intervention) who were advised on diet to lower serum cholesterol, and to stop smoking and on treatment if blood pressure was high. It was the world's first large multifactorial trial of preventing CHD. The trial lasted 6 years. Much was expected from this expensive exercise but the main report in 1982 [561] was a disappointment. In SI serum cholesterol was only 3–2% lower than in UC, though there was more reduction of smokers, 30%. CHD deaths were 7% lower in SI but none of the main end points were significantly different. One problem was that the usual care (UC) group were making spontaneous healthy changes in their risk factors, and CHD mortality was now declining across the USA. After the 6 year intervention stopped, participants were followed up to 10.5 years [562] and 16 years [563]. Differences in outcomes improved post-trial. In those who had been in the intervention group CHD deaths were 11.4% lower and total mortality was 5.7% lower at 16 years. Probably some who had changed in SI continued their new lifestyle; it is also possible that 6 years of reduced risk factors had a longer benefit.

The other multiple risk factor primary prevention trial, the *WHO Collaborative Trial of Multifactorial Prevention of CHD* [564] involved over 60,000 middle aged

men in a total of 80 factories in Belgium, Italy, Poland and the UK, about a quarter of this total in each country. Factories were arranged in matched pairs: one in each pair was randomly allocated to be the control; the other was allocated to intervention, which was cholesterol-lowering diet, advice to stop smoking, reduce overweight, treat raised blood pressure and take regular exercise. The trials ran for 6 years. Overall reduction of total CHD was 10.2%, non-fatal CHD 14.8%. These missed statistical significance because of the disappointingly small reduction of risk factors in the UK factories. No attempt was made to estimate the contributions of the dietary changes but the overall percentage fall in serum cholesterol was only 1% [565].

It had been agreed that each country could report separately as well as collectively. In Belgium there was the best reduction of risk factors and the corresponding best outcomes [563]. 19,409 men took part. In the intervention group serum cholesterol, smoking and blood pressures came down and the result was total mortality in the intervention group minus 17.5% (significant) coronary mortality –20% (NS), CHD incidence –24.5 ($P = 0.03$). Because of the lives that can be saved the Belgian authors suggested: "factory physicians might widen their activities from prevention of specific work-related illness to the prevention of one of the major chronic diseases of our century".

23.3 Four Dietary Trials Assessed by Coronary Angiography

Between 1985 and 1992 four trials were reported that started with coronary angiograms, indicated for stable CHD, e.g., angina. A cholesterol-lowering diet was prescribed and serum cholesterol fell 10–20%. Angiography was repeated after 1, 2 or 3 years: in all four of these trials the amount of coronary narrowing was significantly less (measured "blinded" with computer assistance) than in controls. Each trial differed in details.

In the Leiden trial (Netherlands) the 30 patients were their own controls. Diet was given for two years: vegetarian, with a polyunsaturated/saturated fat ratio 2:1; serum cholesterol fell by 10%. Progression of coronary lesions was not seen in those with good reductions of total/HDL cholesterol [567].

In Ornish's trial (in San Francisco) 42 patients were randomised. The intervention group had a low fat vegetarian diet, were persuaded to stop smoking and exercise for 1 year. Serum cholesterol was down 19%. Diameter of coronary lesions regressed a little in the intervention group (who also had less angina) and progressed in the controls [568].

In Heidelberg (Germany) Schuler et al., randomised 113 patients into usual lifestyle or low fat diet and an exercise programme. Serum cholesterol fell 10% in the latter. At 1 year they had thallium scintigraphy for myocardial perfusion as well as repeat coronary angiography. The intervention group showed less progression or more regression of coronary narrowing [569].

At St Thomas' Hospital, London, 90 patients were randomised to control (C) or cholesterol-lowering diet (D) or (a third group) to the same diet plus cholestyramine

(DC) for 3 years. Mean serum cholesterols were 11% lower on D, 20% lower on DC than on C. There was significantly less progression of coronary lesions in the two intervention groups and fewer cardiac events: 10 (C), 3 (D) and 1 (DC) [570]. .

These four trials were difficult, required high technology and teamwork including cardiologists. They showed what was happening inside the coronary arteries and how cholesterol-lowering diets can modify it.

23.4 The Lyon Diet Heart Study

Lastly came a dietary trial which doesn't fit with the others. de Lorgeril et al. [571], randomised 506 patients who had recovered from a myocardial infarction at a hospital in southern France, to control group or a "Mediterranean-type diet". This provided more bread and fruit, moderate increases in vegetables and fish, less meat and butter. Because subjects would not accept olive oil, a margarine based on canola oil was supplied (containing 48% oleic, 15% saturated fatty acid, 5% trans 18:3, 16% linoleic and 4.8% linolenic acid, etc). During the trial blood pressures did not differ nor did serum cholesterols! The biggest difference in serum fatty acids was 18:3 (linolenic) 0.37 in controls, 0.62 (units not stated) in the diet group. The trial was stopped after a mean follow up of 27 months when there had been 16 cardiac deaths in the control and 3 in the experimental group, 17 non-fatal myocardial infarcts in the control and 5 in the experimental group.

Commentators reactions ranged from puzzlement [572] to enthusiasm [573]. Clearly some of the striking differences after a short trial may be put down to chance and the early stopping decision. The authors themselves later looked for bias and couldn't find anything major [574]. They called back their participants ($R = 423$) at 46 months. The experimental group's survival advantage persisted. Many were still using their trial diet. There was no difference in smokers [575]. de Lorgeril et al., had not measured the dietary habits of the control group so as not to influence them. Serum fatty acids were measured two months into the trial; only α-linolenic acid was significantly associated with improved prognosis.

In one prospective observational study α-linolenic acid estimated from food frequency questionnaires appeared protective against CHD [576], not in another similar (but smaller) study [577]. In rats McLennan and Dallimore showed that dietary canola oil protects against cardiac arrhythmia [578].

Chapter 24
Trials of Better Drugs

In the Lipid Research Clinics – Coronary Primary Prevention Trial, 3,800 men with type II hyperlipoproteinaemia in the USA took cholestyramine resin or placebo for 7 years. In the cholestyramine group LDL-cholesterol was 13% lower and CHD events 19% fewer, but there were more gastro-intestinal side effects (1984).

In Helsinki another large trial tested gemfibrozil for 5 years in half of 4,000 men with raised total/HDL cholesterol. Serum LDL-cholesterols were lower, HDL-cholesterols higher and there were fewer CHD events on the drug but total mortality was not statistically different (1987).

Then in 1987 the first statin, originally discovered by Endo in Japan, was approved for human use by the US FDA. Five large secondary and primary preventive trials with statins were published from 1994 to 1998 (Table 24.1) in over 30,000 subjects. All reported bigger reductions of CHD events and of total mortality than had been achieved in previous prevention trials with diet or drugs. Side effects were usually mild or absent. Statins provided the final convincing evidence that high LDL-cholesterol is an important CAUSE of CHD. Years 1984–1998.

24.1 Cholestyramine (1984)

Two large drug trials reported in the 1980s. *The Lipid Research Clinics – Coronary Primary Prevention Trial* (LRC-CPPT) (1984) was a randomised, double blind trial of cholestyramine resin, given for 7.4 years [579]. The subjects totalled 3,800 asymptomatic middle-aged men with type II hyperlipoproteinaemia in multiple centres in the USA. The control group received a placebo. Both groups followed a moderate cholesterol-lowering diet. The cholestyramine group had reductions of total and LDL-cholesterol 8.5 and 12.6% greater than the placebo group. Definite CHD deaths and/or non-fatal myocardial infarctions were together 19% less in the cholestyramine group (statistically significant). All causes mortality was slightly, though not significantly lower in the cholestyramine group. There were more gastro-intestinal symptoms in the cholestyramine group (and also more violent and

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accidental deaths, assumed to be a chance effect). "The LRC-CPPT findings show that reducing total cholesterol by lowering LDL-c levels can diminish the incidence of CHD morbidity and mortality in men at high risk for CHD because of raised LDL-c levels. This clinical trial provides strong evidence for a causal role for these lipids in the pathogenesis of CHD" wrote the authors (the corresponding author was Basil Rifkind at NHLBI, Bethesda, MD).

24.2 Gemfibrozil (1987)

The other major CHD prevention trial with a drug was the *Helsinki Heart Study*, which used gemfibrozil (1987) [580]. Total subjects were 4,081 men 40–55 years of age, asymptomatic with total cholesterol minus HDL cholesterol at or above 200 mg/dl (5.2 mmol/l). They were randomised to 2,031 placebo or 2,051 gemfibrozil. Gemfibrozil is a fibric acid derivative, i.e. chemically related to clofibrate, but had been found to have somewhat different biological actions, such as raising HDL-cholesterol. The trial lasted 5 years. In the gemfibrozil group total and LDL-cholesterol were some 10% lower, HDL-c was12% higher and triglycerides 40% lower than in the placebo group. There were significantly fewer "cardiac end points" in the gemfibrozil group, notably non-fatal myocardial infarcts, 22 (G) versus 35 (P). All causes mortality was not significantly different, 45 (G) versus 42 (P). There were somewhat more gastro-intestinal side effects on gemfibrozil. The authors concluded that this trial furnished "additional and conclusive evidence of the role of lipid modification in preventing CHD".

24.2.1 Statins Arrive

Before these large, promising trial results could have much effect on practice, a new class of drugs was being developed: the first clinical trials of *statins* reported around the time of the Helsinki trial report. The statins progressed from in vitro experiments in Japan in 1976 [184], to lowering serum cholesterol in dogs (1979) [581], then in human subjects (1980, 1982) [582, 583]. Lovastatin (mevinolin) was approved by the US Food & Drug Administration in 1987, simvastatin in 1988, pravastatin in 1991, fluvastatin in 1994 and atorvastatin in 1997.

Six large preventive trials of statins with disease outcomes were published from 1994 to 1998 (Table 24.1) – others followed, in the twenty-first century. These trials were carried out in Finland, Sweden, Denmark, Norway and Iceland [584], in Scotland [585], USA [586–589], Canada [586], Australia and New Zealand [588]. The five major trials with over 30,000 subjects combined (half on a statin, half on placebo and run for around 5 years) all reported bigger reductions of coronary deaths, non-fatal myocardial infarction and all causes mortality than had been achieved in the previous prevention trials with diet or drugs. [The sixth trial was smaller and specialised; it tested the effect on coronary artery by pass grafts (CABG)

Table 24.1 The first large clinical trials with statins

Name (References)	Year	*N*	Statin	Subjects	Trial type
Scandinavian Simvastatin Survival Study (4S) [584]	1994	4,444	Simvastatin 20–40 mg for 5.4 years	M > F 35–70 years with CHD and serum total cholesterol 5.5–8.0 mmol/l	Secondary prevention trial
West of Scotland Coronary Prevention Study (WOSCOPS) [585]	1995	6,595	Pravastatin 40 mg each evening for 4.9 years	Men 45–64 year with hyper-cholesterolaemia (but no CHD) mean 7 mmol/l	Primary prevention trial
Cholesterol and Recurrent Events trial (CARE) [586]	1996	4,159	Pravastatin 40 mg for 5 years	Men > women 21–75 years, after myocardial infarction with average serum cholesterol below 240 mg/dl (6.25 mmol)	Secondary prevention trial
Effect of aggressive lowering of LDL-c and low dose anticoagulant on coronary artery by-pass graft (POSTCABG) [587]	1997	1,352	Lovastatin 40 mg for 4.3 years	Mostly men average age 61 year with CABG	Some received cholestyramine. All got aspirin
Long-term Intervention with Pravastatin in Ischaemic Disease in Australia & New Zealand (LIDID) [588]	1998	9,041	Pravastatin 40 mg for 6.1 years	Men > women 31–75 years with history of myocardial infarction or with unstable angina serum cholesterols 4–7 mmol/l	Further FU of unstable angina Lancet 2000 secondary prevention trial
Air force/Texas coronary atherosclerosis prevention study (AFCAPS/Tex APS) [589]	1998	6,605	Lovastatin 20–40 mg for 5.7 years	Men (45–73 years) > women (55–73 years), no prior CHD, average cholesterol levels	Primary prevention trial

of lovastatin (and sometimes cholestyramine).] A meta-analysis of the five major trials [590] showed statins reduced major coronary events by 31% and all cause mortality by 21% (both significant). There was little difference between trials (and type of statin) or between men and women or between age groups. This overwhelming result is much more powerful than the bottom line in Table 23.1, the major dietary trials. As Michael Oliver wrote [591] after publication of the West of Scotland Coronary Prevention Trial (WOSCOPS) "The approximately 30% reduction of LDL-cholesterol achieved with statins is a dimension never previously seen with other drugs or diet. These drugs are the most powerful and consistent means available of lowering plasma cholesterol and have no adverse effects (at least within the 10 years of trials) . . ." In the five major trials (in Table 24.1) cancer occurred in 1,021 subjects on placebo and 1,009 on statins [590]. Uncomfortable symptoms, which occur with cholestyramine and nicotinic acid have been largely absent with statins, except for occasional effects on muscles.

Of course the trials tested statins first in people with CHD (previous myocardial infarct) and high serum cholesterol [584] then in people with CHD and average serum cholesterols [586, 588]. They also tested them in people with high cholesterol but no CHD [585], even middle-aged people with average serum cholesterol and no CHD [589]. People 65 years of age and older in the trials seemed to benefit as much as younger people [590].

Medical indications for prescription and use of statins became wider and wider and extend to many people with type 2 diabetes, who are at increased risk of CHD. So many middle-aged people in affluent countries started taking statins that the cost to national prescription subsidies blew out after 2000.

24.2.2 Implications of Statins

The statins provided the final convincing evidence – dietary trials never quite managed to convince everyone – that high LDL-cholesterol is an important CAUSE of CHD, which the WHO's Expert Committee had anticipated back in 1982 [134].

Cholesterol lowering diets, which had been the first line for prevention of CHD for 40 years lost their pride of place in 1995. But Brown and Goldstein point out that people with lifelong and diet-related low serum cholesterols have much lower coronary mortality than western people who take statins for 5 years [592]. They were struck by the very low CHD incidence in people with dysfunctional mutation of the gene for PCSK9 who have very low LDL-cholesterols persisting from birth. Statins taken for a few years in middle age can only do so much. The physiological means to lower LDL concentration is through a stringent diet that is low in cholesterol and saturated fat (e.g., Box 23.1). This should mean that the dietary principles of lowering serum cholesterol need to be applied now in young people – we can hardly give them statins.

Box 24.1 Statins Were Built on Basic Science

K. Bloch (USA) and F. Lynen (Germany) were awarded the 1964 Nobel Prize for Physiology and Medicine for completing the 26 steps of the biosynthesis of cholesterol from acetate. The most important of them is the 4th step because it is the rate-limiting step. This is where 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) is reduced to mevalonate. The enzyme is HMG CoA reductase and its co-factor is NADP. It was discovered independently by Lynen and by Rudney in 1958, obtained in pure state in yeast in 1978 and in human liver in 1982 [593]. Feeding exogenous cholesterol suppresses endogenous cholesterol synthesis and the inhibition occurs at HMG CoA reductase [594]. This explains, at the biochemical level, why dietary cholesterol has little effect in raising human plasma cholesterol concentration, as Keys had pointed out in 1952 [43] (and see Chapter 13).

In 1976 A. Endo in Japan [184] discovered a compound ("mevastatin") in extracts of penicillium citrinum that was a very powerful competitive inhibitor of HMG CoA reductase in yeast. Soon after, at Merck in the USA a similar, even more efficient inhibitor, lovastatin was obtained from aspergillus terreus. This was the first of the "statin" compounds given to humans in large controlled trials to lower high serum cholesterols from 1981 [595]. Simvastatin and pravastatin were also derived from fungal products. Newer statins are synthetic, but the original statins are in effect *antibiotics* against cholesterol via inhibiting HMG CoA reductase. When the structure of the early statins was worked out, they were seen to have an analogue of HMG CoA as part of the molecule [596]. Discovery of these important drugs depended on the basic knowledge of the key role of HMG CoA reductase. There was then a search to find a non-toxic substance that would strongly inhibit the enzyme. Endo has described [597] how he and his team tested 6,000 microbial strains in culture broths. Their screening test was suppression of incorporation of [^{14}C] acetate into nonsaponifiable lipids.

Chapter 25
Linoleic Acid Is Protective

Linoleic acid (18:2 ω-6, LA) has been known since 1956 to be one dietary component that lowers serum total and LDL-cholesterol, hence risk of CHD. It has taken time before becoming clear that LA itself, in diet or in plasma lipids is inversely related to CHD incidence. Evidence has accumulated gradually (Table 25.1), culminating in results from the large US Nurses Cohort (1997). LA also appears to reduce the tendency to thrombosis and risk of dangerous cardiac arrhythmias.

LA is *not* a precursor of arachidonic acid (potentially thrombogenic) at ordinary intake levels and fears that it interferes with conversion of linolenic to EPA have been exaggerated. Years: build up to 1997.

25.1 Cohort Studies Accumulate

In 1997 the major cohort study of US Nurses reported on the relation of dietary fatty acid groups to risk of subsequent CHD risk. They had earlier published adverse findings on trans unsaturated fat (see Chapter 21). Hu et al. had repeatedly included questions about type of fat or oil used for frying, for baking and at the table, and type of margarine in their food frequency questionnaires. Now they reported that CHD risk for the top quintile of polyunsaturated fat (PUFA) intake was low at 0.68, adjusted for other fatty acid types [598]. Intake of PUFA in this top quintile averaged 6.4 en%. Risks went down stepwise across quintiles and were very highly significant. "Polyunsaturated fat" is nearly all linoleic acid (18:2 ω-6, cis) which is the predominant PUFA across the diet.

This was perhaps the date in history when it became clear that higher levels of linoleic acid in diet, in adipose tissue or in serum lipids were associated with decreased risk of CHD. The reports that preceded Hu et al. in Table 25.1 all found significantly lower dietary ω-6 PUFA or linoleic acid in plasma lipids or adipose tissue in CHD cases compared with controls. An early prospective study in 1969 by Kingsbury et al. [599], followed people with arterial disease in the legs. Linoleic acid was estimated as diones by alkali isomerization (before gas chromatography was available). The Western Electric prospective study [600] stood out for some

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Table 25.1 Linoleic acid appears protective

References	Type of study	18:2 in.	Numbers cases/controls	
Kingsbury et al. [599] (St. Mary's Hospital, London)	Pro	Plasma CE	42/146	18:2 expressed as diones by alkali isomerization
Shekelle et al. [600] (Western Electric, Chicago)	Pro	Diet	215/1,900	Extra care (for the time) to estimate dietary fatty acids
Simpson et al. [601] (Oxford, England)	CC	Plasma TG	32/32	Cases also had significantly higher palmitic
Miettinen et al. [602] (Helsinki, Finland)	Pro	Serum PL	33/1,222	Serum TG & CE 18:2 also lower in cases (but NS)
Wood et al. [603] (Edinburgh, Scotland)	Cross sectional	Adipose tissue	28/448	
Valek et al. [604] (Czechoslovakia)	Pro	Serum total lipids	23/107	5-year follow up of men after myocardial infarct. Start 18:2 signify lower in those who died
Joossens et al. [605] (Belgium)		Diet	Used national statistics for the different provinces	Negative reln all causes and CVD mortality and PUF intakes
Roberts et al. [606] (Edinburgh, Scotland)	CC	Adipose tissue	84/292	Cases were sudden cardiac deaths
Goldbourt et al. [607] (Israel)	Pro	Diet	1,098/10,059	Linoleic acid/total fat and P/S both inversely related to CHD mortality
Simon et al. [608] (San Francisco, USA)	Nested CC	Serum CE	94/94	Serum PL 18:2 also lower in cases (but NS)
Hu et al. [598] (US Nurses, Harvard)	Pro	Diet	939/80,082	

Key: Pro = prospective study; CC = case-control; TG = triglyceride(s); CE = cholesterol esters; PL = phospholipid(s).

time as the only cohort study which reported linoleic acid as protective. But unusually for that time the authors had built in estimation of fatty acid intakes and their interviews, with nutritionists, lasted about an hour for each subject. It was supported by several other cohort studies in the 1990s, then the largest was the US Nurses [598]. [Further supportive cohort studies continued after 1999 [609, 610]. No prospective studies showing positive association and increased CHD have been reported.] The case-control studies had to take care that the cases of CHD were

newly diagnosed and had not changed their diet to a cholesterol-lowering one with increased ω-6 PUFA. All the authors of case-control studies in Table 25.1 were aware of this and prevented it.

25.2 Mechanism(s) of Action

Linoleic acid could protect against CHD by lowering LDL cholesterol (as described in Chapter 4). The dietary prevention trials are consistent. The change in the experimental group was increase of ω-6 PUFA along with decrease of saturated fat intake (Chapters 14 and 23). There is some evidence that an increase of dietary ω-6 PUFA can reduce the tendency to thrombosis [343]. Chapter 17 focussed on ω-3 PUFAs reducing the risk of dangerous arrhythmias in animals but the Adelaide workers did report a similar effect to fish oil when they fed sunflower seed oil [388] (rich in linoleic acid), though fish oil was more potent.

25.3 Linoleic (18:2) Not the Usual Source of Arachidonic Acid (20:4)

Textbooks of biochemistry have a diagram showing that linoleic acid is the precursor of arachidonic acid (via two intermediate steps) and arachidonic acid is the parent substance of prostaglandins of the 2 series including thromboxane (TxA2), which stimulates platelet aggregation. Linoleic acid does increase plasma and tissue arachidonic at low intakes, below 3 en%, but at usual intakes this does not happen [611, 612] and linoleic acid intakes around 12 en% may actually decrease tissue arachidonic. The predominant source of arachidonic acid in the body is arachidonic acid per se in foods like meat [613].

25.4 Recommendations

Wood and Oliver concluded their chapter on linoleic acid and CHD [614] with this public health recommendation: “The epidemiological, clinical, and experimental evidence of an inverse relationship between linoleic acid and CHD is sufficiently strong to recommend that populations with a high CHD mortality should supplement their eating habits with more polyunsaturated oil, principally from cereals and vegetables, aiming at a P/S ratio in the region of 0.8 . . ..” This was written before the US Nurses study report [598].

The FAO/WHO Joint Expert Consultation on Fats and Oils in Human Nutrition (1994) considered “Desirable intakes of linoleic acid should provide between 4 and 10% of energy. Intakes in the upper end of this range are recommended when intakes of saturated fatty acids and cholesterol are relatively high” [518].

There has been concern that the increased ω-6 PUFA intake in some developed countries could be interfering with the beneficial effects of ω-3 PUFA (see Chapter 18) [615]. 18:2 ω-6 (linoleic) is present in the body in higher concentration than 18:3 ω-3 (linolenic) and could compete for the Δ-6 desaturase enzyme and reduce conversion of linolenic to 20:5 ω-3. This was a theoretical possibility. Hwang et al. [616] tested it in two human experiments. In the first study subjects were fed a fixed amount of fish oil capsules with varying amounts of PUFAs in the diet. In the second study the ω-6 PUFA content of the diet was held constant while fish oil intake, in capsules, was varied. They found that the absolute amount(s) of fish oil, not the ω-3/ω-6 ratio determined the magnitude of conversion to eicosapentaenoic acid (EPA) and suppression of plasma triglycerides.

Chapter 26
Plant Sterols Fade and Return

Plants also have sterols in their cell membranes, very similar to cholesterol but with one or two extra carbons on the side chain. Those phytosterols (e.g., sitosterol, campesterol) are not absorbed by humans. Pollack in 1953 showed that sitosterol lowers serum cholesterol. Subsequently a sitosterol suspension, "Cytellin" was used in the 1950s mostly in the USA as a serum cholesterol-lowering medicine (dose 3 g three times a day). Mixed crystals of sitosterol and cholesterol are poorly soluble and there is competitive inhibition of intestinal cholesterol esterase. Results were rather variable. It was never widely used and marketing stopped in 1982.

Soon after this, sitosterol and sitostanol were developed in Europe as food additives to enhance the cholesterol-lowering of unsaturated margarines (approved USA 2000). These phytosterols are mostly a product of the wood industry. They are more effective than the earlier suspension, presumably because in ester form and dissolved in food fat. About 1.5 g day lowers plasma cholesterol. Nowadays they appear in premium tub margarines and also in some other lipid-containing foods, e.g., salad dressings, milk. Years 1953–1981 and 1986–2000.

26.1 Phytosterols Not Absorbed

Phytosterols are a natural part of our diet (Box 26.1). People consume about the same 0.25 g/day of phytosterols and 0.30 g of cholesterol (more phytosterols in vegetarians). The most abundant phytosterol in our foods is sitosterol, followed by campesterol. Sitosterol has an additional ethyl group on the cholesterol side chain, in campesterol this is a methyl group. It is a mystery why plants (including trees) use 28 or 29 carbon sterols in their cell membranes, while animals use the 27 carbon, cholesterol.

Schoenheimer, reporting in1929 [620] fed plant sterols to five species of experimental animals and found no increase in liver sterol; they were almost quantitatively excreted, unchanged in the faeces. After feeding cholesterol and phytosterol mixed together only cholesterol was found in the intestinal lymph. He concluded that

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phytosterols are not absorbed. Gould (1955) was first to test this with tritiated β-sitosterol [621]. Its absorption was only about 10% that of cholesterol, and it seemed to be selectively excreted in bile.

Box 26.1 Phytosterols a Natural Part of Our Diet

β-sitosterol is the most abundant dietary phytosterol, followed (in descending order) by campesterol, then stigmasterol, δ5-avenasterol, brassicasterol, and there are others.

The food group that contains most is oils and fats. Among the common vegetable oils corn/maize oil is richest in phytosterols (1,390 mg/100 g), then come sunflower oil (725 mg/100 g) and rapeseed oil (513 mg/100 g, part of which is brassicasterol). Some uncommonly eaten oils contain more: sesame seed oil (2,950 mg/100 g) and wheat germ oil (1,970 mg/100 g) [617]. It is likely that the phytosterols in maize oil, and perhaps others, contribute to the cholesterol-lowering effect of their polyunsaturated fatty acids [618]. After oils and fats, the food groups that provided most phytosterols in the British diet [619] were other cereals, then bread, then vegetables, then sugars and preserves (this includes jam, chocolate, confectionery, potato crisps, tortillas, etc.), with small amounts in nuts, fish, eggs and potatoes.

Extracted phytosterols played a role in human nutrition in two phases: from the 1950s to 1982 as a cholesterol-lowering pharmaceutical, then from the mid 1990s to the present as a cholesterol-lowering food additive, in some margarines and other dietary fats.

26.2 The Pharmaceutical Phase

The pharmaceutical phase started with Petersen's demonstration that addition of soybean sterols to the diet of cholesterol-fed chickens prevented the expected rise of their plasma cholesterol, and in longer experiments it prevented atherosclerosis-like lesions in the aorta [622]. Pollak was the first to show in humans that a plant sterol mixture 5–10 g/day, taken in divided doses lowered blood cholesterol in 24 of 26 subjects [623]. The preparation "was unpleasant to take". Dr RE Shipley at Eli Lilly formulated a 20% suspension of β-sitosterol, along with a suitable placebo suspension. This was given to subjects with hypercholesterolaemia. He usually gave 9 g sitosterol/day, in some cases up to 30 g/day [624]. Most of a small number of subjects responded. Between 1955 and 1957 seven groups in N. America reported reduction of serum cholesterol in nearly all their cases with Eli Lilly's sitosterol suspension, proprietary name "Cytellin". The largest number of cases, 24 were treated by Best and Duncan [625]. Others were reported by Lesesne et al. (1955); Joyner and Kuo (1955); Sachs and Weston (1956); Farquhar et al. (1956); Lehmann (1957);

Levkoff and Knode (1957) [refs in 626]. In Britain there was a solitary (1955) report by Barber and Grant who gave the sitosterol in biscuits [627].

The phytosterol was obtained from tall oil (from coniferous trees) or from soy. There were smaller amounts of campesterol with the sitosterol. It appeared that cholesterol absorption was hindered by formation of mixed crystals of sitosterol and cholesterol, which are poorly soluble [628]. Competitive inhibition of intestinal cholesterol esterase was also likely.

Eli Lilly's medical information (around 1970) described "Cytellin" as a lightly flavoured aqueous suspension of sitosterols that had lowered serum cholesterols in over 85% of cases without side effects. The recommended dose was 3–6 g of sitosterol before meals three times a day.

Sitosterol was moderately effective in lowering LDL-cholesterol in nearly everyone who took it (average fall 15%). There were no adverse effects even when taken for a long time [629] and as little as 3 g was an adequate dose of some preparations [630] . . . and yet it never had the publicity of other drugs that came and went like Atromid and nicotinic acid, and it was not used as much as one could have expected in lipid clinics. Perhaps this was because of variability of response [630], perhaps palatability, perhaps supply problems, perhaps cost. Lilly stopped marketing it in 1982 and it disappeared from pharmacopoeias, about the time that trials with statins were starting.

26.3 Sitosterolaemia

Pharmaceutical sitosterol was not discontinued because of sitosterolaemia (phytosterolaemia). This is a very rare autosomal recessive condition first described by Bhattacharyya and Connor [631] in which phytosterols are *not* poorly absorbed. Tendon xanthomas appear at a young age but, unlike the much more common cause of early tendon xanthomas, familial hypercholesterolaemia, the plasma cholesterol is normal or only moderately raised. Increased plasma phytosterols (around 30 mg/dl) can only be demonstrated with gas chromatography [631]. There is a mutation of two genes ABCG5 and ABCG8 with reduced synthesis of sterolin 1 and sterolin 2 which normally prevent initial entry of nearly all phytosterols at intestinal level and favour excretion of plasma phytosterol into the bile [632]. Sitosterolaemia should be recognised in young people via the xanthomas or family history. *Increased* phytosterol intake, whether pharmaceutical, or in food is aimed at middle-aged people in whom CHD is common.

26.4 The Food Additive Phase

Interest in phytosterols for hypercholesterolaemia returned after rat experiments by Ikeda and Sugano [633] found that sitost*an*ol (i.e., dihydro-sitosterol) was very little absorbed, significantly less than sitosterol, so they suggested it could be a

more effective cholesterol-lowering agent than the sterol. Heinemann et al. (1986) in Bonn gave 0.5 g sitostanol in sunflower oil, t i d to 6 people with hypercholesterolaemia. After 4 weeks serum cholesterol fell by (mean) 15% [634]. Later they treated 9 children with familial hypercholesterolaemia with 2 g, thrice daily: serum LDL-cholesterol fell 30% with sitostanol, 20% with sitosterol [635]. In 1995 there was a major trial by Miettinen et al. (Finland) [636], a one-year, randomised double-blind study in 153 subjects with mild hypercholesterolaemia. Sitostanol was prepared by artificially saturating sitosterol, a product of the wood industry. It was then esterified with rapeseed oil and incorporated in rapeseed oil margarine. The dose of sitostanol was 2.6 g/day (aim 3.0 g) for 6 months, then reduced to 1.8 g/day for half the subjects on experimental margarine. Serum cholesterol was 10% lower at one year on the sitostanol ester margarine ("Benecol", made by Raisio, Finland).

The edible oil industry was now interested in phytosterols or -stanols at a time when the power of statin's was likely to reduce the importance of polyunsaturated margarine for controlling cholesterol.

Westrate and Meijer at Unilever Laboratories compared sitosterol – and sitostanol – enriched margarines and a polyunsaturated margarine control (Flora) in 95 healthy volunteers in a randomised double blind incomplete Latin square design, with 5 treatments in 4 periods [637]. Periods were 3.5 weeks and subjects ate 30 g margarine day, providing about 3 g of sterol. Their margarine with esterified soybean oil sterols (mainly sitosterol) reduced serum cholesterol (8–13%) as much as the sitostanol ester margarine (Benecol) which Miettinen et al., had used [636]. In Finland, Hallikainen et al. also found no significant difference in cholesterol reduction between sitostanol ester and sitosterol ester in rapeseed oil margarine [638]. Hendricks et al. [639] at TNO Nutrition and Food Research, working with Westrate and using similar design compared butter, "Flora" (polyunsaturated) margarine and soy phytosterol ester margarine at three strengths to provide 0.8, 1.6 and 3.2 g phytosterols/day. The middle dose (1.6 g phytosterol/day) reduced LDL-cholesterol 8.5% compared to Flora (12% compared to butter). The top dose of phytosterol reduced LDL-c very little more but reduced serum carotene by nearly 20%.

At the start of the next century, in September 2000 phytosterols received health claim approval from the US Food and Drug Administration. Margarines and salad dressings containing them were marketed as functional foods. In a review on plant sterol and stanol margarines and health, Malcolm Law [640] makes three interesting points:

- Supplies of phytosterols/stanols are limited
- Statins cost about three times as much as plant sterol margarines but they lower serum cholesterol by three times as much
- Phytosterols act in a different way to statins. Effects of the two together on serum cholesterol are additive.

Chapter 27
Soy Proteins Versus Casein

In rabbits casein raises plasma cholesterol but soy protein does not (1965). Extra protein, whether milk, wheat or egg, does not raise the cholesterol in normal human subjects. Sirtori (Milan) reported in 1977 that a large intake (13% energy) of a textured soy protein preparation lowered serum cholesterol substantially in people with hypercholesterolaemia. Then in several university nutrition departments soy proteins were tested, but did not lower cholesterol (in subjects with ordinary cholesterol levels). By 1995 a meta-analysis of 31 trials showed large reductions in 8 trials (7 in Italy) and no or trivial cholesterol fall in 16 trials. It appears that isoflavone naturally present in soy is needed for the effect. Some purified soy protein preparations have had this removed. Years (1965) 1977–1999.

27.1 An Animal Protein Effect on Serum Cholesterol in Rabbits

Ignatowski in 1908 [12, 641] had hypothesized that a toxic metabolite in animal protein led to atherosclerosis. He fed meat to adult rabbits and milk plus egg yolk to weanling rabbits and produced a type of atherosclerosis. Then Stuckey's experiments [13] indicated that egg yolk and brain caused lesions but egg white and meat juice did not [14]. Anitschkow [16] went on from this to demonstrate that pure cholesterol in the diet caused hypercholesterolaemia and aortic lesions. Much of this History followed his discovery. The idea that animal protein might raise plasma cholesterol stayed in the background. In 1923 Newburgh and Clarkson produced atherosclerosis in rabbits with a diet rich in meat [642]. This naturally contains some cholesterol but they subsequently showed by addition of different amounts of cholesterol to the beef protein that the amount present in the meat was not sufficient to cause the atheroma [643].

These arterial lesions were invariably accompanied by hypercholesterolaemia. Freyburg [644] was curious to see if feeding a high vegetable protein ration to rabbits would also raise plasma cholesterol. He used three diets, one with soy bean flour, one with gluten flour and the third with both these, and protein intakes of 13–38%. Blood cholesterols remained in the normal range and there was no atherosclerosis.

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In 1941 Meeker and Kesten showed that casein (as an example of an animal protein) was more atherogenic than soy protein [645]. Later Howard et al. [646] gave groups of rabbits 20 different diets, most including 250 g casein/kg and then they measured plasma cholesterol and sudanophilic lesions in the aorta. All developed high cholesterols and aortic lesions except in the two groups with casein replaced by soya flour. Carroll et al. published a number of studies of this phenomenon in rabbits between 1975 and 1987. They tested eleven different animal proteins: all had cholesterol-elevating effects; casein's was near the middle of the range [647]. It has the benefit for experiments that it can be obtained virtually fat-free and standardised.

27.2 In Humans Soy Protein Compared Against Casein: Variable Results

In humans, serum cholesterol is very low in kwashiorkor and goes up, along with triglyceride and albumin with re-feeding [648]. In untreated cases there is failure of synthesis of apo B-100 in the liver. But in adequately nourished people adding extra protein to the diet does not raise serum cholesterol. Keys and Anderson [649] demonstrated this with 50 g extra protein (as skim milk powder). They also found no difference in cholesterol when the main dietary protein was switched from wheat gluten to egg white (lower to high biological value) isonitrogenously [650].

Sirtori et al. (in Milan) [651] noticed that people with a high intake of vegetable proteins, such as vegetarians in the USA [652] or Seventh Day Adventists in Australia [653] tended to have lower serum cholesterols, and that Carroll had reduced rabbits' cholesterol with soy protein. They carried out a 3 week/3 week metabolic ward crossover trial with a textured soy protein isolate ("Temptein") incorporated in various Italian dishes in 20 patients with type II hyperlipoproteinaemia. 13 en% as soy protein replaced all the animal protein in a low lipid diet. There was a remarkable 21% mean reduction of total cholesterol [651]. Results were supported by a larger trial in 9 Italian (and Swiss) lipid research clinics. "Cholsoy" (only slightly different from the textured soy protein previously used but easier to handle and more palatable) 60–120 g/day was taken by 127 outpatients with stable type II hyperlipoproteinaemia. Serum total cholesterol fell 19% in the first 2 weeks and then a little more by 8 weeks [654].

Elsewhere similar trials were done but little or no reduction of serum cholesterol occurred or there was a slight decrease in LDL-cholesterol (655–658). These trials emanated from leading nutrition research departments, Wageningen, Harvard and Dallas and used good and different experimental designs. Sirtori responded that these disappointing results had been obtained in people with normal cholesterols, with lower soy protein intakes and that the TYPE of soy protein product is important [659]. They have used "textured soy protein" which is defatted but not pure protein; it contains sugars, oligosaccharides and fibre. Purified soy proteins, without non-protein ingredients have been less or not effective.

There was scepticism at national expert committee level. The Nutrition Committee of the American Heart Association concluded that "consumption of vegetable proteins leads to lower cholesterol levels than consumption of animal proteins but not in humans" [660].

Other positive results were also reported, e.g., in Naples, Verillo et al. [661] used the same "Cholsoy" 20 g thrice daily in 55 middle-aged outpatients with plasma cholesterols around 9.0 mmol/l for 4 months. Serum cholesterols fell 29%. A meta-analysis might now be illuminating. James Anderson et al. published it in 1995 in the New England Journal [662]. This thorough study included 37 controlled trials: the bottom line was a decrease in serum total and LDL-cholesterol of 23 and 22 mg/dl. However, the diagram for LDL-cholesterol change in 31 trials shows no or trivial change in 16 trials and large reductions only in 8 (7 of these in Italy). Analysis clearly showed that large cholesterol reductions only happened when the starting level was above 335 mg/dl, as Sirtori had suggested [659]. The amount of soy protein also had an effect, but Anderson et al. did not dissect out the influence of type of soy protein product.

27.3 Isoflavones May Contribute

There was no dispute about the different effects on cholesterol of soya protein versus casein in rabbits (as major dietary proteins). This is easily demonstrated [663], and duplicates of casein diets which did not affect serum cholesterol in van Raaij's human subjects raised cholesterol threefold in their rabbits [655]. Rabbits might serve as a model for mechanism of effects in humans, though this was mainly in people with hypercholesterolaemia. Adjusting the amino acid pattern of soy to that of casein didn't make much difference, nor did removing the saponins from soya. There isn't enough fibre in soya to achieve the effect seen. There have been reports of increased bile acid excretion, of increased pool size of chenodeoxycholic acid and of increased LDL receptor activity on soy protein diets. But none of these appear to have been substantiated [664]. The last mechanism proposed before 2000 was that *isoflavones*, the phytoestrogens in soya add to any serum cholesterol-lowering effect. This was suggested by the finding that soy protein concentrate, which has lost most of its natural isoflavones (unlike textured soy protein) has little serum cholesterol-lowering effect [659]. Anthony et al., fed a moderately atherogenic diet to adolescent rhesus monkeys. Their protein (20 en%) was soy, with or without isoflavones. Only the former lowered serum cholesterol [665]. They later gave male cynomolgus monkeys atherogenic diets with one of three proteins: casein, soy + isoflavones or soy without isoflavones. Serum cholesterols were significantly lower on soy plus [666]. Then in a human trial, Crouse et al. gave healthy people with normal cholesterols NCEP step 1 diets with one of 5 protein variations: 25 g casein or 25 g soya protein containing 3, 27, 37 or 62 mg of isoflavones/day. On soya + 62 mg isoflavones/day serum cholesterol was 4% lower across the group and 9% lower in those with above average LDL-cholesterol [667].

This seems to support the US Food & Drug Administration's rather optimistic (1999) health claim: "25 g of soya protein a day, as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease". This needs qualifications: provided the soya has not lost its inherent isoflavones and there is, of course, no evidence that heart disease is prevented – only that in some cases – serum cholesterol may be reduced, especially if it is high.

Chapter 28
High Homocysteine Associated with Cardiovascular Diseases

Vascular disease is one of the abnormalities in the hereditary condition homocystinuria. In 1969 and in 1976 it was suggested that only moderately raised plasma homocysteine, from any cause might be associated with coronary and other vascular disease. In the 1990s many epidemiological studies reported on this association, at first case-control studies, later prospective studies. The great majority found increased serum homocysteine was associated with CHD, cerebral or peripheral vascular disease. Although homocysteine is a normal metabolite, higher concentrations appear to damage endothelium. Vitamin B-6, folic acid and B-12 are all involved in pathways that metabolise homocysteine. Trials showed that moderate dose supplements of folic acid with or without vitamin B-12 lowered plasma homocysteine. Results of randomised controlled prevention trials, with disease outcome would be reported after 2000. Years 1976–1999.

28.1 Homocystinuria and Arterial Disease

Classic homocystinuria, a rare autosomal recessive condition was first recognised in 1962. Plasma homocyst*ein*e is very high and spills over into the urine as the oxidised form, homocystine. The usual cause is a defect of cystathione β-synthase. The most common clinical features are dislocation of the optic lens, osteoporosis, thinning and lengthening of the long bones, mental retardation, and thromboembolism affecting large and small arteries and veins [668]. McCully (1969), a pathologist described the vascular pathology in two children with this condition who died of massive strokes. Their arteries "looked as though they were from an elderly person" [669]. He suggested that homocysteine might play a part in atherosclerosis, and later demonstrated arterial lesions in rabbits given homocysteine thiolactone parenterally or in their feed [670]. At the time researchers were preoccupied with cholesterol and his theory was ignored or treated with scepticism [671].

However, in Australia Wilcken and Wilcken (1976) moved this idea into its first clinical research [672]. They wondered if heterozygotes for homocystinuria or people with raised homocysteine from other genetic or environmental cause

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were at increased risk of CHD. Plasma homocysteine tends to go up after a load of methionine, the precursor amino acid, especially if homocysteine metabolism is impaired. They gave oral methionine loads to 25 people under 50 years of age with angiographically proved CHD, and to 22 controls. At 4 h homocysteine appeared in 17 of the cases and only 5 of the controls ($P < 0.01$). This was measured as homocysteine-cysteine disulphide on an amino acid analyser.

28.2 Raised Plasma Homocysteine and Arterial Disease

It was nine years before the next case-control study on homocysteine and CHD [673] and only two more studies were published before 1990 [674, 675]. They found higher plasma homocysteines with CHD. Kang et al. (1986) realised that most homocysteine in the blood is albumin-bound [674]. The remaining smaller percentage is in three free forms: the dimer (oxidised form) homocystine, the mixed homocysteine-cysteine disulphide and a little free homocysteine. From then, investigators used laboratory methods which give all these forms, combined as plasma *total* homocysteine, abbreviated tHcy; the methods have been standardised [676]. In 1984 Brattstrom et al., reported raised homocysteine-cysteine mixed disulphide (fasting and post-methionine) in 19 people with minor strokes below 60 years, compared with controls [677]. Peripheral arterial disease [678] was also found to be associated with increased tHcy, as well as deep vein thrombosis [679].

After 1990 there was an exponential increase in the number of publications on tHcy and cardiovascular disease. In 1996 they reached 25 in 1 year [680]. Most were case-control studies, with small numbers of them nested. Cohort studies take much longer to complete. After the discovery of protein-bound homocysteine most investigators measured plasma total homocysteine without methionine loading; this made it possible to collect larger numbers of cases and to use stored plasma. The accumulating number of studies of increased plasma tHcy and cardiovascular disease (not only CHD) were reviewed in 1992 by Kang et al. [681], 1995 by Boushey et al. [682], 1997 by Moghadasian et al. [683] and in 1998 by Refsum et al. [680]. Among the reports was one from Tufts with Framingham Offspring Heart Study subjects as controls [684]. In another, nested case-control from Willett's group, stored bloods from participants in the Physicians Health Study who subsequently experienced myocardial infarction were compared with controls [685]. Then in 1997 a case control study was published from 19 centres in 9 European countries. Cases had recent CHD or cerebrovascular disease or intermittent claudication diagnosed under age 60 years and tHcy was measured fasting and post-methionine. Twice as many cases had high total homocysteine [686]. There was little difference in the frequency of elevated tHcy between CHD, cerebrovascular disease and peripheral vascular disease.

By 1998 Refsum et al. [680] had counted 80 clinical and epidemiological studies, including more than 10,000 patients. The great majority found increased tHcy

associated with CHD, cerebral and peripheral vascular disease which remained strong after adjustments. Editorials in a number of medical journals had titles like "Hyperhomocysteinaemia: a risk factor for vascular disease" [687] and plasma homocysteine started to be measured along with cholesterol by clinical biochemists.

In animal experiments [688], in cell cultures [689] and from the pathology of homocystinuria [668], homocysteine appears to damage endothelial cells and favours thrombosis by increasing thromboxane A_2. But fatty atheromatous plaques are not a common finding either in homocystinuria [668] or in animal experiments [670].

28.3 Environmental Influences on Plasma Homocysteine

Factors other than heterozygosity for cystathione synthase deficiency are more likely causes of mild to moderate increases of plasma tHcy. The 667C→T (thermolabile) mutation of the 5,10 methylenetetrahydrofolate reductase (MTHFR) is quite common and people with this can have modest rises in homocysteine [690]. Old age, male gender, smoking and lack of exercise have small elevating effects [691] and renal failure increases homocysteine [680]. But the exciting environmental influences are nutritional: three B vitamins : B-6, folic acid and B-12 are all involved in pathways that metabolise homocysteine. Folate and vitamin B-12 are needed to change it back to methionine and B-6 is needed to move it on to cystathione and cysteine (Fig. 28.1).

Plasma homocysteine doubled in healthy subjects on an experimental folate deficient diet (25 μg/day) [692] and it was used as a functional biochemical measurement in arriving at the 1998 (North American) Dietary Reference Intake for folate [693]. Higher plasma homocysteine is also one of the biochemical features of vitamin B-12 deficiency [694]. Because of the interest in homocysteine, a number of studies were made in the 1990s, relating levels of these B vitamins to plasma homocysteine in population samples. In some 900 elderly participants in the Framingham study [695], homocysteine concentration had graded inverse relationships with plasma levels and intake of folate and vitamin B-6. Plasma levels, not intake, of vitamin B-12 were similarly related. Boushey et al. [682] included this and eleven other publications in their review. All demonstrated a strong inverse association between plasma homocysteine and serum folate, and there have been other subsequent publications. Nygård et al. (1998) found that folic acid in B vitamin supplements showed a stronger relation to tHcy than dietary intake [696], presumably reflecting the better bioavailability of pure folic acid [697]. In their large study of over 11,000 apparently healthy Norwegians the three major acquired determinants of tHcy were smoking and coffee consumption (which raise it) and folate intake (which lowers it). Low vitamin B-12 is more strongly related to increased plasma homocysteine in vegans [698] and in elderly people with subclinical B-12 deficiency [699].

28.4 Plasma Homocysteine Lowered by Folic Acid and/or Vitamin B-12

Trials showed that homocysteine could be lowered with pharmacological doses of folic acid, 0.6 mg or more [700] but soon the search was on to find a physiological dose that could be used long term or in the form of natural foods. Ward et al. [701] found 200 μg was more effective than 100 μg/day and 400 μg produced little more homocysteine reduction. A "Homocysteine Lowering Trialists Collaboration" [702] analysed 12 trials (though not Ward et al. [701]) and found no difference in effect from 0.5 to 5.0 mg folic acid/day. This range of folic acid dosage should reduce homocysteine by about 25%. Addition of vitamin B-12 produced a further 7% reduction [702]. Schorah et al., showed that even eating breakfast cereal fortified with folic acid, providing an extra 200 μg folic acid/day lowered homocysteine by 10% [703].

The American Heart Association published a Statement on "homocyst(e)ine, diet and cardiovascular disease" at the end of the century (1999) with 101 references [704]. It concluded "Although there is considerable epidemiological evidence for a relationship between plasma homocyst(e)ine and cardiovascular disease, not all prospective studies have supported such a relationship. Moreover, despite the potential for reducing homocyst(e)ine levels with increased intake of folic acid, it is not

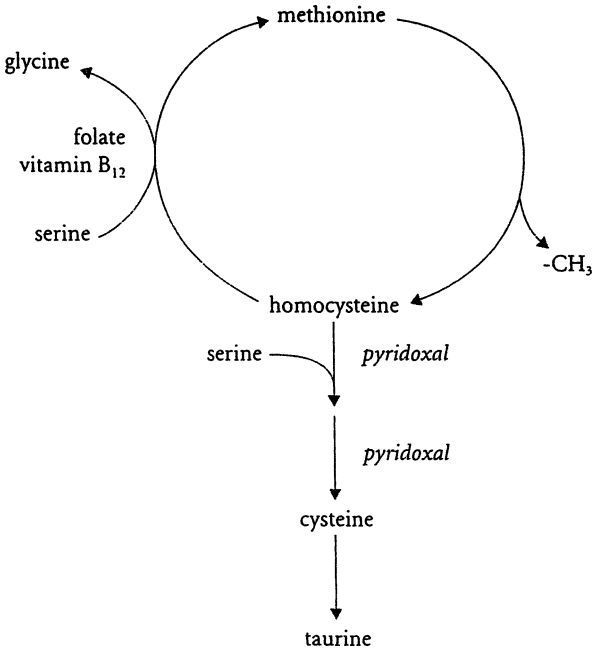

Fig. 28.1 Simplified biochemical diagram, showing how three B vitamins: folate, B-12 and pyridoxine may each lower plasma homocysteine

known whether reduction of plasma homocyst(e)ine by diet and/or vitamin therapy will reduce cardiovascular disease risk. Until results of controlled clinical trials become available, population-wide screening is not recommended, and emphasis should be placed on meeting current RDAs for folate, as well as vitamins B-6 and B-12, by intake of vegetables, fruits, legumes, meat, fish and fortified grains and cereals. . ..”

After the year 2000 the disease outcome results of randomised controlled rials would emerge and it would also be seen whether the homocysteine-lowering effect of folic acid fortification of grain products in North America [705] had a clear effect on any of the cardiovascular diseases.

Chapter 29
And Salt Should Be Included

High blood pressure is one of the three established major risk factors for CHD, and salt (NaCl) has been shown to be an important determinant of hypertension, as described here.

Lewis Dahl first proposed (1954) "if a low salt intake causes a lowering of blood pressure (BP), does a high salt intake cause increased BP?" This met with much scepticism, but evidence that salt is a cause of hypertension built up between 1954 and now:

1. **Isolated communities (formerly) cut off from salt did not experience hypertension (not because their life was stress-free).**
2. **Animal experiments, first salt-sensitive strains of rats (studied by Dahl); later chimpanzees (Denton et al. 1995) given salt develop high BP.**
3. **Human sodium requirements (are much lower) than usual intakes.**
4. **Moderate sodium (salt) restriction *can* lower BP in controlled human experiments.**
5. **Thiazide medicines lower BP, presumably by increasing sodium excretion.**
6. **Poor correlation between sodium intake and BP in individuals is partly the result of regression dilution bias and partly a time delay between change of salt intake and change of BP.**
7. **Humans evolved in a low salt regime of natural foods and breast milk is a low sodium food.**

In the 1970s manufacturers voluntarily removed all added salt from infant weaning foods. Starting 1977 (Dietary Goals for the US) official sets of dietary guidelines have included a recommendation to reduce salt intake, [e.g., to 100 mmol Na (6 g salt) or less] but most of the salt that people eat is put in during processing, not in the home. Years 1954–1995.

29.1 Discovery of Essential Hypertension

"Hypertension" does not appear in the 1904 edition of Osler's textbook of medicine. Estimation of increased tension in the arteries was by palpation of the radial artery at the wrist, a non-quantitative procedure, unreliable because the arterial wall varies in thickness. Increased "tension of the pulse" appears in the textbook mostly under acute and chronic Bright's disease (glomerulonephritis) [706].

A.S. Truswell, *Cholesterol and Beyond*, DOI 10.1007/978-90-481-8875-8_29,

The prototype of the current sphygmomanometer, with cuff inflated round the upper arm and pressure read on a mercury column was invented in Italy by Riva Rocci in 1896 [707]. Harvey Cushing introduced it to North America and Allbutt introduced it to Britain. At this stage physicians who had this instrument noted by palpation the pressure at which pulsation disappeared in the radial artery, i.e., only the systolic arterial pressure.

A Russian vascular surgeon, Nicolai Sergei Korotkoff discovered the auscultatory method of measuring blood pressure [708]. As pressure in the cuff is allowed to slowly fall, the first sounds appear at the maximal blood pressure (i.e., systolic); then when the sounds disappear this is the minimal blood pressure (i.e., diastolic). He presented this at a meeting of the Imperial Medical Academy in St. Petersburg in 1905. There was no journal article but Professor Janowski of the Academy took up the method with interest and Korotkoff's sounds were soon known by specialists in Western countries.

Once systolic blood pressure (at first only systolic) could be quantified, physicians found people with high readings but no obvious renal disease. It was first labelled "hypertensive cardiovascular disease" by Janeway [709] in USA and "hyperpiesia" by Sir Clifford Allbutt in England; these names were later replaced by "essential hypertension" [710]. It took many years before every general practitioner had a sphygmomanometer on their desk and blood pressures were recorded as systolic/diastolic. Price's 1930 textbook of medicine, writing about hypertension gives examples only of systolic blood pressure (BP), usually 170–200 mm without kidney disease [711]. Recording of diastolic pressures became more routine with the growth of life insurance medical examinations.

29.2 Early Diets for Hypertension

The first clinical measurements of BP with low and high intakes of salt, in six patients were by Ambard and Beaujord (in Paris) in 1904 [707, 712]. Low salt was about 3 g/day (50 mmols Na) and high salt 14.5 g/day (240 mmols Na). They measured urinary chloride (with silver nitrate) – sodium could not be measured at that time – and suggested that retention of chloride increases BP.

In America FM Allen[1] in 1920 introduced very low salt diets (0.5–0.75 g salt/day) for people with hypertension. His idea was that avoiding salt would reduce irritation of the kidney and so lessen the pressor activity of the overworked kidney [712]. 180 patients were recorded in their 1922 paper [713]. In the author's judgement, 60% distinctly improved, in 31% the diet was a failure. Some other physicians had similar results, others did not [712]. These very low salt diets were unpalatable and patients lost weight. Where there were useful changes in BP, the measurements

[1]He was the same Allen who pioneered the drastic Allen treatment for type 1 diabetes around 1915, i.e., before insulin. It was a severe weight reducing diet, containing only 5% of calories from carbohydrates.

were usually uncontrolled and interventions were probably too short. Very low salt diets fell into disuse and all but disappeared from the medical literature.

Twenty years later there was a revival of dietary treatment of hypertension. Walter Kempner started reporting his dietary regimen for hypertension in the North Carolina Medical Journal in 1944 [714]. His rice-fruit diet contained rice, fruit, sugar, very little fat plus vitamin supplements and provided about 2,000 calories, 20 g protein, 5 g fat, 850 ml water and 0.15 g of sodium (7 mmol)/day. Kempner believed that its combination of low protein, fat and salt reduced the metabolic load on diseased kidneys and hence BP fell, but he didn't think the very low salt was the main reason for the diet's effect [712]. He published case reports from among hundreds of patients he had treated. In some cases enlarged hearts and abnormal ECGs were restored to normal [715] – just by this diet. Out of 500 patients, hypertension improved in 311 (62%). It did not improve in 163 and 26 patients died.

Kempner monitored urinary nitrogen and electrolytes every day and checked his patients' compliance. He was a very authoritarian physician (like Allen before him). Kempner is quoted as saying "Believe in me, you must. I demand it" [716].

Incidentally, Kempner reported changes in serum cholesterol in his cases [715]. In 200 patients with starting levels 220 mg/dl or more it fell in all but 10; mean change was from 286 to 204 mg/dl. This finding gave Ancel Keys an idea for a more balanced low fat diet to reduce serum cholesterol and help prevent atherosclerosis [56].

Bang et al. [717] in Copenhagen gave 26 patients with hypertension a very low salt diet (262 mg Na), but with 50 g protein and 88 g fat. BPs fell in 23 and in most of them it rose when salt was added, alternating with dummy tablets. They warned that the treatment is contra-indicated in some cases of (salt-losing) chronic nephritis.

The rice-fruit diet was carefully studied by Dole, Dahl et al. on the metabolic ward at the Rockefeller Institute. Restriction of sodium, but not of chloride or protein appeared to be necessary for the clinical effect [718]. After 6 months on this very low sodium diet, previously hypertensive patients showed reversal of x-ray and ECG changes of left ventricular hypertrophy.

Over in Britain a Medical Research Council committee in 1950 reported experiences with Kempner's rice-fruit diet [719] in patients with severe and moderately severe hypertension, studied in five teaching hospitals. Food was still rationed and they had to obtain special supplies of rice and sugar from the Ministry of Food. Forty-one patients managed to take the diet for an average of 41 days; several gave up earlier. BP fell considerably in 70%, patients lost weight and some were in negative nitrogen balance. The committee thought extreme sodium restriction caused the fall of BP. It went up when salt was added, not when protein was added to the diet. "Our experience would suggest that few patients, in this country, could be persuaded to stick strictly to the diet for much longer than six weeks . . . it will not become a popular form of therapy".

Hatch et al. [720] at Columbia University, New York, 1954 confirmed that the hypertensive effects of the rice-fruit diet was prevented by adding 3 g NaCl/day. They devised a "Special Low Sodium Diet" which included low salt meat, vegetables and fat which had about the same BP lowering effect as the rice diet. They were usually able to add 0.5 g NaCl/day without change of BP.

29.3 Is Salt a Cause of Essential Hypertension?

In the mid 1950s a very low salt diet remained one option for treatment of hypertension, among other uncomfortable and not very effective options: sympathectomy, ganglion blockers, reserpine and hydralazine. Lewis Dahl had worked with Dole and others at the Rockefeller Institute in a series of careful metabolic studies that showed the BP lowering on the rice-fruit diet was due primarily to its low sodium content [718]. He began to wonder (as he recalled later [721]) "if a low salt intake causes a lowering of BP, does a high salt intake cause increased BP?" He proposed [722] that some minimum level of sodium intake must be exceeded for the development of essential hypertension but, while necessary, sodium is not of itself sufficient for development of the disease. The pieces of evidence in the 1954 paper by Dahl and Love [722] were: first, production of hypertension in rats by providing saline drinking water [723] or salted rations [724]; secondly, some isolated groups of people at that time (Greenland Eskimos, Australian Aborigines, mountainous Chinese tribes and Cuna Indians in Panama) did not experience hypertension and had little access to salt. Thirdly, the main set of data was from 547 employees at the Brookhaven Laboratory who were classified as "always adding", "sometimes adding", "never adding salt" at table. High blood pressures were found in 10, 7 and 0% of these groups. They subsequently increased the numbers of subjects to 1,346, with much the same result [725] (10.5, 6.8 and 0.7% respectively with hypertension).

Most researchers who have repeated comparison between individuals of salt intakes versus BP like this could not confirm a significant correlation. Miall asked 973 people the same questions in a population sample in south Wales [726]. Adjusted for age there were no differences between "always add salt" and "never add salt". In Framingham, Dawber et al. [727] measured 24 h urinary sodium as an objective representation of total sodium/salt intake. Their plot of urinary sodium against systolic and diastolic BPs is impressively flat. They found that repeat 24 h urine sodiums can differ considerably and that salt intakes at table by dietary history correlated poorly with 24 h urines. Simpson in Otago University, New Zealand also found no correlation between 24 h urine sodium and BP in a population survey of over 500 men and 500 women [728]. They later repeated urine collections in 556 subjects and were surprised to find just how much variation there was between the repeat urine sodiums, averaging 62 mmol/day for men and 51 mmol for women [729]. Single 24 h urinary sodiums, when combined for the group give a reliable estimate of population means, but characterise the individual poorly.

By 1961 Dahl was concentrating on percentages of hypertensives against urinary sodium excretions *in different populations*. He published what came to be called Dahl's line, with Alaskan Eskimos, Marshall Islanders, USA, southern Japanese and northern Japanese on a straight line from 0% hypertensives at 4 g/day NaCl up to 39% hypertensives at 27 g/day NaCl [730] (Fig. 29.1).

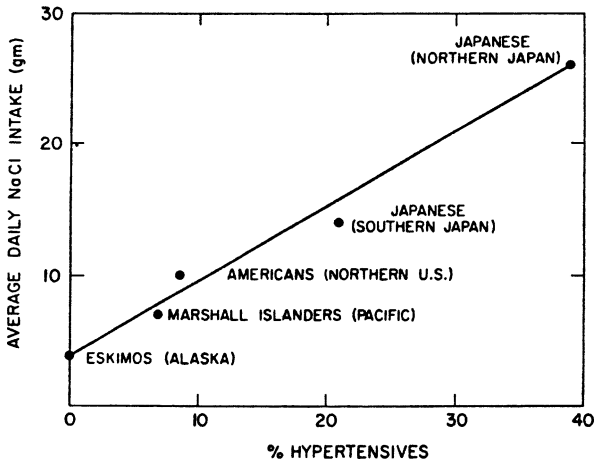

Fig. 29.1 Dahl's line: % hypertensives against average daily salt (NaCl) intake (g/day) in five different communities. From Dahl (1961) [730]

Isaacson et al. added data from 100 healthy Black (Bantu) South African men [731] with 22% hypertensives and mean urinary sodium 18.6 g/day; they fitted well on Dahl's line.

Dahl's laboratory went on to develop a model in white Sprague Dawley rats. By selective breeding they bred two strains of animals: those sensitive to salt in their feed (S) who became hypertensive even on 0.37% salt for some weeks, and those resistant (R), even to 4% NaCl [732]. The rat model helps to explain why some people with hypertension respond better than others to sodium restriction, and in most societies (which consume much more sodium than basal need) hypertension runs in families.

In his 1958 Medical Progress review in the New England Journal of Medicine [733], Dahl reviewed data on human basal sodium requirements, then concentrated on the question whether very low salt intakes are safe. Kidneys normally excrete whatever sodium a person consumes, so there are no body stores. Their patients were studied long term on 100–150 mg sodium (4–6 mmol) and had slightly lower plasma sodium levels; their total exchangeable sodium was only 4.5% lower. They could find no physiological or psychological dysfunction, except for increased aldosterone production. Thus if someone has an acute salt-losing illness (vomiting, diarrhoea, diabetic ketoacidosis, burns) they start from approximately the same plasma sodium concentration.

In a study of Eskimos, who rarely have hypertension in the hunter-gatherer state, urinary sodiums indicated intakes under 5 g/day. So Dahl tentatively recommended this [733] for adults without a family history of hypertension. This is at least 10 times the amount on which adequate sodium balance can be maintained. Then for persons with a family history of hypertension he suggested the early adoption of a diet containing only 500–1,000 mg salt/day (this was before efficient drugs were available).

29.4 Thiazides and Sceptics

At the same time as Dahl wrote those recommendations, orally effective diuretics, thiazides were being introduced and shown to lower BP [734], probably by inducing urinary loss of sodium and chloride. This was more convenient and acceptable treatment than organising and keeping to a very low salt diet. Thiazides reduce BP adequately in many, not all hypertensive patients [735], as can low salt. Other useful medicines appeared in the 1960s, β-blockers and α-methyl dopa.

Appearance of a medicine that increases urinary salt excretion and lowers BP strengthened the importance of salt in established hypertension, but this convenient alternative weakened the clinical case for treating hypertension with the hardship of strict low salt diets.

Sceptics opposed the idea that our usual salt intake is a cause of essential hypertension. They included eminent professors of medicine and experts on hypertension: Sir George Pickering (Oxford University) [736], Professor Swales (Leicester Royal Infirmary) [737], Laragh and Pecker (Hypertension Center, New York Hospital, Cornell) [738], and Brown et al. (MRC Blood Pressure Unit, Glasgow) [739]. Their arguments can be briefly summarised:

1. To lower BP restriction of sodium must be extreme and not everyone responds.
2. Isolated communities who do not experience any hypertension do not have the stresses of modern industrial life. Some may use potash as salt substitute.
3. Most intrapopulation studies of salt and blood pressure have not found a significant correlation between individuals.
4. Dahl's interpopulation studies did not define "hypertensive" and were not adjusted for age. "I regard his diagram as a work of the imagination but of no value" [736].
5. As to the rat experiments, "man is not a rat" [736].
6. We need benefit trials, health outcome trials before asking everyone to give up salt [739].

Box 29.1 Potassium and Blood Pressure

Addison in Toronto [740] in 1928 reported reduction of BP in five people (one himself) with moderate hypertension when given potassium chloride, bromide or citrate. BPs rose on sodium chloride. He tried the potassium because of earlier statements in the literature that potassium chloride causes a diuresis and increases sodium excretion. In 1931 Priddle [741], also in Toronto reported that potassium citrate reduced blood pressure in all of a series of 45 cases of hypertension, some with renal impairment. In a quarter of them BP fell considerably and symptoms improved in all. Priddle continued prescribing a low sodium diet (and later thiazide) with potassium chloride. He introduced the concept of the sodium/potassium ratio in hypertension [742].

Experiments with rats in the 1960s and 1970s showed that hypertension and premature death from high salt rations could be moderated if potassium was given [743, 744].

One large study found that urinary potassium was inversely related to BP in people with pressures ranging from normal to very high [745]. Another study found that total body potassium was inversely related to BP in people with hypertension [746].

When thiazides were introduced for hypertension one side effect was low plasma potassium from increased urinary loss. Oral potassium supplementation corrects the hypokalaemia and there is an accompanying fall of blood pressure [747].

More than 50 years after Addison and Priddle, MacGregor et al. (1982) repeated a trial of potassium in patients with hypertension [748]. But now "Slow K" was used, in a double-blind randomised controlled trial. The hypertension was moderate, patients had no renal failure and received no other medication. Four weeks of 60 mmol/day extra potassium reduced the mean systolic BP by 7 mmHg (and urinary potassium increased by 56 mmol/day). Between that trial and 1997 there were almost 60 reports of the BP-lowering effects of potassium in humans. Thirty-three trials satisfied the criteria for inclusion in a meta-analysis by Whelton et al. [749]. The forest plot shows that there was significant BP lowering, more in hypertensives than normotensives. An earlier meta-analysis (19 trials) by Cappuccio and MacGregor [750] reached the same conclusion (1991). Both concluded that increased potassium intake should be considered in the management of hypertension. Whelton et al. noted that potassium appeared to have an enhanced effect where subjects were exposed to a high sodium intake. The reverse of this is that potassium has less effect in patients who have restricted their sodium intake to around 70 mmol/day [1.6 g Na] [751].

The INTERSALT study (1988) of BP and related variables in over 10,000 people in 52 centres and 30 countries found that 24 h urine potassium excretion was negatively and independently associated with blood pressure of individual subjects within centres after adjustment for sodium excretion, body mass index and alcohol intake [752].

29.5 Answers to the Sceptics; the Salt Case Develops

29.5.1 Moderate Sodium Restriction

BP can be reduced a significant (even if small) amount by *moderate* sodium restriction, around 70 mmol/day. This was demonstrated from 1973 in a series of independent randomised controlled trials in relatively small numbers of people with

moderate hypertension. Sodium intake was checked with 24 h urinary sodium. First of these trials were by Parijs et al. [753] (Belgium), then by Morgan and Myers [754] (Australia) and MacGregor et al. [755] (Charing Cross Hospital, London). By 1986 thirteen of these trials had been done and the fall of BP was greater in older people [756]. MacGregor et al., even demonstrated a dose-response effect [757]. With careful dietetic advice patients with mild hypertension achieved sodium intakes around 50 mmol/day. While adhering to this, in random order they had a month of placebo or 100 mmol sodium or 200 mmol sodium (as "slow sodium" tablets) [757]. Cutler reviewed by now 23 randomised trials of moderate sodium reduction (over 1,500 subjects) in 1991 [758]. Overall mean BP reduction in hypertensive subjects was 4.9/2.6 mmHg; 22/23 trials had a mean reduction, mean urinary sodium was 76 mmol/day lower. These trials lasted 1 month or longer. Law et al. [759] in their review found that reductions have been smaller in trials shorter than this. *They estimated that a 50 mmol/day reduction in sodium intake would reduce the incidence of stroke by a fifth and ischaemic heart disease by a sixth.*

29.5.2 Remote Communities

Further isolated and remote communities have been added to the few that Dahl wrote about. Maddocks [760] suggested there can be four patterns of change in blood pressure with age: (i) Systolic and diastolic rise, (ii) Systolic rises, diastolic flat, (iii) Systolic and diastolic both flat, (iv) Systolic and diastolic both decline with age. The Chimbu in the highlands of Papua New Guinea (in the 1960s) showed the last pattern; urinary sodium excretion was very low and this appeared to be the important environmental determinant. Truswell et al. [761] found reports of 13 communities in which BP does not rise with age in his review and added a 14th, the !Kung of NW Botswana. Their urinary sodiums averaged 30 mmol/day. Then the Yanomamo were discovered in the Amazon jungle. They have low BPs that decline with age [762] and extraordinary low urinary sodium of 1 mmol/day [763]. Intakes may be a little higher than this. Investigators were surprised that people could adapt to such minimal salt intakes and even reproduce (during pregnancy they have exceedingly high urinary aldosterone [763]). Observers do not, however, describe their life as peaceful and stress-free [764].

Many low blood pressure, low salt people no longer have their original way of life. Some now experience hypertension [765, 766]. With globalisation, air transport and four-wheel drive vehicles, salt is light to transport and inexpensive and has probably become universal.

It is instructive to consider isolated communities that *have* experienced hypertension. One striking example is the patients at Dr Albert Schweitzer's mission hospital at Lambarene in Gabon, West Africa "on the edge of the primaeval forest" [767]. Another is Tiree, the most remote and isolated of the Hebridean islands, off the west coast of Scotland, "relatively free from the accepted stresses and strains of modern living" [768]. Experienced observers were surprised by their high prevalence of

hypertension. But it is fairly certain that German mission doctors, and Scots with a regular ferry service would have salt regularly available.

In the Solomon Islands and nearby Bougainville island an anthropological and medical team led by LB Page studied six separate tribal societies in 1966–1970 [769]. They were graded by degree of acculturation to a Western way of life (years and intensity of contact, literacy, Christianity, medical care, cash economy, Western diet); medical examination included BP measurement and estimated salt intake. The Lau tribe ranked fairly low in acculturation but first in height of blood pressures and clearly had the highest salt intake. They lived on islands in a coastal lagoon and cooked their fish, sweet potatoes and greens in seawater.

29.5.3 Regression Dilution Bias

In *intrapopulation* studies, as Frost et al. [770] demonstrated, estimates of the association between blood pressure and sodium intake are affected by systematic under-estimation or "regression dilution bias" because a single BP measurement and one-day's urinary sodium may each be quite far from the individual's long-term mean value. One estimate of the standard deviation of sodium intake within a person was 58 mmol/24 h. Blood pressure readings also vary within the day and between measurements. Consequently there is a flat regression slope, and large numbers are required before the correlation coefficient becomes significant. Frost et al. combined 14 within-population studies (12,773 subjects) of BP and urine sodium: collectively the regression slope was highly significant.

Joossens et al. [753] dealt with this problem by ranking 1,300 subjects by urinary sodium and dividing them into 20 progressive subgroups. Then $r = 0.69$ and $P = 0.001$. Pietinen [771] collected 3 days' urines for sodium and divided subjects by whether first degree relatives had hypertension. Then in the group with a positive family history there was a positive correlation (not in the others). Beard et al. [772] did a secondary analysis of data collected in the 1986/1987 Dietary and Nutritional Survey of British Adults (1,688 men + women). Blood pressure was the mean of the last two of three readings with automated equipment. This was significantly associated with the subject's 24 h urine sodium after controlling for age, obesity, alcohol intake and season of examination.

29.5.4 INTERSALT

INTERSALT was a collaborative standardised international study of electrolyte excretion and blood pressure in over 10,000 men and women in 52 centres in 30 countries (from Argentina to Zimbabwe) in the mid 1980s [752]. This was the answer to criticisms of Dahl's line. The range of sodium excretion was 0.02 (in Yanomanos) to 242 mmol/day (in Tianjin, China). Sodium excretion and systolic BP were positively associated across individuals in 39 centres (significant in 15), negatively in 15 centres (significant in 2). Across the 52 centres there was a significant linear relation between median 24 h sodium excretion and the slope

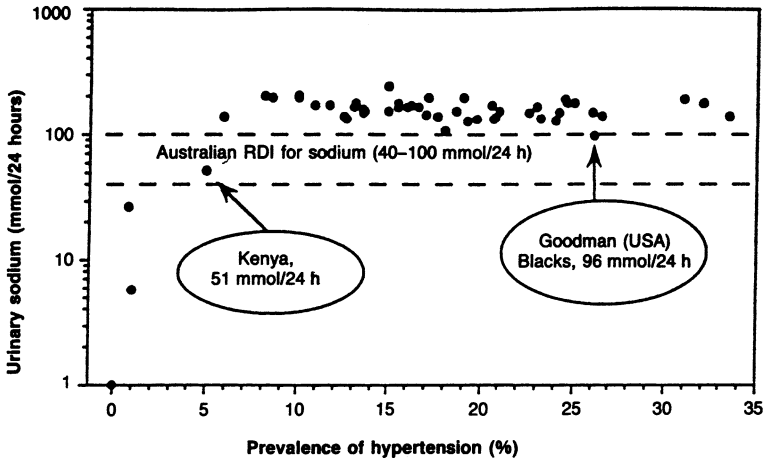

Fig. 29.2 Urinary sodium (mmol/d) against prevalence of hypertension in 47 centres in the Intersalt Study, 1988 [752] plotted by Beard [773]. Note: sodium here is the *vertical* axis

of systolic BP with age. The regression coefficient was 0.003 mmHg/year/mmol sodium ($P = 0.001$). However, when mean sodiums (or Na/K) and BPs for the 52 centres are plotted they do not reproduce Dahl's line (Fig. 29.2).

Except for the four centres with lowest sodiums (two in the Amazon, one in PNG, one in Kenya), the points for the rest of the centres fall roughly along a horizontal line [773]. Median sodium excretion and systolic BP in each centre were significantly associated across the 52 centres, but this was no longer significant if the 4 centres with very low sodium excretion were removed. Strong positive associations of BP with alcohol intake and body mass index were found. Associations with urinary potassium were mostly negative within centres. The INTERSALT results were revised in 1996 after the lead authors realised they had not adequately corrected for the regression dilution problem [774]. The new analysis showed that a sodium intake 100 mmol/day lower could result in systolic BP 3–6 mmHg less (at age 40 year) and a 10 mmHg shallower slope of systolic BP between age 25 and age 55. Higher urine sodium is associated with substantially greater differences in BP in middle age compared with young adults.

29.5.5 Chimpanzee Experiments

The nearest animals to humans are chimpanzees, genetically about 98% the same as us. In a colony of chimpanzees at CIRMF in Franceville, Gabon, Denton et al., gave 13 animals accustomed to their natural vegetarian and fruit diet (low salt) added salt while 13 were controls [775]. The group were 5–18 years old and weighed around 46 kg. They were given an infant formula, in which salt was mixed only for the experimental group. Ten of this 13 took most of the salt ration. The salt intake was worked up, 5 g/day for 19 weeks, 10 g/day for 3 weeks, then 15 g/day for 62

weeks. Mean BP gradually rose by 33/10 mmHg; systolic BP even rose 12 mm on 5 g salt/day. When salt was stopped BPs came down slowly to near the pre-experiment level after 20 weeks. Two of the 10 chimpanzees who took the prescribed salt formula did not share in the increased BP. Animals were anaesthetized for BP measurement. Plasma renin activity and aldosterone went down significantly in the experimental set. This chimpanzee trial was "as close to a decisive experiment as feasible on the influence of salt alone on a young adult hominid population" [775].

29.5.6 Cardiac Effects of Salt Reduction

Reduction of the prevalence of hypertension would be expected to contribute to less CHD in communities with relatively high serum cholesterol [123]. A few large trials have been achieved with modest reduction of blood pressure [776, 777]. TOHP (Trials of Hypertension Prevention) [777] would report cardiac events in 2007.

The best indication of *cardiac effects of salt reduction* in the twentieth century come from randomised controlled thiazide trials. In EWPHE [778] (European Working Party on High Blood Pressure in the Elderly) 65% took only hydrochlorothiazide + triamterene (a potassium sparing diuretic). There were significantly fewer fatal myocardial infarctions in the actively treated group. In SHEP (Systolic Hypertension in the Elderly Program) [779] the rate of myocardial infarction + coronary deaths was 0.73 compared with placebo. Half the patients took only chlorthalidine, half augmented this with atenolol or reserpine. These and three other trials in which the primary treatment was thiazide diuretics were included in Gueyffier's meta-analysis [780]. Coronary events were fewer on active drug than in the placebo groups in all the trials.

In a prospective cohort study He et al., analysed over 14,000 people who had been examined in NHANES 1 in 1971–1972 and followed up for 19 years [781]. Sodium intake was from a single 24 h dietary recall. Among 2,700 overweight people a 100 mmol higher sodium intake was followed by a 44% increase in CHD mortality. However, in 6,800 non-overweight people single recalled sodium intake was not significantly associated with cardiovascular risk.

Box 29.2 Higher Blood Pressures in African Americans

Higher blood pressures in Black people than whites in the USA have been reported recurrently since 1932 [782]. McDonaugh (1964) reported BPs of nearly 3,000 people in a community survey in Evans County, Georgia [783]. Set out in tables and groups by 10 year age group, gender and colour (race), systolic BPs were higher at all ages in Blacks. Three earlier surveys in North America were compared in the same paper. Black people had higher systolic

and diastolic pressures. Differences in social class, occupation, arm circumference, body weight and treatment practices could not explain these racial differences in BP [783]. Black people tend to have slower excretion of sodium after an acute load [784] and have lower renin levels [785]. Their BPs are more sensitive to high sodium intake and more responsive to salt restriction [784]. These different responses are presumably genetically determined. The Middle Passage hypothesis [786] suggests that the slave ancestors of present day African Americans were more likely to survive the horrific transatlantic crossing if they had enhanced ability to conserve salt. But it is not clear whether BPs are lower (by standardised methods) in West Africans or in native Liberians than in the offspring of US slaves transported back there.

29.6 Baby Food Manufacturers Take the Lead

Human milk was found to contain an average of only 15 mg (0.65 mmol sodium)/100 ml by Macy et al. [787] who analysed 300 samples and checked earlier literature. Subsequent analysts have found the same mean value for mature breast milk [788]. Cows' milk contains 3.3 times more, about 50 mg Na (2.2 mmol)/100 ml. Manufacturers of infant formulas have had to demineralise cows milk to make its main chemical components near to human milk, and to avoid contributing to hypernatraemia [789].

In 1968 Dahl wrote about the salt in processed baby *foods* [790]. It averaged 15–16 mEq/100 g in strained meats and vegetables and 28–35 mEq/100 g in dry cereals. There was no obvious benefit from exceeding the infant's sodium requirement 5–10 times. "Added salt in baby foods is neither necessary nor beneficial. It can be harmful. The evidence in rats is so straightforward that, . . . the burden of proof lies with those who add salt to their baby foods rather than with those of us that question this practice".

The 1969 White House Conference on Food, Nutrition and Health [791] echoed this and added "Many foods manufactured for human infants are salted, not in accordance with the sodium content of their ideal food, breast milk, which contains 7 mEq/l, but to please mother's taste".

In 1970 Dahl published "Hypertension and death from consumption of processed baby foods by rats" [792]. These were genetically hypertension-prone rats. Among 25 such rats fed solely processed baby foods all developed BPs around 180–190 mmHg after a few months and half these animals died, but control animals had no hypertension and survived.

The Food Protection committee of the Food and Nutrition Board (NAS-NRC) recommended in 1971 that the salt content of infant foods should be reduced [793]. As Time magazine later reported "In the mid 1970s, in response to public outcry and Government and medical criticism manufacturers stopped adding salt to baby food" [794]. This was a voluntary, unanimous move by a section of the food industry.

All commercial weaning foods and baby foods since then are (by adult standards) very low salt foods, but if baby is given family foods (which mother might think healthier – not out of a can or bottle) his/her sodium intake is much higher [795].

29.6.1 And for Adults

In 1976 Present Knowledge in Nutrition, which in earlier editions had carried brief and perfunctory accounts of sodium gave its longest of 53 chapters to a wide ranging essay on sodium and potassium by Meneely and Battarbee [796]. Meneely had worked with Professor Bill Darby [724, 743], who was now President of the Nutrition Foundation, the publisher. A fuller version of the chapter was also published in American Journal of Cardiology [797]. They presented the evolutionary argument. Early man, our ancestors with no sodium chloride to add to their foods usually consumed around 10 mEq of sodium, but their foods were rich in potassium – processed peas are a good example. In fresh peas the sodium is 1 mg/100 g and potassium 380 mg, but in canned peas with liquid poured off, the sodium and potassium content have changed to 230 and 180 mg/100. The sodium/potassium ratio has gone up 490 times! There has not been enough time for our genetic system to adapt to this. They stated the problem: "It still seems incredible to some, despite a massive body of evidence, that common table salt might be noxious. Since its effects are insidious, it has been difficult to document its toxicity for humans, especially since genetic differences in sensitivity abound and make the literature of population surveys bewildering. The matter is complicated by the protective effect of dietary potassium. . ." [796].

"A unifying and simplifying theory is needed. It is obvious that sodium chloride raises BP in some but does not in others; it is equally obvious that restriction of dietary sodium lowers high blood pressure in some, but not in others. Since excess sodium does not elevate the BP of everyone some conclude it does not have this effect on anyone. Since sodium restriction does not lower the BP in all hypertensives, some think it is not worth restricting sodium intake in any hypertensive. . ." [796].

Patients with essential hypertension who respond to diuretic drugs respond equally well to low sodium diets, but physicians prefer to prescribe pills rather than diets. "Osler noted 'the desire to take medicine is perhaps the greatest feature which distinguishes man from animals" [796]. In two places in the chapter Meneely and Battarbee consider 3.0 g, elsewhere 3.5 g of sodium chloride adequate/more than adequate for adult man (i.e. 50–60 mEq Na).

29.6.2 Dietary Goals for the US

In the 1977 Dietary Goals for the United States [798], produced by George McGovern's Senate committee the 6th goal was "Reduce salt consumption by about 50–85%, to about 3 g/day". The scientific explanation was inadequately brief, relying mainly on Meneely and Battarbee [796]. With estimated salt consumption

of 6–18 g/day, most in processed foods, this brave recommendation was heavily criticised [799] by the Salt Institute ("there is no scientific evidence . . .") but also by the American Medical Association (Mass screening programs are detecting hypertension; in most patients it can be effectively treated . . .). Revised Dietary Goals were published at the end of the same year. "Avoid overweight" was added and the salt goal, now No. 7, was changed to "about 5 g/day" [218] (83 mmol sodium).

29.6.3 Subsequent Official Recommendations

The next official committee to make a similar recommendation (published 1984) was in Australia [800], though it was cautiously worded: "Although the adverse effects of excess sodium intake cannot be stated conclusively, present evidence strongly suggests that the community should be encouraged to reduce consumption of sodium. The aim should be to achieve a community sodium intake of under 100 mmol/day (6 g NaCl)". In 1989, the 749 page review "Diet and Health" by a committee of the US National Research Council's Food and Nutrition Board recommended healthy adults limit total daily salt intake to 6 g or less [801]. "The Committee was aware that a greater reduction of salt intake (i.e., to 4.5 g or less) would probably confer greater health benefits . . . but chose 6 g as an initial goal that can be achieved more readily". WHO followed with a 6 g salt/day population upper limit in 1990 [802]. A committee of the British Department of Health (1994) more cautiously recommended a gradual reduction in average sodium intake by the adult population to about 100 mmol/day (6 g salt) and an increase in potassium intake to about 90 mmol/day [149].

29.7 Most Salt We Eat Was Put in by Food Processors

The big difficulty with advising people to reduce their intake is that most salt is not under their individual control at the table or in the kitchen. Dahl was aware of this in 1972 [721]: to attain a low sodium diet his third rule was "avoid all processed food except fruits and juices". To measure how much of people's salt intake was discretionary, Sanchez-Castillo et al., used lithium fused sodium chloride in the home [803]. Eighty-three (women and men) adult patients of a general practice in a rural town in Cambridgeshire had their 24 h urinary sodium and chloride measured for 12 days. In the middle week subjects used lithium-labelled salt at the table and in home cooking; the lithium was measured in the urine. Table salt usage was measured by loss of weight in each individual's salt cellar. Total salt consumption (estimated from urinary chloride, so sodium bicarbonate and MSG would not be counted) was 10.5 g/day in men and 7.5 g/day in women. Discretionary salt contributed only 15% of this total. Much of the salt used in cooking vegetables was discarded in the cooking water. The conclusion of this research and a review of international data by the same authors [804] is that "Any programme for reducing the salt consumption of

a population should therefore concentrate primarily on a reduction in the salt used during food processing".

The food industry, except the section that makes weaning foods have been defensive; restaurants and celebrity chefs have ignored the research. Food companies have been gradually compelled by food standards authorities to label the sodium content of packaged foods, but the numbers are meaningless to most consumers, confused between sodium and salt, mg and mmol, standard servings versus 100 g. For many foods there is no low salt alternative. Companies that make margarines are an exception. This food is not traditional and its makers have been more tuned in to nutritional science. Unsaturated fat margarines are designed to help prevent CHD.

29.8 How Does BP Increase in Salt-Sensitive Individuals

Human experience, and the chimpanzee experiment [775]; demonstrate that the increase is a slow response. Three systems inside the body are most likely involved in the mechanism: kidneys, arterioles and a postulated humoral factor.

29.8.1 Kidneys

Guyton [805], author of a classic textbook of physiology, produced mathematical (linear) feedback models (1969) indicating that the kidney is the main long-term controller of BP, through adjustments of sodium excretion. Dahl (1974) nephrectomized salt-sensitive rats and transplanted kidneys to them from salt-resistant rats: no hypertension developed. Conversely, salt resistant rats whose kidneys were replaced by kidneys from salt sensitive rats developed hypertension on high salt rations [806]. Isolated blood-perfused kidneys of Dahl salt-sensitive rats excrete less sodium than kidneys of resistant rats perfused at the same inflow pressure [807]. In other hypersensitive strains of rats too, cross-transplantation of kidneys demonstrated that the blood pressure follows the kidney [808]. Experience with human kidney transplants has been consistent with this [809, 810]. It appears that in most forms of hypertension the initiating factor is a restricted ability of the kidney to excrete sodium.

29.8.2 Arterioles

The immediate cause of most hypertension is increased tone in the peripheral arterioles. Tobian (1952) [811] reported increased sodium content of renal arteries (measured post mortem) in patients who had had hypertension. Arterioles may behave differently from large arteries but the muscle cells in arterioles are very difficult to study during life. As proxy, investigators have measured cation content and transport in red cells and leucocytes in people with hypertension. Edmondson

et al., in 1975 [812] reported increased intracellular sodium and reduced sodium efflux in leucocytes of people with uncomplicated hypertension. Others have mostly confirmed this in leucocytes or red cells. By 1986, 20 papers had reported sodium content in red cells. Put together they show it was significantly increased in hypertension [813], with reduced activity of the Na^+, K^+-ATPase sodium pump. Blaustein (1977) [814] reasoned that reduced sodium ion gradient across the plasma membrane promotes entry of more calcium in the cell via the sodium/calcium exchanger. Increased ionised calcium increases vessel wall tension and hence blood pressure (an effect opposed by calcium channel blocker medicines, which are ineffective on low salt diets [815]). Blaustein's 1999 review [816] on sodium/calcium exchange has over 1,000 references!

29.8.3 Humoral Inhibitor of the Sodium Pump

Dahl et al. found that if salt-resistant rats were joined by a skin flap (parabiosis) to salt-sensitive rats they became hypertensive on high salt rations [817]. This was evidence for a transmittable humoral agent. Haddy and Overbeck [818] reviewed the experimental literature and suggested that in animals with volume expanded hypertension there is a circulating agent that suppresses cardiovascular membrane Na^+, K^+-ATPase. Poston et al. [819] found that hypertensive patients' serum reduced sodium efflux from incubated leucocytes; the affected transporter was ouabain-sensitive Na^+, K^+-ATPase. (An inhibitor of sodium efflux, acting on renal tubules will increase urinary sodium excretion, i.e., natriuresis; but in arterioles it will increase intracellelular sodium ion). The inhibitor is not atrial natriuretic peptide: this doesn't affect Na^+, K^+-ATPase [820].

Hamlyn's group used biochemical screening methods, including cellular Na^+, K^+-ATPase activity, and from large volumes of human plasma obtained a tiny quantity of an active compound that matches in a series of 6 different chemical tests with pharmaceutical plant-derived OUABAIN [821, 822]. Antibodies have been prepared, so it is possible to estimate plasma levels of this "endogenous ouabain" in different physiological and disease conditions. Endogenous ouabain by immunoassay was increased in plasma in half of 64 people with essential hypertension [823]. Its activity (effect on ^3H ouabain binding by cultured renal tubular cells) increased with short term increase of salt intake in human subjects [824]. Endogenous ouabain appears to be secreted in the adrenal cortex. Ouabain (from Sigma Chemical Co.), given to rats for 8 weeks (by daily injections) caused hypertension without increased cardiac output [digoxin does not cause hypertension in human patients] [825].

Thus pieces of evidence support the hypothesis that endogenous ouabain might be the link inside the body between salt load and hypertension, but the picture is incomplete and a number of questions remained [822] in 1999.

By 2,000 evidence that sodium chloride is a risk factor for hypertension was strong, but not everyone is salt-sensitive and it is difficult to eat a very low salt diet without avoiding all processed foods. The evidence for salt and CHD was largely indirect, via the hypertension-CHD relationship.

In a 1999 review "Primary prevention of coronary heart disease in hypertension", Professor Sleight (Oxford) wrote "There are several proven strategies, involving both drugs and lifestyle changes. Stopping smoking is the most powerful, but exercise and reduction of fats and salt are also important; the latter will need cooperation with the food industry" [826].

29.9 Into the Next Century

In the next 7 years evidence and opinion about salt and CHD became firmer. A cohort of 2,436 Finnish men and women provided 24 h urine sodiums and were followed up from 1982 or 1987 with the national hospital discharge register. Association between sodium excretion and CHD (98 cases) was significant in men; the trend in women was similar but not significant (only 30 CHD cases) [827]. In a large veterans' retirement home (average age 75 years) in Taiwan the salt used in two of the five kitchens was changed to half KCl, half NaCl (introduced gradually over 3 weeks). Over the next 4 years cardiovascular mortality, including CHD was significantly lower in the experimental group [828].

In a long term trial in the USA, TOHP (trial of hypertension prevention) one group of participants had reduced their salt intake by 44 mmol/day in TOPH One and 33 mmol/day in TOHP Two. The combined late follow-up result was 25% fewer cardiovascular events (most CHD) [829]. The accompanying editorial in the BMJ [830] thought "the battle around the evidence linking salt and heart disease has largely been won ... a population-wide policy of salt reduction will only come through pressure on the food and catering industries".

New major research results on salt and blood pressure were published [831–833] and Health authorities took a stronger line against high dietary salt. Murray et al. at WHO [834] estimated that government action to stimulate a reduction in the salt content of processed foods would be a cost-effective way to limit cardiovascular disease. The British nutrition establishment, in the form of the Scientific Advisory Committee on Nutrition [835] found the evidence stronger than in 1994 that a reduction in average population salt intake would lower BP and contribute to a decrease in cardiovascular disease. It set the population target to 6 g salt/day. In North America the Institute of Medicine (2005) now recommends a sodium intake of 65 mmol/day for adults up to 50 years of age, 55 mmol/day between 51 and 70 years and 50 mmol/day (approx 2.9 g NaCl) for those over 71 years of age [836].

Chapter 30
How It Adds Up

The British Committee on Medical Aspects of Food Policy (COMA) of the Department of Health made detailed recommendations in 1994 in the report "Nutritional Aspects of Cardiovascular Disease" [399]. They reviewed the field before a number of results had been published between 1994 and 1999.

30.1 American Heart Association, 2000

The Nutrition Committee of the American Heart Association published Dietary Guidelines in 2000 [837]. Their major recommendations were the following:

Generally Healthy Eating Pattern
- Consume a variety of fruits and vegetables and whole grain products.
- Include fat free and low fat dairy products, fish legumes, poultry and lean meats.

A Healthy Body Weight
- Match intake of energy (calories) to overall energy needs;
- Limit consumption of foods with a high calorie density and/or low nutritional quality, including those with a high content of sugars;
- Maintain a level of physical activity that achieves fitness and balances energy expenditure with energy intake;
- For weight reduction, expenditure should exceed intake.

A Desirable Blood Cholesterol and Lipoprotein Profile
- Limit the intake of foods with a high content of saturated fatty acids and cholesterol.
- Substitute grains and unsaturated fatty acids from vegetables, fish, legumes and nuts.

A Desirable Blood Pressure
- Limit the intake of salt (NaCl) to < 6 g/day.
- Limit alcohol consumption (to no more than 1 drink/day for women and 2 drinks for day for men).
- Maintain a healthy body weight and dietary pattern that emphasises vegetables, fruits and low-fat or fat-free dairy products.

A.S. Truswell, *Cholesterol and Beyond*, DOI 10.1007/978-90-481-8875-8_30,

This was the product of a 19 person committee and they seem to have specially incorporated elements from Dietary Guidelines for Americans (2000) [838] and results of the DASH trials with blood pressure [839]. The statement had 205 references but these appear in discussion, rather than clear recommendations.

30.2 In "ABC of Nutrition", 1999

A third "Dietary Prescription" published in 1999 [840] was written by an individual, not a committee and more of the results in the previous pages of this history appear in it. Having made the point earlier in the chapter that overweight people tend to have raised plasma cholesterol and that abdominal visceral adiposity is a direct risk factor for CHD, the prescription prescribes the *quality* of the diet when the calorie intake is right for the energy expenditure (Box 30.1).

This is a concise example of how the research in this History has changed people's dietary standards. Except, perhaps, for "moderation with alcohol" none of these elements existed in 1900.

Box 30.1 Dietary Prescription to Reduce the Risk of CHD from "ABC of Nutrition", 3rd Edition (1999) [840]

Total fat. Reduction is not essential for improving plasma lipids but should reduce coagulation factors and day-time plasma triglycerides and contribute to weight reduction.

Saturated fatty acids. Principally 14:0, 16:0 and 12:0 should be substantially reduced from around 15% of dietary energy in many Western diets to 8–10%.

Polyunsaturated fatty acids. Mainly linoleic acid (18:2 ω-6): they should be about 7% of dietary energy (present British level), up to 10%. Omega-3 polyunsaturated fatty acids should be increased, both 20:5 and 22:6 from seafoods and 18:3 from canola (rapeseed) oil, etc.

Monounsaturated fatty acids. Ideal intake if total fat 30%, saturates 10% and polyunsaturated 8% would be 12% of total dietary energy.

Trans fatty acids. With the help of margarine manufacturers these are being reduced. The UK Department of Health recommends no more than 2% of dietary energy. Avoid older hard margarines.

Dietary cholesterol. This boils down to the question of egg yolks. Eggs are a nutritious, inexpensive and convenient food. The UK Department of Health recommends for the general population no rise in cholesterol intake.

Salt (NaCl). Restriction to under 6.0 g/day is advised for the general population (100 mmol Na). It is more important for coronary patients.

Fish. The UK Department of Health recommends at least twice a week, preferably fatty fish. It should not be fried in saturated fat.

Fibre. Oatmeal is recommended.

Vegetables and fruit. These are low in fat, and contain pectin and other fibres, flavonoids and other antioxidants, and they contain folate. Expert Committees in Britain and the USA recommend five servings of different vegetables and fruit per day (400 g/day average weight).

Soy products (not salty soy sauce) recommended.

Alcohol in moderation, two to three drinks per day is beneficial for middle-aged people at risk of coronary heart disease but cannot be recommended for the general population because of the greater danger of accidents in younger people and of all the complications of excessive intake.

Coffee should be instant not filtered.

Chapter 31
The Big Picture

The incidence of CHD and the effectiveness of its treatment have changed with time. Research briefly described in this chapter monitored the big changes, and attempts to explain why they happened. "Disease of the coronary arteries" or later "ischaemic heart disease" did not appear in the International Classification of Diseases until the 4th revision (1931) and WHO annual statistics started reporting non communicable diseases in 1936.[1] From then to 2000 (and beyond) age-standardised mortality (separate for men and women) is the only universally available measure of the impact of CHD in and between countries, and its changes with time.

In some countries there have been very large changes over this period and between countries there have been up to tenfold differences in CHD mortality. In USA, Finland, Australia and New Zealand CHD mortality started to decline in the late 1960s. Other countries showed a later decline or a rise in CHD mortality.

The work of reporting, compiling and publishing these statistics for non communicable disease is valuable research. Years 1950–2000.

31.1 USA and Canada

Figure 31.1 shows CHD mortality for males 35–74 years in North America. The data from WHO were compiled and the graphs drawn for this and Figs. 31.4, 31.5, 31.6, 31.7 and 31.8 by Dr Masoud Mirzaei [841]. In the USA male mortality reached its peak in 1967 or 1968 (there are some irregularities of the line with the changeover to the 8th revision of the International Classification of Diseases in 1968 and the 9th revision in 1977). The mortality rate fell steeply from over 600/100,000 in 1968 to around 200 in the year 2000. Canadian statistics have followed the USA closely, though the peak mortality there was around 550/100,000. Women in N. America

[1] Up till 1948 (when WHO was established) data were collected by a health body of the League of Nations.

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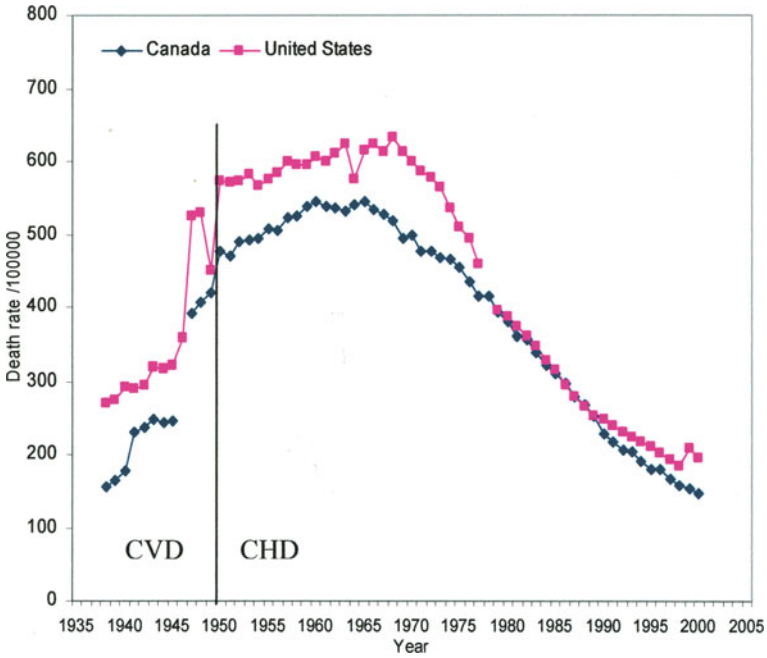

Fig. 31.1 CHD death rates in North America 1937–2002, males 35–74 years [841]

(not shown) have had the same pattern of decline but their (age-standardized) rates have been under half those of US and Canadian men.

No one noticed the decline in CHD mortality until 1974. That year a special communication in JAMA by Weldon Walker [842] opened with some contemporary quotations that "medicine had failed to stop the rising death rate from coronary artery disease". It takes some years for national health statistics to be compiled. Walker only had provisional national statistics up to 1969. They appeared to have come down since 1963. He commented "Could a corporation survive that had not yet completed its 1969 annual report?" Gordon and Thom [843] at NIH in 1975 thought the decrease was probably real and a likely explanation was reduced virulence of influenza. Editorials followed from 1976 [844, 845]. The BMJ thought it possible that the reduction was an artefact caused by changing to the 8th revision of the International Classification of Diseases in 1968.

At state level the decline had started in California in the 1950s, with Maryland and District of Columbia (DC) next [846]. Kentucky, Tennessee and West Virginia were late decliners [847].

In 1978 a Conference on the Decline in Coronary Heart Disease Mortality was held at the National Heart, Lung and Blood Institute, Bethesda [848]. Eighty-four experts participated; the report is over 400 pages long. All they could conclude was that the decrease in CHD mortality was real; both primary prevention and better

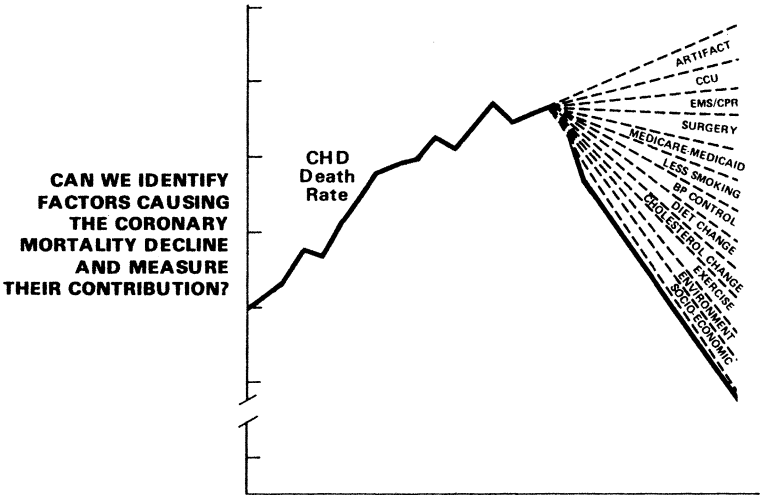

Fig. 31.2 Can we identify factors causing the coronary mortality decline and measure their contribution? S. Blumenthal in Introduction to NIH Conference on the Decline in Coronary Heart Disease Mortality, 1979 [849]

medical care had probably contributed, but they could not fully explain the decline. Which of the 13 likely possibilities in Fig. 31.2 were causing the effect? A useful start would be to quantify the relative contribution of the two possibilities in Fig. 31.3.

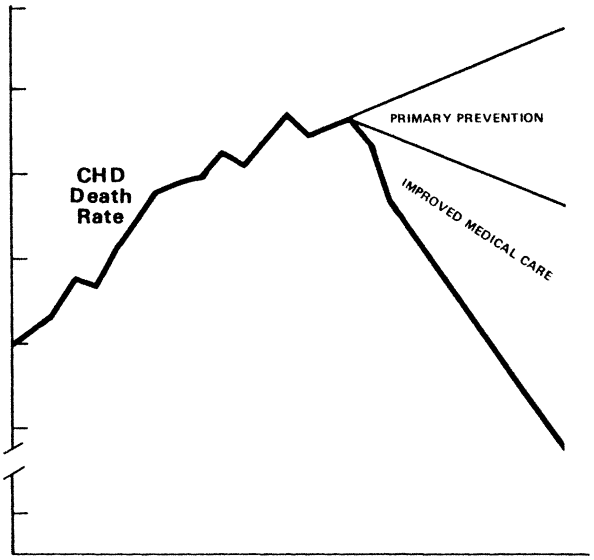

Fig. 31.3 Two main groups of possible reasons for decline in CHD mortality. Also from Blumenthal [849]

CHD mortality rates continued amazingly to come down every year up to 2000 in USA and Canada – and on the other side of the world almost the same phenomenon (in timing and quantities) was occurring in Australia and New Zealand. By the mid 1980s it was clear in several major US health organisations that there had been large reductions in incidence, in admissions for acute myocardial infarction among employees of Du Pont Company [850] and in the Kaiser-Permanente Medical Care Program [851]. At Du Pont the incidence fell faster in white collar than in blue collar workers. Incidence appeared to have decreased more than case-fatality in hospital. In Minneapolis between 1970 and 1980 there was a lage reduction of coronary deaths before reaching hospital and also a reduced case-fatality rate for those admitted with myocardial infarction [852]. Goldman and Cook (1984) made detailed estimates of how much of the decline of CHD mortality could be related to different changes in lifestyle and to medical treatments [853]: lifestyle change accounted for more than half the decline and medical treatments for 40%, the latter included treatment of hypertension. Beaglehole et al., in 1979 [854] noted that the decline in CHD mortality began well before improvements in the medical care of its clinical manifestations. They found data suggesting a modest decline in serum cholesterol in the 1960s, perhaps 5 mg/dl – probably associated with dietary change, but only likely to reduce CHD mortality by 4%.

Which environmental and medical developments were having most effect on CHD mortality must have changed from the 1960s to the late 1990s. Hypertension was more efficiently treated, smoking decreased considerably and statins came to be widely used in the 1990s to lower asymptomatic higher serum cholesterol. Medical management and case fatality greatly improved, with resuscitation in the community, thombolysis and primary angioplasty for acute myocardial infarction; coronary artery by pass grafts for unstable angina; anti-thrombosis treatments and statins for secondary prevention. Ford et al. [855] assessed contributions of these changes to the continuing decline of CHD mortality between 1980 and 2000. They attributed approximately 47% to medical and surgical treatments; approximately 44% to changes in risk factors: reduced serum cholesterol (–24%), blood pressure (–20%), smoking (–12%) less physical inactivity (–5%), but these were offset by increased BMI (+8%) and diabetes (+10%). Thus more of the decline in the USA 1980–2000 was attributable to medical treatments and more of the earlier 1960–1980 decline appears to have reflected incidence and changes in lifestyle – not very clearly defined.

31.2 Australia and New Zealand (Fig. 31.4)

The first report of a turn down in the CHD mortality rate in Australian men was by Ralph Reader [856] Director of the Australian National Heart Foundation, in 1972 (2 years before Walker's communication in USA) [842]. He suggested that intensive coronary care units could be responsible. Christie (1974) was next to write about the decline [857]. As 60% of coronary deaths occur outside hospital, he thought it

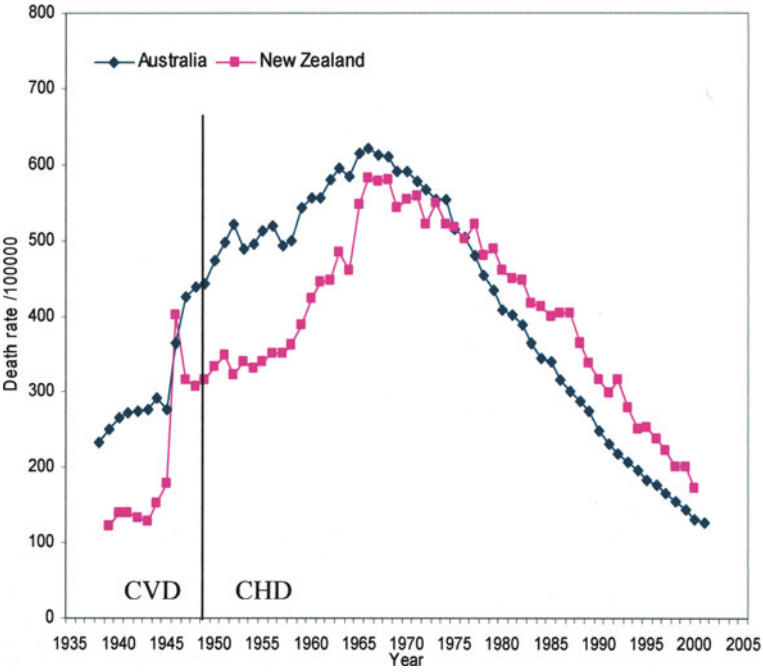

Fig. 31.4 CHD death rates in Australia and New Zealand 1937–2002, males 35–74 years, from WHO [841]

probable that there had been a definite drop in the incidence of CHD. The professional social group showed the greatest decline [858]. In the city of Perth (Western Australia) a CHD register showed that it was mainly due to reduced incidence of sudden death and acute myocardial infarction [859]. Seventy-five percent of all CHD deaths occurred before admission to hospital and it seemed that improved survival in hospital only accounted for about 20% of the decline [860].

Dwyer and Hetzel [861] at CSIRO's Division of Human Nutrition in Adelaide seem to have assumed that there had been little improvement in medical management around 1970 that could account for the reduced mortality. They compared data on risk factors hypertension, smoking and diet in USA, Australia and UK (where CHD mortality was not declining) (Fig. 31.6).

Hypertension and smoking appeared to be coming down in all three countries but only in the USA [862] and Australia was there evidence that consumption of saturated fats was down and polyunsaturated fats had increased. In the same Australian research institute a few years later Charnock, McLennan and colleagues showed in animals that polyunsaturated fats reduce the risk of dangerous arrhythmia in ischaemic hearts [387–391].

In New Zealand CHD mortality declined after 1968, though not quite as fast as in Australia. CHD registers in Auckland (which contains about a quarter of New Zealand's population) showed that the main contribution to reduced mortality in

1974–1981 was a significant reduction of sudden coronary deaths (nearly all outside hospital) [863]. In the environment there had been favourable reductions in butter, increase in vegetable oils, reduction of smoking and more treatment of hypertension. Case fatality rates did not change until later [864].

31.3 Nordic Countries

In the early 1960s *Finland* had the highest CHD mortality in the world and the rate was higher in North Karelia county than anywhere else in Finland. North Karelia had provided the East Finland cohort in the Seven Country Study, in which the median serum cholesterol was 260 mg/dl (6.73 mmol/l) in 1959 [123].

Finland experienced a marked increase in recorded CVD/CHD mortality between 1945 and 1965. The country had been affected by a border war with the Soviet Union from 1937 to 1944. Then after 1968 Finland had a very big fall of CHD mortality from almost 700 to 240/100,000 in the year 2000. Finland has been one of the most active countries in researching [236, 237, 240, 241] and promoting measures to prevent CHD. Figure 31.5 shows that these have been very successful. A major-community based preventive programme started in North Karelia in 1972 and CHD mortality came down more rapidly there than in the rest of the country

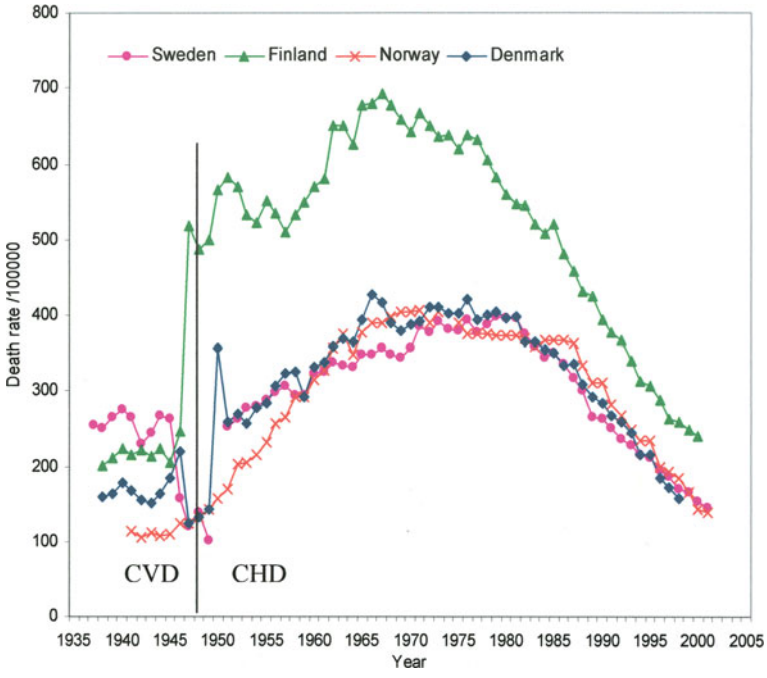

Fig. 31.5 CHD death rates in Nordic countries 1937–2002, males 35–74 years, from WHO [841]

[865]. Vaartiainen et al. [866] examined changes in risk factors: smoking, hypertension and serum cholesterol had all improved nationally and they estimated the 13% reduction of men's cholesterol had the most effect. Between 1972 and 1992 saturated fat intake decreased by a third and vegetable oil and vegetable consumption both increased considerably. They thought these changes could explain most of the decline in CHD mortality. There was also a reduction in case-fatality, but the reduction in incidence was larger [867]. At the end of the century Laatikainen et al., used the IMPACT modelling program to estimate the different contributions to the decline in Finland's CHD mortality between 1982 and 1987 (i.e. the continuing decline, after its first 14 years) [868]. They concluded that improved treatments explained 23% of the mortality reduction, and improved risk factors explained some 53–72%, so up to 24% was unexplained.

In the other Nordic group of countries CHD mortality never reached as high as in Finland. They had less urgency for preventive measures. In Sweden, for example, the decline of CHD mortality started 10 years after Finland and the gradient of the descending curve (Fig. 31.5) was not as steep. A joint paper by professors of medicine in New Zealand and Sweden pointed out the contrast between 1968 and 1977 of consistently falling CHD in New Zealand while mortality was increasing in Sweden [869]. As medical and surgical management, management of hypertension and reduction in smoking were about the same in both countries, they suggested the more active dietary changes occurring in New Zealand could explain the difference.

31.4 British Isles (Fig. 31.6)

It was 10 years after USA, Canada, Australia, New Zealand and Finland before CHD mortality started to turn down in England and Wales (in 1978), though Scotland had reached its (higher) peak in 1973. The first hopeful trends were presented by Florey et al., in the BMJ [870]. During these 10 years several eminent researchers wrote that England and Wales, and Sweden, needed to improve their preventive medicine efforts and suggested why mortality rates had diverged between well informed, affluent countries. Dwyer and Hetzel [861] suggested increased vegetable oil/polyunsaturated fat and decreased saturated fats in USA and Australia was a striking difference (see above) from Britain. Katan and Beynen published a memorable figure showing increase of adipose tissue linoleic acid in the USA and no change in the UK [871].

Geoffrey Rose in a major lecture in 1981 said "We are failing to prevent a preventable disease. If we had shared in the Australian and American decline each year in England and Wales there would be upwards of 25,000 fewer deaths. One can imagine the outcry if some shortcoming in therapeutic services were to cause even a fraction of this number of unnecessary deaths . . ." [872]. Later when CHD mortality was at last heading down in the UK he noted that the national P/S ratio rose from 0.24 in 1980 to 0.35 in 1985. "In the light of all the evidence that has accrued . . . I now think that the P/S ratio is probably one of the most potent determinants of CHD

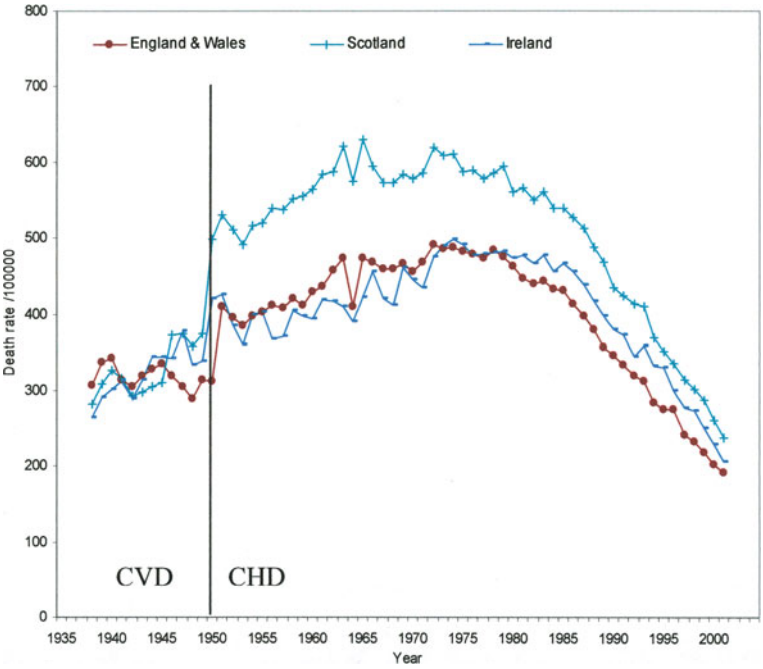

Fig. 31.6 CHD death rates in the British Isles 1937–2002, males 35–74 years, from WHO [841]

rates and trends and that its practical importance should be emphasised accordingly [873]". (He went on to warn that P/S is an oversimplification, mentioning stearic acid, fish oils and trans-fatty acids.)

As CHD rates started to decline in the UK, there was a marked reduction in people in non-manual occupations and no reduction at all among men in manual occupations or their wives [874]. Unal et al., used the IMPACT CHD modelling to investigate factors in the community and medical treatments attributable for the 54% decline of CHD mortality from 1981 to 2000 in England and Wales [875]. They estimated that primary prevention explained 52%, modern cardiological treatments 33% and secondary prevention 13%. Smoking reduction appeared to have had nearly 4 times the effect of serum cholesterol reduction or blood pressure reduction. This method of analysis is, however, blind to any effects of ω-6 or ω-3 polyunsaturated fats other than lowering serum cholesterol.

31.5 Western Europe

On the continent of Europe national CHD mortality has been substantially lower than England and Wales, and only half or less Scotland's rate. In some of these countries there was a dip in the mortality graph with the Second World War. All the countries in Fig. 31.7 have experienced a decline in mortality from around 1975. The

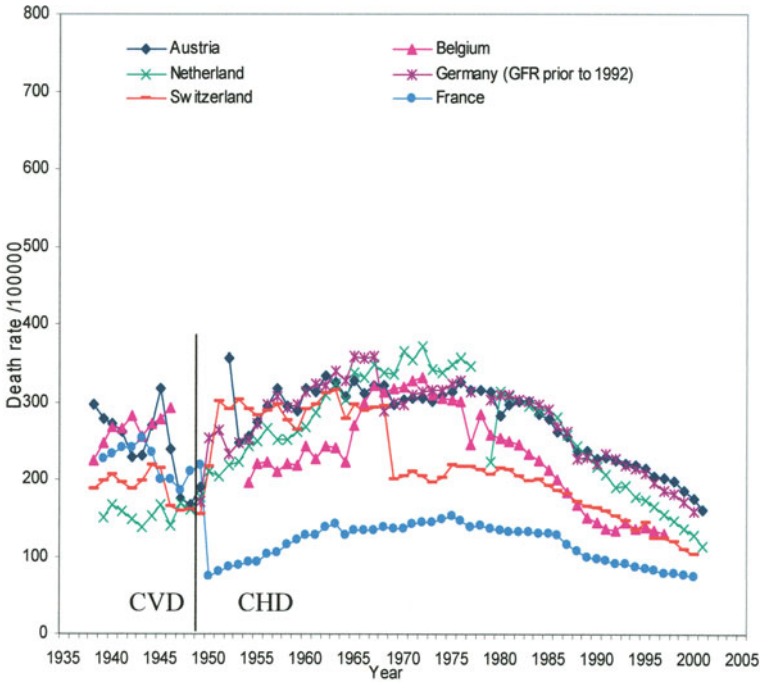

Fig. 31.7 CHD death rates in Western Europe 1937–2002, males 35–74 years, from WHO [841]

fall has been steepest in the Netherlands, from approximately 350 to 150/100,000 by 2000. Katan and Knuiman noted that the Netherlands fall in mortality was preceded by an increasing replacement of "brick" margarines by plant margarines higher in linoleic acid [876]. Abrupt jumps in the lines are presumably due to changeover of ICD codes. The most remarkable was France's rate, that changed from over 200 to under 100 in 1951 – and thereafter stayed well below all the neighbouring countries. By 2000 all these 6 countries had male CHD mortality rates below 200 (for comparison it was approximately 200 in England and Wales, 230 in Finland, 140 in Sweden, 190 in USA, 120 in Australia).

In Southern Europe mortality rates (not shown) have been between Belgium's and France's; Spain's rate has been almost the same as France's. Rates in these countries have trended gradually lower between 1950 and 2000 with no clear peak, except perhaps in Italy. After 1965 rates came down more in Italy (from 250 to 100) but they have slowly risen in Greece (from 100 to 160).

31.6 East European Countries

From about 1960, while CHD was starting to turn down in N. America, Australasia and Finland, mortality was rising in the countries of Eastern Europe; and the rise continued – in Poland it went up 58% in men between 1970 and 1980 – until about

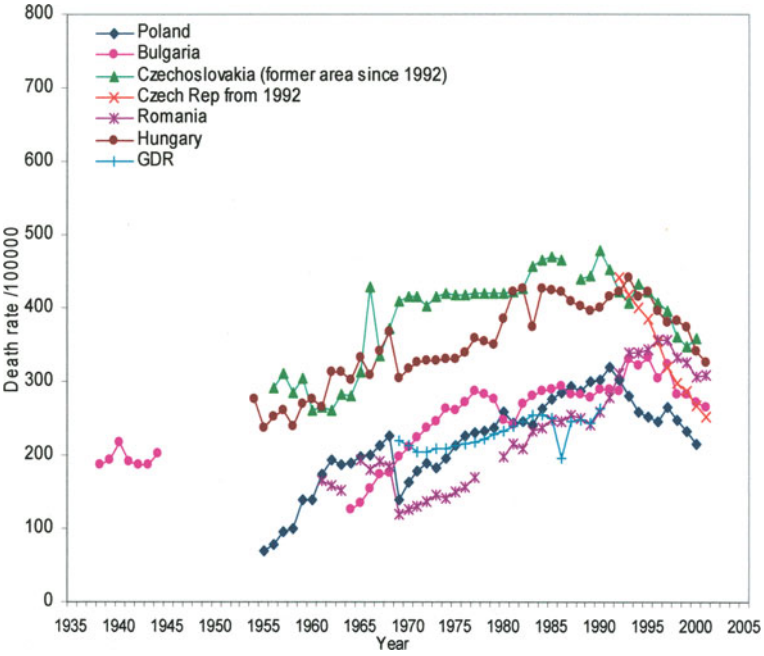

Fig. 31.8 CHD death rates in Eastern Europe 1937–2002, males 35–74 years, from WHO [841]

1990. The Berlin Wall came down in 1989. Since then in all the six countries in Fig. 31.8, CHD mortality has shown a definite decline starting 1990–1994, 20–25 years after USA and Australia. USSR/Russia mortality went down a little between 1980 and 1990 and has climbed higher since (not shown).

In Poland, Czechoslovakia, Hungary, the German Democratic Republic, Bulgaria and Romania there were big political and economic changes, opening up to foods from the west, and changes in food subsidies and prices. It is unlikely that the large falls in CHD mortality (38% down in men in 12 years in Poland) were due to suddenly improved medical facilities. Zatonski and Walter Willett looked into the likely reasons for the 1990s CHD mortality decline in Poland [877]. Changes in smoking were quite small. They commented:

> The experience of Poland is consistent with epidemiological and clinical evidence indicating that mortality due to CHD can be reduced by partly replacing dietary saturated fats with polyunsaturated fats while maintaining a low intake of *trans* fatty acids.
>
> Polyunsaturated fat reduces serum concentration of low density cholesterol, but this cannot account for the size and rapidity of changes in coronary mortality in Poland. A higher intake of polyunsaturated fat also improves endothelial function, reduces platelet aggregability and reduces ventricular fibrillation. The net effect can therefore be appreciated only by evaluating coronary end points. Increased intakes of polyunsaturated fat also probably explain most of the major declines in coronary mortality in the USA, UK and Australia over several decades [861].

> Both ω-3 and ω-6 fatty acids seem to contribute to reductions. According to statistics from FAO the increase in polyunsaturated fat in Poland during the 1990s was primarily from rapeseed and to a lesser degree from soya bean oil; intake of ω-3 and ω-6 fatty acid would therefore have increased, making it difficult to distinguish their relative contributions . . .. A conscious effect was made in Poland to minimise the trans fat content of margarines made from these oils.

In a 1985 symposium, Pyorala et al. [878] did the summing up and prepared a table showing CHD mortality trends in 10 countries and changes of risk factors and medical treatment. In the 6 of the 7 countries that showed reduction of mortality in the 1970s there was a "trend toward better dietary habits" (USA, Australia, New Zealand, Finland, Norway, Belgium, Israel). Three countries that had not then experienced reduced CHD did not show such a dietary trend.

Then in the Workshop on Trends and Determinants of CHD mortality, International Comparisons in Bethesda, MD, August 1988, Epstein [879] reviewed recent changes in CHD mortality in 27 countries: "In almost all of the countries with major falls or rises in CHD mortality, there are, respectively, corresponding decreases or increases in animal fat consumption, with reciprocal changes in the consumption of vegetable fats".

So at the time when CHD mortality turned down from a peak in a number of major countries there was at about the same time a reduced consumption of saturated fats and an increase of polyunsaturated fats. However, as CHD mortality rates continued to be less in the 1970s, the 1980s and the 1990s in North America, Australasia and Finland, this continuing decline was not accompanied by ever increasing intake of polyunsaturated fats at the expense of saturated fats. Into the 1990s there do not seem to have been intensified increased dietary P/S ratios in these countries. The later CHD decline must surely be attributed to greater use of better anti-hypertensive drugs, continuing reduction of smoking rates, increasing use of statins to lower serum cholesterol and a number of advances in the management of acute myocardial infarction and in coronary artery surgery. And these declines have happened despite increased percentages of overweight people in these countries.

Chapter 32
End Notes

32.1 What Happened

Coronary heart disease (CHD) was the outstanding disease of the twentieth century. The name was not used before the century (except in some post-mortem reports). But by mid-century CHD had become the major cause of death in many developed countries and rates were climbing. Then this started to change quite suddenly. In North America and NW Europe CHD mortality peaked from around 1968 and has come down steadily since then. By 2000, rates were only around 30% of their peak. In the same period mortality increased in countries of Eastern Europe. This CHD epidemic has differed greatly in its magnitude and timing between countries with reliable health statistics [880].

Coronary artery thrombosis was assumed to be incompatible with survival until the first case reports in 1910 (in German) [3] and 1912 (in English) [2]. Angina pectoris was associated with coronary artery disease by some leading physicians, but not others. Invention of the electrocardiograph by Willem Einthoven – for which he received the 1924 Nobel Prize for physiology – provided the indispensable objective test that made CHD a reliable diagnosis. Bedford [8] described how between 1926 and 1930 more and more local practitioners in London began to recognise coronary thrombosis and it became a common and familiar illness.

The continuing increase of CHD mortality after World War 2 appears to have been real, not explicable by diagnostic fashion or codes in the International Classification of Diseases. We can only speculate about the reasons for this epidemic, mostly in men, in N America and NW Europe. Smoking had become almost universal in men after the First World War, but the Seven Countries Study showed it is not a sufficient cause [126]; Japanese men smoke but have little CHD. Increased consumption of refined sucrose was suggested [139] but this hypothesis didn't stand up [148]. A plausible suggestion is that there was more saturated fat – and trans fatty acids – for the masses when margarine was developed, the poor man's butter. Hollingsworth [881] records that national fat consumption in the UK rose from 98 g in 1910 to 136 g/day in 1954; hardened vegetable fats first appeared in 1925 and increased progressively. There was more trans fatty acids in hard margarine than in butter. In Norway national household fat consumption rose from 21% in 1890

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to 39% of energy in 1959; saturated fat doubled, trans fatty acids increased, while PUFA remained at 3% of energy [882]. We don't have adequate samples of people's serum cholesterol in the first half of the century, until after the dietary fat-plasma cholesterol-CHD hypothesis was established.

Box 32.1

Cholesterol was discovered in 1770 by Poulletier de la Salle. He dissolved gallstones in hot alcohol: when it cooled cholesterol crystals formed. Cholesterol is an essential component of all cell membranes and myelin. The brain is particularly rich in cholesterol. It is the precursor of sex hormones, adrenocortical hormones, bile acids and vitamin D.

But in the twentieth century serum cholesterol became something for everyone to worry about and try to reduce.

32.2 On the Research Front

On the research front, Anitschkow's lipid-rich arterial lesions in rabbits were a model of interest to pathologists, but Anitschkow himself noted that plasma cholesterols were higher in the rabbit experiments than in humans. He suggested [17] a primary disturbance of cholesterol metabolism in human atherosclerosis. The only human version was the high frequency of CHD in genetic hypercholesterolaemia and xanthomatosis [30], known since the 1930s. There was no expectation in the first half century that change of the environment could stem the increase of CHD.

Then around 1952 there were two breakthroughs:-

1. Case-control studies with larger numbers and using statistical methods showed significantly higher serum cholesterols in CHD patients than matched controls in the same country [35–38].

   Gofman used the ultracentrifuge to study lipoproteins and found one fraction, Sf10-20 significantly more common in people with CHD [39]. This low-density fraction corresponds to β-lipoprotein by simpler paper electrophoresis [40].

   Ancel Keys looked at serum cholesterols beyond the USA. They were lower in Mediterranean countries and black South Africans [44–46] – and so were incidences of CHD. Perhaps average serum cholesterols in Americans were not normal for the arteries in later life.
2. No one had seen expected changes in serum cholesterol when people ate more or less cholesterol (e.g., eggs). Humans have much more efficient feedback control than rabbits [207], it was later shown. Serum cholesterols had fallen considerably on Kempner's rice-fruit diet for hypertension [57] (this was before there was any

effective pharmacotherapy). Noticing this, Keys tried less restrictive diets low in total fat [43]. Serum cholesterols fell promisingly. Then in five years Groen [62], Ahrens [63, 65], Kinsell [64], Bronte-Stewart [66] and soon Keys' group [70] in different laboratories and countries had the idea of testing the effect of different fats: first vegetable versus animal, then by iodine number (i.e. saturated or unsaturated), finally by fatty acid composition. Gas-liquid chromatography had been invented and could do the analyses [84]. Replacement of saturated fat in "Western" diets by poly- and mono-unsaturated fats lowers serum (total) cholesterol.

Case-control (retrospective) studies are not very reliable for finding "risk factors" (a new concept in 1961 [94]) for CHD. Prospective, cohort studies have two big advantages: dietary histories are taken before the disease event in exactly the same way in those who will become "cases" as in those who will remain controls, and they avoid the problem that lifestyle and measurement data can be affected by the disease. Prospective studies require time, patience, organization and funding. They started from around 1950 and reported from around 1960. Framingham, the Seven Countries Study and many others all built up evidence that higher concentrations of serum cholesterol (within the usual adult range in industrial countries) increase the risk of CHD. This greatly strengthened the plasma (total) cholesterol hypothesis. Smoking and hypertension were the other major "risk factors".

To convince the sceptics, and prove the dietary fat – plasma cholesterol – CHD hypothesis, prolonged dietary trials, with disease outcomes were needed. They were very difficult to achieve because compliance was not as good as the scientists hoped, and living conditions for the subjects changed in some trials [236, 556]. CHD events were fewer in most of the intervention groups, not all. In 1994 [883] meta-analysis of all the lower saturated, higher poly unsaturated fat trials showed significantly fewer CHD events on diet.

This confirmation of the diet → CHD hypothesis was not dramatic or widely publicised. Results of large trials with statins appeared from 1994 [584], the first pharmacotherapy that lowers plasma cholesterol reliably without usual side effects. After this, 99% of the scientific establishment and the cardiologists took cholesterol seriously, about 80 years after Anitschkow's first paper.

32.3 Inertia and Opposition

Steinberg in 1989 in an important paper [884] looked back at why it took so long for many physicians to take the cholesterol hypothesis seriously. He describes ten reasons:-

- Undue emphasis on the pathology of the late, complex arterial lesions,
- Misguided search for a single cause,
- Scepticism about validity of animal models,

- Confusion between cholesterol in the diet and cholesterol in the blood,
- Confusion about what constitutes a "normal" plasma cholesterol,
- Resistance of the biomedical community to combining evidence from different sources, notably the overwhelming epidemiology data,
- Weakness of the early clinical prevention trials, when considered individually,
- The different hyperlipidaemias, types 1–5 etc., were too complex for most doctors to deal with,
- Preoccupation of the cardiologists with elegant new diagnostic and interventional technologies,
- No clear explanation as to how hypercholesterolaemia gives rise to the lesion.

We can add to Steinberg's scientific and medical reasons

- Resistance and strong adverse publicity from sections of the food industry, especially cattlemen, dairy and eggs industries, e.g., "Butter is natural, traditional, good for you, tastes better. Cholesterol-lowering products are artificial, may cause cancer", etc.

32.4 In the Public Arena

Nevertheless in the public arena there was action and behaviour change from the end of the 1950s. The fats and oils industry moved to provide vegetable oils rich in PUFA, and semi-solid, tub PUFA margarines which have to be kept in the refrigerator – Fleischman's and "Mazola" in the USA and "Becel" in Europe – were all in some shops by 1960. In 1961 the American Heart Association first suggested low saturated fat, increased PUFA diets for people with, or at risk of CHD [98].

In 1976 in Britain the Royal College of Physicians of London/British Cardiac Society Working Party [148] concluded "There is considerable evidence that the causes of CHD are largely environmental and are rooted in the modern, affluent way of life …". "Dietary recommendations for the whole community involve a reduction in the amount of saturated fats and partial substitution by polyunsaturated fats …"

Dietary Goals for the United States in 1977 [799] recommended for the whole population: Avoid overweight; reduce total fat to 30% total energy intake, reduce saturated fat to 10%, balance with polyunsaturated and monounsaturated fat, about 10% of each; reduce cholesterol consumption to about 300 mg/day. Also increase "complex carbohydrates", reduce refined sugar and limit salt to about 5 g/day.

Although this report was dismissed by some as a poorly referenced document, initiated by a Democrat party presidential hopeful (Senator McGovern), these numbers have more or less persisted in national dietary guidelines since then.

The 1982 WHO Expert Committee [355] considered the relation between plasma total cholesterol and CHD to be CAUSAL. The committee also judged the

diet-blood lipoprotein-CHD relationship to be causal, "considering the evidence as a whole". One paradox needed to be explained: the difficulty of demonstrating an association *within* populations between current diet and current plasma cholesterol of individuals. They could explain this partly by interaction between genes and the environment, partly by within-subject variation of food intake and serum cholesterol.

Time magazine ran a major cover story in 1984 [886] "Cholesterol plays a deadly role in heart disease. The good news is that Americans can prolong their lives by cutting back on their high-fat, high-cholesterol eating habits . . .."

The National Institutes of Health held a Consensus Development Conference in December 1984 [356] "Elevated blood cholesterol is a major cause of coronary artery disease. It has been established beyond a reasonable doubt that lowering definitely elevated blood cholesterol levels (specifically LDL-c) will reduce the risk of heart attacks . . .. We recommend treatment of individuals with blood cholesterols above the 75th percentile. Further we are persuaded that the blood cholesterol level of most Americans is undesirably high, in large part because of our high intake of calories, saturated fat, and cholesterol . . .." All Americans were recommended to reduce total fat intake to 30% total calories, saturated fat to 10% and to increase polyunsaturated fat but no more than 10% total calories.

32.5 What Happened Further

Between the American Heart Association statement (1961) and the Royal College of Physicians report (1976), CHD (IHD) mortality had peaked and was on its way down in North America, Finland and Australasia. It was not noticed for 3 or 4 years; then people found it hard to believe: could it be a statistical artefact? Many different explanations were proposed (Chapter 31). The large mortality reductions in the first 15 years could not be attributed to improved medical technology. In that first phase of declining mortality, incidence and hospital admissions declined more than survival in-hospital. The big advances in intensive care for acute myocardial infarction, coronary artery surgery and statins came in the last 15 years of the twentieth century. Though serum cholesterols did decline between 1968 and 1985 in most countries with early falls in CHD mortality, these changes were small and not enough to explain the size of the mortality reduction. Several researchers thought that other effects of increased PUFA intakes could account for the extra effect [861, 871, 873] Professor Willett joined this opinion in 2005 [877] and 2007 [887]. This is n–6 PUFA, mostly linoleic acid. Linoleic acid might have acted by also reducing the tendency to thrombosis [343] or dangerous arrhythmias [388] or improving insulin resistance [755]. There were no increases of fatty fish intake (i.e., n–3 PUFA) coinciding with the national decreases of CHD mortality. Smoking rates in men were coming down and hypertension treatments were improving, but differences in the decline of CHD between countries suggest these played a smaller role in the striking turnaround.

32.6 Hypothesis and Variations

The original dietary fat → plasma (total) cholesterol → CHD hypothesis had two major twists as it was subjected to research and examination. It has become:

$$\text{dietary}\ \frac{12{:}0\ \text{to}\ 16{:}0}{18{:}2} \rightarrow \underset{\text{(not HDL-c)}}{\text{plasma LDL cholesterol}} \rightarrow \text{CHD}$$

HDL cholesterol is not atherogenic; it is protective. But influences of diet on HDL are relatively small (alcohol raises, overweight and lack of exercise lower HDL-c).

Five of the dietary factors in different chapters of this book work by also affecting plasma LDL-cholesterol:

Chapter 13 *Dietary cholesterol* tends to increase it,
Chapter 26 *Phytosterols* compete with absorption of exogenous cholesterol,
Chapter 20 *Coffee* lipid contains substances that raise plasma LDL-c,
Chapter 21 *Trans* unsaturated fatty acids raise plasma LDL-c, and at higher intakes can reduce plasma HLD-c,
Chapter 27 Some *soy* protein preparations lower LDL-c.

Three other chapters are about dietary components that affect plasma LDL-cholesterol, but have, other effects on the risk of CHD:

Chapter 25 *Linoleic acid* lowers LDL-c but very likely has other actions,
Chapter 15 *Dietary fibre* can lower LDL-c but it is associated with antioxidants, etc in vegetable foods,
Chapter 16 *Obesity* is associated with higher plasma LDL-c levels and also with hypertension but may still increase CHD risk when controlled for LDL-c and BP.

Five more chapters are about dietary components that do not work by altering plasma total or LDL-concentration:

Chapter 18 *Fish oil* provides 20:5 and 22:6 which appear to work by reducing dangerous arrhythmias in ischaemic heart muscle. (n–3 PUFA can lower plasma LDL-c, but only at doses larger than usually present in people's diets).
Chapter 19 *Alcohol* raises HDL-cholesterol. It probably has other actions, e.g., reducing platelet aggregation.
Chapter 28 *Homocysteine*. Increased plasma levels are associated with endothelial damage and arterial lesions.
Chapter 29 *Salt (NaCl)* works by directly affecting blood pressure, another major risk factor for CHD.

CHD rose to be the major cause of death in most developed countries in the twentieth century. Research on its causes, prevention and management was a leading activity in medical research and development. Research on diet and CHD was an integral part of this and the most exciting nutrition research in the second half of the century.

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