[redacted]

The Office Administrator,
ANZFA,
P.O. Box 10559,
Wellington 6036.

Valerie James, [redacted]
November 19th, 2001

ACKNOWLEDGED

ENTERED IN DATABASE

Re: Proposal P242, Application A 417. Public Health and Safety Concerns.

Dear Sir/Madam,

I submit that products should be permitted and marketed only after a complete risk-benefit analysis has been completed and only when health claims made have been established beyond a reasonable doubt.

Cholesterol lowering products have often failed to deliver the promised and touted health benefits. There have therefore been many "disasters of good intent". It is ANZFA's responsibility to prevent another. The following enclosures from responsible medical and scientific publications explain my concerns, via quotes from each document.

1. Report from Committee of Principal Investigators, "A co-operative trial in the primary prevention of ischaemic heart disease using clofibrate" British Heart Journal, 1978.
.... "serum cholesterol was to be lowered by the drug "clofibrate which was considered to be free from serious side effects --- the numbers of deaths and crude mortality in the clofibrate-treated group significantly exceeded those in the high cholesterol control group".
2. "Cholesterol lowering margarines," The Medical letter 1999 ∴ The effect of these margarines on mortality and morbidity from coronary artery disease is unknown, the beneficial effects of lowering cholesterol might be offset by increased plasma concentrations of plant sterols, which may be atherogenic"
3. Myant. "The Biology of cholesterol and related Sterols" William Heinemann Medical Books Ltd, First published, 1981. ∴ none of the points listed above provides proof that the predictive association between plasma cholesterol concentration and IHD (ischaemic heart disease) in the general population is a causal one.... the predictive power of plasma total cholesterol concentration (and by implication of LDL concentration for individuals is not very strong --- the results of intervention

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trials have been controversial (p. 647-649)... In adult mammals 20-25% of the cholesterol in the whole body is present in the whole body is present in the nervous system .... The human placenta forms progesterone from cholesterol... In living systems the rates of metabolic processes are regulated in accordance with the needs of the biological needs of the organism"

4. Oliver, "The Optimum Serum Cholesterol" The Lancet 1982 "Kannel and Gordon state that 'serum cholesterol is not a strong risk factor for CHD. in the sense that blood pressure is a strong risk factor for stroke or cigarette smoking is a risk factor for lung cancer.' I agree. Then let us not overestimate the value of lowering serum cholesterol in those with levels already below the median."

5. Feinleib " on a Possible Inverse Relationship Between Serum Cholesterol and Cancer Mortality" American Journal of Epidemiology 1981 ... "evidence for a statistically significant and potentially important result, viz., a fairly consistent inverse relationship between cholesterol measured at baseline, or early on in studies, and subsequent mortality from various forms of malignancies.... the 'ideal' cholesterol level does not lie at either extreme of the distribution but somewhere towards the mean."

6. Smith, "Diet Blood cholesterol and Coronary Heart Disease: A Critical Review of the Literature" Vector Enterprises, 1988.... "the problem with all these (previous) trials is that none of them have shown a difference in heart attack or death rate in the treated group - only when soft end points were used in fact was there any subjective difference.... first and foremost, the overall death rates (LRC trial results) of the treatment and control groups were essentially identical, indicating that the treatment did not alter life expectancy over the 7.4 year period."

7. Holme "An Analysis of Randomized Trials in Evaluating the Effect of Cholesterol Reduction on Total Mortality and Coronary Heart Disease Incidence" Circulation, 1990. ... total mortality is increased by intervention by about 4 ± 3% compared with control (p? 0.10) ... : the history of cholesterol-lowering trials have shown that they have

not been without specific excess hazards on the part of the participating patients"

8. Ahrens, "Dietary Fats and Coronary Heart Disease: Unfinished Business" The Lancet, 1979. ... "if the public's diet is going to be decided by popularity polls and with diminished regard for the scientific evidence, I fear that future generations will be left in ignorance of the real merits, as well as the possible faults. In any given dietary regimen aimed at prevention of C.H.D. ... any one diet produces different results in different people .... I believe it is anything but a service to the public to postulate one dietary solution for hyperlipidaemia no matter how well-meaning one is in advocating it."

9. Editorial, "Prevention of Ischaemic Heart Disease with Lipid Lowering Drugs." The Lancet, 1988. ... " a risk factor is associated with a disease but it does not necessarily cause it. .... no completely convincing dietary study for the prevention of heart disease has been published .... unwanted effects such as these may become more important if lipid-lowering drugs are given for long periods ... "

10. Ahrens, "The Diet-Heart Question in 1985: Has it really been settled?" The Lancet, 1985 ... "epidemiological surveys will continue to furnish valuable insights into phenomena associated with CHD. But correlations, no matter how strong are never proof ... I know of no evidence that the prudent diet will prevent the development of arterial atheroma at any age ... since many unanswered questions remain about the role of nutrition in C.H.D. prevention, it is remarkable that the press in the U.S.A. has set out to sell the message that the diet-heart question has been solved by the LRC - CPPT."

11. Skrabanek "Nonsensus Consensus" The Lancet 1990. ... "there have been too many 'disasters of good diet' in the history of medicine and people should temper their faith in experts - particularly when they see them coming in droves - with their own informed scepticism."

12. Editorial, Hulley et al "Health Policy on Blood Cholesterol" Circulation 1992. ... "a U shaped association between the level of blood cholesterol and subsequent mortality has been repeated in many studies over the past two

decades ... among women high blood cholesterol is not associated with all-cause mortality nor even with cardiovascular mortality .. the overriding ethical consideration is to do no harm. particularly when considering drugs for people who are in good health, the burden of proof falls on the proponents of the intervention."

13. Forette et al, "Cholesterol as Risk Factor for Mortality in Elderly Women." The Lancet 1989. ... "mortality was lowest at serum cholesterol 7.0 mmol/L. - The mortality peak is much higher in women with the lowest initial cholesterol values than those with the highest values."

14. Schatz et al "Cholesterol and all-cause mortality in elderly people from the Honolulu Heart Program: a Cohort study" The Lancet, 2001 .. "the earlier patients start to have lower cholesterol concentrations, the greater the risk of death .. those individuals with a low serum cholesterol maintained over a 20-year period will have the worst outlook for all-cause mortality"

15. Pedersen et al: Adipose tissue, fatty acids and risk of myocardial infarction - a case-control study. "European Journal of Clinical Nutrition 2000. ... "intake of very long chain n-3 fatty acids as reflected in adipose tissue content is inversely associated with risk of myocardial infarction" (note that plant stanols & sterols are esterified with these long chain fatty acids)

I hope this extensive list of relevant documents is useful.

Yours sincerely,

[redacted]

P.S. Please note especially the evidence that low cholesterol levels in the elderly are a health risk.

British Heart Journal, 1978, **40**, 1069-1118

A co-operative trial in the primary prevention of ischaemic heart disease using clofibrate

Report[1] from the Committee of Principal Investigators

SUMMARY A double-blind intervention trial was started in 1965 to test the hypothesis that the incidence of ischaemic heart disease in middle-aged men can be reduced by lowering raised serum cholesterol levels. It was carried out in 3 European centres—Edinburgh, Budapest, and Prague. Serum cholesterol was to be lowered by the drug clofibrate (ethyl chlorophenoxyisobutyrate) which was considered to be free from serious side effects.

Studies were carried out on 15 745 males, aged 30 to 59 at entry, for an average of 5·3 years, accumulating 83 534 years of experience. The treatment group, of about 5000, Group I, was a randomly chosen half of the men in the upper third of the serum cholesterol distribution in some 30 000 volunteers. The comparable control group, Group II, comprised the other 5000 men of the upper third of the cholesterol distribution, and these were given a placebo. A further control group, Group III, of 5000 men, was selected randomly from the lower third of the cholesterol distribution. These numbers were chosen in order to be 90 per cent certain of detecting a 30 per cent reduction in the incidence of ischaemic heart disease should this occur. Subjects with manifest heart or other major disease were excluded from the trial. No attempt was made to correct other 'risk factors' for IHD, but their presence was monitored and considered in the analysis. Investigators and participants in the trial were unaware of the groups to which individual men belonged.

A mean reduction of approximately 9 per cent of the initial serum cholesterol levels was achieved in the treatment group (ranging from 7 to 11% in the 3 centres); this was less than the 15 per cent fall expected. In Edinburgh, during treatment, serum triglyceride concentrations in Group I resembled those naturally occurring in Group III.

The incidence of IHD was lower by 20 per cent in the clofibrate group compared with the high cholesterol controls ($P < 0{\cdot}05$); this fall was confined to *non-fatal* myocardial infarcts which were reduced by 25 per cent. The incidence of *fatal* heart attacks was similar in the 2 high cholesterol groups and there was no significant difference in the incidence of angina. Group III showed substantially lower rates of ischaemic heart disease.

The reduction of myocardial infarction in the clofibrate-treated group was greatest in men with the highest levels, and greatest reduction in serum cholesterol. Men with a substantial reduction of cholesterol concentration, who smoked, and also had above average blood pressure levels showed the most benefit.

The numbers of deaths, and crude mortality rates from all causes in the clofibrate-treated group significantly exceeded those in the high cholesterol control group ($P < 0{\cdot}05$), though the age-standardised mortality rates did not differ significantly between the 3 groups. The numbers of deaths from 'other

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I. Gyarfas, Hungarian Institute of Cardiology, Budapest (G. Lamm: 1966-1974)
K. G. Green, ICI, Macclesfield
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M. F. Oliver, The Royal Infirmary of Edinburgh
T. Strasser, WHO, Geneva (Z. Fejfar: 1966-1973)

Investigators—
Edinburgh: W. G. Macfie, E. Scott
Budapest: M. Czukas, J. Duba, E. Östor
Prague: D. Grafnetter, Z. Hejl
London: J. Cooper
WHO, Geneva: Z. Pisa, K. Uemura Copenhagen: G. Lamm (from 1974)
ICI: J. M. Thorp

174.

vascular causes' and from 'accidents' as well as IHD were similar in Groups I and II. Excluding these, the excess of deaths in the clofibrate-treated over the high cholesterol control group was 77 *v* 47 ($P < 0.01$). The subgroup with the greatest proportionate excess of deaths is that of conditions related to the liver, the biliary, and intestinal systems, with 19 deaths in Group I *v* 7 in Group II ($P < 0.05$). Deaths from these conditions were commoner, however, in Group III than in Group II (age-standardised rates for the 3 groups being 0·75, 0·17, and 0·65, respectively), and it is possible that Group II had fortuitously *low* rates. The cholecystectomy rate for gall stones was higher in Group I than in Groups II and III ($P < 0.001$).

The results of the trial confirm the basic hypothesis that reduction of high serum cholesterol levels, even in middle-age, can reduce the incidence of IHD. However, the fact that clofibrate increases the incidence of gall stones, and the possibility that it may have even more serious local pathological consequences, indicate that it cannot be recommended as a lipid-lowering drug for community-wide primary prevention of ischaemic heart disease.

By 1965, the association of raised serum lipids, particularly cholesterol, with an increased risk of developing ischaemic heart disease, was well established (e.g. Kannel *et al.*, 1964). Whether raised serum cholesterol actually was causative or, simply, the indication of an underlying metabolic disorder was not known. But on general principles, medical opinion held that it was desirable to reduce serum lipids towards accepted normal levels as far as possible. The means of achieving this were largely based on alterations in diet, and experience at that time suggested that the measures thought necessary were impracticable on any wide scale and were unlikely to be adhered to by most individuals in an affluent society. The possibility of using a cholesterol-lowering drug had been considered but until ethyl chlorophenoxyisobutyrate (later called clofibrate) was introduced (Oliver, 1962; Thorp, 1962; Thorp and Waring, 1962; Symposium on Atromid, 1963) none available satisfied the prerequisites of effectiveness and safety. Clofibrate was known to be capable of reducing plasma concentrations of low density, and very low density, lipoproteins, and thus cholesterol and triglyceride. It was decided, therefore, to initiate a trial in healthy volunteers to find out whether reducing plasma lipids, using clofibrate, would result in a decreased incidence of ischaemic heart disease. No deliberate attempt would be made to change the life style of participants or to rectify other risk factors. A full account of design and procedure has already been published (Heady, 1973). The trial has now been completed, as planned; the present paper summarises the main points of methodology and gives the results.

Trial design—procedure

15 745 healthy men, aged 30-59, selected on the basis of a preliminary determination of serum cholesterol level were assigned to 3 groups as follows: half of the men in the upper third of the distribution of serum cholesterol values were assigned at random to a *clofibrate-treated group* (*Group I*) taking 1·6 g clofibrate daily; the other half of the upper third constituted a *control group* (*Group II*) taking identical capsules containing olive oil. A *second control group* of similar size (*Group III*), chosen at random from the lowest third of the serum cholesterol distribution, also received the olive oil capsules (Fig. 1).

Allocation to the high and low cholesterol groups was carried out every one or two months at each centre, from volunteers 'screened' during the period concerned. Thus, the cut-off levels into thirds varied chronologically.

The essential part of the trial was, thus, the double-blind comparison of the two randomly selected high serum cholesterol groups. The low serum cholesterol group served as a comparison (also double-blind) between men whose untreated cholesterol levels were 'naturally' low and those in Group I whose cholesterol levels fell under treat-

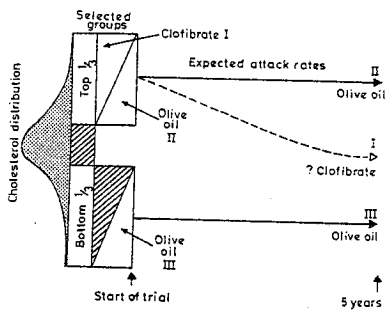

Fig. 1 *Design of the trial (broken line represents hypothetical effect of clofibrate on serum cholesterol and IHD attack rates).*

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containing 400 mg. The daily dose was 1·6 g. The choice of 400 mg antedated the availability of 500 mg capsules for clinical use. There is no evidence that 1·6 g is less effective than 2·0 g. Control capsules were identical in appearance but, as said, contained 400 mg olive oil. Each pack was labelled with the man's name and trial-number in the pharmacy at each centre.

CONCURRENT MEDICATION

There was no restriction on other drugs, but diabetic patients requiring drug treatment and patients receiving anticoagulants were withdrawn from the trial.

DRUG ADHERENCE

Adherence to treatment was assessed by arranging for the blood which was taken for serum cholesterol estimation to be tested also for the presence of clofibrate (chlorophenoxyisobutyric acid, CPIB). This was done by a semiquantitative method (Barrett and Thorp, 1968) in all the men in the clofibrate treated group at every visit and also in a random 10 per cent of the men in the 2 control groups. The purpose of including the control group was twofold: to make the laboratory assessment 'blind' and to assess the level of prescribing of clofibrate in the general population in each centre. 'Blindness' was arranged by compiling a single list of the trial numbers of all subjects on clofibrate and 10 per cent of the controls arranged in numerical order. The blood samples of the men on this list were routinely examined for the presence of clofibrate, the laboratory staff being aware that some men were not in the treated group but not which men.

RECORDING OF DATA

A standard record was completed on admission, and at each follow-up visit. Events occurring during the trial were notified on a separate form. (Copies of forms are available on request—Addendum A.) After completion at the trial centres, the forms were forwarded to the Medical Research Council's Social Medicine Unit in London, for scrutiny and transfer of the information to computer.

CRITERIA FOR ASSESSING THE RESULTS OF THE TRIAL (See Appendix 3)

(1) Ischaemic heart disease (IHD)

The following *major* end-points were defined:

(*a*) Fatal IHD (i) deaths known to have occurred less than 3 hours after onset of symptoms
(ii) deaths occurring more than 3 hours but less than 28 days after onset.

(*b*) Non-fatal myocardial infarction and acute coronary insufficiently surviving more than 28 days.

(Men who died more than 28 days after onset of symptoms were arbitrarily considered to have had a fresh attack. The death was, therefore, not included as a death in the first attack, or 'in the trial', since men with non-fatal infarctions were withdrawn from the trial. If the death occurred within 1 year of the attack it was included in the total of deaths within 1 year of leaving the trial.)

Additionally, the following *minor* IHD end-points were distinguished:

(*a*) Angina pectoris with abnormal electrocardiogram;
(*b*) Angina pectoris without abnormal electrocardiogram;
(*c*) Abnormal electrocardiogram without chest pain.

(2) Deaths from all causes

These were subdivided into deaths from IHD and other deaths. In tables of deaths from all causes, deaths occurring within 1 year of leaving the trial have been included.

Definitions of the terms used are given in Appendix 3, as also are those of other events such as 'claudication' which were notified but were not reasons for withdrawal.

A panel of 2 physicians, not concerned with the day-to-day running of the trial, and situated at WHO headquarters in Geneva, reviewed all events that the participating physicians in the centres considered might be due to ischaemic heart disease, as defined.

Deaths or other events, such as IHD, were deemed to have occurred 'out of the trial' if they occurred after a visit at which the subject was excluded on medical grounds or had, himself, 'opted out', that is had stated his intention of not continuing to take the treatment. If, however, the last visit had been one at which neither of these things had happened, a death (or other event) was considered to have occurred 'in the trial' if it occurred within 9 months of the last visit, at the stage in the trial when visits should have occurred at 6-monthly intervals, or within 18 months at the stage when visits should have occurred at yearly intervals. These periods were chosen to coincide with the definitions of a 'missed visit' or 'failure to attend', which, in turn, were related to the supply of pills which a subject would take away with him at an ordinary visit. Events which, after such visits, did not occur within these time limits were considered to have occurred 'out of the trial'. A special attempt was made to trace all deaths occurring within 1 year

ment. It also, in fact, served as a useful comparison with the other control group.

The study was designed to last 5 years for each subject admitted and it was assumed that (*a*) 70 per cent of the volunteers would remain in the study for that length of time, and (*b*) the untreated incidence of clinical episodes of coronary heart disease in healthy men aged 30 to 59 with moderately raised serum cholesterol levels would be 1 per cent per annum. The number of volunteers was to be large enough to give a 90 per cent chance ($\beta = 10\%$) of detecting, in the clofibrate treated group, a *reduction of one third* in the incidence of heart attacks compared with the high cholesterol control group, at the 1 per cent level of significance (two-sided). To meet these requirements the study would have to admit at least 15 000 subjects.

The figure of one-third reduction in incidence was chosen because available evidence in the early 1960's suggested that clofibrate might be expected to reduce raised serum cholesterol levels by about 15 per cent and that such a reduction might be associated with a fall in incidence of about 30 per cent. It was also felt that if a reduction of 30 per cent were achieved it would be a reasonable basis for prophylactic treatment, whereas with a smaller reduction the value of such intervention would be more problematical.

The men were recruited in ways which differed in the 3 centres (Heady, 1973) from lists of blood donors, electoral rolls, tuberculosis screening registers, and by advertising among the general public. Recruitment started in 1964 but was not complete until March 1972. After primary selection on the basis of serum cholesterol level, an explanation of the purpose of the study was made to the subject, and those who were willing to participate were medically examined before admission to the trial. The men with high serum cholesterol levels were then randomised to the clofibrate treated and the control group at each centre separately. After admission to the trial men were examined at 6 monthly intervals for 2 years, and thereafter at yearly intervals though they were still contacted by post, or otherwise, at 6-monthly intervals. A final follow-up took place a year after cessation of medication.

The trial was closed down from July 1975 onwards, treatment being withdrawn at the man's next scheduled visit after that date. The process took about one year, so that by the late summer of 1976, no more active treatment was being given.

EXCLUSIONS AND WITHDRAWALS

The *criteria for rejection* at admission on medical grounds are detailed in Appendix 1. They consisted mainly of evidence of previous myocardial infarction; other types of heart disease, arterial hypertension as defined, diabetes mellitus requiring drug therapy, or other diseases with a poor prognosis were also reasons for exclusion. *Criteria for withdrawal* from the trial were similar (Appendix 1) but, of course, included adverse reactions. Because of different practice in the 3 centres, and for obvious ethical reasons, the attendant physicians were allowed latitude in withdrawing subjects from the trial, though this was of course done 'blind'. A history of exercise-induced chest pain was not a reason for withdrawal. At first, hypertension was a firm indication for withdrawal but later this was modified and concurrent antihypertensive therapy was permitted.

FOLLOW-UP PROCEDURE

Medical examination at follow-up was similar to that at entry. Questioning about side effects was general. Specific symptoms were not asked for by name, except indigestion, diarrhoea, and skin reactions, which were known side effects of clofibrate.

Men who gave a positive answer to the standard questionnaire on effort chest pain, and whose resting electrocardiogram was normal, were given a further electrocardiographic examination during and after exercise on a bicycle ergometer.

No systematic advice was given on diet, weight reduction, smoking, or exercise.

Failure to keep an appointment was investigated by the use of 'progress chasers' when necessary. Copies of death certificates were obtained.

All men who withdrew from the trial, or were withdrawn for any reason, medical or non-medical, were considered to be still in the trial for the purpose of calculating exposure-to-risk, up to the date of withdrawal (see Appendix 2). The criteria for classifying events such as death, or ischaemic heart disease, as occurring 'in the trial', or otherwise, are described below, under criteria for assessing the results of the trial.

BIOCHEMICAL MONITORING

Blood was taken for estimation of serum cholestero concentrations at each visit. In Edinburgh, from 1972 onwards, trigiycerides were estimated by Auto-Analyzer (Kessler and Lederer, 1965). Urine was examined for sugar and albumin as a routine in Prague and Budapest but not in Edinburgh. Other laboratory tests were carried out as indicated by the history or examination.

DRUG SUPPLIES

Clofibrate for the trial was prepared at Imperial Chemical Industries in opaque white capsules

of leaving the trial, and for the most part, other out-of-trial deaths have not been considered in the analysis.

MONITORING OF RESULTS

The policy was adopted of not reporting results to the investigators so as to avoid influencing their attitude to the trial. The statisticians and an epidemiologist chosen for this purpose alone had access to the results as they came in. The following rules were developed during the study for determining when the main results should be released to the principal investigators:

(*a*) If there were a significant result ($P < 0{\cdot}10$) against the treatment in terms of the main endpoints.

(*b*) If there were a significant result ($P < 0{\cdot}10$) against the treatment for any important side effect where useful discussion would be inhibited by lack of knowledge of the main results.

(*c*) If it became clear that the main result could not became significant ($P < 0{\cdot}01$) in favour of the treatment.

(*d*) If there were a significant result ($P < 0{\cdot}01$) in favour of the treatment in terms of the main endpoints.

A sequential scheme was used with a power of over 90 per cent to detect a difference of 33 per cent between treated and control at the appropriate levels of significance (Appendix 2).

STANDARDISATION OF PROCEDURES

(*a*) *Blood pressure* was taken in accordance with WHO recommendations (1962). (Observer variability was checked periodically within centres.)

(*b*) *Skinfold thickness* (triceps and subscapular) were measured according to the recommendations of Rose and Blackburn (1968).

(*c*) *Electrocardiograms*, at rest, were recorded on 12-lead electrocardiographs (unipolar chest leads). Exercise electrocardiograms were done under conditions which complied with the World Health Organization criteria (WHO, 1968). Classification of electrocardiogram was by Minnesota Code (Blackburn *et al.*, 1960; Rose and Blackburn, 1968), and was carried out 'blind, that is in ignorance of the subject's treatment group. The case records of all infarctions or deaths attributed to ischaemic heart disease were checked by the panel at WHO, Geneva, mentioned earlier. In case of disagreement between the finding of the centre and that of the WHO panel, the electrocardiogram was reviewed at the annual meeting of the investigators and a committee decision was reached.

To maintain comparability between centres, sets of electrocardiograph recordings were circulated for coding at all centres with satisfactory results.

(*d*) *Serum cholesterol:* different methods of cholesterol estimation were used in each centre. The laboratories were subject to both internal and external quality control.

Internal quality control was mainly by the inclusion of a sample of an internal control pool of frozen lyophilised serum in each analytical day (or run). When pools were renewed there was a period of overlap between the new and old pools.

External quality control was by exchange of samples between centres and also by the participation of each centre, first in the programme in the WHO-CDC International Lipid Standardization Laboratory in Atlanta, USA, and later in that of the European Regional WHO Lipid Reference Centre in Prague, which was itself one of the trial laboratories.

Procedures are available on request (Addendum B).

STATISTICAL METHODS

The details of the statistical methods used are in Appendix 2. This deals with age-standardisation, life-table calculations, the definitions and calculation of cholesterol change and mean in-trial cholesterol levels, and with multivariate analysis. A few points only need to be mentioned here.

Life table calculations were carried out by standard methods. Tests of statistical significance of differences in 'survivorship' use a method, similar to the 'log-rank' method (Mantel, 1966; Breslow, 1975), which compares the survivorship curves as a whole rather than at a single time interval after admission.

Statistical significance: in the tables attention is drawn to significant differences between Groups I and II only. Other significant differences are discussed in the text.

Age-standardisation is not necessary for comparing incidence and mortality rates in Groups I and II since the age-distributions in these 2 randomly allocated groups were almost identical. It is, however, necessary in comparing the rates in Group III with those in Groups I and II because the men in Group III, chosen on the basis of lower serum cholesterol levels, were on average slightly younger than those in Groups I and II.

Age-standardisation is also important in comparing other groups whose age-distributions cannot be *assumed* to be the same, for example those with high or low blood pressure, or smokers and non-smokers. Standardisation was confined to the ages 40 to 59 because the number of events at ages 30 to 39 was small (6% of all incidents of IHD) and

TABLE 1

Characteristics at Entry by Group, All Centres

	Group I	Group II	Group III
	Clofibrate	High Cholesterol Control	Low Cholesterol Control
Number of Men	5331	5296	5118
	Mean Values ± S.E. Mean		
Serum Cholesterol (mg/dl) [a]	249 ± 0.5	247 ± 0.4	181 ± 0.4
Age (years)	45.9 ± 0.1	45.8 ± 0.1	44.2 ± 0.1
Height (cm) (H)	172.6 ± 0.1	172.6 ± 0.1	173.3 ± 0.1
Weight (Kg) (W)	79.4 ± 0.1	79.7 ± 0.2	77.1 ± 0.2
Quetelet Index ($\frac{W}{H^2}$ X 10,000)	26.6 ± 0.04	26.7 ± 0.05	25.6 ± 0.05
Systolic blood pressure (mmHg)	135.4 ± 0.2	135.2 ± 0.2	131.7 ± 0.2
Diastolic blood pressure (mmHg)	87.1 ± 0.1	87.2 ± 0.1	84.9 ± 0.1
Skinfold: Triceps (mm)	12.7 ± 0.1	12.8 ± 0.1	11.6 ± 0.1
Subscapular (mm)	18.5 ± 0.1	18.5 ± 0.1	16.4 ± 0.1
	Percentage with Characteristic		
	%	%	%
Age(yrs) 30-	16	17	27
40-	56	55	50
50-	28	27	23
Never Smoked	25	25	33
Ex-smokers	18	18	17
Smokers (cigarettes or other)	56	56	51
Smokers (20+ cigarettes/day)	44	43	36
Father dead	74	73	68
Mother dead	49	49	45
Married	89	90	88
Positive questionnaire for angina [b]	2.1	1.7	1.4
Positive questionnaire for leg pain [b]	1.3	1.3	0.6
Basal murmur	7.6	7.6	6.2
Apical murmur	8.4	7.7	8.8
Arcus	13	12	6.4
Xanthelasma	3.4	2.9	2.2
Xanthoma tuberosum	0.2	0.1	0.1
Xanthoma tendinosum	0	0	0.1
ECG abnormality (Minnesota Code 4-2 or 5-2)	0.8	1.0	0.8

(a) Mean of pre-treatment serum cholesterol values corrected for Centre differences. See Appendix 2.

(b) A modified version of the London School of Hygiene Cardiovascular Questionnaire, Rose & Blackburn 1968. (Addendum A).

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might produce spurious results, and, also, because there were virtually no men in Prague under the age of 40. For the particular purpose of comparing rates *between* centres standardisation was restricted to the age-range 40 to 54 because there were also very few men aged 55 or over in Prague. Equal weightings were given to each 5-year age-group in the standardisation, for simplicity, and because, as it happened, in each centre, the number of men in each of the 4 age-groups, from 40 to 59, in the general population was not very different.

Results

Results are presented in the main part of this report for the 3 centres combined, though attention is drawn to important differences between centres. The main results for IHD for the separate centres individually are shown in Appendix 4 and additional inter-centre differences are available in Addendum C.

COMPARABILITY OF GROUPS

Table 1 shows the characteristics recorded at entry to the trial, for all 3 centres combined, for each Group. There were no important differences between Groups I and II, the high serum cholesterol groups, in any of these characteristics; and this was true also within each centre. Group III, selected on the basis of lower serum cholesterol levels, did however show differences from the other 2 groups. On average, the men in this group were younger, lighter, taller, had lower blood pressures, and smoked less.

There was an important difference between centres in age-distribution of the subjects (Addendum D). In Prague virtually no subjects were recruited under the age of 40, whereas one third of those in Edinburgh and one quarter of the Budapest men were under that age. Budapest, at 32 per cent, had the highest proportion of men over 50 compared with 21 per cent in Edinburgh, and 24 per cent in Prague. Only 57 men in Prague were aged 55 or more. For the trial as a whole, more than half of the subjects were aged between 40 and 50.

MEN AND MAN-YEARS IN TRIAL

Table 2 shows the numbers of men who entered, and completed, or failed to continue in the trial for various reasons. Some 300 had in fact been in the trial for as many as 8 years when it closed. On the other hand, 227 late entrants did not have the opportunity to complete 5 years before it ended. Subtracting these from the total admitted, 69 per cent of those for whom it was possible remained in the trial for 5 years, slightly less than the 70 per cent allowed for in the planning stage. The percentage of men who left the study for any reason was 13 per cent in the first year, and about 5 per cent annually thereafter; the 3 centres behaved similarly.

An attempt was made to establish whether those who 'defaulted' or who were excluded for medical reasons, and those who were in the trial at the end of the study were alive or dead 1 year after leaving the trial. This information was available for 99 per cent of all the men in the trial.

The numbers of men admitted to the trial and the numbers of man-years they experienced in the trial for each centre and each 5-year group of age at admission are available on request (Addendum D).

DRUG ADHERENCE

Group I

In Budapest, 98 per cent of men who were in the trial for at least 5 years showed positive readings on all but 2 occasions and 94 per cent on all but 1 occasion. The corresponding figures for Prague were 94 and 85 per cent. For Edinburgh, during the first 4 years of the trial, the figure was 95 per cent on all but 1 occasion.

Average concentrations of clofibrate in the blood of those men with positive readings in the clofibrate treated group are shown in Appendix 5 (i).

Groups II and III

In Budapest, 1 per cent of men who were in the trial for at least 5 years showed positive readings on more than 1 occasion. In Prague, 19 per cent showed positive readings on more than 1 occasion and 5 per cent on more than 2 occasions. In Edinburgh, 4 per cent showed positive readings on more than 1 occasion. No explanation is available for the higher proportion of positive readings in Prague.

Incidence of IHD and mortality from all causes

Comparing the clofibrate-treated group with the high cholesterol control group the 3 main results of the study are:

(*a*) the overall incidence of major IHD was significantly lower in the clofibrate-treated group;

(*b*) this difference was confined to non-fatal myocardial infarction;

(*c*) total mortality from all causes was significantly higher in the clofibrate-treated group.

Details of these findings are given below, those relating to the incidence and mortality from IHD first, and the mortality from other causes later.

TABLE 2

Numbers of Men at Different Stages

Total Number of Men Screened				52,519
Number of Men invited to attend on basis of their serum cholesterol level				26,259 (a)
Number Rejected for Medical Reasons				725
Number of Men who did not attend for Entry				9,789
Number of Men admitted to Trial				15,745
	Group I Clofibrate	Group II High Cholesterol Control	Group III Low Cholesterol Control	All Groups
Admitted to Trial	5,331	5,296	5,118	15,745
Of these:-				
Withdrawn after entry for medical reasons (including deaths and myocardial infarction)	588	582	390	1,560
Failed to continue for other reasons	1,246	1,188	1,256	3,690
In the Trial when it ended	3,497	3,526	3,472	10,495
Number who completed 5 years	3,586	3,608	3,509	10,703

(a) Half those otherwise eligible were excluded because of the cholesterol levels found at the preliminary screening. (See text and Figure 1).

INCIDENCE AND MORTALITY FROM MAJOR IHD

Table 3 shows the incidence of the first episodes of major IHD in each Group according to the age of the subject at entry to the trial, and distinguishes between fatal and non-fatal attacks. Data for deaths within 3 hours of onset of symptoms are given separately. Rates are standardised at ages 40 to 59.

The *overall incidence of major IHD* (first clinical episodes) in the clofibrate-treated group (5·9 per thousand per annum) was 20 per cent lower than the incidence in the high cholesterol control group (7·4) ($P < 0.05$).

The *mortality from IHD* in first episodes, on the other hand, was virtually the same in the 2 groups (1·3 and 1·2) and, hence, the difference between the 2 groups lies in *non-fatal myocardial infarcts* (4·6 and 6·2 per thousand) ($P < 0.05$).

The incidence of both fatal and non-fatal major IHD events was significantly less in Group III, the low cholesterol control, than in either of the 2 high cholesterol groups ($P < 0.01$ for non-fatal and < 0.05 for fatal, age-standardised).

Deaths known to have occurred less than 3 hours from the onset of symptoms were more common (23), though not significantly so, in Group I than in Group II (17). Again, the numbers in Group III were much smaller (8).

The same information (except for sudden deaths) is shown diagrammatically in Fig. 2 on a life-table

Incidence of M

Event
All Major IHI
Non-Fatal Myocardial Infarction
Fatal IHD
Of these: Deaths within

(a) Standardised being given

(b) Includes 38 18, 16 and 4

(c) The numbers groups respe

* Significant

TABLE 3

Incidence of Major Ischaemic Heart Disease (IHD), Non-fatal and Fatal, by Age at Entry

Rates per 1000 per annum

Event	Age at Entry (years)	Group I Clofibrate No.	Group I Clofibrate Rate	Group II High Cholesterol Control No.	Group II High Cholesterol Control Rate	Group III Low Cholesterol Control No.	Group III Low Cholesterol Control Rate
All Major IHD	30–	3	1.8	3	1.8	2	0.6
	35–	8	3.0	7	2.3	3	0.8
	40–	19	3.0	31	5.0	8	1.4
	45–	61	6.3	84	8.7	18	2.2
	50–	44	8.8	53	10.4	22	4.9
	55–	32	11.5	30	11.6	9	4.6
	Total	167*	5.9*	208*	7.4*	62	2.3
	St.40–59 (a)		7.4		8.9		3.3
Non-Fatal Myocardial Infarction	30–	2	1.2	3	1.8	2	0.6
	35–	6	2.2	7	2.3	1	0.3
	40–	16	2.5	28	4.6	4	0.7
	45–	48	4.9	72	7.5	14	1.7
	50–	35	7.0	42	8.2	18	4.0
	55–	24	8.6	22	8.5	7	3.6
	Total (b)	131*	4.6*	174*	6.2*	46	1.7
	St.40–59		5.8		7.2		2.5
Fatal IHD	30–	1	0.6	0	0.0	0	0.0
	35–	2	0.7	0	0.0	2	0.6
	40–	3	0.5	3	0.5	4	0.7
	45–	13	1.3	12	1.2	4	0.5
	50–	9	1.8	11	2.2	4	0.9
	55–	8	2.9	8	3.1	2	1.0
	Total	36	1.3	34	1.2	16	0.6
	St.40–59		1.6		1.8		0.8
Of these: Deaths within 3 hours (c)		23	0.8	17	0.6	8	0.3

(a) Standardised rates for the four age-groups 40–44, 45–59, 50–54, 55–59, equal weights being given to each age-group.

(b) Includes 38 with Acute Coronary Insufficiency (Intermediate Coronary Syndrome), 18, 16 and 4 in Groups I, II and III respectively.

(c) The numbers of IHD deaths occurring from 3–12 hours were 4, 6, 3, in the three groups respectively.

* Significant difference between Groups I and II ($P < .05$).

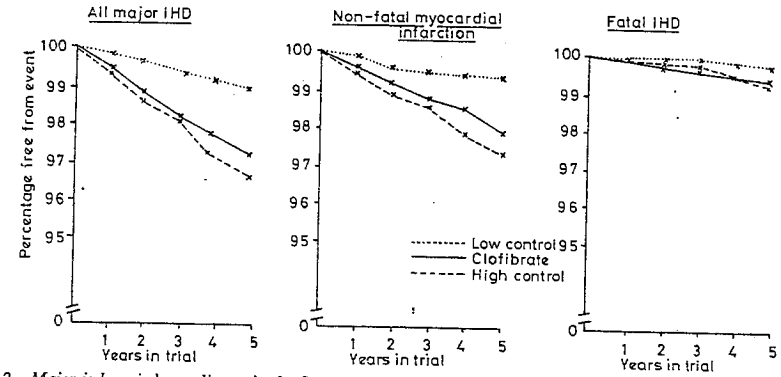

Fig. 2 *Major ischaemic heart disease in the first 5 years (calculated on a life-table basis).*

basis, that is showing the proportions free of IHD at various periods after entry to the trial. The significance tests based on this form of analysis, which take into account the shape of the whole curve, give similar results to those given above based on the comparison of overall rates, that is a significant ($P < 0{\cdot}05$) reduction in non-fatal myocardial infarction in Group I compared with Group II. The diagram itself shows results up to 5 years only, because after that stage rates are based on very small numbers and diagrammatic representation would be misleading. It would be unwise to interpret the apparently greater divergence at 3 years of the curves for the clofibrate and high cholesterol groups as showing that 3 years of treatment has some critical importance. It is more likely to be a random fluctuation. Details of the life-table analysis and of the method used are shown in Appendix 2.

Figure 3 shows the standardised rate *for each centre*, by Group, for all major IHD, for fatal IHD, and for non-fatal myocardial infarction. For the reasons stated above, standardisation is at ages 40 to 54, not 40 to 59. At each centre the rates for all IHD, and for non-fatal myocardial infarction, were higher in Group II than in Group I, and the rates in both Groups were higher than those in Group III. There was no consistent pattern for fatal IHD in the centres, but the numbers of deaths were small.

The incidence of major IHD (taking all groups together) was significantly lower ($P < 0{\cdot}01$) in Budapest than in either of the other 2 centres. Details of numbers and rates are shown in Appendix 4.

In order to test whether adherence to treatment had any important influence on the main result (that is whether, for instance, the difference between the clofibrate-treated group and the high cholesterol control group in the number of major IHD events might have been greater if the treatment had been more strictly observed in Group I than it was) the proportion of negative estimates of chlorophenoxyisobutyric acid (CPIB) in the blood was calculated for the men in Group I who had such an event and compared with the same proportion in all men in Group I. A further test was made, in Group I, of adherence to treatment at the last visit before an IHD event compared with visits at the same stage in the study for all men. The results are shown in Appendix 5 (ii) and (iii). There seems to be no

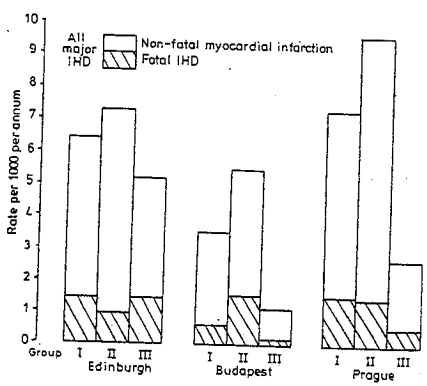

Fig. 3 *Age-standardised incidence and mortality rates of major IHD, 40 to 54 years, per 1000 per annum by centre.*

TABLE 4

Incidence of Minor Ischaemic Heart Disease

Rates per 1000 per annum

Condition[a] (newly arising in the trial)	Group I Clofibrate			Group II High Cholesterol Control			Group III Low Cholesterol Control		
	No.[b]	Rate	Age-St. Rate	No.[b]	Rate	Age-St. Rate	No.[b]	Rate	Age-St. Rate
Angina Pectoris with abnormal ECG [c]	60	2.1	2.6	48	1.7	2.4	31	1.1	1.4
Angina Pectoris without abnormal ECG [d]	212	7.4	9.0	226	8.0	9.6	181	6.7	8.4
Abnormal ECG without chest pain [c]	223	7.9	9.1	260	9.3	10.6	176	6.5	8.8

(a) For definitions see Appendix 3.

(b) Numbers and crude rates relate to men aged 30–59. Age-standardised rates relate to men aged 40–59.

(c) Abnormal ECG = Minnesota Codes: 4.1, 4.2, 5.1, 5.2, 6.1, 6.2, 7.1, 8.3, 11.1, 12.1, 14.1.

(d) As defined by angina questionnaire. (Addendum A).

TABLE 5

Other Vascular Events and Diabetes: Incidence and Withdrawals

Rates per 1000 per annum

Condition[a] (newly arising in the trial)	INCIDENCE Group I Clofibrate			Group II High Cholesterol Control			Group III Low Cholesterol Control			WITHDRAWALS I Clof.	II H.C.C.	III L.C.C.
	No.[b]	Rate	Age St.Rate	No.[b]	Rate	Age St.Rate	No.[b]	Rate	Age St.Rate	No.	No.	No.
Vascular Conditions												
Cardiac Arrhythmia [c]	173	6.1	7.5	166	5.9	6.7	152	5.6	8.1	1	1	0
Hypertension (as defined)	192*	6.8*	7.5*	242*	8.6*	9.9*	146	5.4	6.3	63**	96**	60
Other Heart Disease [d]	-	-	-	-	-	-	-	-	-	9	9	11
Non-fatal Cerebrovascular disease	18	0.6	0.9	13	0.5	0.8	14	0.5	1.0	5	1	2
Intermittent Claudication [e]	184	6.5	7.5	184	6.5	8.3	108	4.0	4.8	43	30 [f]	16 [f]
Venous Thrombosis & Embolism [d]	-	-	-	-	-	-	-	-	-	7	2	1
Diabetes (as defined in each centre)	129	4.6	5.1	102	3.6	4.0	67	2.5	3.4	47*	26*	18

(a) For definitions see Appendix 3.

(b) The numbers and rates relate to men aged 40–59. The age standardised rates relate to men aged 40–59.

(c) Minnesota code 8-1, 8-3.

(d) Incidence figures are not available because these conditions were not notifiable.

(e) As defined by intermittent claudication questionnaire. (Addendum A).

(f) In Edinburgh 6 men in Group II and 3 men in Group III with confirmed peripheral artery disease were not withdrawn from the study. (See text.)

* Significant difference between Groups I and II ($P < 0{\cdot}05$).

** " " " Groups I and II ($P < 0{\cdot}01$).

consistent difference between the CPIB values of men who developed events compared with all men.

INCIDENCE OF MINOR IHD

Table 4 shows the number of minor IHD events in each group together with incidence rates, both crude, for all ages, 30 to 59, and standardised at ages 40 to 59. Minor IHD is classified as angina pectoris with and without abnormal electrocardiogram and as an electrocardiographic abnormality without chest pain, as defined earlier under 'criteria for assessing the results of the trial'. There was no significant difference between Groups I and II in angina pectoris with or without abnormal electrocardiogram, or in those with electrocardiographic abnormalities in the absence of chest pain. The major items of the Minnesota code (4-1, 5-1, 7-1, and 11-1) were analysed separately and together, and again no difference was found between Groups I and II.

OTHER VASCULAR EVENTS AND DIABETES

Table 5 shows the result for other vascular events and diabetes, and includes withdrawals from the trial, as well as incidence.

Apart from hypertension and diabetes there were no significant differences in these conditions between Groups I and II.

Hypertension is defined for the purposes of this trial as a diastolic pressure of 120 mmHg or more on one occasion, between 110 and 119 mmHg more than once, or once only if specific electrocardiographic abnormalities were present (Appendix 3). The incidence and rate of withdrawal in Group I was significantly lower than in Group II. The incidence in Group I was lower than Group II in each centre.

In spite of this, however, mean blood pressure for all subjects during the trial was not materially different in Groups I and II as shown in Table 6 (i). Thus, clofibrate appeared to show no appreciable blood pressure lowering effect overall.

Table 6 (ii) shows that mean blood pressure at the visit immediately before a major event was higher than the mean pressure in men, at the same stage in the trial, who did not have such an event. This was equally true for Groups I and II.

The incidence of hypertension in Group III was significantly lower than in Groups I or II (Table 5) and higher in Prague than in either of the other two centres (Addendum C).

Diabetes was notified more frequently in Group I than in Group II. Though this difference was not statistically significant the number of withdrawals for diabetes requiring drug treatment was significantly higher in Group I than in Group II. Diabetes was much more frequently notified in Prague than in the other centres and, indeed, accounted for more than half the number in the whole trial (Addendum C).

Peripheral artery disease was not a specified reason for withdrawal. Ischaemic leg pain, identified by

TABLE 6

Mean Blood Pressure (mmHg)

	Group	At Entry	Years in Trial 1	2	3	4	5
(i) All Men — Mean Systolic Blood Pressure	I Clofibrate	135	132	131	133	133	134
	II High Cholesterol Control	135	132	131	133	134	134
Mean Diastolic Blood Pressure	I Clofibrate	87	84	83	84	83	84
	II High Cholesterol Control	87	85	84	85	85	84
(ii) Men with a major I.H.D. event (value at visit immediately preceding I.H.D. event)							
Mean Systolic Blood Pressure	I Clofibrate	148	141	139	144	140	137
	II High Cholesterol Control	144	142	140	143	136	149
Mean Diastolic Blood Pressure	I Clofibrate	89	88	89	86	85	87
	II High Cholesterol Control	88	88	88	90	86	92
Men who did not have a major I.H.D. event							
Mean Systolic Blood Pressure	I Clofibrate	135	131	131	133	133	134
	II High Cholesterol Control	135	132	131	133	134	134
Mean Diastolic Blood Pressure	I Clofibrate	87	84	83	84	83	84
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1080 *Committee of Principal Investigators*

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	II High Cholesterol Control	135	132	131	133	134	134
Mean Diastolic Blood Pressure	I Clofibrate	87	84	83	84	83	84
	II High Cholesterol Control	87	85	84	85	85	84

questionnaire (Addendum A), was notifiable to the controlling centre in London. In 1971 notifications in Group I had reached a number which was significantly higher than in Group II (though, as Table 5 shows, the numbers were finally exactly equal) and a special investigation was set up to assess the situation. All cases reported as having ischaemic leg pain were examined by a vascular specialist at each centre, who was unaware of group identity. Blood flow measurements were carried out on doubtful cases. 'Confirmed' cases of peripheral artery disease were withdrawn from the trial and these are the figures shown in Table 5. The difference between Groups I and II is not statistically significant. At Edinburgh the numbers confirmed were small and it was decided to withdraw from the trial only the 6 patients receiving clofibrate. (This was possible without disclosing to the physicians running the clinics the identity of the cases 'confirmed' in the 2 placebo groups.) In comparing Groups I and II in Table 5 this should be borne in mind.

Since 1971, the presence or absence of peripheral arterial pulses was notified to London. The numbers of men, in all centres, with at least one absent pulse

TABLE 7

Serum Cholesterol (mg/dl)

Mean Values during the Trial

Centre & Group	No. of men (c)	Screening Visit(s)	Entry Visit	No. of years after entry 1	2	3	4	5	6	7
Edinburgh										
I Clofibrate	1631	249	242	214	214	220	225	227	229	233
II High Cholesterol Control	1658	249	242	241	240	248	252	255	257	259
III Low Cholesterol Control	1646	179	188	187	190	195	200	204	205	208
Budapest (a)										
I Clofibrate	1193	267	256	228	232	236	228	234	244	243
II High Cholesterol Control	1146	264	255	244	251	254	246	251	257	250
III Low Cholesterol Control	1179	189	194	187	193	197	195	199	201	197
Prague										
I Clofibrate	1884	290	287	255	257	260	262	259	260	264
II High Cholesterol Control	1880	286	282	280	277	279	280	278	275	279
III Low Cholesterol Control	1667	204	205	213	216	217	218	214	214	217
Cholesterol Decrease (b) in the Clofibrate-treated Group relative to the High Cholesterol Control Group expressed as a percentage of starting values			Edinburgh	11.1	10.7	11.5	11.2	11.2	11.6	10.7
			Budapest	6.6	8.0	7.5	7.7	7.0	5.9	3.5
			Prague	10.3	8.7	7.8	7.8	8.1	6.7	6.6
			All Centres (d)	9.7	9.1	9.0	8.9	8.8	8.2	9.0

(a) Throughout this Report, Budapest cholesterol figures refer only to men admitted on the basis of the Watson method of cholesterol estimation. (Addendum B).

(b) Decrease is measured in each Group from the mean pre-treatment levels, i.e. the levels at the Screening Visit(s) and at Entry Visit.

(c) Men who had cholesterol values at screening and entry visits.

(d) Adjusted for differences between Centres, see Appendix 2.

on at least one occasion were: Group I: 257; Group II: 309; Group III: 196 and, with at least two on any occasion: 155, 192, and 114 respectively. The figures for Group III are significantly lower than for Groups I and II ($P < 0.01$), as might be expected. The differences between Groups I and II are also significant ($P < 0.05$).

CHANGE IN SERUM CHOLESTEROL LEVELS DURING THE STUDY

Table 7 shows the mean serum cholesterol levels at screening and at each trial visit for the men in each Group in each centre. The mean starting levels (both at screening and at first visit) are different in the 3 centres and, in particular, the values are somewhat higher in Prague. The greater part of this excess has been shown by tests of control samples (or by the reference system used) to be the result of the methods of cholesterol determination (Addendum B), so that the actual average cholesterol levels before treatment differed little, in fact, between the 3 centres.

There were considerable variations from visit to visit in the mean levels of cholesterol in all groups during the study, particularly in Edinburgh and Budapest. Whatever the reasons for these variations, in the clofibrate-treated group they were, of course, operative in addition to the effects of the drug. To allow for this, when assessing change from mean pretreatment level, at any stage during the trial, the change seen in the high cholesterol control group was deducted from the change seen in the clofibrate-treated group (Appendix 2). This adjusted figure for Group I, therefore, represents that part of the evident change which could, presumptively, be attributed to clofibrate.

The adjusted figures for change in Group I are shown in the lower section of Table 7 for each centre in each year after entry: they are expressed as a percentage of the mean pretreatment levels. The change remains fairly steady after the initial fall measured at the first follow-up visit (that is 6 months) in each centre. In Edinburgh a decrease of about 11 per cent, in Budapest of about 7 per cent, and in Prague of about 8 per cent was maintained; an adjusted figure for all centres is also presented which varies from 9·7 to 8·2 per cent. These figures fell short of the 15 per cent reduction in serum cholesterol envisaged at the outset of the trial.

INCIDENCE OF IHD IN RELATION TO CHOLESTEROL CHANGE

The relation between incidence of IHD and change in serum cholesterol levels can be studied in 2 ways: by comparing the experience of Group I, where the mean serum cholesterol level was reduced by treatment, with that of the 2 control groups, and by relating the individual change in serum cholesterol levels to incidence.

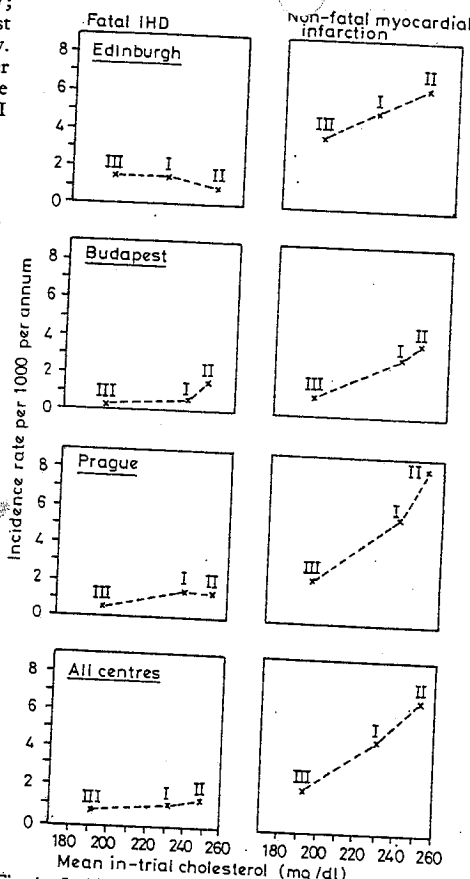

Fig. 4 *Incidence of major ischaemic heart disease and mean in-trial cholesterol by centre and group. Age-standardised rates per 1000 per annum at ages 40-54.*

Incidence related to group changes in cholesterol

The first approach is illustrated in Fig. 4 where the 'mean in-trial cholesterol level' for each Group is related to the age-standardised incidence of IHD, at ages 40 to 54, in that Group. This was done for each centre, and separately for fatal and non-fatal IHD. (Again, this age-range was chosen because centre

comparisons are involved.) The derivation of 'mean in-trial cholesterol level' is described in Appendix 2. It allows for variations from visit to visit in mean cholesterol levels, averages the levels over all visits, and takes account of differences between centres in mean levels. For non-fatal myocardial infarction the point for Group I in each centre lies somewhat below the straight line between the points for Groups II and III. The results are not inconsistent with the possibility of some fairly simple one-to-one relation. The results for fatal IHD are less consistent, possibly because of small numbers of events.

Incidence related to individual changes in cholesterol

For the second approach, individual cholesterol change is defined in an analogous way to group change, that is the change in an individual's pre-treatment cholesterol levels is adjusted by subtracting from it the change in the mean level of cholesterol of all the men in Group II at the same centre and at the same visit. The mean change for an individual is then calculated as the mean of all changes for that individual from his second visit to his last visit: this can be expressed in absolute terms, or as a percentage of starting values. If a man did not have a second visit, that is he left the trial for any reason before then, it was not possible to calculate a change figure for him. This applies, in particular, to those men who had a heart attack in their first 6 months in the trial, and they are therefore excluded from this analysis.

Because men with the greatest reduction have, on average, the highest initial cholesterol levels and, therefore, are initially at higher risk of heart attack than the men with less reduction, it is necessary to study the relation between cholesterol change and the incidence of IHD within broadly similar levels of starting cholesterol.

In Table 8 the men in each of the 2 high cholesterol groups are classified simply (i) into those with a fall in mean cholesterol and those without, and (ii) into those whose initial pretreatment cholesterol was above or below the median of the distribution of pretreatment cholesterol levels. Since these men are all drawn from the highest third of the overall distribution of starting cholesterols, the figures refer to men in the two highest sixths of that distribution. The median was calculated separately in each centre because of the differences between centres in overall levels.

Table 8 is divided into three parts: 8(i), 8(ii), 8(iii), dealing respectively with all major IHD, non-fatal myocardial infarction, and fatal IHD. In each cell there are 3 numbers: the number of events, the rate per thousand per annum (at all ages and unstandardised), and an 'expected' rate. This expected rate, also expressed per thousand per annum, is calculated from a multivariate analysis (see later) and takes into account the 5 factors which were measured at the start of the trial and were shown to have an independent effect on the subsequent incidence of major IHD—namely, age, initial cholesterol level, smoking, systolic blood pressure, and whether the man's father was alive at the start of the trial—and represents the rate of incidence which would be expected in a group of men in Group II whose ages, initial cholesterol levels, etc., were the same as those of the men who are actually included in the relevant cell of the table. Thus any difference between the observed rate and the expected rate in each cell represents, apart from sampling fluctuations, the effect of cholesterol change—that is a rise or fall in mean cholesterol during the trial whether due to clofibrate or not, and is better evidence than that of the crude rate which, of course, makes no allowance for the fact that the men in the different cells of the table almost certainly have different inherent risks because they are of different ages, have different initial cholesterol levels, etc.

The table shows, first, that, both for fatal and non-fatal infarction, the observed rates were higher in the men whose pretreatment cholesterol concentrations were above the median than in the men with values below the median, and the same is true for the expected rates.

Secondly, in terms of cholesterol change during the trial, the observed rates of major IHD for the men in Group I whose cholesterol fell during the trial were substantially below the expected rates whether their pretreatment cholesterols were low or high. Whereas, for the corresponding men in Group II, the difference between observed and expected was neither so clear nor so consistent. For men in both Groups whose cholesterol levels rose during the trial, on the other hand, the observed rates were higher than the expected. The pattern is similar but not quite so clear for non-fatal myocardial infarction alone, while for fatal IHD the picture is not clear.

Although the differences referred to above are not all individually statistically significant, they are consistent with the suggestion that most of the reduction of non-fatal myocardial infarction in the clofibrate treated Group is associated with reduction of cholesterol concentrations.

Effect of other 'risk factors' on the incidence of major IHD

Before proceeding to the multivariate analysis, Table 9 shows how incidence of major IHD is

TABLE 8

Incidence of Ischaemic Heart Disease

by Mean Pre-treatment Cholesterol and Mean Cholesterol Change

Rates per 1000, per annum

(i) Incidence of Major IHD, Fatal and Non-fatal

Mean Pre-treatment Cholesterol(a)		Group I Clofibrate: Fall in Cholesterol	Group I Clofibrate: Rise in Cholesterol	Group II High Cholesterol Control: Fall in Cholesterol	Group II High Cholesterol Control: Rise in Cholesterol
Below Median(b)	Observed Rate	3.7	7.3	6.5	6.1
	Expected Rate(c)	6.2	6.7	6.4	5.9
	No.	37	18	35	43
Above Median	Observed Rate	6.4	12.6	7.6	11.2
	Expected Rate	9.4	11.4	9.2	8.8
	No.	73	14	61	47

(ii) Incidence of Non-fatal Myocardial Infarction

Below Median	Observed Rate	2.7	6.1	6.1	5.1
	Expected Rate	5.3	5.8	5.5	5.1
	No.	27	15	33	36
Above Median	Observed Rate	5.3	8.1	6.0	9.5
	Expected Rate	7.7	9.5	7.6	7.5
	No.	60	9	48	40

(iii) Incidence of Fatal IHD

Below Median	Observed Rate	1.0	1.2	0.4	1.0
	Expected Rate	0.9	0.9	0.9	0.8
	No.	10	3	2	7
Above Median	Observed Rate	1.1	4.5	1.6	1.7
	Expected Rate	1.7	1.9	1.6	1.3
	No.	13	5	13	7

(a) Corrected mean pre-treatment cholesterol (See Appendix 2).

(b) Median values were determined separately for each group and for each centre.

(c) See text.

The following table indicates the mean change in serum cholesterol for all men where a change could be calculated, together with the number of such men in each category

Mean Pre-treatment Cholesterol		Group I Clofibrate: Fall	Group I Clofibrate: Rise	Group I Clofibrate: Total	Group II High Cholesterol Control: Fall	Group II High Cholesterol Control: Rise	Group II High Cholesterol Control: Total
Below Median	Mean change %	-10.2	6.2	-6.7	-6.2	7.4	1.5
	No. of men	1739	479	2218	976	1281	2257
Above Median	Mean change %	-13.6	5.7	-11.6	-7.7	5.5	-3.0
	No. of men	2027	231	2258	1427	779	2206

TABLE 9

Incidence of Major Ischaemic Heart Disease by Factors Present at Entry

All Centres, Age-standardised Rates per 1000 per annum at ages 40–59

(Rates in brackets are based on fewer than 10 events)

Factor	Group I Clofibrate	Group II High Cholesterol Control	Group III Low Cholesterol Control
Serum Cholesterol			
Below Median (a)	5.9	8.4	3.3
Above Median	9.7	11.1	3.6
Systolic Blood Pressure (mmHg)			
Under 120	5.8	4.9	(2.7)
120–139	7.6	7.3	2.5
140–159	7.5	12.6	4.2
160 and over	8.1	11.1	6.0
Diastolic Blood Pressure (mmHg)			
Under 80	6.2	7.8	3.3
80–	7.8	7.3	3.3
90–	6.9	10.4	3.4
100–	7.2	12.5	(3.2)
110 and over	(15.4)	(12.3)	(3.8)
Weight (Kg)			
Under 70	7.6	6.1	3.8
70–	7.4	9.6	3.3
90 and over	7.1	10.0	(2.4)
Height (cm)			
Under 170	8.3	8.9	2.9
170–	6.7	9.4	3.0
180 and over	7.2	5.6	(5.9)
Quetelet Index ($\frac{W}{H^2}$ X 10,000)			
Under 26	8.0	8.4	3.7
26–	6.7	8.4	3.3
29 and over	7.7	11.0	(2.3)
Smoking			
Never smoked	4.2	5.8	(1.3)
Ex-smoker	4.4	5.5	(2.0)
Smoker	10.2	12.1	5.4
Amount Smoked (Cigarette smokers only)			
Under 10	11.4	(3.7)	(1.3)
10–	9.0	10.9	4.9
20 and over	10.1	14.9	6.3
Chest Pain			
Absent	6.8	8.5	3.7
Non-ischaemic	7.6	9.3	(2.1)
Positive questionnaire	(13.2)	(15.9)	(0.0)
Other pain	(13.2)	(9.3)	(6.8)
Leg Pain			
Absent	7.2	8.7	3.5
Non- ischaemic	7.4	9.2	(2.1)
Ischaemic	(22.4)	(19.1)	(0.0)
Arcus - Absent	7.0	8.5	3.3
Present	8.1	11.8	(3.5)
Xanthelasma - Absent	7.0	8.7	3.2
Present	(10.6)	(11.5)	(6.2)

(a) Median values were determined separately for the high cholesterol groups and low cholesterol group within each centre.

related to factors generally recognised as prognostically important in ischaemic heart disease. The rates shown are age-standardised and are surprisingly consistent with those expected from the results of other large population studies. In nearly every category, the incidence rises as the severity of the risk factor increases, and this applies to all 3 Groups, though at different levels. The exceptions are mostly associated with small numbers of events, but blood pressure as a risk factor deserves special mention. Compared with Group II, blood pressure in Group I had much less influence on the incidence of heart attacks.

MULTIVARIATE ANALYSIS

The data shown in Table 9, though striking in their regularity, take no account of the association of one factor with another and hence give no indication of the independent contribution of the various factors to prediction of outcome. For instance, the interesting relation between blood pressure and incidence of IHD might be due, partly or wholly, to a relation between blood pressure and age, smoking, or parental history. The appropriate statistical technique for taking account of these interrelations and producing a statement of the most important independent predictors of future IHD is some form of multivariate analysis. This has been carried out, using the multiple logistic function, as used, for instance, by Keys *et al.* (1972). Details of methods and results are given in Appendix 2.

In this analysis, carried out on men in Group II, in all centres, and excluding the clofibrate treated group for greater comparability with other published studies, the 5 variables which gave independently significant prediction of future IHD (fatal and non-fatal) were age, initial cholesterol, smoking, blood pressure, and whether the subject's father was alive at the start of the trial. Systolic blood pressure was a slightly more significant predictor than diastolic.

To give some idea of the magnitude of these effects (as distinct from their statistical significance) the following table shows the factors by which the risk of future IHD is increased for the indicated change in the variable concerned.

Variable	*Change*	*IHD risks multiplied by*
Age	+ 10 years	1·9
Smoking	present smoker of anything v. never or ex-smoker	2·4
Systolic blood pressure	+ 20 mmHg	1·3
Initial serum cholesterol	+ 20 mg/dl	1·2
Father dead at start of trial	dead v. alive	1·6

The 5 factors which, in the present analysis, give independently significant prediction are very similar to those shown in other prospective studies such as the Framingham Study, already mentioned, the 'Seven Country Study' (Keys *et al.*, 1972), or the study by Medalie *et al.* (1973) in Israel. The regression coefficients concerned are compared in Appendix 2. The coefficients for initial cholesterol are all very similar in these studies and, substituting the average reduction of 9% in cholesterol observed in this study in any of the equations results in a predicted reduction in the incidence of IHD of about 20 per cent, which was the reduction observed in the present study.

Directly to study the effects of treatment, on the other hand, the analysis was carried out on the men in Groups I and II, the treated and untreated high cholesterol groups.

The same 5 variables were included together with a sixth, treatment by clofibrate, that is membership of Group I or Group II. From this analysis treatment emerged as significant ($P < 0{\cdot}05$) and the effect was to reduce the incidence by 27 per cent. This figure is higher than the 20 per cent difference in incidence of major IHD reported above because of an accumulation of small differences in entry characteristics between the men in Groups I and II.

Cholesterol change, as previously defined, was next introduced as a variable (thereby automatically excluding all those who did not have a visit after the first, in whom, of course, no change could be measured). This was done, first, without including treatment and, secondly, including both treatment and cholesterol change. When cholesterol change but not treatment was included in the analysis, cholesterol change was significant and a reduction of 10 per cent in cholesterol (about 20 to 25 mg/100 ml) was associated with a reduction of 22 per cent in the risk. When both cholesterol change and treatment were included the significance of cholesterol change was slightly reduced but the effect of treatment was halved, suggesting that cholesterol change is associated with most (but not all) of the change in incidence resulting from treatment, that is that treatment may have some additional effect beyond that of cholesterol reduction. The excess effect is not itself significant.

OPTIMAL EFFECTS OF CLOFIBRATE ON MAJOR IHD EVENTS

Two approaches were used to find out whether there were any groups of subjects in whom clofibrate treatment was noticeably more, or less, effective than average in influencing major IHD events (fatal and non-fatal myocardial infarction).

The first, for which the results are set out in

TABLE 10

Differential Effect of Clofibrate

(a) By quartile of risk score

Major IHD events. Rates per 1000 per annum

Quartile of risk score	Group I Clofibrate		Group II High Cholesterol Control		Ratio of Rates (I/II)	Difference between Rates (II-I)
	No.	Rate	No.	Rate		
1 (lowest risk)	7	1.11	16	2.51	0.44	1.40
2	29	4.70	33	5.18	0.91	0.48
3	41	6.58	59	9.47	0.69	2.89
4 (highest risk)	78	12.56	93	16.28	0.77	3.72

Table 10(a), divided the men in the 2 high cholesterol Groups into quartiles of 'risk scores'. That is to say, a risk score was calculated for each man, based on the multiple logistic equation derived from Group II, and using the values of the 5 risk factors for that man at entry to the trial. This represented an assessment of his intrinsic risk of having a major IHD event. All the men in both Groups were ranked by this score and divided into 4 equal-sized quartiles of risk. For each such quartile, the number of major IHD events and the total number of man-years in the trial were determined to calculate a rate for each Group. The rates in the 2 Groups were then compared in each quartile. The difference varied from 0·48 per thousand per annum to 3·72, the highest differences being in the 2 quartiles of highest risk. The greatest proportionate change in the rates is in the lowest quartile of risk, whereas the greatest absolute difference in rates is in the highest quartile. From the point of view of achieving the greatest reduction in the number of cases of ischaemic heart disease, the difference is the more relevant index, and it suggests that the greatest benefit would derive from a policy of treating those at highest risk.

The second approach was to evaluate the effects of clofibrate treatment in subjects who had low and high values of the 5 main risk factors (age, smoking, cholesterol, systolic blood pressure, and father alive or dead). For each risk factor, the men in Group I were divided into those with low and high values and the number of events observed are shown in the first line of Table 10(b). In the second line, the 'calculated' number of events is shown. This calcu-

TABLE 10 (b)

Major IHD events in Group I by risk factors at entry to Trial

	Age		Smoking		Father		Systolic BP		Cholesterol		Greatest Effect	Remainder
	< 45	⩾ 45	O+Ex	+	Alive	Dead	<135	⩾135	⩽median	>median		
Observed no. of events	30	125	43	112	14	141	77	78	58	97	45	110
Calculated no. of events	52	158	56	155	27	184	90	119	82	127	68	143
Observed rate/1000 p.a. (o)	2.88	8.63	3.80	8.24	2.25	7.55	5.58	7.02	4.68	7.76	9.46	5.46
Calculated rate/1000 p.a. (c)	5.02	10.93	4.91	11.42	4.36	9.84	6.55	10.71	6.60	10.13	14.30	7.10
R = ratio of rates (o/c)	0.57	0.79	0.77	0.72	0.52	0.77	0.85	0.66	0.71	0.77	0.66	0.77
D = difference between rates (c-o)	2.14	2.30	1.11	3.18	2.11	2.29	0.97	3.69	1.92	2.37	4.84	1.64

lated number, based on the multiple logistic function, is the number of events which would have occurred in the Group I men had they not been given clofibrate. The difference between observed and calculated numbers thus represents the effect of clofibrate treatment, making allowance for all the risk factors used in the logistic analysis. From the observed and calculated numbers, rates were derived and the ratios and differences of these rates are shown in the last two lines. Thus, for instance, in younger men aged under 45 years, the ratio of the rate in the clofibrate-treated group to the rate in similar men in Group II is 0·57 compared with 0·79 in the older men, suggesting a greater benefit of treatment in the younger men independently of other risk factors. On the other hand, the absolute differences due to treatment (2·14/2·30) do not differ appreciably between the younger and older groups. Similar contrasts between conclusions drawn from the ratio and the difference due to clofibrate treatment apply to parental history (ratios 0·52/0·77; differences 2·11/2·29) and to cholesterol (0·71/0·77; 1·92/2·37). For smoking, however, whether the ratios (0·77/0·72) or the difference (1·11/3·18) are taken as the best measure of the benefit of treatment, the result is similar, that is those at higher risk derive more benefit. This is even more so for systolic blood pressure (ratios 0·85/0·66; differences 0·97/3·69). These findings taken together with the results shown in Table 8, suggest that the men likely to benefit most from clofibrate treatment would be those with systolic blood pressure over 135 mmHg, who smoked, *and* who showed a reduction of cholesterol on treatment. This subgroup, comprising men of mean age 46·5 and mean cholesterol of 258 mg/100 ml, are described in Table 10(b) as those with 'greatest effect'. They are compared with the 'remainder', that is all other men in Group I. The ratios of major IHD rates in 'greatest effect' and 'remainder' subgroups were 0·66/0·77 and the differences 4·84/1·64. There is a 34 per cent reduction of events in the 'greatest effect' subgroup, compared with 23 per cent in the 'remainder'. These reductions in incidence are associated with mean cholesterol changes of 12 and 8 per cent, respectively. The 872 men in the 'greatest effect' subgroup represent 18·4 per cent of Group I, which in turn was selected from the upper third of the population. Thus this subgroup represents, at most, 6 per cent of men in the age range concerned.

TABLE 11

Serum Triglycerides (mg/dl)

Last in-trial reading for each man. Edinburgh only.

			Years in Trial		
			5	6	7
Group I	Clofibrate	Mean TG	161	158	162
		No. of men	178	294	592
Group II	High Cholesterol Control	Mean TG	205	211	209
		No. of men	184	276	601
Group III	Low Cholesterol Control	Mean TG	160	163	174
		No. of Men	181	248	658

CHANGE IN SERUM TRIGLYCERIDES DURING THE STUDY

As mentioned earlier under biochemical monitoring, serum triglycerides were measured in one centre only (Edinburgh) and then only from 1972. Since no baseline analysis was made, it is not possible to produce serial measures of individual change of triglyceride concentrations. Nevertheless, Table 11 shows that the concentrations after 5, 6, and 7 years of treatment with clofibrate were much lower than in the untreated high cholesterol group, and even slightly lower than in the low cholesterol control group.

Deaths from causes other than ischaemic heart disease

In the analysis of many conditions other than ischaemic heart disease there are good reasons for including deaths that occur after leaving the trial with those that occur in the trial. Deaths from cancer form an obvious example. The centres

TABLE 12

Deaths in the Trial and Within 1 year of Leaving it

Main Cause Groups. Numbers of Deaths & Rates at Ages 30–59, and

Age-standardised Rates per 1000 per annum at ages 40–59

Cause of Death	Group I Clofibrate			Group II High Cholesterol Control			Group III Low Cholesterol Control		
	No. (All Ages)	Rate (All Ages)	St. Rate 40–59	No. (All Ages)	Rate (All Ages)	St. Rate 40–59	No. (All Ages)	Rate (All Ages)	St. Rate 40–59
Ischaemic Heart Disease	54	1.6	2.1	48	1.4	2.1	20	0.6	0.8
Other Vascular	14	0.4	0.5	14	0.4	0.6	9	0.3	0.5
Neoplasm: malignant	58	1.7	2.2	42	1.3	1.7	41	1.3	2.5
Neoplasm: benign	3	–	–	–	–	–	1	–	–
Other Medical Causes	16*	0.5*	0.8*	5*	0.2*	0.2*	7	0.2	0.4
Accidents and Violence	17	0.5	0.6	18	0.5	0.6	15	0.5	0.6
All causes other than IHD	108*	3.2*	4.1	79*	2.4*	3.1	73	2.3	3.9
All causes other than IHD, Vascular and Accidents & Violence	77**	2.3**	3.1*	47**	1.4**	1.9*	49	1.5	2.9
TOTAL ALL CAUSES	162*	4.9*	6.2	127*	3.8*	5.2	93	2.9	4.7

* Significant difference between Groups I and II ($P < 0.05$).

** " " " Groups I and II ($P < 0.01$).

differed, for valid local reasons, in their policies for excluding men from the study who developed malignant disease. On the other hand, it was not possible to be sure that all deaths occurring out of the trial were included. As mentioned earlier, a thorough search was made for all deaths occurring within one year of leaving the trial and, almost certainly, all but a few of these have been found. The figures in the report include all such deaths notified to the statistical centre up to the end of 1977. In this section, therefore, dealing with deaths from all causes, Table 12 shows the number of deaths and the unstandardised and age-standardised death rates for the principal causes of deaths occurring 'in the trial' and within 1 year of leaving it. Table 14 shows the numbers of deaths in more detailed categories of causes, and also distinguishes between deaths in the trial and those occurring within 1 year of leaving it. Such information as is available about deaths occurring out of the trial but *more* than 1 year after leaving it is available on request (Addendum E).

The total number of deaths was significantly greater in Group I (162) than in Group II (127, $P < 0.05$), and the life-table analysis also shows a significant ($P < 0.05$) excess in Group I. There was a slight excess of deaths from ischaemic heart disease in Group I but nearly all the difference between Groups I and II lies in the deaths from causes other than IHD. Here again the differences in total numbers (108 and 79) and in crude rates (3·2 and 2·4) are significant ($P < 0.05$) and the life-table analysis gives a similar result ($P < 0.05$, Fig. 5).

In terms of the individual cause groups other than IHD, shown in Table 12, the numbers of deaths from 'other vascular' conditions and from 'accidents and violence' are almost exactly the same in both groups, so that virtually the [illegible] difference lies in deaths from cancer and from [illegible] medical causes', which are shown in detail in Table 14. However, when considering deaths from conditions not usually considered to be related to serum cholesterol level (which applies to the deaths in these 2 categories), it is appropriate to compare the mortality in Group I with that in Group III (the low cholesterol control group) as well as Group II, provided that in so doing allowance is made for the younger age at entry in Group III. (There are other differences between Group III and the other groups, but age is probably the most important. Appendix 2, multivariate table, Nos. 10, 11, and 12.) In Table 12 the age difference is taken into account by standardisation, and the effect of this adjustment is, of course, to increase the death rates in Group III compared with those in Groups I and II.

A comparison of the age-standardised rates in Groups I, II, and III for all causes other than IHD (4·1, 3·1, 3·9) suggests that part of the difference between Groups I and II is partly or largely the result of a low mortality in Group II rather than a high mortality in Group I. For cancer the corresponding rates are 2·2, 1·7 and 2·5 respectively, with a stronger indication of the same point.

Because of its importance, a detailed follow-up of all in- and ex-trial morbidity and mortality due to cancer was carried out. Table 13 shows that there was little difference between Groups I and II (66 cases against 61) in ex-trial deaths and cases still surviving. The main difference between these Groups thus lies in the in-trial mortality. Comparing all 3 Groups, the standardised rates for all malignant neoplasms are 3·6, 2·8, and 3·4, and for mortality,

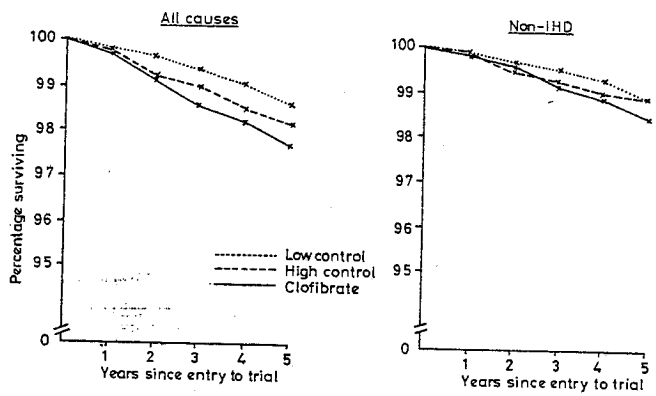

Fig. 5 *Total mortality. In-trial and within 1 year of leaving. Life-table basis.*

differed, for valid local reasons, in their policies for excluding men from the study who developed malignant disease. On the other hand, it was not possible to be sure that all deaths occurring out of the trial were included. As mentioned earlier, a thorough search was made for all deaths occurring within one year of leaving the trial and, almost certainly, all but a few of these have been found. The figures in the report include all such deaths notified to the statistical centre up to the end of 1977. In this section, therefore, dealing with deaths from all causes, Table 12 shows the number of deaths and the unstandardised and age-standardised death rates for the principal causes of deaths occurring 'in the trial' and within 1 year of leaving it. Table 14 shows the numbers of deaths in more detailed categories of causes, and also distinguishes between deaths in the trial and those occurring within 1 year of leaving it. Such information as is available about deaths occurring out of the trial but *more* than 1 year after leaving it is available on request (Addendum E).

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In terms of the individual cause groups other than IHD, shown in Table 12, the numbers of deaths from 'other vascular' conditions and from 'accidents and violence' are almost exactly the same in both groups, so that virtually the whole difference lies in deaths from cancer and from 'other medical causes', which are shown in detail in Table 14. However, when considering deaths from conditions not usually considered to be related to serum cholesterol level (which applies to the deaths in these 2 categories), it is appropriate to compare the mortality in Group I with that in Group III (the low cholesterol control group) as well as Group II, provided that in so doing allowance is made for the younger age at entry in Group III. (There are other differences between Group III and the other groups, but age is probably the most important. Appendix 2, multivariate table, Nos. 10, 11, and 12.) In Table 12 the age difference is taken into account by standardisation, and the effect of this adjustment is, of course, to increase the death rates in Group III compared with those in Groups I and II.

A comparison of the age-standardised rates in Groups I, II, and III for all causes other than IHD (4·1, 3·1, 3·9) suggests that part of the difference between Groups I and II is partly or largely the result of a low mortality in Group II rather than a high mortality in Group I. For cancer the corresponding rates are 2·2, 1·7 and 2·5 respectively, with a stronger indication of the same point.

Because of its importance, a detailed follow-up of all in- and ex-trial morbidity and mortality due to cancer was carried out. Table 13 shows that there was little difference between Groups I and II (66 cases against 61) in ex-trial deaths and cases still surviving. The main difference between these Groups thus lies in the in-trial mortality. Comparing all 3 Groups, the standardised rates for all malignant neoplasms are 3·6, 2·8, and 3·4, and for mortality,

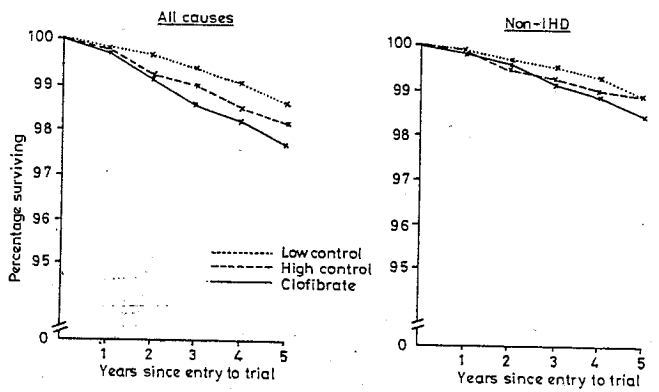

Fig. 5 *Total mortality. In-trial and within 1 year of leaving. Life-table basis.*

TABLE 14

Deaths in the Trial and Within 1 year of Leaving it[a]

Detailed Causes, distinguishing deaths in the Trial from those out of the Trial

Numbers of deaths at ages 30–59

Cause of Death	Group I Clofibrate			Group II High Cholesterol Control			Group III Low Cholesterol Control		
	In-Trial	Out of Trial within 1 year	Total	In-Trial	Out of Trial within 1 year	Total	In-rial	Out of Trial within 1 year	Total
Ischaemic Heart Disease									
Within 3 hours	23	9	32	17	8	25	8	2	10
More than 3 hours	13	9	22	17	6	23	8	2	10
Total	36	18	54	34	14	48	16	4	20
Other diseases of the Circulatory System									
Cerebrovascular disease	8	1	9	7	3	10	3	2	5
Thrombophlebitis	4	–	4	3	1	4	–	–	–
Chronic pulmonary heart disease	1	–	1	–	–	–	1	2	3
Other diseases of circulatory system	–	–	–	–	–	–	–	1	1
Total	13	1	14	10	4	14	4	5	9
Malignant Neoplasms									
Oesophagus, stomach	6	3	9	4	1	5	6	2	8
Small intestine, colon, rectum	6	5	11	3	3	6	3	2	5
Liver, gall bladder, pancreas	6	1	7	3	2	5	5	–	5
Larynx, bronchus and lung	13	4	17	7	4	11	10	4	14
Skin	2	–	2	2	–	2	2	1	3
Brain	4	1	5	2	3	5	1	1	2
Haematopoietic tissue	2	–	2	1	2	3	1	–	1
Other, or not known	1	4	5	2	3	5	2	1	3
Total	40	18	58	24	18	42	30	11	41
Benign Neoplasms	3	–	3	–	–	–	1	–	1
Other Medical Causes									
Pulmonary Tuberculosis	1	–	1	1	–	1	–	–	–
Diseases of the Central Nervous System	–	–	–	–	1	1	–	–	–
Diseases of the Respiratory System									
Influenza	1	–	1	–	–	–	–	–	–
Bronchopneumonia	1	–	1	–	–	–	2	–	2
Chronic Bronchitis	1	–	1	1	–	1	–	–	–
Peptic Ulcer	2	–	2	1	–	1	2	–	2
Liver & Gall Bladder Diseases									
Acute Hepatitis	1	–	1	–	–	–	–	–	–
Liver cirrhosis	–	–	–	–	–	–	1	–	1
Cholecystectomy (see Table 16)	3	–	3	–	–	–	1	–	1
Pancreatitis	2	1	3	–	–	–	–	–	–
Diseases of the Genito-urinary System									
Chronic pyelonephritis	1	–	1	–	–	–	–	–	–
Calculus of kidney	1	–	1	–	–	–	–	–	–
Hyperplasia of Prostate	1	–	1	–	–	–	1	–	1
Chronic Osteomyelitis	–	–	–	–	1	1	–	–	–
Total	15	1	16*	3	2	5*	7	–	7
Accidents & Violence									
Injuries, homicide, accidents	13	–	13	13	1	14	11	1	12
Suicide	4	–	4	2	2	4	3	–	3
Total	17	–	17	15	3	18	14	1	15
TOTAL all Causes other than IHD	88	20	108*	52	27	79*	56	17	73
TOTAL ALL CAUSES	124	38	162*	86	41	127*	72	21	93

(a) Deaths occurring more than one year after leaving the Trial, so far as they are known, are shown in Addendum E.

* Significant difference between Groups I and II ($P < 0.05$).

TABLE 15

Withdrawals for Medical Reasons other than Major Ischaemic Heart Disease

Reason for Withdrawal	I.C.D. No.	Group I Clofibrate	Group II High Cholesterol Control	Group III Low Cholesterol Control
Minor IHD		10	9	5
Other Vascular Events (See Table 5)		128	139	90
Infective & Parasitic Diseases	000-136			
Pulmonary tuberculosis	011	3	5	6
Viral hepatitis	070	9	9	11
Other		2	-	1
Neoplasms (malignant & benign)	140-239	13	12	9
Endocrine & Metabolic Diseases	240-279			
Diabetes (See Table 5)	250	47*	26*	18
Weight gain	277	9*	2*	2
Other		5	9	5
Mental Disorders	290-315			
Psychoses	290-299	2	-	2
Neuroses & other non-psychotic disorders	300-309			
Impotence	305.6	14	7	6
Other		1	2	4
Diseases of the Nervous System	320-389	4	7	4
Diseases of the Respiratory System	460-519	1	-	1
Diseases of the Digestive System	520-577			
Peptic Ulcer	533	5	11	6
Indigestion, nausea, etc.	536.9	20	30	37
Diarrhoea, etc.	561	9	8	11
Other, including diseases of liver and gall bladder		24	19	22
Diseases of the Genito-urinary System	580-629	3	2	4
Diseases of the Skin	680-709			
Hair loss	704	-	1	-
Other		1	3	2
Diseases of the Musculo-skeletal System	710-738	1	2	-
Symptoms & Ill-defined conditions	780-796			
Dizziness	780.5	4	1	1
Pains in the joints & muscles	787	1	2	1
Rash	788.2	7	13	10
Fatigue	790.1	7	2	3
Other		15	5	10
Accidents	800-999	1	1	1
TOTAL		346	327	272

* Significant difference between Groups I and II ($P < 0.05$).

TABLE 16

Cholecystectomies for Gallstones

		Group I Clofibrate	Group II High Cholesterol Control	Group III Low Cholesterol Control
(i) Numbers and Rates				
No. of men admitted to trial:-		5331	5296	5118
No. of cholecystectomies for gallstones in:-				
Edinburgh		22	4	10
Budapest		18	11	9
Prague		19	9	6
All Centres: No.(a)		59***	24***	25
Rate		2.1***	0.9***	0.9
Age-stand. rate (b)		2.0	1.2	1.6
(ii) Numbers in relation to Time in Trial				
Years in Trial:	<2	11	2	5
	2-	17	7	6
	4-	18	11	10
	6 & over	12	4	4
	Not known	1	-	-
	Total	59	24	25

(a) Includes 4 deaths (see Table 14).

(b) Age-standardized 40-59.

*** Significant difference between Groups I and II ($P < 0.001$).

American Coronary Drug Project Research Group (1975) that clofibrate may have led to thromboembolic complications, a special examination of the death-record forms in the present study was carried out, under 'blind' conditions, to study whether deaths occurring after surgery, for example, might have been more commonly associated with thrombotic or embolic phenomena in Group I. The numbers for Groups I and II were not significantly different (6 *v* 3).

The differences in total mortality between Groups I and II were most obvious in Budapest and least in Edinburgh (Appendix 4) and this was most noticeable in death from causes other than IHD. As with deaths in the trial as a whole, however, the age-standardised mortality in Group III exceeded that in Group II.

WITHDRAWALS FOR MEDICAL REASONS

Table 15 shows the variety of conditions, some of

TABLE 17

Regional Pathology

Numbers of deaths in the trial and within 1 year of leaving it, at ages 30–59

Site of Pathology	Deaths from "Other Medical Causes"(a)			Deaths from Malignant Neoplasms			All Deaths at these sites		
	I	II	III	I	II	III	I	II	III
Liver	1	0	1	3	1	1	4	1	2
Gall bladder	3	0	1	1	0	0	4	0	1
Small intestine	0	0	0	1	0	0	1	0	0
Colon	0	0	0	6	5	3	6	5	3
Rectum	0	0	0	4	1	2	4	1	2
Total	4	0	2	15	7	6	19*	7*	8
Rates per 1000 per annum	-	-	-	-	-	-	0.57*	0.21*	0.25
Age-standardised rates (40–59)	-	-	-	-	-	-	0.75**	0.17**	0.65

(a) See Table 14.

* $P < 0.05$

** $P < 0.01$

which were suspected of being adverse reactions, which caused withdrawal from the trial. Cholelithiasis was not known as an adverse effect of clofibrate until half-way through the trial and was not listed as such. A preliminary report on the incidence of cholecystectomy for gall stones occurring in the trial has already been published (Cooper *et al.*, 1975). Table 16 (i) shows the final position, confirming beyond doubt the previous reports. Four of these men died in the perioperative period from sepsis or other complications, 3 in Group I and 1 in Group III.

'Indigestion', 'diarrhoea', and 'skin reactions' were specifically recorded at follow-up visits because they are well recognised side-effects of clofibrate. Table 15 shows that none of these three caused withdrawal from the trial more frequently from Group I than from the other groups. Weight gain, hair loss, muscle cramps, and cardiac arrhythmia are also listed because they have been questioned as possible adverse effects of clofibrate. Only weight gain accounts for a significant difference ($P < 0.05$) between Groups I and II, but the numbers are very small (9 *v* 2).

The category 'other' includes a variety of complaints such as thirst, insomnia, vertigo, headache, and intolerance to alcohol. The numbers were too small to allow for statistical appraisal of individual conditions.

The centres differed considerably in the number of subjects withdrawn for medical reasons (Addendum C). Numbers were greater in Prague than in either of the other two centres; the conditions where this excess was most noticeable were diabetes,

hypertension, 'other digestive' conditions, and infective hepatitis.

CONDITIONS NOT NECESSARILY CAUSING WITHDRAWAL FROM THE TRIAL

Such conditions, recorded as possible side-effects of treatment, are shown in Appendix 6.

Approximately 13 per cent of men reported one of the conditions listed on at least one occasion (distribution between Groups was 15, 12, and 12 per cent ($P < 0.01$)). Specific conditions are mentioned in the discussion. There were, however, substantial intercentre differences.

Various bizarre effects were attributed to the trial capsules, there being no significant difference in incidence between groups. Interestingly, increased sweating was reported as being more common in Group I than in Group II, but only at Edinburgh, where the difference in incidence was statistically significant (24 *v* 7, $P < 0.01$). No cases had to be withdrawn from the trial because of this symptom.

Discussion

PRINCIPAL FINDINGS

The trial has shown that healthy men, aged 30 to 59, with moderately raised serum cholesterol levels who were treated with the cholesterol-lowering drug clofibrate had significantly fewer major IHD events than comparable controls ($P < 0.05$). This reduction was confined to non-fatal myocardial infarcts ($P < 0.05$). The incidence of fatal first IHD events, whether within or after 3 hours of onset of symptoms, did not differ significantly in the two groups. There were in the trial and within 1 year of leaving it significantly more deaths from all causes in those treated with clofibrate compared with the control groups (162, 127, and 93). This excess occurred mainly in the deaths from causes which were not due to IHD, other vascular disease, or accidents (77, 47, and 49 deaths).

Reviewing the assumptions made in planning this trial, the actual fall in serum cholesterol was less than the initial forecast of 15 per cent; the incidence of IHD at ages 30 to 59 in the high cholesterol control group was 0·74 per cent compared with a predicted 1 per cent; the reduction in incidence of IHD achieved in the treated group was 20 per cent compared with 30 per cent on which the original calculations were based. This was significant at the 5 and not the 1 per cent level. The total number of men recruited, however, was 15 745 compared with 15 000 and the number of man-years experienced 83 534 against the postulated 75 000. The period of study was, of course, not 5 years for each individual though the average length of time in the study was 5·3 years: some were in the trial [illegible] years and a few for only 4. The actual percentage of men lost to the trial for all reasons in the first 5 years was 31 per cent compared with the assumed 30 per cent.

ISCHAEMIC HEART DISEASE

(a) Mortality

The failure of clofibrate to reduce the incidence of fatal heart attacks is disappointing. The prevention of non-fatal infarction, however, might be expected to result in improved long-term mortality, since men with a history of myocardial infarction have a worse prognosis than those without such a history. Mortality from IHD, particularly early death, was not reduced in other primary prevention trials using a diet rich in polyunsaturated fats (Dayton *et al.*, 1969; Miettinen *et al.*, 1972); and the Albany-Framingham study has shown that the incidence of sudden death bears no relation to prior serum cholesterol levels (Kannel *et al.*, 1975). The mechanisms responsible for such deaths are probably less directly related to serum cholesterol change and the amount of coronary atheroma than those leading to non-fatal myocardial infarction. Deaths within 3 hours of the onset of symptoms are mostly the result of primary ventricular arrhythmias, probably resulting from acute myocardial ischaemia. Deaths more than 3 hours and less than 28 days after onset of symptoms are mostly related to the extent of the infarct itself and are the result of secondary arrhythmias, cardiogenic shock, heart failure, and rupture of the ventricle.

(b) Non-fatal myocardial infarcts

The results reported relate only to events occurring during the trial, when the men were taking the capsules. No systematic attempt was made to ascertain non-fatal myocardial infarcts after the end of treatment. Available data were examined, however, to identify any 'rebound' effect after withdrawal of clofibrate and there was no difference between Groups I and II in the incidence of total IHD after 3 months, 6 months, or 1 year.

Most of the difference in incidence of non-fatal myocardial infarction between Groups I and II appears to be related to cholesterol reduction. The effect of reducing cholesterol with clofibrate was to lower the incidence whatever the initial concentration and this is well illustrated by comparing Groups I and II in Table 8. Men with high initial serum cholesterol concentrations had a worse prognosis in both Groups, whatever changes occurred during the trial period. The highest incidence rates for all IHD in the high cholesterol control group occurred in those with initial concentrations above

the median, that is in the upper sixth of the total cholesterol distribution for the populations studied. Cholesterol 'non-responders' comprised a substantial minority (about a sixth) of Group I, and the higher incidence rates of IHD events in these partly dilutes the results for the Group as a whole. Under conditions of ordinary medical practice such patients would be considered as 'failures', and an unsatisfactory regimen would be replaced with a potentially better one.

The extent to which concurrent reduction of elevated serum triglycerides could have contributed to the reduction in non-fatal IHD cannot be assessed from our data.

(c) Optimal effects of clofibrate on major IHD events

An attempt was made to identify those most likely to benefit from clofibrate treatment. In this trial, they were men who had a reduction of serum cholesterol on treatment and had a systolic blood pressure of over 135 mmHg and who smoked. This subgroup, comprising men with a mean age of 46·5 years and a mean initial serum cholesterol of 258 mg/100 ml had a 34 per cent reduction in major IHD events compared with a 23 per cent reduction in the remainder in Group I. These reductions in incidence were associated with mean serum cholesterol changes of 12 per cent and 8 per cent, respectively. This subgroup represents, at most, 6 per cent of the men in the age range concerned.

(d) Angina and myocardial ischaemia

The incidences of angina pectoris occurring during the trial, whether with or without supporting electrocardiographic evidence, and of electrocardiographic signs of myocardial ischaemia in the absence of chest pain, were similar in Groups I and II. A decreased rate of development of the earliest symptoms of IHD might have been expected if clofibrate slowed the pace of narrowing in the coronary-arteries. This was suggested in the results of the Scottish and Newcastle trials of clofibrate in patients with angina (Dewar and Oliver, 1971). But neither was specifically designed to test the possibility and a new trial was proposed. Results of the current study do not help to answer this question.

MORTALITY FROM ALL CAUSES

There were 162 deaths in the clofibrate-treated group during and in the first year after the trial compared with 127 in the high cholesterol control group and 93 in Group III (Table 12). The excess of 35 deaths between Groups I and II is significant ($P < 0·05$); 6 of the 35 were due to IHD and 29 to causes not usually associated with serum cholesterol concentrations or changes. The difference in overall crude rates was also significant ($P < 0·05$). The design of the trial provided a second control group (Group III) which, though chosen on the basis of relatively low initial cholesterol levels, should also be used for such comparison provided that correction is made for the fact that men in this group were slightly younger than those in Groups I and II. Age-standardised rates have been used for comparison for this purpose, since the multiple logistic analysis shows that correcting for other factors as well as age adds very little. There was no significant difference in age-standardised mortality from all causes between any of the Groups.

Numbers of deaths due to other vascular causes and from accidents and violence were similar in Groups I and II. The greater part of the excess in Group I was distributed over a wide range of medical conditions classified in Table 12 under 'malignant neoplasms' and 'other medical causes' and detailed further in Table 14. These two main categories, taken together, show an excess in Group I over Group II (77 *v* 47; $P < 0·01$), and the crude rates were also significantly in excess ($P < 0·01$). Though there was a significant difference in age-standardised rates (3·1 and 1·9; $P < 0·05$) between Groups I and II, there was virtually none between Groups I and III (3·1 and 2·9), suggesting that the rates in Group II may be spuriously low.

(a) Malignant neoplasms

The excess in Group I over Group II of deaths due to cancer occurred mostly during the trial (40 *v* 24), and not in ex-trial deaths either within or after 1 year (38 *v* 31) or in those with diagnosed cancer still alive at the end of the trial (28 *v* 30). While neither the numbers nor the crude rates are significantly in excess, this might suggest that, while not being carcinogenic, clofibrate could have accelerated pre-existing cancer and have led to earlier death. But the cumulative percentages of cancer deaths occurring in Group I compared with Group II at different times after entry are almost identical (Fig. 6) and what difference there is suggests a later, rather than an earlier, mortality due to cancer in Group I. The age-standardised death rates in the three groups were 2·2, 1·7, and 2·5. The corresponding age-standardised rates for deaths from malignant neoplasms calculated from the official mortality statistics for men of the same age in the Edinburgh area, Budapest, and Prague are as follows: 2·2 (East Central and South Scotland, 1969-73), 2·6 Budapest (1972-74), 2·8 Prague (1968-73). These figures are surprisingly close to the rates observed in trial subjects in Groups I and III.

Thus the data for all cancer do not give rise to special concern.

(b) 'Other medical causes'

There was a significant excess in deaths and crude death rates from 'other medical causes' in the clofibrate-treated group compared with the high cholesterol control group (16 and 5; 0·5 and 0·2; $P < 0·05$). This excess was most evident in Budapest and virtually absent in Edinburgh (Appendix 4). The age-standardised death rate in the clofibrate-treated group was significantly higher than in the high cholesterol control group but not significantly greater than in the low cholesterol control group.

Though the numbers are small, these results have caused much concern and particular care has been given to accurate assessment of necropsy reports, which were interpreted without knowledge of treatment groups by 2 observers. Of the 28 patients dying from 'other medical causes', 25 had necropsies performed.

Regional pathology

There were more deaths from diseases of the liver, gall bladder, and intestines, including malignant neoplasms of these sites, in the clofibrate-treated group than in the high cholesterol control group. Taken together with the significant excess in the treated group compared with both control groups of cholecystectomies for gall stones, there was therefore a possibility that clofibrate might be producing pathology in this area. The relevant mortality data are collected in Table 17. The difference in numbers of deaths between Groups I and II (19 *v* 7) is significant ($P < 0·05$) as also is the difference between age-standardised rates ($P < 0·01$). On the other hand, the similarity of the age-standardised rates in Groups I and III gives some reassurance, suggesting that the low rate in Group II may have been fortuitous. The numbers concerned are small. This shows up in the contrast between the crude and age-standardised rates for Group III. The proportions of all deaths not due to IHD but due to pathology of the liver, gall bladder, and intestines were 19/108 = 18 per cent in Group I, 7/79 = 9 per cent in Group II, and 8/73 = 11 per cent in Group III. On entry, men who subsequently died from the causes shown in Table 17 were, on average, a few years older, slightly heavier, and more likely to have fathers who have died than the rest of the men in the same treatment group.

Although the statistical impropriety of retrospectively selecting a particular subgroup for analysis is fully recognised, investigators have to draw attention to any unexpected biological finding which might relate to the conclusions as a whole. A plausible biological hypothesis can be stated in support of the subgrouping shown in Table 17, and no other subgrouping of data of major endpoints (other than IHD) has shown significant differences between the clofibrate-treated and high cholesterol control groups. The hypothesis is that clofibrate, through mobilisation of cholesterol from tissue pools (including arteries) resulting in excretion of cholesterol and related sterols, could contribute to liver, biliary, and intestinal pathology.

The mechanisms through which clofibrate might do this are not understood. It has been shown that after 3 years of daily administration of the drug, there is continued increase in excretion of cholesterol into the bile. There is also *enhanced* excretion of endogenous neutral sterols and this exceeds a *decrease* in acid sterol excretion (Grundy *et al.*, 1972). No study has been reported of the relation of increased neutral sterol excretion to large bowel pathology, though *raised* faecal acid sterol concentrations have been implicated in the pathogenesis of cancer of the colon (Hill *et al.*, 1975). Other possibilities are that clofibrate has some effect in altering intestinal bacterial flora; or that directly or indirectly it causes aromatisation of certain bile acids (Hill, 1977), leading to tissue damage. Another potent hypolipidaemic drug, nicotinic acid, also increases neutral sterol excretion (Einarsson *et al.*, 1977).

In the Los Angeles Veterans Administration Study (Dayton *et al.*, 1969), there were more deaths from non-atherosclerotic causes in the group receiving a polyunsaturated fat diet than in an untreated control group. In particular, there were more deaths caused by malignant disease in the treated compared with the control group (31 *v* 17), but further analysis of the results of this and other dietary studies disclosed no significant excess incidence of cancer in the experimental groups (Ederer *et al.*, 1971). S. Dayton (1978, personal communication) has recently reanalysed his data and there was no evidence of excess pathology, including cancer, specifically in the liver, biliary, and intestinal systems in the diet group compared with controls. In the Helsinki Mental Hospital Study of the primary prevention of IHD (Miettinen *et al.*, 1972) there was also a non-significant excess in total mortality among the experimental group taking a polyunsaturated fat diet. Age-adjusted death rates/1000 person-years for malignant neoplasms (5·02 diet *v* 3·96 control) and 'other diseases' (15·45 diet *v* 13·03 control) were both greater in men receiving the cholesterol-lowering diet. No data are available concerning regional pathology.

MORBIDITY

Cholecystectomies

The figures for cholecystectomy operations for gall

stones shown in Table 16 confirm our own (Cooper *et al.*, 1975) and other (Coronary Drug Project, 1975) reports that gall stone formation is an adverse effect of clofibrate therapy. Interestingly, the difference in incidence of cholecystectomy between Groups I and II appears to be greater, though not significantly, in the first 4 years of clofibrate treatment than later and does not increase with time. (Table 16 (ii)).

No attempt was made in the trial to determine the prevalence of gall stones, but clofibrate is known to decrease bile solubility leading to greater lithogenicity (Pertsemlidis *et al.*, 1974). Diets with a high polyunsaturated/saturated fat ratio have also been reported to increase the incidence of gall stones (Sturdevant *et al.*, 1973). Nicotinic acid also increases cholesterol saturation of bile and may increase the risk of gall stone formation (Leijd *et al.*, 1978). Perhaps any procedure that promotes cholesterol excretion can lead to gall stone formation.

Hypertension

There were significantly fewer notifications ($P < 0{\cdot}05$) and withdrawals ($P < 0{\cdot}01$) because of hypertension in Group I compared with Group II (Table 5). The explanation of this is not clear, though clofibrate may have prevented the development of hypertension in some patients. Support for this suggestion comes from the relatively weak relation in Group I between systolic or diastolic blood pressure at entry to the trial and the incidence of IHD. This might be expected if the beneficial effects of clofibrate were stronger than the adverse effects of developing hypertension as shown by the expected relation between blood pressure and prognosis in Group II. However, clofibrate did not appreciably lower blood pressure in Group I as a whole.

Intermittent claudication

There was no significant difference in the reported incidence of intermittent claudication. A clinical study, conducted without knowledge of treatment groups, confirmed that there was no difference between the groups in signs of peripheral arterial disease.

Cardiac arrhythmias

The incidence of cardiac arrhythmias was not significantly different in Groups I and II. Electrocardiograms, however, were recorded only from the time taken to complete a 12-lead record and not for longer periods for rhythm detection.

Cerebrovascular events

There was no significant difference in fatal or non-fatal cerebrovascular events between Groups I and II. The combined figures for the 3 Groups were 27, 23, 19.

Thromboembolic events

The slight excess in Group I compared with Group II of cases of non-fatal thromboembolism causing withdrawal from the trial (7 *v* 2) was not reflected in an excess in fatal thromboembolism (4 and 4).

Diabetes mellitus

The apparent excess in Group I compared with Group II in 'diabetes mellitus requiring drug treatment' requires comment. Glucuronic acid, which can result from clofibrate glucuronide, can cause false positive urine tests but would not affect glucose tolerance tests. A special investigation during the terminal stages of the trial was carried out in Prague, which accounted for more than half of the cases. All men showing glycosuria in Prague had been referred to a department of diabetes for glucose tolerance tests and a decision regarding treatment, and possibly this accounted for part of the higher number of cases there. On final review a few cases were not confirmed. However, withdrawals at Budapest were also more numerous in Group I than Group II, though not significantly so.

These findings are puzzling in view of the number of published reports indicating that clofibrate has a beneficial effect on glucose tolerance and probably on insulin sensitivity (Barnett *et al.*, 1977; Enger *et al.*, 1977; Lithell *et al.*, 1977). The latter two studies involved men with clinically latent diabetes discovered during a health screening programme and are, therefore, perhaps comparable with our own. The long-term effects of clofibrate treatment on diabetes in 50 hyperlipidaemic patients followed for 6 to 8 years were favourable (Berkowitz, 1971).

Withdrwals and reported side effects

Significantly more of the few men who were withdrawn from the trial for weight gain were in Group I than in Group II (Table 15), and significantly more men in Group I reported weight gain (Appendix 6). In fact, however, differences between mean weights during the trial for all men in the 2 groups were trivial. Impotence was also reported significantly more often by men in Group I than in Group II but no attempt has been made to establish its real incidence. Diabetes has been discussed above. Gastrointestinal symptoms and diarrhoea were also reported significantly more commonly in Group I but were transient or quickly reversed on stopping medication.

TRIGLYCERIDES AND LIPOPROTEINS

The absence of analyses of serum triglycerides or of lipoprotein fractions at the start of the trial requires comment. This trial was initiated in the 3 centres between 1965 and 1968 and methods for serum triglyceride and lipoprotein analysis were still being devcloped at that time; automated systems for estimation of plasma lipids were not available to the centres at the start of the trial. The clinical importance of specific lipoprotein types was first described after the start of the trial (Frederickson *et al.*, 1967) and internationally standardised by the World Health Organisation only in 1970. It is one of the fallibilities of long-term trials that methodology can improve to such an extent that the original design appears inadequate.

As mentioned earlier, serum triglycerides were measured in one centre only and the results confirm the well-known observation that clofibrate reduces serum triglycerides even more than serum cholesterol. It is particularly interesting that the concentrations in the treated group approximate to those in the low cholesterol control group. In view of the suggested importance of raised serum triglycerides as a risk factor for IHD (Brown *et al.*, 1965; Carlson and Böttiger, 1972), their obvious reduction by clofibrate may also have contributed to the reduction of non-fatal IHD events.

CHARACTERISTICS OF CONTROL GROUPS

A low and a high cholesterol control group were included in the trial to provide additional means of appraising the response of the treatment group. For example, the importance to the community of changes in IHD incidence would be enhanced if the difference between treated and control high-risk groups was such that the incidence in the treated group approximated that of a low-risk control group. The inclusion of putatively high and low risk control groups also provides actual evidence for the community under study that the groups chosen do have a spectrum of risk.

The incidence of IHD in the high cholesterol control group was less than predicted. A degree of 'placebo response' is to be expected but this might not affect high and low risk groups equally. Attention to their health by attendance at clinics, blood tests, and the daily swallowing of capsules is likely to lead to 'correction' of more adverse habits in the group with the most adverse habits. Thus, the high cholesterol control group might be likely to alter the diet, stop their higher cigarette smoking, and increase physical activity more than the low cholesterol control group. Such changes could militate against being able to show highly significant differences compared with a treated high-risk group. Though we suspect that the rates in Group II for some major end-points not related to IHD and vascular events may be unrepresentatively low, this cannot be proved because the actual rates for these end-points are not known specifically for high cholesterol populations.

The men in Group III—as well as having low serum cholesterol levels—were younger, lighter in weight, had lower blood pressure, and smoked less. As expected, their risk of IHD was relatively low. To some extent, the mortality figures for IHD for this group are reassuring when assessing the meaning of the differences between Groups I and II, and the age-standardised rates for deaths from causes other than IHD are more closely related in all categories to those for Group I rather than Group II.

CONDUCT OF THE TRIAL

From an early phase of the study, there was an unfavourable trend in mortality from all causes. By the end of 1972, this came near to the 10 per cent significance level on a sequential basis and the principal investigators were informed. During 1973 the figures improved somewhat, but by the end of 1974 the unfavourable trend in overall mortality was resumed though still not reaching the 10 per cent level. Because of this, and the fact that an interim assessment suggested that there were disappointing benefits in terms of the main objectives of the study—reduction of the incidence of all IHD events—the possibility was seriously considered in 1975 that the trial should be terminated prematurely and the findings reported.

It was agreed, however, that an intensive study should first be made into the deaths from cancer, as there was a suggestion at that time of an excess in the mortality figures in Group I. This involved a major investigation into available information on morbidity as well as mortality of men in the trial, and men who had left the trial, including reviews of all relevant clinical and necropsy records, searches of national and local cancer registers, and reappraisal of all the death certificate data. The results of this inquiry reported in Tables 12 and 13 and Fig. 6 are reassuring.

The investigators were agreed that premature closure of the trial and release of results was inadvisable until the special study of cancer was complete. Meanwhile, the adverse overall trend of mortality in Group I had continued and it neared the 5 per cent significance level late in 1976. By this time, the trial was already being closed and this process was accelerated.

These developments presented the investigators with difficult and anxious decisions and they emphasise that it is mandatory in such long-term trials to apply rigorous monitoring of all supposedly therapeutic regimens.

OTHER PRIMARY PREVENTION TRIALS

The results of our study are in some accord with those of the only other published trial of the primary prevention of ischaemic heart disease using clofibrate. But this was an incompletely randomised study, not conducted 'blind', and the first report (Krasno and Kidera, 1972) has been severely criticised (Feinstein, 1972). The greater reduction in incidence of non-fatal myocardial infarction was associated with a reduction in serum cholesterol of 40 mg/100 ml (L. R. Krasno, 1978, personal communication). This was achieved by a high degree of rapport between the physicians and the subjects, who were fully aware of their individual cholesterol levels.

The results of the only other large long-term primary prevention trial (Miettinen *et al.*, 1972) are relevant to the findings of the present trial. Mean serum cholesterol reduction in middle-aged men and women in two mental hospitals, given a diet with polyunsaturated/saturated (P/S) ratio of 1·42 to 1·73 on a cross-over design, ranged from 12 to 18 per cent compared with the control periods when they received a normal hospital diet. When the results for the treated groups were pooled, there was a significant reduction in IHD mortality ($P < 0·02$): no data are available concerning morbidity. There are, however, weaknesses in the design and conduct of the Helsinki Mental Hospital Study and the authors refer carefully to some of these. Others have been emphasised by Halperin *et al.* (1973), who concluded that the data 'may not be of sufficient strength . . . to establish the diet hypothesis and to draw the authors' conclusions'.

Another relevant carefully controlled long-term prevention trial is the Los Angeles Veterans Administration Study (Dayton *et al.*, 1969). This used a double-blind design but was not exclusively a primary prevention study and included, as a minority, patients with pre-existing complications of atherosclerosis. It was conducted in an elderly male population and the effects of a diet high in polyunsaturated fats and low in saturated fats were compared with a control diet comprising fat calories mostly of animal origin. The difference in serum cholesterol between the treated and control groups was 12·7 per cent. There was no difference in the incidence of fatal events, whether sudden or not, but there was a 31 per cent decrease in non-fatal myocardial infarction. This was not statistically significant, but pooling of the data in all subjects (primary and secondary prevention) for all other vascular events, particularly cerebral infarction, showed significant reduction in the 8-year incidence rates in the diet group ($P = 0·01$). There were 85/424 deaths resulting from non-atherosclerotic causes in the diet group and 71/422 in the control group.

SECONDARY PREVENTION TRIALS USING CLOFIBRATE

The results in terms of IHD of secondary prevention diet and drug trials are not directly relevant to those of this trial and to the primary prevention trials briefly described above, because the natural history of the development of IHD has probably already been determined by the occurrence of myocardial infarction and because of the inevitable selection of the populations studied. But brief mention is made of non-cardiovascular deaths in the only two which used clofibrate.

In the Coronary Drug Project (1975), the percentages (based on 5-year rates) who died from a non-cardiovascular event was 2·1 in the clofibrate-treated group, 2·1 in the nicotinic acid-treated group, and 1·5 in the placebo group. Cancer deaths were equally distributed, giving a slight excess of non-cardiovascular and non-cancer deaths in the treated groups (1·5% and 1·5%) over the placebo group (0·9%); the slight and non-significant excess in the clofibrate group only became evident during the seventh year of treatment.

The secondary prevention trials using clofibrate (Physicians of the Newcastle-upon-Tyne Region, 1971; Research Committee of the Scottish Society of Physicians, 1971) did not give any data concerning non-cardiovascular deaths.

GENERAL COMMENTS

Clofibrate produced only a modest reduction in the mean serum cholesterol level and this was matched by a similarly modest but significant reduction in the number of IHD events, confined to non-fatal myocardial infarcts. There are a number of factors which might have militated against a more striking preventive effect than shown by this trial.

(1) Reduction of plasma lipids might have been too small.

(2) The partial control of one risk factor might not be expected to produce a significant yield in the face of persistence of other risk factors.

(3) The duration of lipid lowering might not have been long enough to influence long-standing coronary artery disease.

(4) Reduction of plasma lipids might have been started too late in life.

(5) Adherence to treatment might have been incomplete, though plasma levels of clofibrate do not suggest this. It is widely assumed and is part of the basis of many national health education campaigns that a greater reduction in serum cholesterol would be associated with a greater effect on IHD incidence.

But this is difficult to achieve. Unfortunately, there is a sharp division in experience between those who treat patients or individuals through the aegis of a major hospital or lipid clinic, where it is possible to achieve good control of raised plasma lipids, and those who have attempted to control them over long periods in the community. Perhaps it is realistic to conclude that only partial control is readily obtainable in any setting other than one where there is close and regular surveillance, and that this may not be enough.

The excess in total mortality has much concerned us. The mortality from diseases of the liver, biliary tract, and intestines may be a fortuitous association, and, indeed, the number of deaths involved is small, but it could also be due either to clofibrate itself or relate to the effects of the drug in promoting excretion of sterols by way of these tissues. If this speculation is true, the implications for other lipid-lowering regimens are plain. Attention should be paid, therefore, to the possiblity of pathology arising in these tissues with the long-term administration of other regimens that promote excretion of bile sterols.

The investigators are grateful to the men who have participated in this long trial for their willing and patient co-operation.

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The names of technical, computing, and clerical staff are available on request (Addendum F).

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[1]LIST OF ADDENDA AVAILABLE ON REQUEST

A. Clinical data forms and questionnaires
B. Serum cholesterol methods
C. Centre differences
D. Population at risk by 5-year age-group and centre
E. Deaths more than one year out of trial
F. List of technical, computing, and clerical staff

LIST OF APPENDICES

1. Criteria for rejection and withdrawal
2. Statistical methods
3. Definition of end-points
4. Centre differences
5. Drug adherence
6. Conditions not necessarily causing withdrawal but recorded as possible side-effects

APPENDIX 1

CRITERIA FOR REJECTION AND WITHDRAWAL

Rejection from Entry into the Trial

1. History of treated myocardial infarction with ECG signs (see 2a and b below) and/or enzyme changes (using local methods and standards). Previous ECG and hospital records must be examined. An unsubstantiated history of myocardial infarction is not a cause for rejection.

2. ECG evidence of heart disease (Minnesota code system):
   (a) ECG evidence of myocardial infarction or widespread myocardial ischaemia; i.e. category 1-1, 1-2, 4-1, 5-1, 6-1 or 9-6 (as defined).
   (b) Complete left bundle branch block: 7-1.
   (c) "Lone" atrial fibrillation or flutter.
   (d) Multiple (more than 4 in 12 complexes) or bifocal ventricular extrasystoles.

3. Systemic hypertension:
   (a) A diastolic blood pressure of 120 or greater on any one occasion.
   (b) A diastolic blood pressure of 110-119 on any two occasions.
   (c) A diastolic blood pressure of 110-119 on any one occasion if accompanied by ECG signs of left ventricular hypertrophy, or strain; i.e. 3-1, or 3-3 + 5-2 or + 5-3.
   (d) If the diastolic blood pressure is within the accepted limits but only on account of treatment with anti-hypertensive drugs and the ECG shows signs of left ventricular hypertroply or strain; i.e. 3-1, or 3-3 + 5-1 or + 5-2 or + 5-3.

4. Clinical evidence of rheumatic heart disease.

5. Congenital heart disease.

6. Pulmonary heart disease - as defined by the World Health Organisation (1961). bronchitis, emphysema or kyphoscoliosis when associated with ECG signs of right ventricular hypertrophy or strain - 2-2, 3-2 , 7-2 and 7-3.

7. Other heart disease associated with cardiomegaly or heart failure.

8. Diabetes mellitus requiring drug treatment.

9. Co-existing disease with an unfavourable prognosis reducing likelihood of completion of trial:
   (1) Malignant disease.
   (2) Residual paralysis due to cerebral damage with or without hypertension.
   (3) Chronic advanced renal disease with systemic manifestations.
   (4) Cirrhosis of liver with systemic manifestations.

NOTE: A positive answer to the effort chest pain questionnaire alone or coronary insufficiency (as defined) are not indications for rejection.

Withdrawal after Admission to the Trial

1. Myocardial infarction - see "Definition of end-points" in Appendix 3.

2. Hypertension - as for the criteria for rejection, until November 1973. Thereafter hypertensive men were kept in the trial and treated where necessary.

3. Other heart disease - which has previously not been recognised - as defined under rejection criteria 5, 6 and 7.

4. Diabetes mellitus - requiring drug treatment.

5. Contraindications for taking capsules:
   (a) Side-effects which cannot be tolerated by individual.
   (b) Infective hepatitis and cirrhosis.
   (c) Advanced renal disease.
   (d) Agranulocytosis or thrombocytopenic purpura.

APPENDIX 2

STATISTICAL METHODS

Incidence and Mortality Rates are expressed per 1000 per annum i.e. as

$$\frac{\text{the number of new events (or deaths)}}{\text{man-years of exposure}} \times 1000$$

Man-years of exposure were calculated separately, for each man, as the time, in years and fractions of a year, from admission to withdrawal from the trial for in-trial rates, and from admission to withdrawal + 1 year for rates of events occurring in the trial or within one year after withdrawal.

Date of Withdrawal was defined as the date of the last trial visit for men who were in the trial when it stopped, but otherwise as the date of the event which removed the man from the trial - e.g. death, myocardial infarction, withdrawal for medical reasons, or of explicitly "opting out". For men who were "lost to follow-up" the date was taken as 9 months from the date of the last trial visit, during the period when visits were at 6-monthly intervals, and as 1½ years from the date of the last trial visit in the period when visits were at 12-monthly intervals. As stated in the text, a similar principle was adopted for deciding whether a death occurred "in the trial" or "out of the trial".

Age-standardisation was by simple averaging of the death-rates in five-year age-groups. "All-centre" rates were standardised over the range 40-59 years, but because there were only 57 men aged 54-59 on admission in Prague (Addendum D), individual centre rates were standardised over the range 40-54 years, thus enabling them to be compared.

This method of standardisation was adopted, after much discussion, as the simplest and clearest way of dealing with a situation made difficult by the difference in age-structure of the trial populations in the several centres. The use of equal weights for the 5-year age-groups corresponded reasonably closely with the age-distributions of the populations from which the men were drawn (as opposed to the trial populations) in each centre. Any more detailed weighting system (e.g. by centre and age distributions of the trial population) would have produced very variable rates in the individual cells and thus high standard errors. This also was one of the main reasons for excluding from the age-standardisation deaths in the range 30-39. The other reason was the absence of any men in Prague in this age-range.

Life Table The life-table data are calculated by standard methods, using intervals of 1 year from admission. The tests of significance between survivorship curves is a χ^2 test with 1 degree of freedom and follows the approach of Mantel (1966) and Cox (1972), using a constant hazard rates model. That is to say it tests the difference between the shapes of the entire curves rather than the percentage surviving at a particular period after admission. In this it is similar to the log-rank test but uses grouped intervals rather than precise times.

The following tables give the percentages free from various events at different stages in the study, calculated on a life-table basis and shown in Figures 2 and 3 or mentioned in the text, together with the standard errors of these rates and the results of the overall tests of significance.

Significance tests The significance of the difference between the numbers of events in Groups I and II were calculated by the binomial formula on the expectation that they should be equal. Slight differences in numbers exposed to risk were ignored.

In comparing standarised rates the variance of the standardised rate was assumed to be the sum of the variances in the separate 5-year age-groups, appropriately weighted. Thus, for standardisation at ages 40-59, let r_i be the rate and d_i the number of events in age-group i, where i = 1,2,3,4, corresponding to ages 40-44, 45-49, 50-54, 55-59, respectively. The variance of r_i was taken to be approximately r_i^2/d_i. The standardised rate is, thus, $\frac{1}{4}\sum_{i=1}^{4} r_i$

and its variance $\frac{1}{16}\sum r_i^2/d_i$

Formulae for Cholesterol Change Let C_{1x} indicate the serum cholesterol level of a man in Group I at the xth visit, and C_{1p} the mean of his pre-treatment cholesterol levels. Let C_{2x} and C_{2p} be the corresponding values for a man in Group II. Let $\overline{C}_{1x}$ be the mean of the serum cholesterol levels at the xth visit for all men in Group II in the same centre, and $\overline{C}_{2p}$ the corresponding mean of pre-treatment cholesterol levels.

Life Table "Survivorship" Data

The figures are percentages free of the event indicated ± the standard error of this percentage.

Years in Trial	IN-TRIAL								
	All I.H.D.			Fatal I.H.D.			Non-fatal I.H.D.		
	I	II	III	I	II	III	I	II	III
0	100	100	100	100	100	100	100	100	100
1	99.5±0.10	99.4±0.11	99.9±0.05	99.9±0.05	99.9±0.03	99.9±0.03	99.6±0.08	99.5±0.10	99.9±0.04
2	98.9±0.15	98.7±0.16	99.6±0.09	99.7±0.07	99.8±0.06	99.9±0.04	99.1±0.13	98.8±0.15	99.6±0.09
3	98.3±0.19	98.2±0.19	99.3±0.12	99.6±0.09	99.7±0.08	99.9±0.05	98.7±0.16	98.5±0.18	99.5±0.11
4	97.9±0.21	97.3±0.24	99.2±0.13	99.6±0.10	99.6±0.10	99.8±0.07	98.3±0.19	97.8±0.22	99.4±0.12
5	97.3±0.24	96.7±0.27	99.0±0.15	99.5±0.11	99.4±0.11	99.7±0.08	97.9±0.22	97.2±0.24	99.3±0.13
6	96.8±0.27	95.7±0.31	98.8±0.17	99.3±0.13	99.3±0.13	99.7±0.08	97.4±0.24	96.4±0.29	99.0±0.15
7	95.8±0.35	94.8±0.37	98.3±0.23	99.0±0.17	99.2±0.16	99.5±0.14	96.7±0.31	95.6±0.35	98.8±0.19
8	95.1±0.46	94.0±0.50	98.1±0.28	99.0±0.17	99.0±0.21	99.5±0.14	96.0±0.44	94.9±0.46	98.6±0.24
Comparison of Groups	χ^2	P		χ^2	P		χ^2	P	
I & II	4.583	< 0.05		0.056	> 0.05		6.173	< 0.05	
I & III	52.787	< 0.01		7.280	< 0.01		46.522	< 0.01	
II & III	94.363	< 0.01		5.945	< 0.05		94.497	< 0.01	

Years since entered Trial	IN-TRIAL + 1 YEAR AFTER LEAVING					
	Total Mortality			Non I.H.D. Mortality		
	I	II	III	I	II	III
0	100	100	100	100	100	100
1	99.7±0.08	99.8±0.05	99.8±0.06	99.8±0.05	99.9±0.04	99.9±0.05
2	99.2±0.13	99.3±0.12	99.7±0.08	99.6±0.09	99.5±0.10	99.7±0.07
3	98.6±0.17	99.0±0.15	99.4±0.12	99.2±0.13	99.3±0.12	99.5±0.10
4	98.3±0.19	98.5±0.18	99.1±0.15	98.9±0.15	99.0±0.14	99.3±0.13
5	97.7±0.22	98.2±0.20	98.6±0.18	98.5±0.18	98.9±0.16	98.9±0.16
6	97.0±0.27	97.6±0.24	98.2±0.21	98.0±0.22	98.4±0.20	98.5±0.20
7	95.7±0.37	96.9±0.30	97.4±0.29	97.1±0.30	98.0±0.24	98.0±0.25
8	95.0±0.47	95.9±0.47	96.5±0.46	96.4±0.43	97.6±0.35	97.3±0.41
Comparison of Groups	χ^2	P		χ^2	P	
I & II	4.280	< 0.05		4.553	< 0.05	
I & III	17.033	< 0.01		5.849	< 0.05	
II & III	4.025	< 0.05		0.082	> 0.05	

Then the mean percentage change at the xth visit in all the men in that centre in Group II is

$$\frac{\bar{C}_{2p} - \bar{C}_{2x}}{\bar{C}_{2p}} \times 100,$$

and the cholesterol change at the xth visit of an individual in Group I is

$$\frac{C_{1p} - C_{1x}}{C_{1p}} \times 100$$

Thus the man's cholesterol change at visit x as defined in the text is

$$\left[\frac{C_{1p} - C_{1x}}{C_{1p}} - \frac{\bar{C}_{2p} - \bar{C}_{2x}}{\bar{C}_{2p}}\right] 100$$

or

$$\left[\frac{\bar{C}_{2x}}{\bar{C}_{2p}} - \frac{C_{1x}}{C_{1p}}\right] 100$$

The man's mean cholesterol change (or response) is the mean of this function for all the man's visits from the second onwards.

Individual changes in Groups II and III, similarly defined in terms of the mean change in Group II are:-

$$\left[\frac{\bar{C}_{2x}}{\bar{C}_{2p}} - \frac{C_{2x}}{C_{2p}}\right] 100 \quad \text{and} \quad \left[\frac{\bar{C}_{2x}}{\bar{C}_{2p}} - \frac{C_{3x}}{C_{3p}}\right]$$

by obvious extension of the notation to Group III.

Similarly mean changes (over all men at a given centre, at visit x) in Groups I and III were defined as

$$\left[\frac{\bar{C}_{2x}}{\bar{C}_{2p}} - \frac{\bar{C}_{1x}}{\bar{C}_{1p}}\right] 100 \quad \text{and} \quad \left[\frac{\bar{C}_{2x}}{\bar{C}_{2p}} - \frac{\bar{C}_{3x}}{\bar{C}_{3p}}\right] 100$$

respectively.

Mean In-trial Cholesterol Level The mean level (for all men in a treatment Group at a given centre and visit) in Groups I and III was derived by applying to the mean pre-treatment level the mean percentage change figure, derived as above to allow for changes in the average value of cholesterol levels from time to time in Group II. For Group II the observed levels were used.

These values were then averaged over all visits to give a mean in-trial cholesterol level for that Group and centre.

In order to be able to use the same scale of cholesterol values a further correction was made to adjust for initial differences between centres in the pre-treatment cholesterol levels. (As has been stated, the results of the exchange of serum samples shows that differences between centres in observed values were mainly methodological.)

This correction was made as follows:-
A single factor, f, was derived for each of the two centres (Budapest and Prague) relating its mean values to those for Edinburgh.

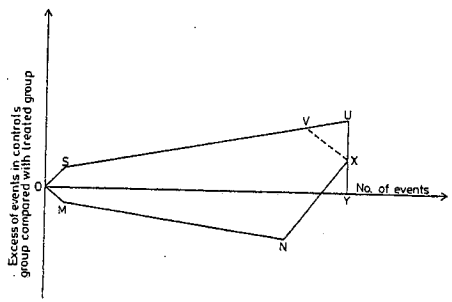
$$f = \frac{2x_1 + x_3}{2y_1 + y_3}$$

where x_1, y_1 = are the mean pre-treatment levels, at ages 40-59 for the combined Groups I and II for the centre (x_1) and for Edinburgh (y_1)

and x_3, y_3 = are the mean pre-treatment levels, at ages 40-59 for Group III for the centre (x_3) and for Edinburgh (y_3)

The values of f were 0.948 for Budapest and 0.874 for Prague and were used throughout to convert centre values to a common scale where this was required - e.g. in Figure 4, or in the multivariate analysis. These figures were also used to denote the "all centres" figures in the bottom line of Table 7.

Sequential Scheme for Monitoring of Results It was agreed that the results of the trial should be released to the principal investigators if, according to the sequential diagram in Appendix 2, Figure 1, a path crossed the line SVX indicating a result in favour of the treatment: or if one crossed the line MN, the early warning line, indicating a result against the treatment or if a line crossed NX representing a non-significant result. Any of these would be reported in the first instance to the Committee of Principal Investigators who would decide, on the basis of this and other information, whether the result should be further divulged and whether the trial should be stopped. This scheme applied to any class of event - e.g. all deaths, deaths from ischaemic heart disease, I.H.D., or any adverse side-effect.



Appendix 2: Fig. 1 *Excess of events in control group compared with treated group.*

The line SVU is taken from Armitage's set of closed sequential designs (Armitage, P. 1957, Biometrika, 44, 9-26) with $2\alpha = 0.01$, $\beta = 0.90$ and set to detect a 1.5:1 difference. The line MN is taken from the same set of designs, but with $2\alpha = 0.10$, $\beta = 0.90$ and set to detect the same difference. The point Y is set at 400 events, chosen to give a fixed sample result with $2\alpha = 0.01$ and $\beta = 0.90$ and able to detect a 1.5:1 difference and X is the point on the line YU such that any path which cuts YU above X gives a significant result. VX is at 45° to the vertical so that any path which cuts VX must cut YU above X. XN is also at 45° to the vertical so that no path which cuts NX can cut YU above X (and thus give a significant result).

The characteristics of this scheme are as follows:

(1) Effective (two-sided) significance level for a favourable result - 1.4% (347).

(2) Effective (two-sided) significance level for an unfavourable result - 9.4% (347).

(3) Power to detect an actual reduction of 33% in incidence in the treated group - 93% (218).

(4) Power to detect an unfavourable result of the same magnitude - 91% (110).

The figures in brackets are the median number of events required before a decision is reached (Heady, 1974).

Multivariate Analysis

The multiple logistic model was used (Walker & Duncan, 1967) the end-points being either any episode of major I.H.D., of non-fatal myocardial infarction or of fatal I.H.D., or death from causes other than I.H.D.

The following variables were used in the equations:

Variable	Units
Log_e(age)	Log (years)
Smoking	0 = never or ex- 1 = present
Father alive/dead	0 = alive 1 = dead
Treatment	1 = control (Group II only) 2 = clofibrate
Systolic blood pressure	mmHg
Corrected mean pre-treatment Cholesterol (defined in text)	mg/dl
Mean cholesterol change (defined in text)	percent

Except for the last, all these variables refer to entry characteristics.

In addition, the following variables were also examined in various combinations: age, number of cigarettes smoked, whether the subject's mother was alive, presence of I.H.D. in siblings, weight, height, diastolic blood pressure, presence of basal or apical heart murmur, arcus senilis, xanthelasma, xanthoma tuberosum, triceps and subscapular skinfold thickness, Quetelet index and positive chest-pain questionnaire, but their contribution to prediction was either non-significant or less than that of the selected variables (e.g. in the case of highly correlated measurements such as systolic and diastolic blood pressure).

The Table gives details of the coefficients (B), t value, standardised (B), and intercepts (α) fitted to the multiple logistic equations for the relationships between some or all of the main variables and various end-points in various treatment groups. The statistical significance of any coefficient is indicated by the "t" value (roughly, if t is greater than 2, or less than -2, the coefficient may be regarded as significant at the 5% level). To take account

of the different units used in comparing results with other studies and in assessing the relative importance of the variables in any one equation standardised β's should be used. They are derived by multiplying the β's by the standard deviation of the variables. In Tables 8 and 10, comparisons (e.g. between different sub-groups of high and low levels of 'risk' factors) have been based on the 'expected' incidence of events for a given set of baseline characteristics using the data derived from GroupII. Thus, for example, the expected probability of an individual experiencing (over the duration of the study) a given event (e.g. any I.H.D.), based on the data from Group II, would use the coefficients and intercept shown at No. 1 of the Table. The sum of such 'risk scores' for each individual in any group or sub-group, provides an estimate of the total number of specified events to be expected from the independent contributions of each of the baseline characteristics. This figure, because of the exponential distribution of individual risk scores, (and the iterative fitting procedure), overestimates (by 3-5%) the true incidence, and derived values have been corrected accordingly.

In the example quoted above, the calculated 'risk score' for I.H.D. of two individuals with the following baseline characteristics:

	Age	Smoking	Father	Systolic BP	Cholesterol
A:	40	0	Alive	125	220
B:	55	+	Dead	160	260

would be:- p(A) = 0.0072

p(B) = 0.1324

Where $p = \frac{1}{1 + e^{-(\alpha + \Sigma \beta_i x_i)}}$

x_i = value for each of i baseline characteristics

β_i = regression coefficient for baseline characteristic x_i

α = Intercept

In this (very limited) example the sum of the risk scores (which should be multiplied by the appropriate factor) gives the (uncorrected) expected total number of events (0.1396) expected to occur if there had been no effect of intervention.

Results from other studies

The unstandarised coefficients (β) for serum cholesterol in multivariate equations for the "prediction" of ischaemic heart disease from three other published studies are given below. In each study, men previously free of ischaemic heart disease have been followed for a number of years. These coefficients should be compared with the corresponding value from the first line of the Table in this Appendix. i.e, 0.008.

	Age range	β
Seven country study (Keys et al., 1972)	40-59	0.010
Israel (Medalie et al., 1973)	40-49	0.014
	50-59	0.007
Framingham (Kannel et al., 1964)	40-62	0.008

A cholesterol reduction of 9% (the mean value observed in this study) would correspond to a reduction of 20% in the incidence of I.H.D. if β is 0.10, to 16% if β is 0.008, and to 24% if β is 0.014.

It will be seen that the range of values of β quoted above is similar to the range seen in the first 7 lines of the Table in this Appendix where the equations for different combinations of treatment groups and variables included in the equations are shown.

Acknowledgement

The help and advice of Mr. David Clayton lately of the Department of Clinical Epidemiology and Social Medicine of the Royal Free Hospital School of Medicine in connection with the technical aspects of the life-table and multivariate analysis is gratefully acknowledged. The test of significance in the life table analysis was developed by him, and he wrote the associated computer programme as well as the programme to handle the multivariate analysis. In both respects his statistical advice was also invaluable.

No.	End-point	Group(s)	β, (t-value) and standardised β for variables							Intercept
			Log_e(Age) (log(yrs))	Smoking (0/1)	Father History (0/1)	Systolic BP (mmHg)	Cholesterol (mg/dl)	Cholesterol Change (%)	Treatment (1/2)	
1	All I.H.D.	II	2.76 (4.4) 0.40	0.87 (5.3) 0.43	0.48 (2.1) 0.21	0.014 (3.7) 0.25	0.0084 (3.4) 0.22			-18.707
2	All I.H.D.	III	5.21 (4.4) 0.85	1.08 (3.6) 0.54	-0.03 (-0.1) -0.01	0.018 (2.8) 0.31	0.017 (1.6) 0.21			-29.416
3	All I.H.D.	I	3.32 (4.5) 0.48	0.79 (4.3) 0.39	0.68 (3.0) 0.38	0.0064 (1.4) 0.11	0.011 (4.6) 0.29			-21.042
4	All I.H.D.	II/III	3.40 (6.1) 0.53	0.94 (6.5) 0.47	0.35 (1.8) 0.16	0.0153 (4.6) 0.23	0.012 (8.1) 0.51			-22.292
5	All I.H.D.	I/II	3.09 (6.1) 0.44	0.83 (6.5) 0.41	0.67 (3.6) 0.29	0.0126 (4.2) 0.22	0.010 (5.1) 0.27	0.023 (3.9) 0.24		-20.430
6	All I.H.D.	I/II	3.00 (6.3) 0.44	0.83 (6.8) 0.41	0.64 (3.6) 0.28	0.011 (3.7) 0.19	0.0095 (5.6) 0.26		-0.32 (-2.9) 0.16	-19.282
7	All I.H.D.	I/II	3.09 (6.1) 0.22	0.84 (6.5) 0.21	0.67 (3.6) 0.29	0.013 (4.2) 0.22	0.0099 (5.0) 0.26	0.019 (3.0) 0.20	-0.18 (-1.4) -0.09	-20.145
8	Non-fatal M.I.	II	2.02 (3.0) 0.29	0.89 (4.9) 0.44	0.71 (2.8) 0.31	0.014 (3.3) 0.24	0.0072 (2.6) 0.19			-15.875
9	Fatal I.H.D.	II	7.46 (4.1) 1.07	0.80 (2.1) 0.40	-0.64 (-1.4) -0.28	0.015 (1.7) 0.26	0.013 (2.5) 0.33			-39.128
10	Non-I.H.D. Deaths (in- and<1 yr ex- trial)	III	6.79 (5.8) 1.11	0.72 (2.8) 0.36	0.28 (0.8) 0.13	0.010 (1.6) 0.17	-0.012 (-1.8) -0.23			-30.347
11	Non-I.H.D. deaths (in- and<1 yr ex- trial)	III	6.88 (6.1) 1.13	0.70 (2.7) 0.35		0.0099 (1.5)				-32.472
12	Non-I.H.D. deaths (in- and<1 yr ex- trial)	III	7.10 (6.4) 1.16							-31.576

APPENDIX 3

DEFINITION OF END-POINTS OF VASCULAR DISEASES

Ischaemic heart disease

The term ischaemic heart disease (I.H.D.) as used in the trial comprises major and minor events defined as follows:

A. Major Events

(1) Fatal events - these include deaths from myocardial infarction (as defined below) and deaths where it is not possible with certainty to diagnose myocardial infarction, but surrounding circumstances suggest that this is the cause.

Fatal events have been sub-divided into those occurring within 3 hours of the onset of symptoms, and those occurring between 3 hours and 28 days of the onset of symptoms

(2) Non-fatal myocardial infarction (see below) surviving more than 28 days.

(3) Acute coronary insufficiency (see below) surviving more than 28 days. These were classified with non-fatal myocardial infarction in the final analysis.

B. Minor Events

(1) Angina pectoris (see below) with an abnormal ECG (Minnesota codes 4.1, 4.2, 5.1, 5.2, 6.1, 6.2, 7.1, 8.3, 11.1, 12.1, 14.1).

(2) Angina pectoris without one of the above codable abnormalities.

(3) An abnormal ECG (as defined above) developing during the course of the trial without symptoms of chest pain to warrant classification by questionnaire as angina pectoris.

Myocardial infarction

Myocardial infarction was considered to have occurred when:

(1) The resting ECG developed Q-wave changes, classified by the Minnesota code as 1.1 or 1.2, or 9.6 (a new category defined as follows: ST junction (J) and ST segment elevation of 1mm or more with an upward sloping ST segment and a negative or diphasic T wave, with the negative phase of at least 1mm. These changes must be present in at least two leads from V_2-V_6 and last for at least 3 days. The changes described were regarded as indicating recent intramural anterior myocardial infarction).

OR

(2) The resting ECG developed equivocal Q-wave changes (codable as 1.3) with elevated serum enzymes or with classical symptoms (prolonged chest pain of ½ hour or more, acute breathlessness or syncope).

OR

(3) There was a typical clinical picture with classical symptoms (see above) and elevated serum enzymes, but without definite ECG evidence. Consensus had to be reached on these cases at the Annual Review Meeting.

Elevated serum enzymes

These were interpreted in each centre according to the locally accepted ranges of normal and abnormal.

Acute coronary insufficiency

This was defined as classical chest pain lasting more than ½ hour at rest, with ECG abnormalities codable as 4.1, 5.1 or 7.1, but without elevation of serum enzymes.

Angina pectoris

This was said to be present when appropriate positive answers were given to item 23, "effort chest pain", on the follow-up form. (It was notifiable as newly arising in the trial only if the corresponding questions on the admission form were negative.) The answers to question 23 were said to be positive when positive answers were given to the first or second questions, "Have you ever had any pain or discomfort in your chest?" or "Have you ever had any pressure or heaviness in your chest?" and the third question, "Do you get any of these when you walk uphill or hurry?" In addition the following questions must have been completed in the manner shown below:-

(1) What do you do if you get it while you are walking?
Answer: stop or slow down.

(2) If you stand still what happens to it?
Answer: relieved.

(3) How soon?
Answer: 10 minutes or less.

(4) Will you show me where it is/was?
Answer: sternum (upper middle), sternum lower, left anterior chest plus left arm.

Exercise ECG

This was conducted on a bicycle ergometer using graded work loads of 50, 100 and 150 watts, each of 4 minute duration, according to W.H.O. standards.

An exercise test was done in all cases of angina (positive effort chest pain questionnaire) with normal resting ECG. An intra-exercise ECG was said to be positive if the horizontal or downward sloping segment was depressed 2mm or more; a post-exercise ECG was considered positive when codable as 11-1, 12-1 or 14-1 at any time in the period of 1 to 10 minutes after the test had been stopped. Intra- or post-exercise ECG's were also considered positive if extrasystoles appeared exceeding 10% of the number of observed complexes (based on all ECG readings during the exercise test).

Intermittent claudication

This was said to be present when appropriate positive answers were given to item 24, "leg [illegible] on the follow-up form (Addendum A). (It was notifiable as newly arising in the trial only if the corresponding questions on the admission form were negative.)

Hypertension

This was defined as:

(a) A diastolic blood pressure of 120 or greater on any one occasion.

(b) A diastolic blood pressure of 110-119 on any two occasions.

(c) A diastolic blood pressure of 110-119 on any one occasion if accompanied by ECG signs of left ventricular hypertrophy or strain (coded as 3.1, or 3.3 + 5.2, or as 3.3 + 5.3).

Cerebrovascular disease

No trial definitions were specified. Centres used their own conventions.

APPENDIX 4 CENTRE DIFFERENCES (See Table 3)

Incidence of Major Ischaemic Heart Disease (IHD), Non-fatal and Fatal, by Age at Entry

Rates per 1000 per annum

Edinburgh

Event	Age at Entry (years)	Group I Clofibrate		Group II High Cholesterol Control		Group III Low Cholesterol Control	
		No.	Rate	No.	Rate	No.	Rate
All Major IHD	30-	1	1.0	3	3.0	0	0.0
	35-	6	3.7	6	3.3	2	1.0
	40-	5	2.4	18	8.3	3	1.6
	45-	14	7.1	18	9.7	6	3.4
	50-	11	9.7	5	4.0	10	10.6
	55-	13	14.0	9	12.2	5	9.3
	Total	50	5.7	59	6.7	26	2.9
	St.40-54[a]		6.4		7.3		5.2
Non-Fatal Myocardial Infarction	30-	0	0.0	3	3.0	0	0.0
	35-	5	3.1	6	3.3	0	0.0
	40-	4	1.9	16	7.4	0	0.0
	45-	10	5.1	16	8.6	5	2.9
	50-	9	7.9	4	3.2	8	8.5
	55-	8	8.6	8	10.8	4	7.4
	Total [b]	36	4.1	53	6.0	17	1.9
	St.40-54		5.0		6.4		3.8
Fatal IHD	30-	1	1.0	0	0.0	0	0.0
	35-	1	0.6	0	0.0	2	1.0
	40-	1	0.5	2	0.9	3	1.6
	45-	4	2.0	2	1.1	1	0.6
	50-	2	1.8	1	0.8	2	2.1
	55-	5	5.4	1	1.4	1	1.9
	Total	14	1.6	6	0.7	9	1.0
	St.40-54		1.4		0.9		1.4
Of these: Deaths within 3 hours [c]		8	0.9	4	0.5	4	0.4

(a) Standardised rates for the three age-groups 40-44, 45-59, 50-54, equal weights being given to each age-group.

(b) Includes 4 with Acute Coronary Insufficiency (Intermediate Coronary Syndrome), 2, 2 and 0 in Groups I, II and III respectively.

(c) The numbers of IHD deaths occurring from 3-12 hours were 1, 1, 2, in the three Groups respectively.

APPENDIX 4 CENTRE DIFFERENCES (See Table 3)

Incidence of Major Ischaemic Heart Disease (IHD), Non-fatal and Fatal, by Age at Entry

Rates per 1000 per annum

Budapest

Event	Age at Entry (years)	Group I Clofibrate		Group II High Cholesterol Control		Group III Low Cholesterol Control	
		No.	Rate	No.	Rate	No.	Rate
All Major IHD	30-	2	2.9	0	0.0	2	1.4
	35-	2	1.9	1	0.8	1	0.7
	40-	4	2.1	1	0.5	1	0.6
	45-	9	4.3	11	5.1	2	1.2
	50-	6	4.3	15	10.6	2	1.7
	55-	17	9.7	21	12.2	3	2.3
	Total	40	4.5	49	5.5	11	1.2
	St.40-54[a]		3.6		5.4		1.2
Non-Fatal Myocardial Infarction	30-	2	2.9	0	0.0	2	1.4
	35-	1	1.0	1	0.8	1	0.7
	40-	4	2.1	1	0.5	0	0.0
	45-	5	2.4	6	2.8	2	1.2
	50-	6	4.3	12	8.5	2	1.7
	55-	14	8.0	14	8.1	2	1.5
	Total [b]	32	3.6	34	3.8	9	1.0
	St.40-54		2.9		3.9		1.0
Fatal IHD	30-	0	0.0	0	0.0	0	0.0
	35-	1	1.0	0	0.0	0	0.0
	40-	0	0.0	0	0.0	1	0.6
	45-	4	1.9	5	2.3	0	0.0
	50-	0	0.0	3	2.1	0	0.0
	55-	3	1.7	7	4.1	1	0.8
	Total	8	0.9	15	1.7	2	0.2
	St.40-54		0.6		1.5		0.2
Of these: Deaths within 3 hours [c]		5	0.6	6	0.7	2	0.2

(a) Standardised rates for the three age-groups 40-44, 45-49, 50-54, equal weights being given to each age-group.

(b) Includes 7 with Acute Coronary Insufficiency (Intermediate Coronary Syndrome) 2, 4 and 1 in Groups I, II and III respectively.

(c) The numbers of IHD deaths occurring from 3-12 hours were 1, 3, 0, in the three Groups respectively.

APPENDIX 4 CENTRE DIFFERENCES (See Table 3)

Incidence of Major Ischaemic Heart Disease (IHD), Non-fatal and Fatal by Age at Entry

Rates per 1000 per annum

Prague

Event	Age at Entry (years)	Group I Clofibrate		Group II High Cholesterol Control		Group III Low Cholesterol Control	
		No.	Rate	No.	Rate	No.	Rate
All Major IHD	30-	0	0.0	0	0.0	0	0.0
	35-	0	0.0	0	0.0	0	0.0
	40-	10	4.3	12	5.6	4	2.0
	45-	38	6.7	55	9.8	10	2.0
	50-	27	11.0	33	13.5	10	4.2
	55-	2	19.5	0	0.0	1	9.8
	Total	77	7.3	100	9.7	25	2.6
	St.40-54 (a)		7.3		9.6		2.7
Non-Fatal Myocardial Infarction	30-	0	0.0	0	0.0	0	0.0
	35-	0	0.0	0	0.0	0	0.0
	40-	8	3.5	11	5.1	4	2.0
	45-	33	5.8	50	8.9	7	1.4
	50-	20	8.1	26	10.6	8	3.4
	55-	2	19.5	0	0.0	1	9.8
	Total (b)	63	5.9	87	8.4	20	2.1
	St.40-54		5.8		8.2		2.3
Fatal IHD	30-	0	0.0	0	0.0	0	0.0
	35-	0	0.0	0	0.0	0	0.0
	40-	2	0.9	1	0.5	0	0.0
	45-	5	0.9	5	0.9	3	0.6
	50-	7	2.8	7	2.9	2	0.8
	55-	0	0.0	0	0.0	0	0.0
	Total	14	1.3	13	1.3	5	0.5
	St.40-54		1.5		1.4		0.5
Of these: Deaths within 3 hours (c)		10	0.9	7	0.7	2	0.2

(a) Standardised rates for the three age-groups 40-44, 45-49, 50-54, equal weights being given to each age-group.

(b) Includes 27 with Acute Coronary Insufficiency (Intermediate Coronary Syndrome) 14, 10 and 3 in Groups I, II and III respectively.

(c) The numbers of IHD deaths occurring from 3-12 hours were 2, 2, 1 in the three Groups respectively.

APPENDIX 4 CENTRE DIFFERENCES (See Table 12)

Deaths in the Trial and within 1 year of leaving it

Main Cause Groups. Numbers of Deaths at Ages 30-59 and Age-standardised Rates per 1000 per annum at ages 40-54

Cause of Death	Centre	Group I Clofibrate		Group II High Cholesterol Control		Group III Low Cholesterol Control	
		Number (All ages)	Rate (40-54)	Number (All ages)	Rate (40-54)	Number (All ages)	Rate (40-54)
Ischaemic Heart Disease	Edinburgh	18	1.6	12	1.4	9	1.2
	Budapest	13	1.0	18	1.3	5	0.7
	Prague	23	2.0	18	1.5	6	0.5
Other Vascular	Edinburgh	1	0.0	6	0.5	1	0.2
	Budapest	7	1.0	3	0.2	5	0.6
	Prague	6	0.4	5	0.6	3	0.2
Neoplasm: malignant	Edinburgh	12	1.5	12	1.0	9	0.6
	Budapest	24	2.9	14	1.1	14	1.8
	Prague	22	1.9	16	1.2	18	1.5
Neoplasm: benign	Edinburgh	1	-	-	-	1	-
	Budapest	-	-	-	-	-	-
	Prague	2	-	-	-	-	-
Other Medical Causes	Edinburgh	2	0.0	1	0.1	0	0.0
	Budapest	8	0.5	2	0.2	1	0.2
	Prague	6	0.6	2	0.2	6	0.5
Accidents & Violence	Edinburgh	5	0.4	1	0.1	1	0.0
	Budapest	8	0.9	6	0.8	9	1.3
	Prague	4	0.3	11	1.0	5	0.4
All causes other than I.H.D.	Edinburgh	21	2.0	20	1.8	12	0.8
	Budapest	47**	5.3	25**	2.3	29	3.9
	Prague	40	3.4	34	3.0	32	2.8
All causes other than I.H.D., Vascular and Accidents & Violence	Edinburgh	15	1.6	13	1.2	10	0.6
	Budapest	32*	3.4	16*	1.3	15	2.0
	Prague	30	2.7	18	1.5	24	2.1
TOTAL ALL CAUSES	Edinburgh	39	3.6	32	3.2	21	2.0
	Budapest	60	6.3	43	3.5	34	4.6
	Prague	63	5.4	52	4.5	38	3.2

* Significant difference between Groups I and II ($P<0.05$).

** Significant difference between Groups I and II ($P<0.01$).

APPENDIX 5

Drug Adherence Group I

(i) Average concentrations of Clofibrate (CPIB) (μg/ml)

Centre	Years in trial				
	1	2	3	4	5
Edinburgh	142	141	151	157	-
Budapest	132	141	134	129	124
Prague	156	165	159	171	171

(ii)

Centre	Percentage of negative estimates in:	
	Men with a major I.H.D. event	All men
	%	%
Edinburgh	3	6
Budapest	12	6
Prague	13	9

(iii) Average concentrations of clofibrate (CPIB)

	Centre	Visit number						
		2	3	4	5	6	7	8
Men with a major IHD event (value at visit immediately preceding IHD event)	Edinburgh	172	145	158	132	154	145	150
	Budapest	128	135	116	160	144	114	91
	Prague	148	145	156	159	141	166	164
All men	Edinburgh	129	142	142	141	151	157	157
	Budapest	132	132	125	141	134	129	124
	Prague	151	156	140	165	159	171	171

APPENDIX 6

Conditions not necessarily causing withdrawal from the trial but recorded as possible side effects

Condition	Edinburgh			Budapest			Prague			All Centres			
	I	II	III	I	II	III	I	II	III	I	II	III	Total
Endocrine, Metabolic Diseases													
Weight gain	66**	24**	36	25	15	13	8	2	1	99**	41**	50	190
Mental Disorders													
Impotence	13	7	14	18	16	7	27**	10**	10	58**	33**	31	122
Diseases of Circulatory Sys.													
Cardiac Arrhythmia	-	-	2	-	-	-	-	-	-	-	-	2	2
Diseases of Respiratory Sys.													
Catarrh, coryza	2	2	2	-	-	-	-	-	-	2	2	2	6
Diseases of Digestive System													
Aggravation of peptic ulcer	-	-	1	-	-	-	-	-	-	-	-	1	1
Indigestion, abdom. symptoms	168	163	119	43*	23*	21	109**	55**	59	320**	241**	199	760
Constipation	20	19	24	5	4	1	3	3	2	28	26	27	81
Intestinal hurry	130**	90**	121	9	16	13	31	20	23	170*	126*	157	453
Diseases of the Skin													
Pruritus	2	-	-	6	5	4	4	-	3	12	5	7	24
Alopecia	2	2	1	1	1	1	-	-	-	3	3	2	8
Hirsutism	1	-	-	-	-	-	-	-	-	1	-	-	1
Symptoms & Ill-defined Conditions													
Dizziness	7	6	7	6	4	12	3	7	5	16	17	24	57
Sleep Disturbance	6	2	4	7	7	6	8	11	4	21	20	14	55
Ocular symptoms	-	2	-	-	-	-	-	-	-	-	2	-	2
Dental symptoms	1	1	-	-	-	-	-	-	-	1	1	-	2
Paraesthesia	2	6	5	6	7	12	6	5	8	14	18	25	57
Epistaxis	15	18	21	1	3	1	6	2	2	22	23	24	69
Chest discomfort	1	2	-	-	-	-	-	-	-	1	2	-	3
Anorexia	1	1	2	-	-	-	-	-	-	1	1	2	4
Nausea	2	-	1	-	-	-	-	-	-	2	-	1	3
Urinary symptoms	3	1	3	6	3	-	1	-	1	10	4	4	18
Muscular cramps	10	7	5	4	5	4	3	2	-	17	14	9	40
Arthralgia	1	7	5	-	-	1	3	3	2	4	10	8	22
Hyperhidrosis	24**	7**	7	4	7	3	1	3	-	29	17	10	56
Rash	29	24	21	9	12	5	16	12	17	54	48	43	145
Increased appetite, thirst	7	3	2	8	2	10	7	2	2	22	7	14	43
Fatigue, depression	31	30	24	7	5	5	13	13	11	51	48	40	139
Headache	4	4	6	9	6	1	2	3	11	15	13	18	46
Coldness	8	10	6	2	3	-	-	1	-	10	14	6	30
Reduced tolerance to alcohol	2	6	6	-	1	2	-	-	-	2	7	8	17
Other	-	-	-	-	1	-	-	-	-	-	1	-	1

* Significant difference between Groups I and II ($P < 0.05$).

** Significant difference between Groups I and II ($P < 0.01$).

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ORLISTAT FOR OBESITY

Orlistat (*Xenical* – Roche), a lipase inhibitor that decreases absorption of fat from the gastrointestinal tract, is now available for treatment of obesity.

MECHANISM OF ACTION — A pentanoic acid ester, orlistat binds to gastric and pancreatic lipases, inhibiting their activity and preventing absorption of about 30% of dietary fat. This loss of calories is the main cause of weight loss, but drug-induced adverse effects may also contribute by causing a decrease in food intake.

PHARMACOKINETICS — Absorption of orlistat is minimal. Eight hours after a 360-mg dose of ^{14}C-labelled orlistat, plasma concentrations were barely detectable. Virtually all of the radioactivity was excreted in feces.

INDICATIONS — The package insert says that orlistat should be reserved for patients with a body mass index (BMI) of at least 30 kg/m^2, or 27 kg/m^2 for those with hypertension, diabetes or dyslipidemia. A 5'10" person weighing 210 pounds has a BMI of 30, and one weighing 190 pounds has a BMI of 27.

CLINICAL TRIALS — Two large placebo-controlled trials tested the effect of orlistat 120 mg t.i.d. or placebo in addition to a low-calorie diet for one year, followed by a second year on the drug or placebo in addition to a weight-maintenance diet (L Sjöström et al, Lancet, 352:167, 1998; MH Davidson et al, JAMA, 281:235, 1999). The results of the two studies were similar; after one year, subjects on orlistat had lost an average of 9 to 10 kg (10% of baseline weight) with the drug, compared to a 6-kg (6%) loss with placebo. In the second year, patients who stayed on the drug regained 1.5 to 3 kg, compared to a 4- to 6-kg regain in patients switched to placebo. Patients on orlistat showed statistically significant but clinically trivial improvements in cardiovascular risk factors compared to those on placebo; after one year, diastolic blood pressure decreased by an average of 2 mmHg and after two years, total cholesterol decreased by 5 mg/dL.

A double-blind trial of orlistat for weight maintenance in 729 patients who had lost a mean of about 10 kg by dieting alone found that those treated with orlistat 120 mg t.i.d. for one year regained a mean of 2.6 kg, compared to a mean regain of 4.4 kg with placebo. About 24% of patients taking orlistat did not regain weight, compared to 16% of patients on placebo (JO Hill et al, Am J Clin Nutr, 69:1108, 1999).

A one-year double-blind trial in 391 obese patients with type 2 diabetes found that patients treated with orlistat 120 mg t.i.d. in addition to diet lost a mean of 1.9 kg more than those given placebo plus diet. Orlistat-treated patients showed a mean decrease in HbA1c of 0.18%, compared to a mean increase of 0.28% with placebo (PA Hollander et al, Diabetes Care, 21:1288, 1998).

No studies have been published on use of orlistat concomitantly with phentermine (Medical Letter, 36:107, 1994) or with sibutramine (*Meridia* – Medical Letter, 40:32, 1998), the only other drug approved by the FDA for long-term treatment of obesity.

ADVERSE EFFECTS — Symptoms such as flatulence with discharge, oily spotting and fecal urgency occurred in 20% to 40% of subjects taking orlistat, twice as many as in the placebo group. Presumably these effects would be more frequent and more severe in patients who did not adhere to a diet providing 30% or less of calories from fat. Decreases in mean serum concentrations of vitamins A, D and E and beta-carotene have occurred; taking supplements of fat-soluble vitamins will correct the deficiency of vitamins, but will not make up for the loss of fat-soluble carotenoids from fruits and vegetables. Pooled data from seven controlled trials showed that 9 of 747 patients taking orlistat developed breast cancer, compared to 1 of 579 on placebo. All the cases of breast cancer occurred in women more than 45 years old. In the two published two-year clinical trials, no difference was detected in the incidence of breast cancer with orlistat compared to placebo. The long-term effects of chronic steatorrhea on neoplastic or inflammatory bowel disease are unknown. The drug is contraindicated in patients with chronic malabsorption syndrome or cholestasis.

DOSAGE AND COST — *Xenical* is supplied in 120-mg capsules. The manufacturer recommends taking one capsule three times a day with each main meal containing fat. If the meal contains no fat, the dose can be omitted. Fat-soluble vitamin supplements should be taken at least two hours before or after taking orlistat. The wholesale (AWP) cost of 90 120-mg capsules of *Xenical* is $118.80, according to *Drug Topics Red Book Update*, June 1999.

CONCLUSION — Orlistat is modestly effective in helping obese patients lose weight. As with other weight-loss drugs, patients tend to regain at least some of the lost weight even if the drug is continued. Orlisat causes some uncomfortable, potentially embarrassing adverse effects and interferes with absorption of fat-soluble vitamins, beta-carotene and other carotenoids. No data are available on the effectiveness or safety of combining orlistat with other anti-obesity drugs.

CHOLESTEROL-LOWERING MARGARINES

Two cholesterol-lowering margarines have been marketed in the USA. One (*Benecol* – McNeil Consumer Health Care) contains hydrogenated sterols, mainly sitostanol derived from pine tree wood pulp. The other (*Take Control* – Unilever) contains naturally occurring unsaturated sterols, mainly sitosterol from soybean oil. Both have been approved by the FDA as foods. *Benecol* has been available in Finland since 1995.

CHEMISTRY — Major plant sterols are similar in chemical structure to cholesterol with a double-bond at the 5 position but they differ in their side-chain configuration. The stanol esters in *Benecol* are produced from sterols by hydrogenation of the double bond at the 5 position to 5 α-stanols and subsequent esterification with unsaturated fatty acids.

Oral administration of plant sterols decreases intestinal absorption and increases fecal excretion of both dietary and biliary cholesterol. The poorly absorbed sterols apparently compete with cholesterol for incorporation into mixed bile salt micelles, a prerequisite for uptake of cholesterol by enterocytes. Other steps in the intestinal absorption of cholesterol may also be inhibited. The smaller supply of intestinal cholesterol to the liver results in decreased production and increased removal of low-density lipoprotein (LDL) precursors, decreasing LDL production. Decreased availability of cholesterol leads to increased hepatic *de novo* cholesterol synthesis, which may be the reason why some patients do not respond. As with other cholesterol-lowering drugs, plant sterols must be taken indefinitely; when they are stopped, cholesterol levels return to baseline.

CLINICAL STUDIES — Sitostanol Ester Margarine – A double-blind trial in mildly hypercholesterolemic women and men randomized 51 subjects to use 24 g daily of margarine without sitostanol, and 102 to use 24 g of the same margarine containing 3 g of sitostanol ester. After six months, half of the actively treated subjects decreased their sitostanol intake to 2 g daily. After 12 months, 3 g of sitostanol had decreased average LDL cholesterol from 160 to 134 mg/dL, compared to virtually no reduction in the control group. The daily dose of 2 g of sitostanol for the second six months decreased LDL from 153 to 138 mg/dL at 12 months. Twelve people who used the margarine with sitostanol showed no decrease in LDL. The effect of sitostanol on HDL cholesterol and triglycerides was not statistically significant (TA Miettinen et al, N Engl J Med, 333:1308, 1995).

A double-blind crossover trial in 22 women with a previous myocardial infarction found that 3 g of sitostanol in 21 g of unsaturated margarine, taken daily for seven weeks in addition to a cholesterol-lowering diet, reduced total and LDL cholesterol by 13% and 20% compared to 5% reductions with margarine alone. In 10 women who were already taking simvastatin 10 to 20 mg/day, the sitostanol-containing margarine produced a further LDL cholesterol reduction of 16% (H Gylling et al, Circulation, 96:4226, 1997). A seven-week study in eight hypercholesterolemic, non-insulin-dependent diabetic men showed that sitostanol ester (3 g/day) margarine and pravastatin (40 mg/day) individually decreased LDL cholesterol by 14% and 38%, while the combined effect was a 44% reduction (H Gylling and TA Miettinen, J Lipid Res, 37:1776, 1996).

Sitosterol Ester Margarine – A crossover trial in 80 normal or moderately hypercholesterolemic women and men who were given, for 3.5 weeks each, 0.85, 1.62 and 3.26 g per day of a sterol mixture containing 48% sitosterol in a full-fat (70%) margarine (daily dose 25 g) found that LDL cholesterol decreased, compared to a control margarine, by 6.7%, 8.5% and 9.9% with increasing sterol dosage. HDL cholesterol and triglyceride levels were not affected (HFJ Hendriks et al, Eur J Clin Nutr, 53:319, 1999).

Comparison of Sitosterol and Sitostanol – A double-blind randomized trial compared plant sterol esters and sitostanol esters (3 g/day) in margarine (30 g/day) with a control margarine in 80 normal or mildly hypercholesterolemic women and men. After 3.5 weeks the two enriched margarines had both lowered LDL by about 12% compared to the control (JA Weststrate and GW Meijer, Eur J Clin Nutr, 52:334, 1998).

ADVERSE EFFECTS — Sterol and stanol esters in margarine are well tolerated and do not affect the taste or texture of the margarine. Short-term studies have shown no effects on routine laboratory tests. While parenteral administration of beta-sitosterol has estrogen-like effects in animals, no estrogenic activity was detected *in vitro* or *in vivo* after oral administration of sitosterol ester margarine to experimental animals.

High serum concentrations of plant sterols have been associated with premature coronary artery disease (CJ Glueck et al, Metabolism, 40:842, 1991). Patients with inherited sitosterolemia develop xanthomas and premature ischemic heart disease. Since stanols are absorbed less than sterols, plasma sterol concentrations should be lower with *Benecol* than with *Take Control*. Both sitostanol and sitosterol ester margarines may decrease plasma concentrations of antioxidants such as alpha- and beta-carotene, lycopene and alpha-tocopherol. Phytosterol is found in breast milk and in the plasma of nursing infants.

DOSAGE — *Take Control* contains 1120 mg of sitosterol per one-tablespoon (14 g) serving. The manufacturer recommends one to two tablespoons per day. Each serving also contains 6 g of fat including 0.5 g of saturated fat, and 50 calories. *Benecol* contains 1.5 g of plant stanol ester (sitostanol) per 1 1/2-teaspoon (8 g) serving. The manufacturer recommends three servings per day. Each serving contains 5 grams of fat and 45 calories; a "lite" version contains 3 grams of fat and 30 calories. Both *Take Control* and *Benecol* cost about five times as much as ordinary margarine.

CONCLUSION — Sterol-enriched margarines can lower LDL cholesterol by 10% to 15%. The effect of these margarines on mortality and morbidity from coronary artery disease is unknown; the beneficial effects of lowering cholesterol might be offset by increased plasma concentrations of plant sterols, which may be atherogenic.

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THE BIOLOGY OF CHOLESTEROL AND RELATED STEROIDS

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some of the more interesting of these related compounds is considered in this chapter. The two groups of metabolites of cholesterol of major biological importance in vertebrates—bile acids and steroid hormones—are considered separately in Chapter 5.

2 VERTEBRATES

2.1 General

Cholesterol is by far the most abundant sterol in vertebrates. Most of the cholesterol in the whole body is present in free (unesterified) form in the plasma membranes and subcellular membranes of cells and in the myelin of nervous tissue. However, in certain specialized tissues, and under some pathological conditions, substantial quantities of cholesterol are esterified with long-chain fatty acids. In the plasma of all mammals, for example, the bulk of the cholesterol is esterified. The presence of cholesteryl sulphate and of other steryl sulphates has been demonstrated in several mammalian tissues and body fluids, including the adrenal cortex, brain, kidney, liver, plasma, bile and urine; meconium and faeces from newborn infants also contain cholesteryl sulphates and the disulphate esters of several hydroxylated derivatives of cholesterol. (For references to steryl sulphates, see Drayer and Lieberman, 1967; Gustafsson and Eneroth, 1972; Iwamori *et al.*, 1976.)

The probable *functions* of cholesterol in higher animals are: (a) to serve as an essential stabilizing constituent of cell membranes, plasma lipoproteins and myelin, and (b) to act as a precursor of bile acids and steroid hormones and, in the unusual case of toads, as a precursor of poisonous substances. One of the functions of esterified cholesterol is to act as a store of cholesterol for steroid-hormone synthesis; another may be to act as a reservoir of surplus cholesterol in a form in which it cannot interact with membranes.

In addition to cholesterol and its esters, other sterols and related steroids are present in measurable amounts in many animal tissues. These occur either as intermediates in the biosynthesis of cholesterol or as products of its enzymic or non-enzymic modification. Strictly speaking, all the steroidal precursors of cholesterol should be present at finite concentrations in all tissues in which cholesterol is synthesized. However, the concentrations of many intermediates is such that their presence in the tissues can only be demonstrated by special techniques involving the use of radioactive tracers. Although plant sterols are poorly absorbed from the diet, many tissues of omnivorous and herbivorous animals contain detectable amounts of β-sitosterol and other phytosterols.

are also present, often in surprisingly large amounts. In wool fat, the sebum secreted by the sheep's skin, lanosterol and agnosterol account for as much as 10% of the total sterol. The lipids of human sebum contain up to 17% of squalene (Nicolaides *et al.*, 1968), as well as small amounts of lathosterol and 7-dehydrocholesterol. In the surface lipids of the human forehead, cholesterol accounts for more than 90% of the total sterol (Nikkari *et al.*, 1974). The sterols of human preputial sebum (smegma) contain a high proportion of 5α-cholestanol, mainly in esterified form. Squalene is present only in traces in the sebum of most species other than man (Nicolaides *et al.*, 1968). Squalene is present in the human sebaceous secretions of ear wax, hair fat and vernix caseosa (see Goodman, 1964).

2.4 Nervous tissue

In adult mammals 20–25% of the cholesterol in the whole body is present in the nervous system, including the brain, spinal cord and peripheral nerves. Most of the cholesterol in the nervous system is present in unesterified form as a major constituent of myelin, but small quantities of esterified cholesterol and related sterols are found in other cellular constituents of brain. Several sterols other than cholesterol have been identified in brain. These include lanosterol, 14α-desmethyllanosterol, 5α-cholestanol, lathosterol, desmosterol, 26-hydroxycholesterol and 24β-hydroxycholesterol. Desmosterol is present in considerable amounts in the brains of immature birds and mammals and may be an intermediate in the main pathway for cholesterol biosynthesis in brain tissue during the early stages of myelination. Table 3.3 shows the percentages of cholesterol and other sterols in the total sterol fraction of immature rat brain. In mature rat brain, desmosterol is present only in traces, possibly because of its conversion into cholesterol during maturation.

Owing to the high cholesterol content of myelin, there are marked regional differences in the sterol composition of brain. Furthermore, since myelination of the central nervous system continues after birth

Table 3.3
Sterols of developing rat brain (% total free sterols)

Sterol	%
Cholesterol	91.4
Desmosterol	8.3
14α-Desmethyllanosterol	0.19
Lanosterol	0.12

(From Ramsey and Nicholas (1972)).

sequence from which all the carbon of cholesterol is derived. On a strict interpretation of this definition, β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) is the primary intermediate in sterol biosynthesis (see Fig. 4.1). However, it is more usual to regard the acetyl unit (acetate or acetyl-CoA) as the starting-point for the biosynthetic sequence, since it is the final common pathway through which most of the carbon contributing to sterols is channelled. Note that in this discussion the alkyl residue of the phytosterol side-chain is not considered to be part of the sterol skeleton. More generally, the above definition of a primary intermediate is not applicable in cases where a molecule is synthesized *via* two or more separate pathways, each contributing to a specific portion of the final product; for example, the alkyl residue at C-24 of plant sterols is derived from methionine.

As shown in Fig. 4.1, the acetyl unit lies at the junction of several metabolic pathways. Moreover, several minor tributaries add to the carbon flow between acetate and mevalonic acid. Hence many substances that are sterol precursors, in the sense that they supply carbon for sterol synthesis, are not intermediates in this pathway in any useful sense of the term. The relevance of these metabolic interconnections to the design and interpretation of experiments with isotopically labelled compounds is considered in Section 1.2.

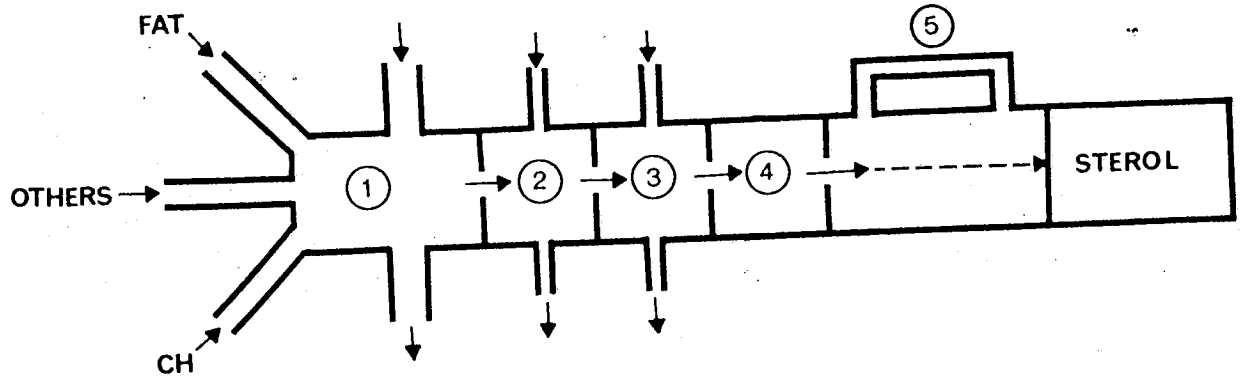


Figure 4.1
Diagram to show the sources of carbon in the critical steps in the biosynthesis of sterols. Arrows show the direction of flow of carbon. CH, carbohydrate; OTHERS, sources of carbon other than carbohydrate and fat; (1) acetate or acetyl-CoA; (2) acetoacetyl-CoA; (3) HMG-CoA; (4) mevalonic acid; (5) indicates the existence of alternative pathways in the later stages of sterol biosynthesis. No carbon enters the pathway after mevalonic acid, other than that used for alkylating the side-chain of a phytosterol. Routes from mevalonate to non-sterol polyisoprenoids are omitted.

2 REACTION MECHANISMS

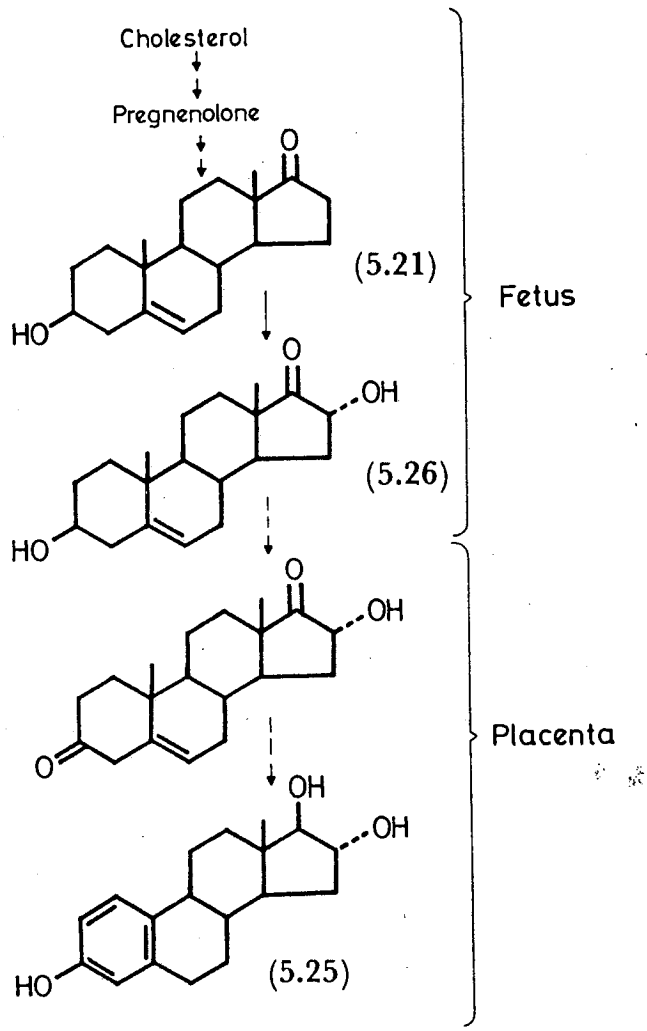
2.1 Some definitions

Although most, if not all, of the chemical reactions in sterol biosynthesis are catalyzed by enzymes, it is safe to assume that the mechanisms of these reactions conform to the well-established laws

Testosterone (**5.22**) is formed from androstenedione by reduction of the 17-keto group to give the 17β-hydroxysteroid. In some species, testosterone may also be formed by an alternative route in which (**5.21**) is converted into (**5.20**).

In the ovaries, oestrogens are formed from cholesterol *via* androstenedione (**5.20**) and testosterone (**5.22**), the two androgenic hormones arising by the pathway shown in Scheme 4. Oestrone (**5.23**) and oestradiol-17β (**5.24**) are formed from androstenedione and testosterone, respectively, by removal of the C-19 methyl group and conversion of ring A into the aromatic form ('aromatization'). Scheme 5 shows the probable sequence of reactions involved in the aromatization of androgens to form oestrone and oestradiol-17β.

The human placenta forms progesterone from cholesterol by the pregnenolone pathway (Scheme 3) but lacks the enzymes required for conversion of C_{21} steroids into C_{19} or C_{18} steroids. However, 16α-hydroxydehydroepiandrosterone (**5.26**), formed in the fetal adrenal cortex from cholesterol and pregnenolone and transported thence to



Scheme 6 The conversion of cholesterol into oestriol by the combined action of the fetus and placenta.

(**5.21**), Dehydroepiandrosterone; (**5.25**), oestriol; (**5.26**), 16α-hydroxydehydroepiandrosterone.

shunt in female rats is about twice that in male rats and that this sex difference is due to a difference in the ability of the kidneys to metabolize mevalonic acid through the shunt pathway. By measuring the recovery of $^{14}CO_2$ in the breath after an intravenous injection of (3*R*)-[5-^{14}C]mevalonate, Fogelman *et al.* (1975) have shown that the shunt accounts for up to 12% of the metabolism of mevalonate in normal human subjects. A pathway of this magnitude could conceivably participate in a mechanism for regulating sterol synthesis in the liver by diverting a variable proportion of the carbon of mevalonic acid from its flow along the pathway to sterol.

4 REGULATION OF STEROL SYNTHESIS

4.1 General considerations

In living systems the rates of metabolic processes are regulated* in accordance with the biological needs of the organism. In the mature animal, the general effect of metabolic regulation is to maintain homeostasis within each tissue and within the body as a whole. But homeostasis is by no means the only function of biochemical regulatory mechanisms. In some tissues, considerable deviations from the steady state may be essential to the success of the organism. An example of a long-term deviation is the marked increase in the rate of synthesis of cholesterol that occurs in the immature central nervous system during the deposition of myelin; once myelination is complete, the rate of synthesis falls irreversibly to a negligible level. Examples of short-term deviations from the steady state are the diurnal rhythm in cholesterol synthesis in the livers of some animals that feed intermittently (presumably, this is related to the need for bile acids in the intestine during the absorption of fat), and the very rapid increase in cholesterol synthesis in the adrenal glands in response to the need for a sudden increase in the secretion of steroid hormones.

The regulation of metabolic processes in a multicellular organism may be thought of in terms of two levels of complexity: that of the individual cell and that of the organism as a whole.

* The term 'regulation' (L. *regulare*, to direct or control), as used in relation to biological systems, is hard to define in a way that would satisfy all biologists. By derivation, the word implies a purposive process, since one would not control something without reference to some desirable or optimal state, though trying to define what is optimal for a particular system is, as Riggs (1967) has remarked in relation to feedback control, 'all too often an exercise in futility'. But if one discards altogether the idea of purpose from the meaning of regulation, one might as well not use the word at all in discussions about living systems. The word 'purpose', as used here, does not, of course, imply consciousness of the state that will result from regulation. Those who dislike any reference to purpose in a scientific context should note that few modern biologists have difficulty in accepting the possibility that purposive mechanisms could arise by natural selection in a purposeless universe.

the effects of these experimental procedures on sterol metabolism. Weis and Dietschy (1969) were able to reverse the effects of a bile fistula on hepatic synthesis of cholesterol in rats by intravenous infusions of chylomicrons, the form in which absorbed cholesterol enters the circulation via the intestinal lymphatics, but not by intravenous or intraduodenal infusions of bile salts. They concluded that bile salts influence cholesterol synthesis in the liver not by acting *in situ*, but by enhancing the transport of biliary and intestinal cholesterol into the circulation and thus promoting feedback inhibition of cholesterol synthesis. On this interpretation, the stimulatory effect of a bile fistula on hepatic HMG-CoA reductase activity is mediated by release from the inhibition normally exerted by cholesterol transported to the liver *via* the intestinal lymphatics, and the inhibitory effect of bile-salt feeding in intact animals is due to enhanced absorption of cholesterol. In support of the view that bile salts themselves do not suppress HMG-CoA reductase in the liver by an action *in situ*, Weis and Dietschy drew attention to the fact that hepatic synthesis of cholesterol is markedly increased by ligation of the bile duct (Fredrickson *et al.*, 1954), a procedure which increases the concentration of bile salts in whole liver.

Decreased absorption of cholesterol may well contribute to the effect of a bile fistula on sterol synthesis, since complete diversion of bile from the intestinal lumen is known to abolish the absorption of cholesterol from the intestine (Siperstein *et al.*, 1952). However, it is difficult to explain the effects of cholestyramine on hepatic synthesis of cholesterol simply in terms of an effect on absorption of cholesterol from the intestine, since cholestyramine acts by interfering specifically with the reabsorption of bile salts from the ileum and has generally been found to have little effect on the absorption of cholesterol. That bile salts have a direct effect on cholesterol synthesis in the liver is shown by the experiment of Hamprecht *et al.* (1971), who found that if rats with thoracic-duct fistulae are adapted to a regulated cycle of light and darkness, the subsequent administration of cholic acid by stomach tube decreases the activity of hepatic HMG-CoA reductase during the dark phase (when enzyme activity is maximal). Since all the cholesterol absorbed from the intestine is diverted by a thoracic-duct fistula, the inhibitory effect of cholic acid observed in these experiments cannot have been mediated by increased absorption of cholesterol. In an experiment in some ways analogous to that of Hamprecht *et al.*, Mosbach (1972) showed that in rats in which hepatic HMG-CoA reductase activity has been stimulated by feeding β-sitosterol (a plant sterol which interferes with the absorption of cholesterol), the further addition of taurocholate to the diet decreases the activity of the enzyme to the control level observed in animals not fed β-sitosterol. This result is difficult to explain other than by supposing that taurocholate decreases the activity of HMG-CoA reductase in the liver by an action *in situ*.

The effect of bile acids *in situ* upon hepatic HMG-CoA reductase is apparently not due to inactivation of preformed enzyme, since the addition of physiological amounts of taurocholate to the perfusing fluid of an isolated perfused rat liver has no immediate effect on the incorporation of $[^3H]H_2O$ into liver sterols (Liersch *et al.*, 1973). In agreement with this, the activity of HMG-CoA reductase in liver homogenates is not inhibited by adding pure bile acids to the homogenate at physiological concentrations. This suggests that the modulation of HMG-CoA reductase activity in the liver by bile salts acting *in situ* is due to repression of enzyme synthesis. This could be wholly or in part the consequence of the effect of bile acids on the activity of cholesterol 7α-hydroxylase. By repressing this enzyme, bile acids might lead to an increase in the concentration of free cholesterol in the smooth endoplasmic reticulum (the substrate for cholesterol 7α-hydroxylase) which might, in turn, affect the activity of HMG-CoA reductase by one or other of the mechanisms discussed in Section 4.3.2. Additional evidence that bile salts act within liver cells to suppress the synthesis of HMG-CoA reductase has been obtained by Barth and Hillmar (1980), who showed that taurocholate at physiological concentrations inhibits the glucocorticoid-induced rise in HMG-CoA reductase activity in monolayer cultures of rat hepatocytes.

4.3.6 Hormones

Although there is an extensive literature on the effects of hormones upon sterol synthesis in the liver (see Rodwell *et al.* (1976) for references), much of it is contradictory and hard to interpret. Part of the difficulty arises from the complexity of the interactions between different hormones, which may be antagonistic, complementary or permissive, and also from the ability of some hormones to produce effects in one tissue as a result of an action in another tissue. These factors almost certainly account for many of the discrepancies between hormonal effects observed in intact animals, those observed in animals from which various endocrine glands have been removed and those observed in perfused livers or in other types of liver preparation.

All this is, of course, familiar to every experimental endocrinologist, but much of the early work on the effects of hormones on hepatic synthesis of cholesterol is questionable for two other reasons. First, in most of the work in this field carried out before about 1970, the experiments were designed without taking account of the diurnal rhythm in hepatic HMG-CoA reductase activity, itself capable of causing a five-fold variation in the rate of sterol synthesis. Secondly, in studies based on measurement of the rate of incorporation of $[^{14}C]$acetate into liver sterols, the results are subject to considerable error due to variable dilution of the acetyl-CoA pool from endogenous sources (see 2.2). Since several hormones (e.g.

fall in adrenal cholesterol concentration, suggesting that X-irradiation acts like other forms of acute stress by stimulating the conversion of cholesterol into glucocorticoids in the adrenal cortex. It is difficult to explain the increased synthesis of cholesterol in the liver entirely in terms of increased output of hormones from the adrenal glands, since the stimulatory effect of catecholamines on hepatic cholesterol synthesis is not nearly as great as that of X-irradiation, and since corticosteroids are thought by most workers to have an inhibitory, rather than a stimulatory, effect on cholesterol synthesis in the liver.

At least one other form of acute stress—hind-limb ischaemia in the fasted rat—has been shown to stimulate cholesterol synthesis in the liver (De Matteis, 1969). This, too, cannot be mediated by a primary effect on the adrenal glands, since the rise in synthesis in the liver is not prevented by removal of the adrenals.

The intravenous injection of nonionic detergents such as Triton WR-1339 (a polyoxyethylene polymer) causes marked hypercholesterolaemia and an increased rate of sterol synthesis in the liver (Frantz and Hinkelman, 1955) and in many other tissues, particularly the adrenals (Andersen and Dietschy, 1977). The increase in sterol synthesis in the liver is accompanied by increased activity of HMG-CoA reductase (Bucher *et al.*, 1960). Although the hypercholesterolaemia is due partly to accumulation of triglyceride-rich lipoproteins in the plasma owing to the inhibition of lipoprotein lipase by the detergent, it seems likely that there is also a redistribution of cholesterol from the liver into the plasma, since the liver cholesterol concentration falls while the rate of synthesis of cholesterol is rising (Hirsch and Kellner, 1956). If the redistribution involves a net loss of free cholesterol from the subcellular membranes to which HMG-CoA reductase is attached, this might be expected to lead to an increase in enzyme activity, as discussed in Section 4.3.2. A mechanism similar in principle to this could also be responsible for the observation of Jakoi and Quarfordt (1974) that intravenous infusions of lecithin into rats lead to a fall in the concentration of microsomal cholesterol in the liver, with a concomitant rise in hepatic HMG-CoA reductase activity.

The effects of some drugs on sterol synthesis in the liver may be mentioned briefly in this Section. Clofibrate (the ethyl ester of p-chlorophenoxyisobutyrate) and nicotinic acid are used clinically for lowering the plasma cholesterol concentration in hypercholesterolaemic patients. Both drugs inhibit cholesterol synthesis in the liver, but in neither case is this the complete explanation of the effect of the drug on the plasma cholesterol (for reviews see Steinberg (1970) and Gey and Carlson (1971)). Phenobarbital, a drug known to induce several enzymes associated with the endoplasmic reticulum of the liver, enhances hepatic synthesis of cholesterol from acetate (Jones and

stimulatory effect of cholestyramine upon sterol synthesis in the liver has already been referred to (Section 4.3.5). β-Sitosterol, also used in the treatment of human hypercholesterolaemia, has a similar effect upon sterol synthesis in the liver by interfering with the absorption of dietary and endogenous cholesterol from the small intestine.

In the course of a general search for drugs likely to be of use in the treatment of hypercholesterolaemia, many compounds which inhibit various steps in the biosynthesis of cholesterol have been discovered. Although the great majority have been found to be unsuitable as therapeutic agents, usually because of their side-effects or because they are inactive in the whole organism, some have been useful in the study of sterol biosynthesis (see, for example, 2.5). Some of these inhibitors are discussed in a review by Dempsey (1969). Some other physiological factors that influence cholesterol synthesis in the liver are considered in the section dealing with developmental aspects of sterol metabolism (Chapter 6).

4.4 Regulation in the intestinal wall

A diurnal rhythm in HMG-CoA reductase activity is detectable in the rat's intestine (see Section 4.3.1).

In mammals, cholesterol synthesis in the small intestine differs from that in the liver in that intestinal synthesis is inhibited only to a small extent by fasting and is uninfluenced by cholesterol feeding, even if the feeding is prolonged for several weeks. In non-mammalian vertebrates, however, cholesterol feeding readily inhibits cholesterol synthesis in the intestine (Siperstein, 1970). Bile acids in the lumen of the gut inhibit the intestinal synthesis of cholesterol (see Wilson (1972) for review). In bile-fistula rats the rate of incorporation of [^{14}C]acetate into cholesterol in all segments of the small intestine is several times greater than that in intact animals and the increased rate of cholesterol synthesis in the bile-fistula animals is suppressed by intraduodenal infusion of whole bile or of taurocholate or other bile salts. A similar inhibitory effect of biliary bile acids on cholesterol synthesis in the wall of the small intestine occurs in man and in monkeys. Cholesterol synthesis in the wall of the rat's intestine increases after an intravenous injection of Triton 1339 or when the plasma lipoprotein concentration is lowered by administration of 4-aminopyrazolopyrimidine (4-APP). The possible relevance of these effects to the regulation of cholesterol synthesis under physiological conditions is discussed in Section 4.5.

Phenobarbital has a specific stimulatory effect on the incorporation of [^{14}C]acetate into cholesterol in the rat's small intestine *in vitro*, though the effect is less than that seen in the liver (Middleton and Isselbacher, 1969).

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Cholesterol and Atherosclerosis

1 INTRODUCTION

A full account of the pathology, causes and clinical consequences of atherosclerotic lesions in the arterial wall would take us far beyond the proper limits of a book on cholesterol. Nevertheless, there has been so much discussion of the plasma cholesterol in relation to ischaemic heart disease* (IHD) that we cannot ignore atherosclerosis altogether. Whether or not the current preoccupation with cholesterol as a possible cause of IHD will turn out to have been wholly justified, it is undeniable that cholesterol is a major component of the lipids of all atherosclerotic lesions and that the plasma cholesterol concentration is, in a statistical sense, predictive of heart attacks. In this chapter I shall consider the morphology and chemistry of the lesions of human arteries, the experimental production and regression of lesions in animals, the role of the plasma cholesterol in the development of atherosclerosis and the mechanisms by which the plasma cholesterol acts as a risk factor for IHD in man. Throughout, the emphasis will be on the coronary arteries, since IHD is by far the most serious outcome of atherosclerosis. So as to avoid confusion between *risk factors* and *causes* of IHD, these two aspects of the atherosclerosis question are considered separately.

2 THE ATHEROSCLEROTIC LESION

2.1 The normal artery

Fig. 13.1A shows, in diagrammatic form, the main features of a normal medium-sized artery, such as the coronary artery, of a young human

* In the context of this chapter the term *IHD* is preferable to the more usual *coronary heart disease* because the study of the plasma cholesterol as a risk factor for heart disease in man is concerned largely with clinically detectable ischaemia of the myocardium and not with the underlying coronary atherosclerosis (which is present in many apparently healthy adults).

hypothesis that there is a positive correlation between IHD and the antecedent plasma total cholesterol concentration. They are broadened to include the measurement of other variables, such as body fatness, blood pressure and diet which, in the light of all the knowledge available at the start of the trial, might be thought to influence the tendency in a given healthy individual to develop IHD. Variables that can be shown to be associated with an increased risk of IHD have come to be known as *risk factors* (Stamler, 1967). In its widest sense, a risk factor may be defined as any characteristic of the host or environment that is associated with an increase (*positive* risk factor) or decrease (*negative* risk factor) in susceptibility to a disease. This definition is arbitrary to the extent that it does not specify the magnitude or time-scale of the change in susceptibility. A factor associated with a 1% change in the 10-year incidence of overt IHD in men aged 80 years would obviously not be worth considering. Stamler (1973) suggests that a positive risk factor for IHD should be defined as one associated with a doubling of the incidence rate before age 65. It may be noted that a risk factor cannot be detected unless its frequency or magnitude can be measured and unless it varies within the population under investigation.

It must be clearly understood that a risk factor for IHD is not necessarily a cause of the disease. Prospective studies cannot reveal the causes of coronary atherosclerosis, but they do provide a short list of candidates for further examination (epidemiological investigation combined with common sense will often take us a long way, but to establish the truth we need evidence of a different kind). In addition to this, they provide information of the kind that is of interest to life-insurance companies, irrespective of its relevance to the cause of IHD. Thus, statistical information on one or more risk factors in a population can be used to estimate the probability that a given individual in that population will develop IHD within a specified time. These estimates may be used to identify members of the population who are 'at risk' and, hence, for whom preventive measures are indicated. They are also essential in the design of primary prevention trials of measures directed against IHD in a well population.

4.4 The plasma cholesterol as a positive risk factor

Shortly after the end of the Second World War, prospective trials of the type discussed in Section 4.2 were initiated in the United States. By 1963, Keys *et al.* (1963) were able to point to four such studies, one of them continued for 15 years before completion and all showing a significantly higher incidence of clinical events in subjects whose plasma cholesterol concentration was above, than in those in whom it was below, the mean or median value at the first examination. Keys *et*

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Letters to the Editor

THE OPTIMUM SERUM CHOLESTEROL

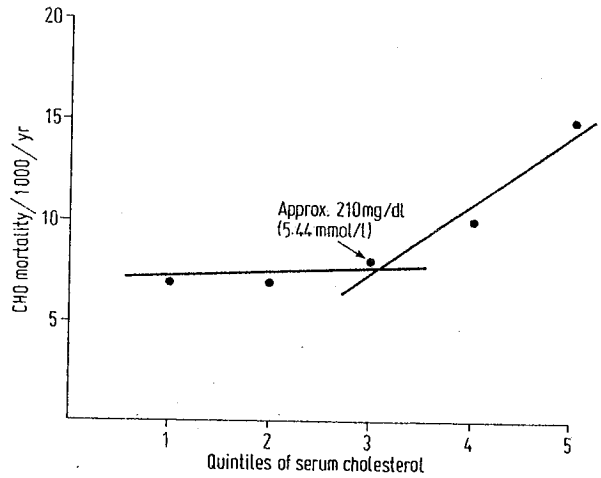
SIR,—It is excellent that the debate for the search for the optimum serum cholesterol has been joined by Dr Kannel and Mr Gordon (Aug. 14, p. 374-5) with a careful and thoughtful appraisal of the problem and the presentation of new data from the 10 and 20 year follow-up in men initially under 50 in the Framingham Study. But surely it is a specious argument to put greater emphasis on these new figures because they happen to show a stepwise gradient of coronary heart disease (CHD) risk through the quintiles of serum cholesterol from the lowest to the highest than on those from the much larger (8422 men) Pooling Project Research Group[1] which did not show any gradient of CHD risk across the lowest three quintiles (univariate logistic function), even although the Framingham population contributed. If we are to select one of the five pooled populations for particular scrutiny in the search for the optimum serum cholesterol, it is equally legitimate to look at the results of another of the pooled populations, the Chicago and Peoples Study[2] of 1233 men, which did not show any CHD mortality increase across the lowest three quintiles of serum cholesterol. While I accept that pooling of the results of five populations might, for the reasons stated by Kannel and Gordon, make them more unstable, such pooling is more likely to reflect the real situation in the population at large, and this is what we are trying to assess.

Support for the view that there is a non-linear discontinuous relation between serum cholesterol and CHD is gained from four other very large surveys outside the U.S.A. The Whitehall Study of British Civil Servants,[3] which reported a five-year mortality follow-up of 14 863 men aged 40–64, showed no significant difference in CHD mortality between the lowest three quartiles of serum cholesterol in men without features of CHD on admission to the study. In the Oslo Study[4] of 14 816 men aged 40–69 the hypothesis of a linear risk curve was specifically rejected and the incidence of myocardial infarction only became sharply increased when levels were in the region of 300 mg/dl. In the Stockholm Prospective Study[5] of 3486 men, followed up for 14½ years, the numbers of deaths from CHD were the same in the lowest three quintiles of serum cholesterol, and even the slight increased incidence in the top quintile was not significantly different from that for the first four quintiles ($Z = 1 \cdot 17$). The Israel Ischaemic Heart Disease Study[6] of 10 059 men aged 40–65 showed a non-significant increase in CHD mortality through the lowest four quintiles of serum cholesterol (with decreasing high density lipoprotein levels operating as a greater determinant of risk).

Kannel and Gordon's presentation of univariate logistic regression coefficients for various clinical manifestations of CHD presenting in Framingham 20 years after baseline serum cholesterol level is also welcome. They point out that the association of serum cholesterol with angina, myocardial infarction, and sudden death is of a similar order but, when earlier data from Framingham were merged with those from Albany,[7] no significant relation was found between serum cholesterol at the initial examination and the incidence of sudden death. Indeed, when multivariate regression coefficients were applied, systolic blood pressure, ECG evidence of left-ventricular hypertrophy, relative weight, and cigarette smoking all contributed significantly to sudden death, but serum cholesterol made no independent contribution. Is this another indication of instability or is it, once again, a manifestation of increasing the size of the population under study and getting a more representative result?

Lest it be thought from this correspondence, which follows my review[8] last year, that I have any profound disagreement with Kannel and Gordon, I would like to take this opportunity to state clearly that the evidence is convincing, in my opinion, that serum cholesterol should be reduced when it is definitely raised. Perhaps a useful working definition of a raised cholesterol is one above one standard deviation over the mean of the population under study. Further, the evidence that reduction of CHD incidence, possibly excluding sudden cardiac death, will occur as a result of reducing high serum cholesterol levels is strong.[9]

The difference in opinion relates to the extent we are justified in advising the population to change their dietary habits to reduce serum cholesterol below levels of about 200 mg/dl or 5·17 mol/l, recently recommended by a W.H.O. committee[10] as the optimum, when there is no strong evidence of a relation with CHD incidence, when there is at present nothing to indicate that CHD rates will be lowered, when there might even be adverse effects, and when the necessary dietary changes can be tiresome. The possibility should be considered that there is a threshold of serum cholesterol beyond which CHD risk increases and below which little will be gained in terms of reduction of CHD (figure).



Discontinuous relation between serum cholesterol and CHD mortality: is there a threshold effect?

Based on Pooling Project, Israeli, Stockholm, and Whitehall studies (32 470 men).

Kannel and Gordon state that "serum cholesterol is not a strong risk factor for CHD, in the sense that blood pressure is a strong risk factor for stroke or cigarette smoking is a risk factor for lung cancer". I agree. Then let us not overestimate the value of lowering serum cholesterol in those with levels already below the median.

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carcinogenic. But, as has been indicated, much more needs to be done before such an etiologic inference is derived from existing epidemiologic information. Because of the great importance of these observations for public health policy and for personal medical care, a well-defined program of epidemiologic and other types of investigation is necessary.

These data, however, do provide a lesson for epidemiologists. These reports clearly indicate that we should not become so specialized in our research endeavors with respect to one disease entity so that other entities are ignored. Prospective studies in cardiovascular diseases have been in progress for many years. This possible relationship of cancer to cholesterol may have been ascertained earlier. A way must be found for incorporating the consideration of a variety of diseases in studies of any specific disease whenever the situation so permits. This is particularly true in prospective studies where one starts not with a specific disease, as in retrospective studies, but with a population group who, during the course of the study, develop and die from many diseases. Also, more collaboration between agencies that provide funds for research on individual diseases is clearly necessary to broaden the benefits of epidemiologic research.

But, when all is said and done, the issue becomes one raised by David Hume 250 years ago, "How do we know?" (16).

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ON A POSSIBLE INVERSE RELATIONSHIP BETWEEN SERUM CHOLESTEROL AND CANCER MORTALITY

MANNING FEINLEIB[1]

During the last thirty years the National Heart, Lung, and Blood Institute has sponsored several long term epidemiologic studies to elucidate the natural history of coronary heart disease and the risk factors which predispose certain people to develop this disease. These and similar prospective studies have had tremendous success in documenting the precursors of coronary heart disease including age, gender, high blood pressure, elevated blood cholesterol, and cigarette smoking. However, until recently there has been little attention given to the analysis of the precursors of non-cardiovascular illness and death. In rectifying this problem, the accompanying papers (1–3) present evidence for a statistically significant and potentially important result, viz., a fairly consistent inverse relationship between blood cholesterol measured at baseline, or early on in the studies, and subsequent mortality from various forms of malignancies.

In conducting epidemiologic studies, the investigator usually focuses his analyses on the specific hypotheses under investigation and tends to avoid the diffuse global "fishing expedition." In general, this approach is sound, but, after a positive relationship has been found which suggests the need for some public health action, it is often necessary to look for possible side effects or unexpected ramifications in regard to other health outcomes and parameters. Since scientific research is an iterative process, as data continue to accumulate, new insights are obtained which may cause modification of current beliefs and extension of one's concerns to more complex relations and interactions. This seems to be the current situation with regard to the prognostic meaning of various levels of serum cholesterol to future risk of disease. Virtually all of the major prospective epidemiologic studies which measured cholesterol at the beginning of the follow-up period were concerned with its relation to risk of cardiovascular disease. Now the time has come to consider its relation to other diseases.

The tendency for most epidemiologists and public health practitioners to think in terms of monotonic risk functions is also generally sound so long as one is concerned with a relatively specific disease outcome. However, when the totality of disease outcomes is considered, the rule tends to be for a U- or J-shaped relationship. Thus, although hypertension is clearly a risk factor for coronary heart disease and stroke, clinicians are acutely aware that hypotension may also be a serious clinical problem. Likewise, excessive body weight has been related to excessive mortality from heart disease and other conditions, and, although millions of Americans constantly strive to keep their weights under control, it is clear that being excessively underweight also has adverse consequences. For body weight we have long been accustomed to think of an optimal range of values for which total morbidity and mortality might be minimized. Why should cholesterol levels behave any differently? Evidence is now beginning to accumulate that indeed it does not. It is still too early to talk of optimal levels of cholesterol be-

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cause there is as yet no appropriate cost function which can summarize the possible effects on mortality, morbidity and general well-being of various levels of blood cholesterol. Moreover, until now there has been a lack of the basic risk data in relation to endpoints other than the cardiovascular ones.

Although the accompanying papers (1–3) tend to show a fairly homogeneous inverse relationship between the occurrence of malignancies and serum cholesterol levels, the data are not entirely consistent and other possible explanations for the reported relations might be put forward. In February, 1980, the National Heart, Lung, and Blood Institute conducted a small workshop to look into the available data (4). Much of the data presented, including those portions in the accompanying articles, showed an inverse relationship between cancer mortality and cholesterol among men and a U-shaped relationship between total mortality and cholesterol (5, 6). But some of the data showed no trends among men, none showed any significant trends for women, and several other studies (7, 8) which did show the inverse relationship for men have suggested alternate explanations.

Before examining some of the possible explanations for these associations, it must be emphasized that the observations reported to date are just associations—none of the investigators claims a causal relationship between low cholesterol and subsequent development of malignancies, although it is the fear of this possibility that has given rise to grave concern in many quarters. All of the studies deal with naturally occurring variations in cholesterol levels and not with potential effects of altering cholesterol levels through intervention. At the present stage of knowledge we are concerned simply with assessing the consistency of the associations in various epidemiologic settings both in the United States and abroad and with determining whether the

TABLE 1

Factors to be considered in assessing the epidemiologic relationship of serum cholesterol to cancer occurrence

1. Chance phenomenon.
2. Biases in assessment of baseline cholesterol.
3. Time sequence of events—Does early cancer cause low cholesterol?
4. Biases in ascertainment of cause of death.
5. Competing risk of death from coronary heart disease.
6. Confounding with other risk factors.

associations can be explained by vario artifacts of data collection or analysis by associations with other confoundi factors. Some of the items to be consider in assessing the epidemiologic relatio ship of plasma cholesterol to cancer a listed in table 1.

Each of the items shown in table 1 h been dealt with to some extent in t three studies presented in this issu However, it may be of interest to through each of them with regard to t data from the Framingham Heart Stud which have been presented in deta elsewhere (9). The age-adjusted 20-ye mortality from all causes, from corona heart disease, and from cancer amo men in the Framingham cohort is sho in figure 1. Whereas there is a strong a consistent direct relationship betwe cholesterol level and the risk of dea from heart disease over the 20-year p riod, there is an almost symmetric inver relationship for death from malignanci so that the overall relationship betwe cholesterol and total mortality tends to a U-shaped curve. Mortality was me sured from the 4th biennial examinati done at Framingham (approximate 1954–1957) because this was the exam nation at which the most complete info mation was available for other variabl which might be confounding factors wi serum cholesterol level and subsequen disease experience.

To assess whether this was just

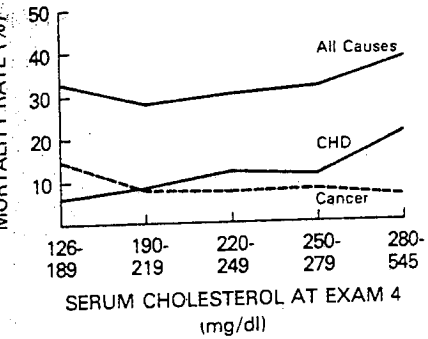

FIGURE 1. Age adjusted mortality rates (20 years) for all causes, coronary heart disease (CHD) and cancer, by serum cholesterol measured at Exam 4 of the Framingham Study in men aged 35–64 years. Source: reference 9.

chance observation for this particular examination, the experience with regard to other baseline examinations was analyzed and is shown in table 2. For examination 2, the first one at which complete cholesterols were determined, the relationship did not reach statistical significance. But for exams 3, 4 and 5, with 22, 20 and 18 years experience, respectively, the relationships were remarkably consistent and significant for men. For women, none of the relationships between cancer mortality and serum cholesterol level was significant for any of the baseline examinations.

The possibility of biases in assessment of baseline cholesterol values is exceedingly important in the present context, not so much from the point of view of selection biases or measurement biases, but from the possibility that those who are destined to die from malignancies during the follow-up period already had early malignancies when first examined and that their baseline cholesterols were depressed due to the presence of the cancers. This problem has been discussed by others (6–8) with somewhat different conclusions. In the Framingham mortality data this question was analyzed in two ways. First, elimination of all those deaths that occurred during the four years after the baseline examination showed virtually no changes in the relationships previously shown. Second, the examination of long-term mortality in those who entered with low cholesterol (below the median) versus those who entered with high cholesterol showed that the differential mortality between the two groups was not due to early excess mortality from malignancies. Rather, the excess cancer deaths accumulated steadily during the entire follow-up period.

It is, of course, possible that elimination of the first four years' experience does not compensate for the effects of early or occult malignancies and that their effect may last for many years before the tumor is diagnosed or results in death. A more direct assessment of this hypothesis has recently been presented in terms of the incidence of cancer in the Framingham Cohort. Here, too, a marked inverse relation was found between cholesterol level

TABLE 2

*Age-adjusted logistic function coefficients of cancer mortality on serum cholesterol at Exams 2–5 of the Framingham Study**†*

Time of measurement of serum cholesterol (follow-up)	Men		Women	
	Coefficient	*t*-value	Coefficient	*t*-value
Exam 2 (24-year incidence)	−0.00218	−1.09	0.00149	0.81
Exam 3 (22-year incidence)	−0.00595	−2.62	0.00052	0.27
Exam 4 (20-year incidence)	−0.00630	−2.90	−0.00174	−0.83
Exam 5 (18-year incidence)	−0.00431	−2.01	−0.00070	−0.35

* Previously unpublished data.

† Population at risk is free of a cancer diagnosis at measurement of serum cholesterol.

and the incidence of cancer. Again, the elimination of the new cancer occurring during the first four years of follow-up did not diminish the inverse relationship. It is still possible that the malignancy may exert an effect on cholesterol levels even four years or more prior to the first clinical appearance of the disease, but this hypothesis now seems more tenuous.

The role of possible biases in the ascertainment of cause of death has not been specifically dealt with in any of the published studies other than for each of them to document their procedures for ascertainment and coding of death certificates and other evidence with regard to underlying cause of death. To some extent, this problem is minimized in incidence studies such as that reported elsewhere for Framingham and Evans County (6, 9). Since the results from the incidence data parallel those for mortality data, it would indicate that the ascertainment biases are minimal.

However, the possibility that competing risks of death from other causes, in particular coronary heart disease, may uncover a spurious inverse relationship to cancer must be considered. The contention here is that those who have high cholesterol tend to die from heart disease so that a relative proportionate increase in cancer death shows up among those w low cholesterol levels. This compet risk model was evaluated in the Fram ham data by means of an actuarial table method for competing risks (10). T analysis showed that the overall can mortality for 20 years follow-up w about one-third higher among those w low cholesterol than for those with h cholesterol whether the coronary he disease deaths were included or exclud in the competing risk model.

Another possibility which had to explored was whether the inverse lationship between cancer and choleste level could be explained by other risk f tors which were associated with both, t is, the role of confounding variables. T was explored by means of the multi logistic model which had been used ve successfully for more than a decade look at the role of several factors acti simultaneously upon the occurrence coronary heart disease. The results of t analysis with regard to cancer mortal are shown in table 3. This table shows t *t*-values for the seven variables that we considered in the model, both singly the univariate case, and for all of the simultaneously in the multivariate ca For women, in the multivariate mod none of the coefficients other than that

TABLE 3

*t-values for logistic function coefficients for cancer mortality during 20 years following Exam 4 of the Framingham Study**

Variable at Exam 4	Multivariate analyses		Univariate analyses	
	Men	Women	Men	Women
(Cases/population)	(151/1836)	(146/2242)	(151/1836)	(146/2242)
Age	6.05	4.28	6.60	5.12
Alcohol	4.27	0.84	4.82	0.19
Serum cholesterol	−3.10	−1.09	−3.25	0.88
Cigarettes	2.80	−0.38	2.17	−1.52
Education	−1.31	−0.80	−3.95	−2.20
Metropolitan relative weight	−1.26	0.28	−1.69	1.67
Systolic blood pressure	0.85	0.23	2.34	2.75

* Previously unpublished data.

age was statistically significant. However, for men, four of the variables were significantly related in the combined model—age, alcohol consumption, cigarette smoking, and an inverse relationship for serum cholesterol. Similar analyses for the other populations studied show that the inverse relationship for cholesterol and cancer mortality cannot be accounted for by any confounding factors for which data were available.

There are still many gaps in the information currently available. In addition to the obvious need to investigate these relationships in more populations and with regard to specific cancer types and sites, other, perhaps more difficult, questions are still unresolved. The determinants of low serum cholesterol, as well as the determinants of high serum cholesterol, are still not precisely known (11). Genetic factors, nutrition and energy expenditure have been implicated. Various disease states are associated with low cholesterol levels and, as mentioned previously, some suspect that early, asymptomatic malignancies may induce low cholesterol levels. If the association between low cholesterol and subsequent occurrence of malignancies is replicated consistently in a variety of populations, is this due to a cause-and-effect relationship or to an indirect association through some other pathway?

It is probably much too early to speculate about biological mechanisms but already there have been reports of a possible role of low vitamin A (a fat-soluble vitamin) in this process and of an association between certain immune mechanisms and reduced cholesterol (6, 12, 13). Undoubtedly, other speculations will be forthcoming as more investigators dwell on this important problem. What, for example, are the roles of the specific cholesterol-bearing lipoprotein fractions? It is now recognized that with regard to the risk of coronary heart disease, the various lipoprotein fractions do not have the same strength of association or direction of association: low density lipoprotein cholesterol is positively ated to coronary heart disease while high density lipoprotein cholesterol seems to have a strong negative relation. Do similar relationships obtain with regard to these fractions and the risk of malignancies?

In the few investigations which have studied the relationship of cholesterol and malignancies in women, no inverse association has been found. Is this because different cancer sites tend to dominate in women as compared to men, or are other mechanisms involved? Are there different relations for hormone-dependent tumors and cholesterol than there are for other types of cancers? There is a paucity of information from animal experiments on the relationship between cholesterol and malignancies. This omission must be rectified before detailed conclusions about mechanisms can be drawn.

Although the available data on cancer incidence and cholesterol tends to parallel the data on mortality, this has not yet been confirmed by very many studies. It is possible that cholesterol levels do not affect cancer incidence but may influence the growth and spread of tumors. Evidence for such a relation would have significant implications for the nutritional management of cancer patients. Thus, there is a wide variety of important questions that must be answered before the full meaning of the reported associations can be assessed.

Although the papers in this issue and those already published by no means resolve the complete nature of the relationship between plasma cholesterol and the risk of non-cardiovascular diseases, certain tentative conclusions are possible. One conjecture is that the "ideal" cholesterol level does not lie at either extreme of the distribution but somewhere towards the mean. From several directions, the indication is that for optimal mortality rates the ideal cholesterol may be somewhat below that of the average US level,

but probably not at the very low levels advocated by some.

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Original Contributions

SERUM CHOLESTEROL AND MORTALITY IN A JAPANESE-AMERICAN POPULATION

THE HONOLULU HEART PROGRAM

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Kagan, A. (Honolulu Heart Program, Honolulu, HI 96817), D. L. McGee, K. Yano, G. G. Rhoads and A. Nomura. Serum cholesterol and mortality in a Japanese-American population: the Honolulu Heart Program. *Am J Epidemiol* 1981;114:11-20.

The authors have examined the relation of baseline serum cholesterol level to subsequent 9-year mortality in a cohort of Japanese-American men. The baseline serum cholesterol level was positively related to coronary heart disease mortality. It was inversely related to total cancer mortality and to mortality from cancers of the esophagus, colon, liver and lung and to malignancies of the lymphatic and hematopoietic system. When mortality in the first two years after examination was removed from consideration in an attempt to allow for existing clinical or subclinical disease, the inverse relation to cancer persisted but was statistically significant only for colon cancer and lung cancer among the common sites as well as for malignancies of the lymphatic and hematopoietic system. When known prevalence cases of cancer were eliminated from the computation, the inverse relation between cholesterol level and cancer mortality persisted significantly only for colon among the common cancer sites. The relation of baseline serum cholesterol level to total mortality in this cohort was quadratic; that is, there was an excess of deaths associated with serum cholesterol level at the high end of the distribution (mainly due to coronary heart disease) and at the low end (mainly due to cancer).

cholesterol; coronary disease; mortality; neoplasms

Recent findings have raised questions about the relationship of low serum cholesterol to subsequent morbidity and mortality. One question is whether low serum cholesterol, while associated with decreased risk of coronary heart disease, might be associated with increased risk of other diseases, cancer in particular (1–10). Another question is whether low serum cholesterol is a manifestation of subclinical disease (10). The present report examines these questions in the Honolulu Heart Program cohort.

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Abbreviation: CHD, coronary heart disease.

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Supported by National Heart, Lung, and Blood Institute Contract 62955 and by the National Cancer Institute Contract NO1 CP 61060.

Methods

The Honolulu Heart Program is a prospective study of coronary heart disease and stroke among a target population of American men of Japanese ancestry who were born between 1900 and 1919 and

DIET, BLOOD CHOLESTEROL AND CORONARY HEART DISEASE: A CRITICAL REVIEW OF THE LITERATURE.

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7. THE "CONCLUSIVE" TRIALS

"The problem with all these (previous) trials is that none of them have showed a difference in heart attack or death rate in the treated group. Only when soft-end points were used in fact was there any subjective difference."

Robert Levy (1977[288])a

"Most of the dietary (clinical trial) studies can be criticized in terms of study design, observer bias or improper end point [soft] end points."

Robert Levy (1980[300])a

"...all causes of mortality is the acid test...because...(the)...concordance among trends of CHD, cardiovascular and all-causes mortality rates strongly supports the conclusion that the decreases reported in CHD mortality rates are real, not spurious."

Jeremiah Stamler (1977[1600])a

INTRODUCTION

This chapter is devoted primarily to the NHLBI sponsored trial known as the Lipid Research Clinics Coronary Primary Prevention Trial, commonly referred to as LRC-CPPT or simply LRC. NHLBI claimed that the trial provided the conclusive proof that lowering blood cholesterol reduces the risk of CHD. The results of this trial formed the basis for the multimillion dollar National Cholesterol Education Program, designed to convince every American to change his/her diet.

This chapter will focus secondarily on two other trials published after the LRC study which NHLBI refers to as further evidence supporting its program.

Not only will it be shown that the trials produced results based entirely on soft endpoints and failing to pass the all-causes mortality acid test, it will also be seen that NHLBI seriously abused scientific ethics in its analyses and interpretations of the trial data. Very importantly, it will also be seen that NHLBI staff and supporters published statements which, together, constitute a logical impossibility. Finally, it will be shown that NHLBI has indirectly admitted that diet has little or no effects on risk of CHD.

THE PURPOSE AND DESIGN OF LRC-CPPT

The design (protocol) of the LRC trial was published in 1979.[503] ~~589~~ It stated that "The inconclusive findings of the many studies to date can be mainly attributed to their inability to meet requirements such as the use of a randomized, double-blind design; the need to achieve a sufficient cholesterol differential between treatment groups to affect the course of CHD significantly; the need for sufficient participants; satisfactory statistical methods to analyze the data; selection of participants with potentially reversible lesions (atherosclerosis). In some trials a further

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a As will be seen, the trials considered "conclusive" by Levy and others, i.e., the LRC and Helsinki II trials, were based entirely on "soft" end points and did not pass Stamler's all-causes mortality acid test.

problem has been the occurrence of significant morbidity (increase in rate of disease) and mortality (increase in rate of death) associated with the use of cholesterol-lowering agents which negated any hypothetical benefit of lowered cholesterol." The facts are, however, that many previous trials did meet the above requirements but still did not show any benefits of lowering blood cholesterol. Furthermore, the so-called requirement of selecting "participants with potentially reversible lesions" is totally absurd because no one had shown that atherosclerosis was reversible in humans, so how could anyone select participants with reversible lesions?

Of great importance is the fact that the protocol report fully acknowledged that some previous trials found higher disease and death rates in groups treated with cholesterol-lowering agents, particularly drugs. As any statistician knows, the possibility that a drug may have either beneficial or harmful effects means that a statistical test used on trial results should be "two-tailed." A two tailed test was, in fact, employed in the 1975 NHLBI sponsored trial, the Coronary Drug Project, which found higher death rates in some groups treated with drugs.[490] Authors of the protocol report nevertheless rejected the two-tailed test, thereby reducing the differences necessary between treatment and control groups to gain statistical significance. They did, however, emphasize the need for selecting a high level of significance, as was selected in the Coronary Drug Project. On page 3 of the protocol report it is stated that "a significance level of .01 was chosen as the standard for showing a convincing difference between treatment groups." And in Appendix D, the need for the .01 level for significance is stressed even more, namely, "since the time, magnitude and cost of the study make it unlikely that it could ever be repeated, it was essential to be sure that any observed beneficial effect of cholesterol-lowering was a real one. Therefore,...(significance) was set at .01 rather than the usual .05."[a]

University of Southern California professor and statistician, J.P. Guilford, noted that "some investigations adopt a standard (level) of significance in advance of the study or experiment. This lays down the rule for decision-making beforehand ... One disadvantage is that there may be temptation to modify the adopted standard after the results are in."[1165] This is precisely what the LRC investigators did. Not only is lowering significance level after-the-fact universally considered scientifically unethical, they also falsely stated that they had not originally selected the higher level of significance.

In sum, the use of a one-tailed rather than a two-tailed test and the use of the .05 rather than the .01 level of significance, greatly increased the likelihood that very small differences between treatment and control groups would become statistically significant.[506,509] Ironically, it was precisely this kind of biased analysis and reporting that led one NHLBI staff member, Shiela Mitchell, to criticize earlier studies.[488] To further dilute the importance of the LRC's findings, the investigators used very unconventional statistical tests on their data, indicating that even the substantial lowering of the significance level and use of a one-tailed test was not sufficient for achieving significance with conventional tests.

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[a] Authors of the Coronary Drug Project stated that "... a result should not be judged statistically significant unless it achieves at least the .01 level of significance or perhaps even the .005 significance level."[490] Before the results of the LRC trial were published, Stamler and Dipalma stated that "...CDP has become a model and has set standards for benefit-to-risk ratio studies of long term therapy of serious disease."[1351] If so, the LRC authors lowered those standards substantially.

These unethical tactics virtually transformed nonsignificant findings into significant findings.[a] In view of the fact that this study provided the cornerstone for NHLBI's multimillion dollar campaign to change everyone's diet and undoubtedly influence millions to begin taking cholesterol-lowering drugs for life, these tactics resulted in tragic consequences and not simply abuses of statistical ethics.

LRC TRIAL DESCRIPTION

The LRC trial began in 1973 and ended in 1983. Subjects participated in the trial for durations of 7 to 10 years, with an average of 7.4 years. Like most previous trials, women and nonwhites were excluded and men were selected whose blood cholesterol levels were among the highest in the male population. The subjects ranged in age from 35 to 59, with an average of 47.8, and they were clinically free of major illnesses and "notable" obesity. Thus, at the outset the sample of men was purposely intended to be completely unrepresentative of the American population. In fact, 480,000 men had to be screened to find the atypical 3,806 men who ultimately participated in the trial.[147] Yet, the LRC investigators neglected to tell the public this fact and insisted that the trial results were applicable to all Americans, including women.

The subjects were randomly distributed into two groups. The treatment group of 1906 subjects received daily doses of a blood cholesterol-lowering drug called cholestyramine, while the control group of 1900 subjects received an inactive (placebo) substance. Cholestyramine was selected because it had been known to reduce blood cholesterol and because of "...its nonabsorbability from the gastrointestinal (GI) tract (that is, it does not enter the body's blood system), its few systemic effects, and its low level of significant toxicity." It should be noted, however, that there is evidence that the drug promotes GI cancer in animals.[147,194] It also has numerous side effects.

Over the course of the study the blood cholesterol levels of drug treated subjects averaged 8.5% lower than those of the control subjects.

The report of the LRC trial was published in two parts in the Journal of the American Medical Association in January, 1984.[500,501] The authors, headed by Basil M. Rifkind, Chief of the LRC program, NHLBI, prefaced the results of the study by stating:

"plasma total (cholesterol) and LDL-C levels may be reduced by diets and drugs. However, before such treatment can be advocated with confidence and before it can be concluded that cholesterol plays a causal role in the pathogenesis of CHD, it is desirable to show that reducing cholesterol levels safely reduces the risk of CHD in man. Many clinical trials of cholesterol lowering have been conducted, but their results, although often encouraging, have been inconclusive."

In addition to the term "often encouraging" being a gross exaggeration of reality, as was seen in Chapter 6, the entire statement is most inconsistent in view of the fact that NHLBI and AHA have been strongly advocating for many years that Americans should alter their diets to reduce blood cholesterol. This statement, therefore, clearly implies that the NHLBI's and AHA's previous recommendations to the public have been made without confidence.

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[a] As will be seen, further after-the-fact changes in protocol were actually necessary to finally achieve significance.

There is little reason to believe that the LRC trial was not well-designed and properly conducted. The data analysis, interpretations of results and conclusions drawn by the authors, however, were not examples of unbiased and objective scientific reporting. As will be seen, numerous other medical researchers expressed similar criticisms.

Perhaps the first unethical act the LRC investigators committed was the changing of the variables to be measured. On Page 5 of the protocol report it is clearly stated that "The primary end points (plural) in the CPPT are CHD death and non-fatal myocardial infarction (heart attack)."[503] These end points, of course, were common to nearly all previous clinical trials. In the 1984 report of results, however, the LRC investigators indicated a single end point, namely, "The primary end point (singular) for evaluating the treatment was the combination of definite CHD death and/or definite nonfatal myocardial infarction." This protocol change was obviously accomplished after examining the results and finding no significant differences between the groups for CHD deaths alone or nonfatal heart attacks alone. In response to later criticisms of this change, the LRC authors stated that "The study was not designed to detect the effect of treatment on fatal or nonfatal CHD events alone, and statistical tests for these subcategories were not considered appropriate."[504] But this response was totally preposterous because it is completely inconsistent with the protocol report and previous trials. It is also inconsistent with the fact that NHLBI has emphasized for many years that CHD is the number one killer disease and that the reduction of CHD deaths is a major goal of its research program. It is also inconsistent with the former NHLBI director's (Levy) statement before the 1977 Senate Select Committee on Nutrition and Human Needs that "...the end points will be heart attacks and heart attack deaths."[288] To say that it is not "appropriate to analyze the number of deaths in the treatment and control groups independently of non-fatal heart attacks is simply absurd and a back-door admission that there were no significant differences between the groups. In this regard, the reader is referred to the quotes by Levy preceding the introduction to this chapter.

There were 38 and 30 CHD deaths recorded for the control and treatment groups, respectively. The LRC authors claimed, therefore, that the drug produced a 24% reduction in risk of CHD death. In the first place, this value is misleading because the difference between the groups was 8 and 8 ÷ 38 = 21%, not 24%. Of far more importance, however, is the fact that this percentage is rather meaningless, independent of sample size. In reality, the death rate in the control group was 38/1900 or 2.00%, while the rate in the treatment group was 30/1906 or 1.57%. The actual percentage reduction was 2.00 - 1.57 or 0.43%. Third, the LRC authors should not have stressed a difference between groups in death rates because they reported no statistical test for these data. Had they performed such a test, the difference would have been nonsignificant.[a] Obviously the LRC authors wanted it both ways -- to report a difference but not to report the nonsignificance of that difference.

The identical argument can be presented with respect to nonfatal heart attacks. The LRC authors reported 158 and 130 of these incidents for the control and treatment groups, respectively, for a percentage reduction in risk of 19% (although the difference between 158 and 130 is 28 and 28 ÷ 158 = 17.7%). The actual rate reduction was 1.5%. Similarly, the authors

[a] A common statistical test (Chi square, X^2) reveals that the difference was not remotely close to significance.

should not have again stressed a difference between groups because they also reported no statistical test for these data.[a]

Finally, the LRC authors reported 187 and 155 CHD deaths and nonfatal heart attacks combined for the control and treatment groups, respectively, for a percentage reduction in risk of 19% (but 187 - 155 = 32 and 32 ÷ 187 = 17.1%). Again, the actual rate reduction was only 1.7%. The LRC authors reported the difference between groups as significant using highly unconventional tests. Whether or not one wishes to accept their tests, one must remember that their "significance" was achieved only after substantially lowering their criteria for achieving significance.

To more clearly appreciate the full impact of the altering of protocol and the use of unconventional statistical tests, consider the example of an outstanding college football player who must achieve a score of 70 on a test to remain on the football team. He takes the test and scores 55. The school them simplifies the test so that the athlete's answers now achieve a score of 60. The school simplifies the test again, elevating the score to 65. Finally, the school changes the passing score criterion from 70 to 65. Not so surprisingly, the athlete "passes."

Now let us turn to other data presented in the LRC report that were not given to the public. Using the LRC authors' procedure of showing differences between groups, Table 7-1 lists the diseases and deaths for each group for which differences were 5 or more cases. The second to last column of the table indicates whether the difference was positive or negative with respect to the treatment group and the last column indicates whether the difference was significant, using the statistical test, Chi Square.

First and foremost, the overall death rates of the treatment and control groups were essentially identical, indicating that the treatment did not alter life expectancy over the 7.4 year period. While Gotto announced in 1988 that this trial "was not designed to show differences in all-causes mortality,"[1425] such a remark was a ludicrous way of attempting to explain away the most important finding of this trial. How can a trial not be designed to show differences in all-causes mortality? In effect, this trial failed to pass the important "acid test" emphasized by Stamler before the 1977 Senate Select Committee.

As can be seen in Table 7-1, the percentage differences of all nonCHD diseases and deaths were greater than CHD heart attacks and deaths, averaging 80.4% for nonfatal disease and 312.5% for deaths. The reader should first note that most of the effects were associated with the treatment. Second, nonfatal GI cancer and skin cancer and fatal GI cancer, yielded significant differences, not heart attacks or CHD deaths. However, as was previously pointed out, percentage differences can be grossly deceiving. For example, suppose there were two CHD deaths in the control group (2 in 1906) and one in the experimental group (1 in 1900). Using the LRC authors' method of calculation, the percentage difference would be 100% and yet the actual difference would only be one. Rates are vastly more meaningful because they uniformly relate incidents with the total sample. Thus, even the 700% increase in GI cancer deaths shown in Table 7-1 is a rate increase of only 0.37%. Nevertheless, it is statistically significant and it is consistent with evidence that cholestyramine promotes GI cancers in animals (see Chapter 8). The director of the LRC trial, Basil Rifkind, elsewhere referred to the GI cancers in the treatment group as a "slight increase" and concluded that

[a] The Chi square test again revealed that the difference was not significant.

TABLE 7-1

Nonfatal and fatal diseases in the LRC trial
(Adapted from 500)

NONFATAL CONDITIONS	CON	EXP	% DIFFERENCE	POS/NEG	SIGNIFICANT
Heart Attacks	158	130	17.7	+	NO
GI Cancer	11	21	90.9	-	YES
GI Ulcer	20	30	50	-	NO
GI Gastritis	5	12	140	-	NO
GI Appendicitis	4	11	175	-	NO
Respiratory Cancer	13	7	46.2	+	NO
Skin Cancer	11	3	72.7	+	YES
All Other Cancers	22	27	22.7	-	NO
FATAL CONDITIONS					
CHD	38	30	21	+	NO
GI Cancer	1	8	700	-	YES
Respiratory	8	3	62.5	+	NO
Trauma	4	11	175	-	NO
TOTAL DEATHS	71	68	4.2	+	NO

"...they came about by chance and had nothing to do with treatment."[191] Since the difference between groups on GI cancer deaths was 7 the difference between groups on CHD deaths was 8, why, then, did not kind consider the latter difference to be due to chance as well?[a] Instead, he reduced a significant finding to "chance" and elevated a nonsignificant finding to "significance."

The March 26, 1984 issue of Time Magazine, whose cover read, "Cholesterol: and now for the bad news," stated in the early part of an article purporting to describe the LRC study, "lowering cholesterol levels markedly reduces the incidence of fatal heart attacks."[757] The LRC authors did not make such a statement in their report but they did make an announcement to the media that the results of the LRC trial "... have the potential to markedly reduce the large number of heart attack deaths..." Thus, the media translated potential into actual heart attack deaths, based on a statistically nonsignificant finding.

The fact is that all the numbers and differences reported in the LRC study were pathetically small and of little or no practical significance. They were, in fact, remarkably similar to the results of the many previous clinical trials, as can be seen in Tables 6-1 and 6-2 of Chapter 6. Twenty-three of 27 U.S., British and European trials failed to show a difference in total deaths between treatment and control groups. The LRC trial was one of those 23. The reader may recall that the remaining four trials were either scientifically flawed, obtained results opposite to others or were conducted by a drug company.

RESPONSE TO THE LRC TRIAL'S RESULTS

The response to the LRC trial by the media and other medical researchers was enormous. The media accepted the LRC authors' press releases without question, but many medical researchers were highly critical of the LRC study. The media were apparently unaware of the massive criticisms and the public, therefore, once again received a completely one-sided view. To illustrate how the NHLBI misled the press, the editor of one journal compared the following statements by the LRC authors in the Journal of the American Medical Association and in their subsequent press release.[200]

In the Journal

"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."

To the Press

"In summary, the LRC-CPPT is the first study to demonstrate conclusively that the risk of coronary heart disease can be reduced by lowering blood cholesterol. For each 1% fall in cholesterol, a 2% reduction in heart attack risk can be expected.[b] These results have widespread implications for many millions of Americans and, if applied, have the potential to

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a Even though the cancer death difference was one less than the CHD death difference, significance is based, in part, on how different a ratio is from 1/1. Thus, 30/38 is nearly 1/1, while 1/8 is, of course, quite different.

b As noted by others, there is no valid evidence to support this statement.[509,510,1180] As will be seen below, it derives from a very elementary error in statistical reasoning.

An Analysis of Randomized Trials Evaluating the Effect of Cholesterol Reduction on Total Mortality and Coronary Heart Disease Incidence

Ingar Holme, PhD

The primary aim of this study was to estimate the relation between cholesterol reduction and total mortality and coronary heart disease (CHD) incidence. Secondarily, the clinical issues of whether the efficacy of cholesterol lowering is dependent on the treatment modality, presence of CHD at baseline, or the simultaneous introduction of other interventions was explored. All randomized clinical intervention trials of cholesterol reduction were used in an overview analysis of total mortality rate and CHD incidence; analysis was performed with weighted linear regression. The trials include those that used primary and secondary intervention, diet and drugs, and single or multifactor design. Nineteen trials were analyzed for total mortality, and of the 19, 16 were analyzed for CHD incidence rate. Net difference in cholesterol change between study groups was used as the independent variable, and the three previously mentioned dichotomous design characteristics were used as additional independent variables. For every 1% reduction in cholesterol, an estimated 2.5% reduction in CHD incidence is indicated (95% CL: 1.1, 3.9). With regard to CHD drug trials tended toward better efficiency in cholesterol lowering than did dietary trials. With regard to total mortality, this efficiency was higher in secondary than in primary preventive trials. The efficiency was also somewhat dependent on the baseline cholesterol level. This study shows that cholesterol reduction is effective in lowering CHD incidence, but cholesterol reduction must be at least 8–9% to be effective in lowering total mortality. (*Circulation* 1990;82:1916–1924)

In the prevention of early occurrence of coronary heart disease (CHD), various strategies have been implemented based on the three most important coronary risk factors, that is, total cholesterol, blood pressure, and cigarette smoking. The evidence of cholesterol lowering as an efficient method of CHD risk reduction has been tested through a series of intervention trials. These include single and multifactor trials, and primary and secondary diet and drug trials.[1–19]

Two overview analyses have evaluated the effects of cholesterol lowering in clinical trials.[20,21] They show that for every 1% cholesterol reduction a 2% CHD risk reduction is achieved. This 1:2 ratio is subsequently referred to as "the cholesterol benefit ratio." These overviews generally exclude multifactor trials or analyze them separately, and they do not address the question of whether the effect of cholesterol lowering is dependent on design characteristics such as primary and secondary or diet and drug intervention.

The primary aim of this study was to estimate the relation between cholesterol reduction and total mortality and CHD incidence. Secondarily, the clinical issues of whether the efficacy of cholesterol lowering is dependent on the treatment modality, presence of CHD at baseline, or the simultaneous introduction of other interventions was explored.

These questions are addressed through an overview analysis of all randomized controlled clinical trials involving designed cholesterol lowering. Total mortality and CHD incidence are the only end points under investigation. Trials with angiographic end points will not be discussed.

Methods

The criteria for inclusion of trials in this overview were 1) designed cholesterol lowering, 2) randomized design, and 3) total mortality or CHD incidence reported as end points. The criteria for exclusion of

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TABLE 1. Design Characteristics for Included Cholesterol-Lowering Trials in Overview Analysis

Trial	Diet/ drug	Primary/ secondary	Single/ multifactor	Open/ blind	M/F	Age range (yr)	Mean age (yr)	Follow-up (yr)	Baseline serum cholesterol (mg/dl)
MRFIT[1]	Diet	Primary	Multi	Open	M	35–57	46	6–8	253
Hjermann et al[2]	Diet	Primary	Multi	Open	M	40–49	45	6–7½	325
WHO fact[3]	Diet	Primary	Multi	Open	M	40–59	48	5–6	216
Acheson and Hutchinson[4]	Drug	Secondary	Single	Blind	M+F	–	...	≦7	288
Carlson et al[5]	Drug	Secondary	Single	Open	M+F	≦70	59	3½	247
RC of Scottish Society[6]	Drug	Secondary	Single	Blind	M+F	40–69	52	6	266
Coronary Drug Project[7]	Drug	Secondary	Single	Blind	M	30–64	54	4½–8	249
Newcastle upon Tyne[8]	Drug	Secondary	Single	Blind	M+F	≦65	52	5	249
Dorr et al[9]	Drug	Secondary	Single	Blind	M+F	18+	54	3	307
Dayton et al[10]	Diet	Secondary	Single	Open	M	55+	66	≦8	234
Leren[11]	Diet	Secondary	Single	Open	M	30–64	56	5	296
MRC[12]	Diet	Secondary	Single	Open	M	≦60	...	2–7	272
MRC[13]	Diet	Secondary	Single	Open	M	≦65	...	6	263
Rose et al[14]	Diet	Secondary	Single	Open	M+F	≦70	55	2	260
Woodhill et al[15]	Diet	Secondary	Single	Open	M	30–59	49	2–7	282
LRC-CPPT[16]	Drug	Primary	Single	Blind	M	35–59	47	7–10	279
WHO (clofibrate)[17]	Drug	Primary	Single	Blind	M	30–59	45	5.3 (average)	248
Frick et al[18]	Drug	Primary	Single	Blind	M	40–55	47	5	289
Frantz et al[19]	Diet	Primary	Single	Open	M+F	All	...	5	–

trial end points were 1) termination of trial or trial subgroups because of unforeseen side effects, and 2) data gathered after end of planned trial duration.

Nineteen randomized cholesterol-lowering trials with CHD incidence or total mortality as end points fulfill the inclusion requirements, and they are listed in Table 1.

The trials include both untreated and placebo-treated control groups with open and blinded design. They vary with respect to drugs or diet, primary or secondary intervention, single or multifactor intervention, follow-up period (from 2 to 10 years), age span, and sample sizes (from less than 100 to 50,000). The coronary risk at entry also varies widely between the trials. Because of this heterogeneity, which cannot be totally controlled by statistical modeling, a major random component is bound to be present in such an overview analysis. In the analysis, all trials are equally handled disregarding differences in quality of methods and conduct.

The multifactor trial by Miettinen et al[22] was both a diet and a drug trial in which some intervention group patients received a dietary regimen alone, and others received a drug regimen in addition. For this reason, the trial has been excluded from the analyses. Its inclusion would not have influenced global results noticeably because it reported only a few events.

A particular problem with respect to inclusion and exclusion is present for the Coronary Drug Project,[7] which had to discontinue three of its six trial regimens before the scheduled completion of the project. This was due to an excess in fatal complications in the actively treated groups compared with the placebo-treated group. A further technical complication from a statistical point of view is the problem of treating five actively treated groups against only one matching placebo-treated group. All odds ratio statistics for those five trial arms would be dependent and would seriously complicate the overview analysis (see below). The decision was made to exclude the three discontinued groups and only include the pooled results from the niacin- and the clofibrate-treated groups and compare that with the placebo-treated group. It is realized that this procedure probably biases the overview results somewhat by showing a greater benefit from cholesterol lowering on total mortality. However, the alternative of collapsing all treatment arms results in one treatment group would probably also have a biassing effect in the other direction because of the frequent overestimation of effects seen in trials stopped prematurely.

In the Scottish Research Committee Study,[6] the data from both blinded and open phases has been included.

The end points used are total mortality and CHD incidence. The definitions of CHD may vary, but fatal and nonfatal acute myocardial infarction including sudden deaths as reported in each trial have been adopted without adjustments. Three trials have not reported CHD incidence data,[4,9,15] and they will only be included in the analysis of total mortality. In the trial by Rose et al,[14] probable infarctions are not included as a CHD end point. The morbidity data from the Multiple Risk Factor Intervention Trial (MRFIT) trial[23] has been included using electrocardiographic criteria.

Some trials have produced long-term follow-up information after completion of the scheduled trial

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period, for instance, the Coronary Drug Project[24] or the Oslo Study.[25] It was decided not to include the additional end points in these studies. An argument about increased precision is surely relevant. However, the validity of such data is harder to judge in the context of the relation to cholesterol reduction because the cholesterol difference between treatment groups are no longer fully controlled as in the trial itself.

In all trials, intention-to-treat analysis data have been used whenever a choice appeared. The small trial of Acheson and Hutchinson[4] has only published on treatment data but is included in this overview. The WHO multiple risk factor trial[3] used factories as units of randomization, but the odds ratio (OR) for that trial will be treated in the same way as the other person-based randomized trials.

The calculations of net difference in cholesterol levels between treatment groups were reported differently in the various trials. Sometimes percent average differences between baseline and postrandom follow-up values of cholesterol in each group were computed, and then the absolute differences between the groups based on these averages were calculated. In other (most) trials, the difference was calculated as the percent net difference between average postrandom value in each group. The difference between the two methods should be small because of randomization.

Statistical Analysis

Each trial was used as a unit for statistical analysis. OR for treatment versus control group was calculated. To obtain an adjusted OR for the entire set of trials, pooling of the various trials by ignoring the independent variables was performed with the Mantel-Haenschel method in the Peto framework with observed and expected cases.[26] To determine which trials dominate the overview analysis, a radial plot of standardized logOR according to a method of Galbraith[27] was used (Figure 1). In a radial plot, a regression line through the origin will pass through the global point estimated at the radial segment. The line will be dominated by points with high x values, that is, high precision. The use of logOR instead of OR overcomes some problems with the interpretation along the OR scale. First, the length of its confidence interval is dependent on the level of OR. This means that with a given sample size a confidence interval around an estimated OR of 1.5, for instance, will be much wider than a confidence interval around an OR of 0.67. This is not the case with a confidence interval of logOR. Second, confidence intervals will be preferably symmetrical on both sides along a logOR scale but not on an OR scale. Third, a unit of change in logOR will give the same percent change at any point of OR. This will not be the case if OR changed.

The logarithm of OR was used as the dependent variable in various weighted linear regression models given below with percent net difference in cholesterol

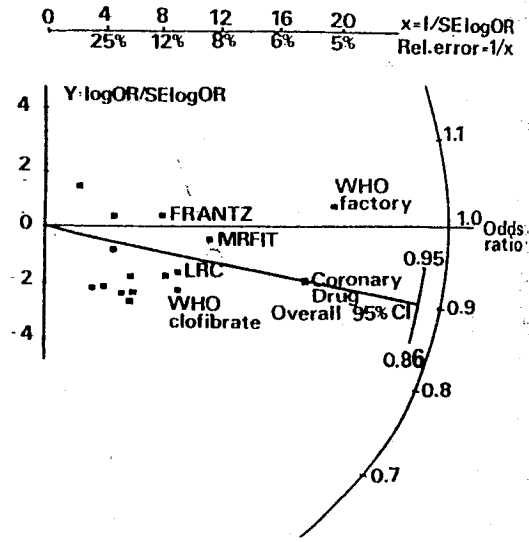

FIGURE 1. *Radial plot of CHD odds ratio (OR) for all trials from Table 2. Each point has unit standard error in the y-axis direction. OR for each trial is found by extrapolating a line from origin to the global estimate on the circular scale.*

between the treatment groups as one independent variable. The weight used for each trial was the variance of OR, suggested by the fact that the overview logOR can be written as a weighted average of each trial's logOR where the weights are (relative) variances of OR.[28] Design characteristics such as single-factor or multifactor, primary or secondary prevention, and diet or drug were defined by three separate dichotomized independent covariates. Interaction terms between cholesterol and the three design variables were defined by multiplying percent cholesterol difference with each binary variable.

The criteria for classifying the various trials with respect to the design characteristics were as follows. Trials aiming at changing other CHD risk factor levels in addition to cholesterol are classified as multifactor trials, whereas trials with cholesterol-lowering alone are classified as single-factor trials. Trials including patients without known cardiovascular disease at baseline are classified as primary preventive trials, whereas all others are classified as secondary preventive trials (mostly on infarction patient populations). Cholesterol reduction by diet alone or by drug (possibly with addition of diet in both treatment groups) distinguishes the diet or drug values.

Average baseline cholesterol (mg/dl), male gender (1–0), and average age at entry (years) were three other independent variables used in some supplementary analyses.

The strategy of analysis was first to search for interactions between cholesterol reduction and each design characteristic. If interactions were not present, this indicates that none of the design characteristics had any modifying effect on the logOR cholesterol relation.

An example of a model for testing interaction between single-factor or multifactor intervention on the cholesterol and CHD relation is

$$\log OR = \beta_0 + \beta_1 \Delta chol + \beta_2 \Delta chol \cdot SM + \epsilon$$

Usually, a linear term with SM (single-factor or multifactor intervention) would also be included in such a model, but the question raised and the appropriateness of two regression lines (SM=0 or 1) starting at the same point on the y axis makes it undesirable to add such a term. The model fit was not improved by such an addition.

The null hypothesis to be tested first would be $H_0: \beta_2 = 0$. Here, Δchol equals percent net difference in cholesterol between treatment groups. SM is 1 for single-factor and 2 for multifactor trials; ϵ is an error term. Similarly, the term DD (diet or drug) would be 1 for diet and 2 for drug, whereas the term PS (primary or secondary intervention) would be 1 for primary and 2 for secondary prevention trials. The software package GLIM[29] was a practical tool for the analysis.

One would think that if there is no net difference in cholesterol between treatment groups logOR should be zero (neglecting multifactor intervention effects). Therefore, β_0 ought to be zero, a hypothesis that will be tested separately after assessing the final model. If the hypothesis is well accepted, the omission of β_0 seems natural.

The interpretation of the slope β_1 in case of $\beta_2 = 0$ is that it expresses the benefit ratio of cholesterol reduction. That is for every 1% net reduction in cholesterol between treatment groups, a risk reduction of β_1 multiplied by 100% occurs, ignoring the influence of the design variables. The Lipids Research Clinics Program, for instance, claimed $\beta_1 \times 100\% = 2.0\%$ unadjusted.[20]

In a supplementary analysis, the ratio logOR/Δchol was calculated from each trial and regressed (weighted as before) against the three supplementary baseline variables.

Tests of significance are performed with χ^2 tests. A χ^2 statistic of residuals between observed and expected cases is computed for the model under the alternative and under the null hypothesis. The difference between the two is the χ^2 test statistic for the null hypothesis. Adequacy of model fit was judged by comparing the χ^2 residual squared difference between observed and expected end points according to the model with its degrees of freedom, with the realization that these should be about equal in case of a good model fit.

Results

Table 2 presents number of deaths and CHD end points in the various trials of the overview analysis as well as OR and 95% confidence intervals and percent net difference in cholesterol between treatment groups. Total mortality is increased by intervention by about 4±3% compared with control ($p>0.10$). Likewise, CHD incidence is reduced by about 10±2.5% ($p<0.001$). This corresponds to a weighted average net difference in cholesterol of 5–6%; that is, the 1:2 CHD benefit ratio of cholesterol reduction is confirmed across all trials. However, the χ^2 residual for a model with the constant term alone is far above the number of degrees of freedom ($p<0.01$), indicating heterogeneity across trials for the CHD end point.

To determine which trials dominate the overview, a radial plot of standardized CHD logOR by its precision measured by $x = 1/\text{SE logOR}$ was developed (Figure 1). The larger the x, the greater the dominance of the drawn regression line through origin and global estimate on the radial segment. Each estimate has unit standard error on the y axis. Six trials in the figure dominate the regression line because of their high precision, and their influence somewhat reduces the beneficial effects of cholesterol lowering compared with the trend in the other trials.

Relation to the Degree of Cholesterol Reduction

Total mortality. Even if cholesterol reduction could not be shown to significantly reduce total mortality in all trials combined, Figure 2 shows that it is associated with a weak downward trend in total mortality. The figure suggests that the treatments used in these trials may have a small adverse effect on mortality when cholesterol levels are not reduced and that cholesterol lowering appears to offset this effect.

Table 3 shows the regression coefficients with standard errors and χ^2 error term for the models that test interaction between cholesterol reduction and design characteristics with respect to total mortality. Model 1 presents estimated coefficients for percent net difference in cholesterol without interaction terms, and models 2, 3, and 4 give the cholesterol interaction coefficient for each particular design characteristic. The fit was not quite good for models 1 and 2, indicating that some important, unexplained variation is omitted. The question of a different efficacy of cholesterol lowering by diet or drugs is not supported by the data in model 2, which shows no sign of an interaction effect. However, in model 3, the interaction term for primary or secondary intervention is of borderline significance ($Z=1.97$, $p=0.05$), and the model fits very well with its degrees of freedom. This indicates that secondary preventive trials have shown a stronger relation between total mortality reduction and cholesterol lowering than have primary preventive trials, an observation not unexpected when one considers the dominance of CHD deaths in secondary preventive trials.

Single-factor or multifactor trials could not be shown to have different regression slopes because the interaction term in model 4 was not significant ($Z=1.34$, NS). With regard to the effect of cholesterol lowering on total mortality, multifactor trials showed a weak tendency to be more efficient than

TABLE 2. Number of Deaths and Instances of Coronary Heart Disease, Odds Ratios and 95% Confidence Intervals, and Percent Difference in Cholesterol Levels Between Treatment Groups

Study	n T/C	Death T/C	CHD T/C	Death OR	Death 95% CL	CHD OR	CHD 95% CL	%ΔChol	CHD Z logOR	CHD logOR±SE logOR
MRFIT[1]	6,428/6,438	265/260	277/280	1.021	1.217 0.856	0.990	1.173 0.835	2	−0.12	−0.01±0.087
Hjermann et al[2]	604/628	16/24	19/36	0.693	1.289 0.366	0.534	0.951 0.331	10	−2.13	−0.627±0.294
WHO fact[3]	24,615/25,169	997/924	773/756	1.103	1.213 1.011	1.047	1.156 0.946	1	0.91	0.046±0.051
Acheson and Hutchinson[4]	47/48	23/20	...	1.174	2.987 0.599	...	... ...	9	...	... ...
Carlson et al[5]	279/279	24/26	41/62	0.923	1.950 0.611	0.603	0.979 0.453	17	−2.05	−0.506±0.247
RC of Scottish Society[6]	264/273	34/38	59/76	0.934	1.520 0.574	0.778	1.131 0.535	14	−1.31	−0.251±0.191
Coronary Drug Project[7]	2,222/2,789	554/709	596/839	0.981	1.108 0.857	0.892	0.990 0.804	8	−2.15	−0.114±0.053
Newcastle upon Tyne[8]	244/253	27/48	55/89	0.583	0.883 0.331	0.646	0.897 0.467	13	−2.60	−0.437±0.168
Dorr et al[9]	1,149/1,129	37/48	...	0.751	1.158 0.487	...	...	10	...	...
Dayton et al[10]	424/422	174/177	60/88	0.978	1.267 0.733	0.682	0.941 0.494	13	−2.34	−0.383±0.164
Leren[11]	206/206	44/51	61/81	0.764	1.237 0.498	0.755	1.049 0.544	14	−1.68	−0.281±0.167
MRC[12]	199/194	28/32	40/39	0.853	1.437 0.479	1.000	1.553 0.644	16	0	0.000±0.225
MRC[13]	123/129	20/24	43/44	0.874	1.627 0.444	1.038	1.560 0.674	6	0.18	0.037±0.208
Rose et al[14]	54/26	6/1	13/4	2.890	12.669* 0.472	1.744	4.097 0.546	4	1.28	0.556±0.436
Woodhill et al[15]	231/237	39/28	...	1.429	2.533 0.901	...	... ...	5	...	...
LRC-CPPT[16]	1,906/1,900	68/71	155/187	0.955	1.337 0.679	0.811	1.022 0.669	9	−1.78	−0.210±0.118
WHO (clofibrate)[17]	5,331/5,296	128/87	167/208	1.462	1.921 1.119	0.791	0.977 0.640	9	−2.18	−0.235±0.108
Frick et al[18]	2,051/2,030	45/42	56/83	1.060	1.624 0.694	0.658	0.936 0.481	10	−2.33	−0.419±0.180
Frantz et al[19]	4,922/4,853	268/256	134/129	1.034	1.233 0.867		1.304 0.804	13	0.20	0.025±0.123
Total				1.037	1.096 0.981	0.905	0.954 0.858			
				χ^2(het)=24.0 df=18. NS		χ^2(het)=35.7 df=15 $p<0.01$				

*Uncertain due to small numbers.
logOR±SE logOR and Z score added for CHD.
CHD, coronary heart disease; OR, odds ratio.

single factor trials, but model fit is also somewhat poorer in this case.

CHD incidence. Figure 3 displays the relation between CHD logOR and percent net difference in cholesterol reduction. The estimated β_0 term of the linear model with net cholesterol difference alone is 0.061±0.052 (NS). Table 4 shows the model estimates with and without interaction terms for CHD incidence. The coefficient for cholesterol difference between treatment groups is highly significant in model 1 ($Z=4.1$, $p<0.001$), but the model fit is not quite satisfactory. For all trials combined, a benefit ratio of cholesterol lowering is estimated to be 1.25 with 95% confidence interval ranging from 1.1 to 3.2. In model 2, the fit is better, and the interaction term for diet and drug terms approaches statistical significance ($Z=1.74$, $p=0.08$). This may indicate that drug trials are more efficient than dietary trials in reducing CHD incidence by cholesterol lowering. Unlike the reduction of total mortality, reduction of CHD inc

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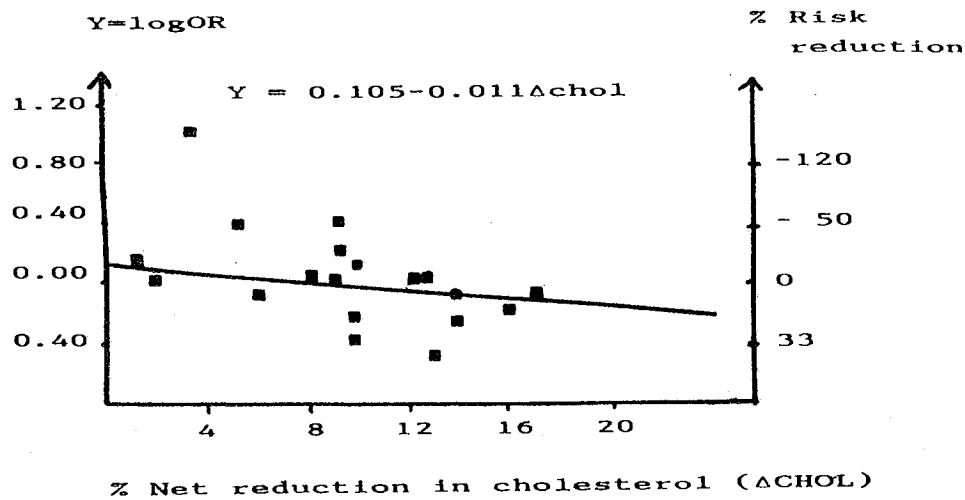

FIGURE 2. *Plot of log odds ratio (OR) of total mortality by percent net difference in cholesterol (ΔCHOL) with weighted regression line. All trials.*

dence by cholesterol lowering does not seem to differ between primary and secondary trials. Also, single-factor and multifactor trials did not differ significantly in this respect.

A supplementary analysis was performed to search for possible covariates to the variation in the cholesterol benefit ratio. This was achieved by regressing the ratio (weighted) between CHD logOR and Δchol in each trial against the variables of baseline cholesterol level, baseline age, and whether the trial was conducted in male patients (see Table 1). Figure 4 presents a plot of the relation to baseline cholesterol. The Y variable, which should be an estimate of β_1, shows a clear downward trend in reducing CHD incidence by starting with an increased level of cholesterol, that is, an increasing efficiency of cholesterol lowering ($p<0.001$). Table 5 gives details of the equations for each of the three independent variables. Neither age nor all-male trials showed a significant relation to the cholesterol benefit ratio.

Discussion

Between-trial comparisons will always suffer from a series of methodological shortcomings due to differences in protocols, quality of conduct, incomplete reporting, and so on.[30] Despite these limitations, the overview analysis showed that a substantial amount of the heterogeneity in the trial outcomes on CHD was attributable to the cholesterol reduction attained by their participants. The model fits indicated also that the remaining variability could be attributable to chance. In fact, the weighted regression line had an intercept close to zero, which is consistent with the hypothesis of no CHD risk reduction if not preceded by a cholesterol reduction.

The LRC-CPPT reported that for every 1% cholesterol reduction one should expect a 2% CHD risk reduction. This was evident from an overview analysis of long-term epidemiological follow-up studies, between-trial comparisons, and for various end points in that trial.[20] Epidemiological follow-up studies usually operate with a single baseline measurement of cholesterol. Therefore, the slope of the regression should be multiplied by $1/r$ due to measurement errors, individual short-term variations, and so on, where r is the correlation coefficient between two independent cholesterol readings (taken for the same individual some time apart). For cholesterol, r is approximately 0.70, so the ratio of 1:2 should be 1:3 as reported by MacMahon et al.[31]

The crude estimate of the CHD benefit ratio in this overview was 1:2.5 with a 95% confidence interval ranging from 1.1 to 3.9. The broadness of this interval reflects the major impact of uncontrolled variability in this type of analysis. However, the supplementary analyses showed that the efficiency of CHD preven-

TABLE 3. **Estimated Regression Coefficients (SE) for Total Mortality in Four Models for Testing Interaction Between Cholesterol Reduction and Design Characteristics Including All Trials With Model χ^2 Residual and Degrees of Freedom**

Model	Constant β_0	ΔChol β_1	ΔChol · DD β_2	ΔChol · PS β_2	ΔChol · SM β_2	χ^2	df
1	0.105 (0.048)	−0.011 (0.006)				20.1	17
2	0.106 (0.050)	−0.008 (0.013)	−0.002 (0.008)			20.0	16
3	0.104 (0.045)	+0.010 (0.012)		−0.014 (0.007)		16.2	16
4	0.157 (0.061)	+0.027 (0.029)			−0.043 (0.032)	18.0	16

DD, drug/diet; PS, primary/secondary preventive; SM, single-factor/multifactor trials.

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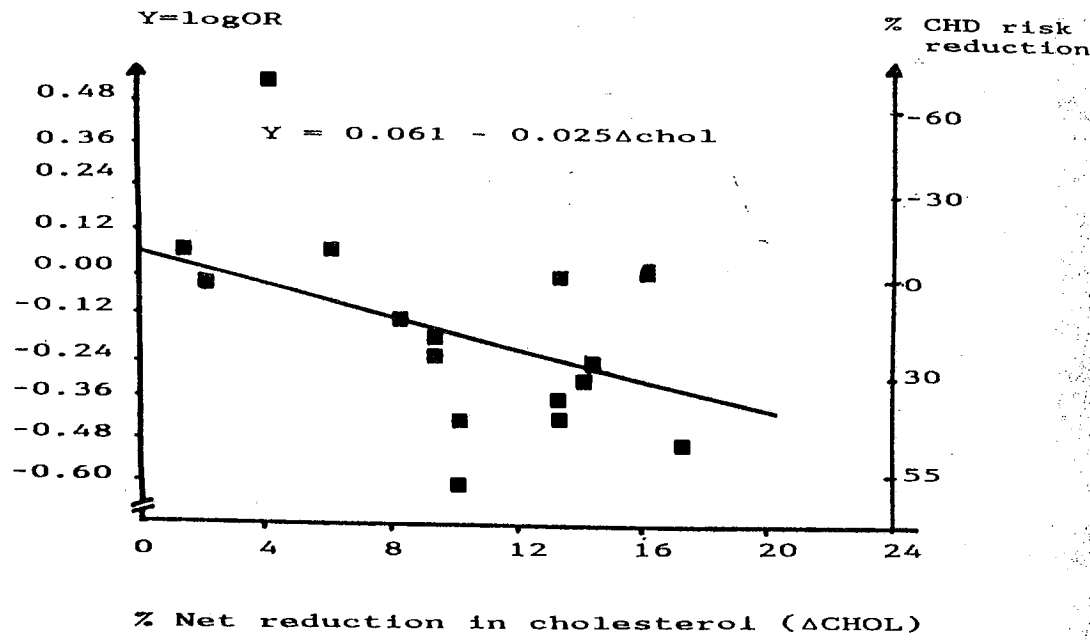

FIGURE 3. *Plot of log odds ratio (OR) of coronary heart disease (CHD) incidence according to percent net difference in cholesterol (ΔCHOL) with weighted regression line. All trials with CHD incidence data included (n=16).*

tion by cholesterol lowering could be dependent on the baseline level of cholesterol. At 240 mg/dl cholesterol, the estimate of β_1 was 0.0068 but was −0.036 at 280 mg/dl cholesterol. These estimates still have wide confidence intervals and should be interpreted with care. The relation to age at baseline was nonexistent within the age span observed; that is, cholesterol lowering did not seem to be less efficient in reducing CHD incidence in higher than in lower age groups. A weak tendency for all-male trials to show a more efficient relation between cholesterol lowering and CHD prevention than the trials with both sexes could be seen, but this issue cannot be answered with these data.

A word of caution should be given because these discussions are only relevant for small and moderate cholesterol reductions starting from (mostly) elevated levels in a rather short-term perspective. The 1:2.5 ratio will hardly be valid for drastic cholesterol reductions (above 20%) in the "normal" population. Also, preferential publication of positive trial results is a problem inherent in such a discussion.

The data indicate that cholesterol lowering by drugs more effectively reduced CHD incidence than did that by diet. Strong contributors to that indication are the dominant Frantz and MRFIT trial results. Also, drug trials are performed more often in patients at high risk of CHD than are dietary trials that "should" provide a better reduction CHD incidence. Any significant effect on total mortality, however, could not be traced.

Primary or secondary preventive trials did not significantly differ with respect to the efficiency of cholesterol lowering on CHD incidence. This is consistent with the notion that the distinction between primary and secondary intervention is rather artificial. What is really aimed at in these trials is a reduction in the progression or a regression of atherosclerosis in these patients, and average degree of atherosclerosis was probably not much different for patients in the two types of trials.

Single-factor and multifactor trials also show some slight tendency toward a difference in cholesterol reduction slopes; that is, multifactor trials are more

TABLE 4. **Estimated Regression Coefficients (SE) for Coronary Heart Disease Incidence in Four Models for Testing Interaction Between Cholesterol Reduction and Design Characteristics: Sixteen Trials Included With Model χ^2 Residual and Degrees of Freedom**

Model	Constant β_0	ΔChol β_1	ΔChol · DD β_2	ΔChol · PS β_2	ΔChol · SM β_2	χ^2	df
1	0.061 (0.052)	−0.025 (0.006)				17.0	14
2	0.065 (0.048)	−0.005 (0.013)	−0.012 (0.007)			14.0	13
3	0.063 (0.053)	−0.029 (0.014)		0.003 (0.008)		16.8	13
4	0.110 (0.059)	−0.011 (0.025)			−0.040 (0.027)	14.5	13

DD, drug/diet; PS, primary/secondary preventive; SM, single-factor/multifactor trials.

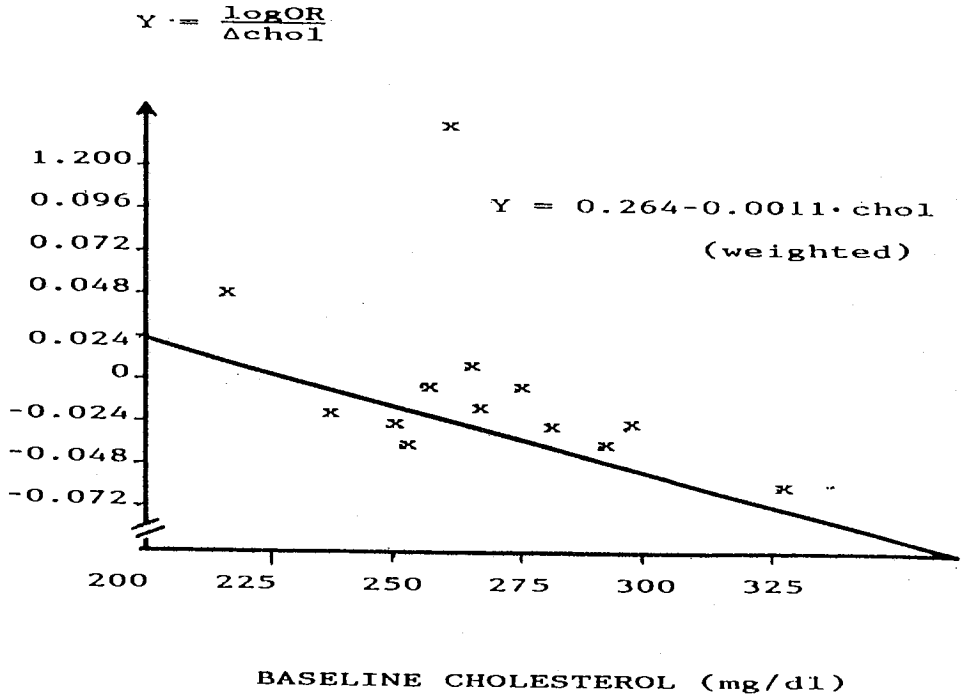

FIGURE 4. *Plot of coronary heart disease (CHD) cholesterol benefit ratio (logOR/Δchol) by baseline cholesterol level (chol). All trials with CHD end point except Frantz et al[19] are included (n=15).*

effective than single-factor trials with regard to cholesterol reduction. However, statistical significance was not reached on a conventional level.

A finding in this study was that total mortality logOR was lower in secondary than in primary preventive trials, adjusting for cholesterol reduction, but this was not seen for the CHD end point. Patients in secondary prevention trials have a much higher probability of dying from CHD than those in primary preventive trials. Thus, for total mortality, there is a built-in "advantage" for secondary prevention trials of cholesterol lowering, assuming that non-CHD end points do not counterbalance the beneficial effects more in secondary than in primary prevention.

The history of cholesterol-lowering trials have shown that they have not been without specific excess hazards on the part of the participating patients. The most dramatic side effect was seen in the WHO clofibrate trial where total mortality was increased by 35% in the treated compared with the control group.[17] The global estimate from all trials of logOR in total mortality was positive despite an overall 6% cholesterol reduction across trials. The adjusted analysis showed that total mortality logOR was significantly above zero without cholesterol reduction. Thus, to be of benefit, cholesterol lowering should have been at least 8–9% to outweigh the hazards involved, assuming that the hazards are not dose–response dependent of the cholesterol-lowering drug regimen. Hopefully, the future experience with new drugs and strategies will show improvements.

Summary Conclusions

The 1:2 benefit ratio of percent cholesterol lowering on CHD incidence may be a slight underestimate according to this across-trial overview analysis that adjusted for design characteristics. Cholesterol lowering seems equally effective in primary and secondary trials but is possibly more effective in drug than in dietary trials with CHD as an end point.

What was gained by cholesterol lowering with respect to CHD risk reduction was mostly lost on other fatal end points so that at least 8–9% choles-

TABLE 5. Regression Equations (Weighted by Reciprocal of logOR Variance) Between Cholesterol Benefit Ratio (logOR/ΔChol) and Three Independent Variables: Trials with Coronary Heart Disease End Points, Excluding the Frantz et al[19] Trial

Model	Constant γ_0	Baseline cholesterol γ_1	Baseline age γ_1	Male trial γ_1
1	0.264 (0.055)	−0.00107 (0.00022)*		
2	−0.012 (0.065)		−0.00019 (0.0013)	
3	−0.021 (0.032)			0.024 (0.033)

*$p<0.001$

terol reduction had to take place before an associated reduction of total mortality was indicated.

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KEY WORDS • total mortality • coronary heart disease • cholesterol • clincial trials

Occasional Survey

DIETARY FATS AND CORONARY HEART DISEASE: UNFINISHED BUSINESS

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Summary In the scientific and lay press, dietary recommendations that are aimed at prevention of coronary heart disease in the general public are appearing more and more frequently. The dietary pattern that is now most widely advocated is a low-fat, low-cholesterol diet with a polyunsaturated/saturated ratio of 1. The argument for such a dietary change is supported mainly by extrapolations from epidemiological data and from animal experimentation. Reasons are given for concluding that the recommendations are unwise, impractical, and unlikely to lead to a reduced incidence of arteriosclerotic disease. Since complacency is equally inappropriate, a few practical questions are outlined that should be settled before the public is assured that a low-fat diet will lead to a reduced risk of coronary heart disease.

INTRODUCTION

THE debate over the optimal quality and quantity of fat in the diet of the Western world has become more and more heated in the past 2 years, but hardly more illuminating. The issue has long since moved out of the scientific press and into the public arena: in the U.S.A. it seems to have turned into a political skirmish between politicians, the Department of Agriculture, and the National Institutes of Health (NIH),[1,2] in which the wages of war include territorial claims, the profitability of agricultural and food processing interests, and the balance of international trade.

THE VARIOUS SIDES OF THE DEBATE

On the scientists' side the debate is waged by proponents of several points of view: those who think the "diet-heart question" is utter nonsense, totally without scientific basis;[3,4] others who promote a low-fat, low-cholesterol diet for the general public;[5] those who favour a national diet low in cholesterol and saturated fats but moderately high in polyunsaturated fats;[6] some who conclude that refined sugar is mainly to blame for the high prevalence of coronary heart disease (CHD) in Western countries,[7] and others who point to suboptimal intakes of fibre.[8] There is also a minority group, to which I belong, that believes that the time is not yet ripe for drawing up national guidelines and dietary recommendations for the general public, but wishes to see the public fully informed on the progress made as well as the questions remaining.

REASONS FOR THE DEBATE

The debate is fired by the sheer magnitude of the numbers of CHD deaths; by the advances in understanding of fat and sterol metabolism gained over the past 30 years; by the belief that almost everyone in affluent societies is hyperlipidæmic; and by a medical ethos that is uneasy with inaction.

REPORTS AND RECOMMENDATIONS

If we knew precisely which diet can prevent or allay the development of CHD, there would be no debate. The fact that the available evidence is soft ensures that opinions are divided. The fact that the argument has recently become less objective seems to me to be proof-positive that the experts do not yet know—for sure—what to advise or when to advise it. This is not to say that panels of informed and conscientious medical scientists have not tried to reach valid conclusions: indeed, there have been more than fifteen reports over the past 10 years emanating from official and quasi-official organisations in Britain, Germany, Holland, Scandinavia, Australia, New Zealand, Canada, and the United States. Committees convened there have almost uniformly warned against the increasing obesity in affluent societies; some have concluded that our Western diets should be lower in cholesterol and saturated fats, while others haves opted for increased intakes of polyunsaturated fats.

The McGovern Report

In the U.S.A. the most recent and highly publicised recommendations to the public were the six "dietary goals" set forth by a Senate Select Committee chaired by Senator McGovern. The first report (February, 1977) implicitly promised that a national shift to a diet lower in saturated fat and cholesterol, lower in refined sugar and higher in starches, and very much lower in salt would surely lead to a reduced incidence of CHD, stroke, hypertension, diabetes, and cancer.[5] A revision undertaken in reply to widespread objections (December, 1977), stated more conservatively that such a diet would decrease the *probability* of premature incidence of disease.[9] No one really doubts that the McGovern diet can be translated into a feasible diet that the public can learn to like, but at least one commentator[10] has calculated that U.S. agriculture does not have the physical means to provide it to the entire nation.

A Report of the American Society of Clinical Nutrition

In the McGovern report and other official statements the scientific basis for the recommendations set forth was neither documented nor critically analysed. Rather, these documents presented a codification of opinions and beliefs. The weakness of this approach has recently been redressed by the American Society for Clinical Nutrition. In 1978 its directors called together a panel of 9 medical scientists with wide experience in clinical medicine, human metabolism and nutrition, epidemiology, and animal experimentation. The panellists were intentionally selected as representatives of a spectrum of viewpoints, often with opposing biases. They were asked to weigh the quality of all published scientific evidence relating to six dietary issues—dietary fat, cholesterol, carbohydrates, alcohol, excess calories, and salt. Each of 6 panellists was persuaded to assemble an annotated review on one of the six issues; 24 outside advisers assisted in this labour. The assembled panel debated the

reviews for completeness and balance. Then it measured the quality and strength of the evidence against eleven criteria: the consistency of the epidemiological data *among* and *within* population groups: the strength, independence, and temporal relationships of the epidemiological data; the effects of new exposure to or removal of the putative factors in already affected as well as in apparently unaffected individuals; necropsy data; the results of animal experiments; and finally the plausibility of the postulated biological mechanisms. Six brief Consensus Statements were fashioned out of these measurements.

These Consensus Statements and the supporting reviews have been published as a supplement to the December, 1979, issue of the *American Journal of Clinical Nutrition*. An introduction[11] describes the panel's attempt to put numbers to the strength of the evidence on each of the several dietary issues. What is truly novel is that it also measures the cohesiveness of the views of the 9 panellists on each of the issues—a factor that has rarely been quantified and almost never displayed in previous reports on diet and health. These measures of cohesiveness were made after the panellists had reviewed, debated, and dissected the evidence on each of the issues over a period of 15 months, and only after a consensus on each of the issues had been reached.

In regard to dietary fats, cholesterol, and arteriosclerotic disease, the panel concluded that the association of these two factors to arteriosclerotic disease *among* population groups was strong, but *within* population groups weak; that the strength and independence of the association were weakened by the confounding effects of a number of genetic, environmental, and socioeconomic factors; that proof from necropsy evidence was either lacking or confounded by other factors; that the effects of changing the intakes of saturated fats and cholesterol both in primary and in secondary prevention trials offered suggestive but not definitive proof of the association; that animal experimentation had succeeded in producing models of arteriosclerotic disease in some but not all species, with wide differences in responsiveness to the intake of cholesterol and saturated fat even within species; and that the underlying biological explanations were not well understood.

The panel as a whole voted that the evidence relating the intake of saturated fat and dietary cholesterol to atherogenesis in man was convincing; further, they agreed that a reduction of saturated fats and cholesterol would lead to reduced levels of plasma cholesterol. On the other hand the evidence on disease prevention by diet modification was considered unconvincing. Thus, the panel could not guarantee that lipid-lowering by dietary means would necessarily lead to a reduced incidence of new events of CHD. Although these conclusions represented a consensus, the spread of opinion (or lack of cohesiveness) of the 9 panellists' individual views on these issues was considerable—far greater, for instance, than on salt and hypertension.

THE LIPID HYPOTHESIS

In a recent talk in Houston, Texas, before the 5th International Symposium on Atherosclerosis, Dr R. I. Levy, director of the National Heart, Lung and Blood Institute of the NIH expressed his view that a diet lower in cholesterol and saturated fat (a "prudent diet") could be expected to lead to a reduction of CHD. The basis for this view is the as yet unproven hypothesis (the lipid hypothesis) that a reduction of the level of plasma cholesterol by whatever means (dietary, drug, or otherwise) will lead to a reduced incidence of CHD.

I have previously described my reasons for concluding that this hypothesis is a valid working theory.[12] However, it has only once been properly tested. In a large clinical trial of the lipid-lowering drug clofibrate,[13] involving 15 000 hypercholesterolæmic but otherwise well males in a 6-year experiment in Edinburgh, Prague, and Budapest, there was a highly significant decrease in new events of non-fatal myocardial infarction in the drug-treated group. There was, however, no reduction in total mortality: indeed, a disturbing increase in incidence of pathology of the intestinal tract was noted. All questions of drug toxicity aside, it was clearly shown that the greater the reduction of plasma cholesterol, the greater the reduction of new events of non-fatal myocardial infarction.

MY RESISTANCE TO GENERAL RECOMMENDATIONS

In view of these results, why oppose the issuance of dietary recommendations to the general public at this time? Let it be clear at the outset that my laboratory will consider itself bountifully rewarded for its efforts if a reduction of plasma lipids, achieved by dietary means, can be shown clearly to reduce the incidence of CHD: we published the first clear proof that plasma lipids can be predictably altered by changes in the quality of dietary fat 25 years ago,[14,15] and since then we have been, amongst others, in the forefront of a parade of research that has added substantially to our present understanding of disordered lipid metabolism in man. Thus, we are intellectually and emotionally involved in the outcome of any proper test of the lipid hypothesis. However, if the public's diet is going to be decided by popularity polls and with diminishing regard for the scientific evidence, I fear that future generations will be left in ignorance of the real merits, as well as the possible faults, in *any* given dietary regimen aimed at prevention of CHD.

My reasons for resisting the current advocacy of a low-fat national diet are four in number.

1. There has been no previous test of the "prudent diet". The only dietary trials carried out with proper controls and with minimal confounding biases—namely, the 12-year Finnish trial in two mental institutions[16] and the 8-year Veterans Administration Hospital trial in Los Angeles[17]—compared two diets with moderately high fat intakes (35–40% of total calories) in which the degree of unsaturation of the two fat mixtures varied as widely as was feasible under the test conditions. As noted above, these primary-intervention trials resulted in suggestive but not conclusive evidence that the incidence of new events of cardiovascular disease was reduced on the more highly unsaturated fat regimen. In neither experiment was total mortality reduced.

On the other hand, a trial of the low-fat dietary regimen recommended by the McGovern Committee[9] and the American Heart Association[18] has never been carried out. It seems that the proponents of this dietary change are willing to advocate an untested diet to the nation on the basis of suggestive evidence obtained in tests of a *different* diet. This illogic is presumably justified by the belief that benefits will be obtained, vis-a-vis CHD prevention, by *any* diet that causes a reduction in plasma lipid levels.

2. The "prudent diet" will have only a small effect on plasma lipid levels. How great a reduction in lipids can be anticipated if the nation is persuaded to adopt the low-fat dietary guidelines? To approach an answer to this question, let us use the most recent data on food consumption in the U.S.A.[19] and calculate the effect of reducing the nation's fat intake from 44% to 30% of a mean daily food intake of 2500 kcal/day, raising the polyunsaturated/saturated (P/S) ratio of the fat from 0·44 to 1·0, and lowering the cholesterol intake from 500 to 300 mg per day. The Keys formula[20] predicts a mean decrease in plasma cholesterol from 220 to 194 mg/dl*, a 12% decrease. But 100% compliance with the dietary guidelines will certainly not be attained, any more than it has been in the anti-cigarette campaign waged by the Government over the past 15 years. Even 50% compliance seems unlikely to me; this translates to a mean decrease in plasma cholesterol of 6%—that is, from 220 to 207 mg/dl.

Is this small decrease worth the enormous effort required to achieve it? The answer is uncertain, for the risk/benefit ratio depends on facts we simply do not have. Thus, I believe the burden of proof rests on those who claim that real benefits will be derived from this minor change in our national dietary pattern.

3. Any one diet produces different results in different people. The argument for a one-diet approach assumes that all members of the population react similarly to diet changes, like pure-bred laboratory animals. This assumption is simply incorrect; the more closely we look, the more often we identify the wide individual differences that clearly reflect our heterogeneous genetic endowment.

We have amply documented these differences in our studies of human responses to changing intakes of fat and cholesterol. This laboratory has had more than 25 years' experience in more than 800 inpatients, each fed for at least 12 weeks on orally administered formulas in which wide variations in carbohydrate/fat calories, saturated/unsaturated fat quality, and cholesterol/plant-sterol intakes have been tested in our studies of the key factors in cholesterol homœostasis—absorption, transport, synthesis and its feedback control, conversion to bile acids, and tissue storage. I need no further evidence to convince me, first, that I cannot predict in advance how and by how much any given individual will respond to a given dietary challenge.

Let me cite examples that bear directly on the question of changes in cholesterol intake. 4 patients were put on diets in which the cholesterol content was increased two to four times over that accepted as the mean daily intake of 500 mg in U.S.A. adults today. The first patient experienced a continuous rise in plasma cholesterol levels from 250 to 450 mg/dl over a 5-week period; the other 3 showed only trivial changes in plasma levels. However, one of them demonstrated complete compensation for absorbed cholesterol by an increase in neutral sterol excretion; the second also compensated fully by a reduction in endogenous cholesterol synthesis that matched the amount of cholesterol absorbed, milligram for milligram; and the third showed no change in excretion or synthesis, but reacted by retaining one-third of the daily cholesterol intake in tissue stores. We do not know to this day what proportions of the general population fall into these four response-groups.

Let me also cite examples that bear on the question of exchanges of dietary fats differing markedly in degree of unsaturation, such as corn oil and lard. In such experiments, carefully monitored on a metabolic ward, we measured the plasma levels of cholesterol that were attained after 6–8 weeks' ingestion of each dietary mixture, always at eucaloric intake levels and at fat intakes of 40% of total calories. If the corn oil period is taken as the baseline in each patient, the substitution of lard (P/S=0·43) for corn oil (P/S=8) in 13 patients caused plasma cholesterol levels to rise 5, 15, 16, 17, 18, 19, 20, 23, 27, 28, 32, 33, and 39%; coconut oil (P/S=0·03) exchanged for corn oil in 6 patients caused rises in plasma levels of 5, 34, 37, 40, 60, and 77%; butter (P/S=0·06) exchanged for corn oil in 9 patients caused increases of 18, 30, 30, 32, 35, 36, 53, 60, and 84%. We could not have predicted the extent of these variations, nor can we today identify who will respond how.

It is clear from these data that individuals vary widely in their responsiveness to changes in quality of fat and in cholesterol intake. Equally convincing are the variations we have seen in substitution of carbohydrate for fat calories.

On the basis of these studies I can confidently predict that adoption of the "prudent diet" will cause various reactions in different segments of the population. No one can say today what proportion of the general population will experience a decline in plasma cholesterol levels, nor of what degree; how many will increase cholesterol synthesis to match a decrement in cholesterol absorption, nor to what degree; how many will experience a flux of cholesterol into or out of tissue stores, nor to what degree; and how many will be totally unaffected by the change.

Moreover, it is absolutely certain that no one can reliably predict whether a change in dietary regimens will have any effect whatsoever on the incidence of new events of CHD, nor in whom. All of these caveats bring me to my fourth reason for objecting to general recommendations at this time.

4. Crucial questions remain to be resolved. Any broad-scale change in the dietary patterns of the Western world should follow and not precede a resolution of the many unsolved problems that bear directly on our understanding of lipid and lipoprotein metabolism in man, and their relationships to atherogenesis. A Working Group Report from the National Heart, Lung and Blood Institute of the NIH[21] urged in 1977 that the first priority of effort should be directed at the prevention and control of arteriosclerotic disease. But is it not obvious that the success of prevention depends on the thoroughness of our understanding of the root causes of disease? Lacking that understanding, are we in danger of launching an all-out war against the wrong foe?

Hyperlipidæmia is only one of the risk factors predisposing to CHD, and the genesis of hyperlipidæmia in more than 95% of the general population is simply not understood: according to Goldstein et al.,[27] less than 1% of the hyperlipidæmia in the U.S.A. is monogenic in origin.

Thus, the uncertainties are numerous. But let me identify just three of the immediate problems that demand solution before any dietary guidelines are issued to the general public.

The choice between a low-fat diet with P/S=1 and a moderate-fat diet with P/S>2.—Diets taken at eucaloric levels will affect the fatty-acid composition of the tissues in predictable ways: the lower the fat intake and the higher the carbohydrate intake, the more saturated the body's fatty acids. By contrast, the higher the intake of polyunsaturated fats, the more unsaturated the fatty-acid composition of the lipids in plasma and in all formed elements of the blood; in adipose tissue and other

* Conversion factor, mg/dl to mmol/l=0·026.

stored lipids; and in all membrane lipids. Given these facts, on what basis do we choose to produce tissues rich in saturated fatty acids ("hard fats") when the choice is available of creating tissue lipids that are highly unsaturated ("soft fats")? Scientists are only now beginning to test whether the fatty-acid composition of plasma lipoproteins affects the transport of cholesterol into and away from the bulk tissues (adipose tissue, muscle, and connective tissue), into or away from arterial wall tissues, endocrine organs, and the central nervous system. Does the "softness" of fat in cell membranes affect the immunoresponsiveness of lymphocytes? What about the permeability of cell membranes to solutes, anæsthetic agents, drugs, foreign bodies, bacteria, viruses, metastatic tumour cells? Is the ageing of cells affected by their "softness"? What about the thrombotic potential of the blood in "hard fat" versus "soft fat" patients: is the balance between aggregating and antiaggregating prostaglandins and their oxidation products altered? Why is the incidence of spontaneous mammary tumours in undernourished C_3H mice so markedly reduced, and the rate restored by sustituting fat for carbohydrates in the same subcaloric diet? Are cell receptors modified by the degree of saturation of cell membrane lipids?

Even if low-fat diets and highly unsaturated-fat diets cause the same amount of cholesterol-lowering in the plasma of most of the general population (which we do not know), how can we make the choice now, in the face of the unanswered questions in the paragraph above, to request the general public to adhere to a low-fat diet?

HDL/LDL ratios and dietary fat quality.—The recent resurgence of interest in the ratio of high-density to low-density lipoproteins (HDL/LDL) as predictors of CHD risk is only now leading investigators to define the effects of diet on this ratio in plasma lipoproteins. There is only one published report[23] on this question today, but this deficiency will doubtless be remedied within the next few years.

The VLDL and chylomicron cascade.—Similarly, there are no published data on the question whether the rates of conversion of very-low-density to low-density lipoproteins (VLDL to LDL) and of chylomicrons to remnants is affected by the fatty-acid composition of the structural elements of these lipoproteins. Nevertheless, the catabolism of VLDL, LDL, and chylomicrons appears to be critical in control of their circulating levels; indeed, some workers consider them to be the key determinants in the process of atherogenesis.

CONCLUSIONS

In view of these many considerations and uncertainties, I feel it is irresponsible to make the dietary recommendations that are being so widely proposed to the general public at this time. Let no one conclude that I am complacent about the diet of the Western world, or about our sedentary way of life. I have no interest in preserving the status quo. Indeed, I can forsee both economic and health benefits in adopting a style of living that emphasises the output side of our energy balance while simultaneously reducing food intakes, both of which can be expected to diminish the incidence and impact of hypertension and diabetes; the incidence of CHD itself may decrease as the population adopts a lifestyle characterised by reduced cigarette-smoking and reduced total body-weight.

I am truly sympathetic to the need for speed in settling the many unresolved questions that bear on the diet-heart proposition, for I believe that their solution will point the way to a series of rational dietary approaches to the prevention of CHD, a disorder that seems to have many causes, hence many solutions. Hyperlipidæmia is certainly associated with and may even be one of those causes, but it is important to recognise that hyperlipidæmia also has many causes and hence many solutions. I believe it is anything but a service to the public to postulate *one* dietary solution for hyperlipidæmia, no matter how well-meaning one is in advocating it. Let us address the unanswered questions and demand the means to solve them quickly.

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Christmas Quiz

ANYONE READ THE LANCET?

How well have you read *The Lancet* in the past year?

1. Why are hobbits extinct in England now?
2. What was Brunel doing upside down?
3. Where did A. C. Dornhorst publish his memorable chesty phrase?
4. What replaced weaver's bottom?
5. Where did *male oscuro* strike?
6. Who carried an alarming iron load?
7. Fleeting intercostal pain has an eponym. What is it?
8. With the prize in mind, whose initials were J.B.P.A. de M. de L.?
9. What smiled in response to viral exposure?
10. Areca nuts, burnt seashells . . . what is missing in the recipe?
11. When was V day?
12. *Cholo coquero* is not a Mexican chicken dish in chocolate sauce, but what is it?
13. Where would you find the "county of the three impasses"?
14. Cystisine is associated with what sort of poisoning?
15. What was Moses' cure for asthma?

A prize, The Illustrated Origin of Species, *Richard Leakey's presentation of Darwin's book, will be awarded to the three most nearly correct entries. Entries should be sent to the London office of* The Lancet *and will be held until Monday, Feb. 4, to allow overseas readers to enter.*

THE LANCET

Primary Prevention of Ischaemic Heart Disease with Lipid-lowering Drugs

A HIGH level of plasma cholesterol is a major risk factor for heart attacks: the risk correlates with the amount of cholesterol carried on the low-density lipoproteins (LDL-C) but is inversely related to the cholesterol carried on the high-density lipoproteins (HDL-C).[1,2] A risk factor is associated with a disease but does not necessarily cause it. In large populations there is a relation between dietary fat intake, mean plasma cholesterol level, and ischaemic heart disease[3] and it is mainly for this reason that British,[4] European,[5] and American groups[6] have recommended that any patient with a raised plasma cholesterol should be treated. The ideal total plasma cholesterol level is usually said to be 5·2 mmol/l (200 mg/dl) but since the mean total cholesterol level in British middle-aged men is 6·3 mmol/l with a standard deviation of 1·0 mmol/l,[7] such a policy would imply the treatment of a large proportion of the healthy population.

Although both total cholesterol and LDL-C can be reduced by an appropriate diet, within individuals there is little correlation between dietary fat intake and plasma cholesterol level, so diet cannot be the only factor controlling plasma lipids. Proof of a causal relation between diet, plasma cholesterol level, and ischaemic heart disease could only be obtained with a randomised and double-blind trial comparing two diets. No completely convincing dietary study for the prevention of heart disease has been published and it is unlikely that a perfect study will ever be conducted: the number of patients needed would be too large; the cost would be too high; dietary studies cannot be double-blind; and it is impossible to prevent a control population from changing its dietary habits. Since the dietary evidence "falls short of proof",[8] the next step is to look to studies in which plasma cholesterol is modified by drug treatment. The publication of the Helsinki Heart Study of gemfibrozil in the primary prevention of ischaemic heart disease is therefore of considerable interest.[9]

In this study, nearly 19 000 men aged 40–55 were screened, and 6000 were found to have a non-HDL-C (ie, LDL-C plus very-low-density lipoprotein cholesterol [VLDL-C]) greater than 5·2 mmol/l. Of these, 4081 were free of overt vascular disease and were randomised to receive either placebo (2030 patients) or gemfibrozil 600 mg twice daily (2051 patients). In both groups the initial plasma total cholesterol level was 7·5 mmol/l and all patients were advised to diet. Gemfibrozil treatment led to an 11% reduction in total cholesterol, a 14% reduction in non-HDL-C, and a 10% increase in HDL-C. The trial endpoints were cardiac deaths plus fatal and non-fatal myocardial infarction. After five years of follow-up, on intention-to-treat analysis, there had been 56 endpoints in the gemfibrozil group and 84 in the placebo group. This 34% reduction in endpoints (95% confidence interval 8·2–52·6%) was statistically significant ($p < 0·02$). The difference between the groups was entirely due to a reduction in non-fatal myocardial infarction in the gemfibrozil group (48 *vs* 71). The hypothesis that gemfibrozil treatment would reduce ischaemic heart disease therefore appears to be supported, and strength is added to the view that a raised plasma cholesterol level is causally related to ischaemic heart disease. However, the total numbers of deaths in the two groups were virtually the same (gemfibrozil 45, placebo 42), so if the object of screening and treatment is mainly to prevent death, it is not immediately obvious that the widespread

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prescription of gemfibrozil should be recommended for patients with hyperlipidaemia.

The results of this latest study are remarkably similar to those of the two other large primary prevention trials with lipid-lowering drugs. In the WHO study of clofibrate[10] (like gemfibrozil, a fibric-acid-derivative that increases cholesterol excretion) some 10 000 men with a raised plasma total cholesterol (mean 7·5 mmol/l) were randomly allocated to receive clofibrate or placebo. After five years, active treatment had reduced the total cholesterol level by 9%, and there had been 167 cardiac endpoints in the clofibrate group and 209 in the placebo group. However, there were 162 total deaths among the patients given clofibrate and 127 among those given placebo. The adverse effect was due to an excess of deaths from various causes, but the excess was still evident when the patients were followed for a further four years.[11]

In the Lipid Research Clinics (LRC) Coronary Primary Prevention Trial with the bile-acid sequestrating resin cholestyramine,[12] 3800 men with an LDL-C greater than 4·5 mmol/l and a mean total cholesterol level of 7·0 mmol/l who had not shown adequate cholesterol reduction on diet alone were randomly allocated to treatment with cholestyramine or placebo. Cholestyramine caused a 13% reduction in total cholesterol and after seven years there had been 158 cardiac events in the group given active treatment and 187 in the placebo group. The difference was mainly attributable to non-fatal infarction; the slight reduction in cardiac deaths was offset by an increase in deaths from other causes, so the total numbers of deaths were 68 in the patients given cholestyramine and 71 in those given placebo.

The fact that three different cholesterol-lowering drugs reduced non-fatal myocardial infarction in three groups of patients with different patterns of hyperlipidaemia certainly suggests that cholesterol reduction is of itself beneficial. Cholestyramine has no effect on HDL-C, so perhaps the increase in this component with gemfibrozil is unimportant. But the balancing effect of an increase in deaths from other causes seen in association with all three drugs cannot be ignored simply because it did not form part of the hypothesis that these trials were designed to test. Clinicians, as opposed to experimental pathologists interested in the aetiology of atherosclerosis, will very properly ask what cost is incurred with the drug treatment of hyperlipidaemia.

Apart from the financial cost (which for both cholestyramine and gemfibrozil is considerable) there are other unwanted effects. In the gemfibrozil study, cholesterol-lowering treatment was associated with more gastrointestinal operations (81 *vs* 53); more gallstones (18 *vs* 12); more cancer (31 *vs* 26); and more eye operations, particularly for cataract (17 *vs* 12). Although none of these differences is individually statistically significant, the trend is worrying; the excess of cataracts is particularly disturbing since cataracts may also be associated with lipid reduction by the new class of hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors that block cholesterol synthesis. In the LRC trial, 70% of patients given cholestyramine had unpleasant gastrointestinal symptoms; gallstones (which were also more frequently seen in association with clofibrate treatment in the WHO trial) occurred in 16 patients on cholestyramine compared with 11 on placebo, and there were more operations on the central nervous system in the group given active treatment (40 *vs* 23).

Unwanted effects such as these may become more important if lipid-lowering drugs are given for long periods, and it is possible that treatment may be needed for more than the five to seven years of these trials. In the Coronary Drug Project trial of lipid-lowering drugs in patients who had had a myocardial infarction, three regimens were abandoned early because of side-effects. One regimen (clofibrate) had no effect, and the fifth (nicotinic acid) led to a reduction in non-fatal infarction without affecting total mortality. When the patients who had been in the trial were reviewed nine years later,[13] the fatality rate in the first four groups was similar to that in the placebo group, but in the nicotinic acid group 52% of patients had died compared with 58% in the placebo group—an 11% reduction, which was statistically highly significant. This observation has to be viewed with caution because it did not form part of the study itself, but nevertheless it may indicate the timescale over which the effects of lipid reduction have to be observed.

One reason why it is difficult to document any benefit there may be in reducing plasma lipids is the generally good prognosis of symptomless patients with hyperlipidaemia: the placebo-treated patients in the gemfibrozil trial had an annual event rate (mainly non-fatal) of 0·8%. Although a low plasma cholesterol level may be a desirable objective, it is by no means certain that drug therapy is a good way of achieving it.

Combating Undernutrition in the Third World

IN many countries of Africa, Asia, and Latin America, the combined effects of undernutrition arising from poverty and infections associated with insanitary environment continue to be the major determinant of morbidity and mortality. Ultimately it is true that durable nutritional improvement of populations can be achieved only as part of their

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Point of View

THE DIET-HEART QUESTION IN 1985: HAS IT REALLY BEEN SETTLED?

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ON Dec 12, 1984, the National Institutes of Health (NIH) Consensus Development Conference on Lowering Cholesterol to Prevent Heart Disease held a press conference to publicise the agreements reached by a fourteen-person panel that met and heard evidence from twenty-two experts on the preceding 2 days. This press conference represents the beginning of a massive publicity campaign in the USA that is aimed at the public, all segments of the health profession, and the food industry. The major recommendations contained in the 23-page draft statement handed to the press (now published in *JAMA* April 12, p 2080) are based on the view that high plasma cholesterol levels are causally related to the high rate of coronary heart disease (CHD) in the western world. On this there was indeed a consensus. The panel thus recommended that plasma cholesterol levels in the USA be reduced towards those in countries where CHD is not a major health problem, and that dietary changes aimed at producing these reductions be undertaken by everyone over 2 years of age. In the diet recommended (often referred to by the American Heart Association as its prudent diet) total fat intake is reduced from its present level of about 40% of total calories to 30%, with no more than 10% of calories from saturated and 10% from polyunsaturated fats, and dietary cholesterol intake is reduced from about 450 mg/day per person to 250–300 mg.

Several dissenting opinions were voiced by invited speakers and by commentators from the floor. The main questions were whether the USA public can be promised a reduced incidence of CHD if the prudent diet is widely adopted; whether the prudent diet is safe and effective for everyone over the age of 2 years; and whether the diet recommended is the best choice of several known to reduce plasma cholesterol levels.

The panel evidently leaned heavily on epidemiological evidence and public health considerations rather than on clinical viewpoints and data obtained through studies in man. I wish to examine the nature and implications of the differences in these two approaches, and to address the three questions above.

EPIDEMIOLOGY AND HYPERCHOLESTEROLAEMIA

An epidemiological approach is based on events in large populations rather than on individual case-histories—on the distributions (including ranges and means), of these events and on time-trends of these averages, rather than on trends in individuals. Conclusions are based on probabilities, and on traditional (but nevertheless arbitrary) definitions of the power of chance in deciding whether a correlation is strong or weak.

Retrospective surveys of CHD in large populations have revealed that fat intake in countries with low CHD rates is considerably lower than in regions with higher intakes. Prospective surveys have shed light on the relation between new events of CHD and plasma levels of total cholesterol. Much of what we know today about risk factors has been derived from such studies, in which developments over time have been carefully measured.

Thus, epidemiological studies provided strong evidence for linkage between cholesterol levels and CHD. Metabolic ward studies that showed that cholesterol levels could be modified by the quality of dietary fat intake led to the lipid hypothesis—that CHD rates will drop if plasma cholesterol levels are reduced. Over the past 25 years this hypothesis has been put to the test in more than 20 trials which attempted to lower plasma cholesterol levels by dietary manipulations or by the administration of plasma-cholesterol-lowering drugs. Only the Lipid Research Clinic's coronary primary prevention trial (LRC-CPPT)[1] produced evidence for benefit that was any more than suggestive. The CPPT was a drug trial. However, as argued at the consensus meeting, by pooling the results of all dietary and drug trials, using CHD deaths and definite non-fatal myocardial infarctions as end-points, the degree of benefit far exceeded what might have occurred by chance. In contrast, a similar aggregate analysis of all non-CHD mortality showed an inverse relation (ie, less CHD but more non-CHD deaths). Although this latter outcome might have been due to the play of chance alone it is disconcerting, to say the least.

To sum up, the panel seems to have been strongly influenced in its final decisions by data from epidemiological surveys and by controlled clinical trials of the lipid hypothesis, such as the LRC-CPPT.

CLINICAL MEDICINE, CLINICAL SCIENCE, AND HYPERCHOLESTEROLAEMIA

Clinicians are consulted by individuals. They can be guided in the management of that patient by epidemiological findings only to the extent that they have come to recognise the presence of known CHD risk factors and accordingly attempt to modify them. For each patient there must be an evaluation of known risk factors, and of contributory factors such as lifestyle, social and economic pressures, and underlying disease (renal, thyroid, and liver). The clinician will try to correct any overweight, then hypercholesterolaemia, first by dietary means and later by use of drugs if necessary. The physician cannot be precisely guided by what he knows of any previously described group response to a given regimen that has been tested in a large-scale controlled study. Means, standard deviations, and probability scores will not be useful to him: his patient is only one individual.

The panel recognised these many important aspects of individual patient management but chose to follow the public health approach of reducing the mean cholesterol level (now 210 mg/dl for the USA) of the entire population.

PROS AND CONS OF THE TWO APPROACHES

Epidemiological surveys will continue to furnish valuable insights into phenomena associated with CHD. But correlations, no matter how strong, are never proof. Moreover, surveys totally lack control over factors that may eventually prove to be strongly operative: for instance, to what degree are the CHD rates of Irish migrants to Boston (or Japanese to Hawaii) due to changes in lifestyle or to economic and social changes, rather than (as now assumed) to dietary changes?

Prospective controlled clinical trials, on the other hand, are designed to isolate the relative contributions of single factors in CHD incidence: this can be successful if the questions are clearly defined, if sufficient individuals are recruited into the

trials, if end-points are hard, and if everyone is blinded to the progress of the study (except the data-evaluation committees). Such trials can ascertain whether the benefits and risks are due to chance, and within what limits of confidence. However, the investigators cannot predict at the start which individual in the experiment will survive, or who will suffer a "countable" new event. Because the incidence rate of CHD is low, most of the recruits will survive, therefore such trials are extremely costly.

The pragmatic approach implicit in any clinician/patient interaction, by contrast, is likely to have maximum advantages for the patient because his treatment is tailored to suit him. However, the clinical approach today cannot be expected to offer a scientific test (in the epidemiological sense) of the effectiveness of any particular intervention in individual patients; and controlled clinical trials cannot be expected to offer strong experimental evidence of benefit to individual patients, but only to groups, and then only in probability terms. Both approaches have their strengths and weaknesses; and neither can assess risks in terms of absolute numbers, only as changes of risks in proportional terms.

I firmly believe that the decision to undertake the approach recommended by the consensus panel should be based, not on faith or zeal or alarm, but on hard scientific evidence. I am one of a minority that believes that existing evidence is far from convincing. To justify that view, let me describe where I agree and where I disagree with the panel's recommendations.

CONSTRUCTIVE ASPECTS OF THE CONSENSUS STATEMENT

Increasing Public Awareness

The NIH statement has already caught the attention of the media and the better-informed segments of the public. Confused by the medical to-and-fro on cholesterol, the public may well say, "There they go again". But it is possible that the statement may prompt individuals to pay attention to assess whether they are at high risk, and to do something for their own benefit.

Achieving Better Weight Control

The adoption of the prudent diet in the USA may lead to lower body weight in a nation that is generally obese, and perhaps to diets that are more balanced than are at present being taken. A weight reduction of about 10% in Americans is likely to produce major health benefits.[2]

Screening for Hypercholesterolaemia

The statement asks everyone to be aware of his/her plasma cholesterol level and to recognise where on the distribution curve for the general population this level falls, so that remedial action can be taken if necessary. However, first there must be reliable and economical screening services; this may lead to efforts to make standardisation procedures easy to set up and to monitor frequently. Also, physicians may make themselves better informed and interested in intervening effectively when plasma cholesterol levels are found to be undesirably high. The level of medical attention to hypercholesterolaemia in patients with proven myocardial infarctions (MI) and in patients who have been subjected to coronary artery bypass grafts is deplorable.

QUESTIONABLE ASPECTS OF THE CONSENSUS STATEMENT

Unjustifiable Extrapolations

All recent controlled clinical trials of the lipid hypothesis have been undertaken in males, and usually in those at highest risk of CHD because of hypercholesterolaemia, since they are the ones who stand to gain the most if the hypothesis is proved correct and who are more apt to adhere to trial protocols. Besides, since most recruits would survive, the economics dictate that trials be mounted in persons with the highest CHD rates. However, the relation between risk and plasma cholesterol is not linear: the risk curve starts flat, then becomes a rapidly increasing slope.

The LRC-CPPT, on which the panel based its recommendations, was a drug trial in high-risk males that showed that cholestyramine taken daily for the 7·4-year mean duration of that trial was beneficial (but only marginally) in males aged 35–59 years belonging to the top 5% of the cholesterol distribution curve. The report[1] closed with these words: "The trial's implications . . . could and should be extended to other age groups and women and, since cholesterol levels and CHD risk are continuous variables, to others with more modest elevations of cholesterol levels".

I seriously doubt that the benefits of cholesterol-lowering seen in the highest risk males can be expected to occur also in men, women, and children with lower plasma cholesterol levels, especially when a different and untested method of intervention—namely, the prudent diet—is applied. My first objection to the extrapolation is that the mode of action of cholestyramine is different from that of any cholesterol-lowering dietary regimen. Indeed, how the prudent diet may reduce plasma cholesterol levels is still largely unexplored. Whether the same degree of cholesterol-lowering by cholestyramine and by the prudent diet would lead to the same health benefits has yet to be shown by actual experimentation. Secondly, the shape of the risk/plasma cholesterol curve means that the decrease in risk for a given percentage decrease in plasma cholesterol in those persons at the flatter end of the curve will be very much smaller than that for those whose plasma cholesterols fall on the rapidly rising slope. Thus, the largest part of the general population (perhaps the bottom two-thirds) have the least to gain by lowering cholesterol levels. Whether the risk/cholesterol curve is continuous or broken at some threshold level is irrelevant. The benefits of any given cholesterol reduction for those at the left end of the curve are small, however that reduction may be achieved; the considerable frequency of CHD in this large segment of the population is due to factors other than hypercholesterolaemia.

I cannot accept the recommendation that the prudent diet be adopted by everyone over the age of 2 years. That viewpoint was rationalised on two grounds: (1) the generalisation by the CPPT authors that any 1% reduction in plasma cholesterol level will lead to a 2% reduction in CHD incidence in all segments of the population; and 2) the public health view that dietary interventions are more feasible if adopted by a whole family than singly by a high-risk member of a family. The panel argued for starting intervention early before lesions develop, and for striving to establish desirable eating habits in childhood. However, I know of no evidence that the prudent diet will prevent the development of arterial atheroma at any age: the hypothesis is reasonable but unproven, especially in children considered to be at high risk by the panel criteria. And, why start the diet at age 2, when worldwide studies of aortas obtained at necropsy have shown that fibrous plaques first become manifest at the age of 15–19 years?[3] There is also no evidence that optimum growth and good health will be maintained in growing children on such a diet, although a test of this proposition could have been recommended.

Choice of Dietary Regimen

Sizeable reductions in plasma cholesterol levels can be achieved by a diet in which polyunsaturated fats are substituted for saturated fats. There also is abundant evidence that the quality of fat ingested is a far stronger determinant of that phenomenon than is the amount of cholesterol ingested. Why is a vegeterian diet with a high polyunsaturated/saturated ratio not offered as a feasible dietary regimen? Another substitute for the prudent diet could be a diet rich in fat of monoenoic structure, which in Mediterranean countries has been correlated with low plasma cholesterol levels and low rates of CHD; furthermore, recent metabolic ward studies have shown[4] that the substitution of monounsaturated fat for polyunsaturated fat causes equivalent decreases in plasma cholesterol levels without lowering high-density lipoprotein levels. A third option could be a diet containing fish oils; such a diet has been associated with major reductions in plasma cholesterol and triglyceride levels and low rates of CHD in Eskimos.

Why then did the consensus panel recommend the prudent diet? Hjermann et al[5] obtained a 13% difference in plasma cholesterol levels between his intervention and control groups of very high-risk Norwegian males (final levels 263 and 341 mg/dl) when they examined the effects of a 28% fat calorie diet over a 5-year period. In contrast, in the Multiple Risk Factor Intervention Trial (MRFIT) only an 8% reduction in plasma cholesterols was obtained in high-risk American males on a 30% (then 25%) fat calorie diet.[6] Hjermann et al reported a reduction in total CHD events in his diet group by lumping together all sudden deaths (explained and unexplained plus fatal and non-fatal MIs), but the statistical validity of that procedure and conclusion has been questioned.[7] I know of no other evidence from controlled trials that the prudent diet will reduce CHD incidence, and no evidence on long-term acceptability, practicality, or frequency of undesirable side-effects.

Aside from the unconvincing evidence for benefit of any of the four dietary regimens listed above, there is so far no convincing evidence as to whether any of these regimens affect the risk of other non-CHD disorders. The only diet tested to date in large numbers of patients in which both risks and benefit have been assessed is that in which fat intake provided about 40% of calories and the P/S was of about 2: several types of statistical analysis of 5 such trials[8] allowed the conclusion that "(these) cholesterol-lowering diets do not influence cancer risk". One of the weaknesses of the consensus statement is the failure to acknowledge the paucity of data available to us with regard to risk/benefit ratios of the prudent diet or of the several alternatives discussed above.

Candour on Present State of Evidence

I would have been content with the consensus statement if it had confined itself to what we do know and what we do not. It promises benefits without giving the evidence to back up that promise. By failing to emphasise what we do not know, the statement sweeps these weaknesses in our evidence under the rug, as if they were trivial. I have disagreed with that position.[9]

A Barrier to Future Inquiry

Since many unanswered questions remain about the role of nutrition in CHD prevention, it is remarkable that the press in the USA has set out to sell the message that the diet-heart question has been solved by the LRC-CPPT. (In this process the press has had more than tacit support from many of the scientists concerned in the trial.) If this atmosphere prevails, there will be little encouragement for young medical investigators to probe more deeply into the mysteries of nutrition/CHD relations, and there will be increasing resistance by peer reviewers to approve funds for further study of these unsolved questions.

The Consensus Process

I am dismayed by the imbalance between the importance of the issues at stake, on the one hand, and, on the other, the manner in which the consensus development conference considered these issues. In a companion commentary (this page), Professor Oliver describes his reactions to this conference, at which he was a participating speaker; I fully agree with his criticisms and his analysis of ways in which such matters should be addressed in future.

CONCLUSION

I believe that as scientists we are expected by the public to render scientifically sound advice. Policy-makers must come to their own conclusions, and will do so for a complex of reasons—political, social, and economic. That is their affair; ours is to be sound, as sound as current evidence permits, stating clearly where the gaps in knowledge exist.

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CONSENSUS OR NONSENSUS CONFERENCES ON CORONARY HEART DISEASE

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TWO public meetings were held towards the end of last year to review existing evidence regarding areas of uncertainty concerning coronary heart disease and to make recommendations about future policy. One was organised in the United Kingdom by the King's Fund and was on the advisability and need for increasing coronary artery bypass surgery here. The other was held in the United States by the National Heart, Lung and Blood Institute and was on the practicability and value of lowering blood cholesterol in the general population.[1]

Both were called consensus development conferences. This is an ambiguous description: is the emphasis to be placed on consensus or on development? The purpose of a conference designed to develop and reach a consensus view in an area where there are numerous and disparate opinions is quite different from that of a conference which is a learned debate about whether it is possible to reach any consensus on how an

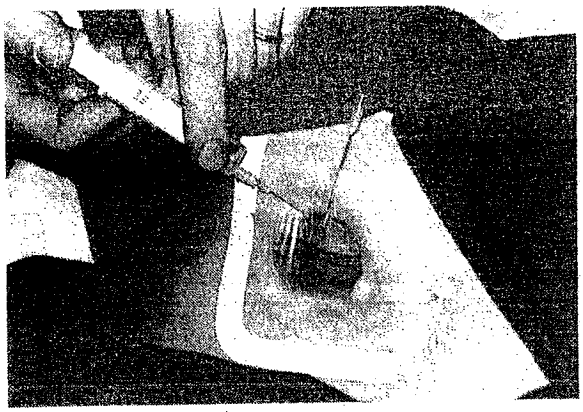

Fig 1—Injection of saline to measure pressure-sore volume.

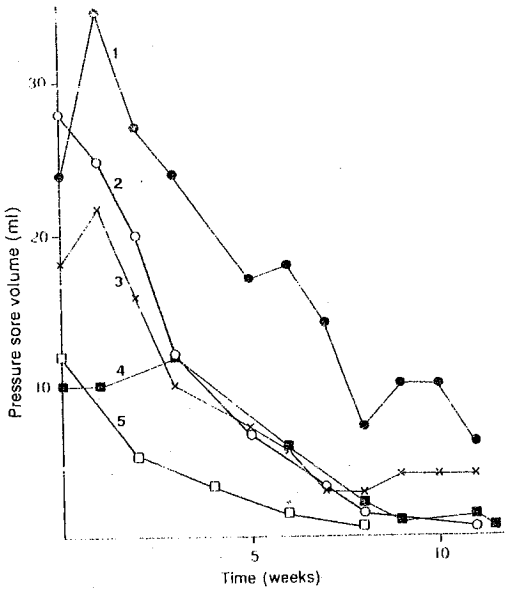

Fig 2—Pressure-sore volume against time for 5 patients.

placed at the highest point of the sore to allow air to escape (fig 1). If there is subcutaneous necrosis the skin should be gently massaged to ensure complete release of air. The volume of solution required to fill the sore is recorded, and before the film is removed the circumference of the sore can be traced with a pen so that an accurate assessment of surface area can be made.

Results

The technique has been used to monitor pressure sores under treatment, with satisfactory reproducibility (see table). Fig 2 shows a chart of sore volumes against time for 5 patients treated by different regimens; volumes initially increased in 3 patients because of surgical or chemical debridement.

REPRODUCIBILITY OF MEASUREMENT TECHNIQUE

	Sore volume (ml³)					
	Day 1		Day 2		Day 3	
—	A	B	A	B	A	B
Sore 1	0·6	0·7	0·6	0·6	0·4	0·5
Sore 2	1·3	1·2	1·5	1·5	1·6	1·4
Sore 3	1·8	1·7	1·9	1·8	1·7	1·7
Sore 4	14	19	15	17	19	18

Observers A and B measured volumes independently at different times of day.

Discussion

Pressure sores have three dimensions: a simple and reproducible technique by which their volume can be measured would enable more accurate monitoring of treatment in an individual, and comparison of different regimens between individuals. However, for superficial sores on parts of the body with little subcutaneous tissue, surface area assessment alone is probably a satisfactory guide to the rate of healing—and the technique we describe may less accurately measure the volume of such sores because the transparent film may be more difficult to apply. Our technique is especially useful for pressure sores over the sacrum and ischial tubercle or trochanter, where the skin surface area is a particularly poor guide to size, and for which the best management is most controversial.

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VIEWPOINT

Nonsensus consensus

PETR SKRABANEK

When in 1974 the American Psychiatric Association declared that homosexuality was no longer a disease, the new consensus was the result of a vote among the members. Similarly, if a group of religious functionaries were to cast a vote on whether homosexuality is still a sin, the majority could give their assent and the consensus would be upheld. It would be a mistake to equate such consensuses with a democratic decision, as *demos* has no say in the matters. Neither is anyone the wiser when a consensus is reached.

Consensus conferences on health issues are a recent phenomenon. Since 1977, the National Institutes of Health in the USA have organised almost 100 consensus conferences, at a cost of about $10 million. Most doctors are unaware of what these conferences were about and in many instances the practice of medicine has been unaffected. As recommendations from consensus conclaves are issued *ex cathedra*, without any reference to original data, lawyers may use them in malpractice suits against doctors who have not followed them. The careful selection of participants guarantees a consensus. A token dissident, coopted to maintain the semblance of impartiality, is, as a rule, not given space to ruffle the smoothness of the consensus report. Yet the very need for consensus stems from a lack of consensus. Why make an issue of agreeing on something that everyone (or nearly everyone) takes for granted? In science, lack of consensus does not bring about the urge to

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hammer out a consensus by assembling participants whose dogmatic views are well known and who welcome an opportunity to have them reinforced by mutual backslapping. On the contrary, scientists are provided with a strong impetus to go back to the benches and do more experiments.

Uncertainty in medicine, as in theology, is intolerable and a consensus conference, like a synod of bishops, is convoked to settle the matter. A recent example was the report of the National Cholesterol Education Program Expert Panel on detection, evaluation and treatment of high blood cholesterol, issued by a committee of thirty experts.[1] There is strength in numbers and it silences the critics. Among many recommendations, this report endorses a diet for which there is not a scrap of evidence that it is capable of changing the risk of dying from coronary heart disease, but there is reasonable evidence that it does not. The agreement on dietary treatment and on the meaning of "high" cholesterol is achieved by an old Chinese consensus method employed in settling the question of the length of the Emperor's nose. As Richard Feynman recalled, since no one was allowed to see the Emperor's face, this precluded direct measurement, but a consensus could still be reached by going around the kingdom and asking experts on the length of the Emperor's nose what they *thought* it might be and by averaging all the answers. Since the number of questioned imperial rhinosophists was rather large, the standard error of the mean was very low, and the precision of the estimate was good.

Medical fashions come and go, but now that the world has become a global village, they reap hecatombs of victims. People have developed a new love-hate relationship with medicine: they dream about "alternatives" but they pay through the nose for "health checks". The financial exploitation of the worried-well and of the sick whom doctors cannot cure is no longer verbally denounced by the leaders of the profession; it is the order of the day. To make it easier for "consumers" to opt for buying "health", the "product" is neatly packaged and advertised with slogans that have a scientific ring—computerised diagnosis, automated cholesterol measurement, the latest pharmaceutical breakthroughs, and other quick technological fixes for humans ills and woes. The risks of new technologies are not evaluated; and since there is no evidence of risks it is assumed that there is evidence of no risks. When finally the risks can no longer be ignored and exceed the benefits by a wide margin, a new fashion takes over. It took over ten years for neonatologists to question why over 10 000 premature babies in incubators became blind. The cause of the blindness was retrolental fibroplasia induced by the routine use of oxygen. The possibility that something so good and natural as oxygen could become a leading cause of childhood blindness did not cross anyone's mind for a long time. It is easy to be wise with hindsight. But what about the "prudent" diet, recommended by experts who claim that if ingested daily it would conquer the number one killer—coronary heart disease? Surely it could do no harm, or could it?

Consensus experts do not put any cost on their recommendations since everyone would live longer and who are we to put a price on human life? Money spent on the crusade will not be available for other, more effective uses. For this very reason it would be unwise for a single-cause enthusiast to delve too deeply: other experts could cheat him out of his budget. To make their case, the consensus experts are tempted to inflate the importance of their cause by jumbo-jet statistics. This is done by enumerating how may lives would be lost, which otherwise would be saved if the experts got hold of the money, in the next 10, 20, 50 years, in a population of 100, 200, 500 million. As such numbers are large, and become larger by multiplication, they can be expressed suitably as the number of jumbo jets crashing in the national airspace daily. These statistical massacres stun both politicians and the public. Once the bandwaggon starts moving downhill the prestige, power, and credibility of the experts are at stake. Various ruses must be employed to suppress, dismiss, or distort new information which undermines the premises of the consensus.

There have been too many "disasters of good intent" in the history of medicine and people should temper their faith in experts—particularly when they see them coming in droves—with their own informed scepticism. After all, it is the public who will carry the cost both physically and financially. William Silverman pointed out that the ultimate test of any medical innovation should be, Is life any sweeter? "Criticism must come from sceptics in the community if we are to separate 'halfway' technical solutions from solid claims of improvement in general welfare".[2] Knowing that someone is eager to sell you a cholesterol number, and keep the proceeds of the lottery, could put off even the hardened gambler.

The oldest consensus among the vendors of health, and other traders along the valley of the shadow of death, was that people want to be deceived and should be pleased accordingly. In the past, mountebanks were distinguishable from their more respectable colleagues at least in appearance and manners, if not by the effectiveness of their cures. Nowadays, the convergence of medicine and its "alternatives" is an ominous foretaste of the ultimate consensus that all will be healthy by the year 2000, with the WHO blessing, provided they don't die by then, eat plenty of fibre, and promise never to use their reason again.

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From The Lancet

A sharper *Lancet*

Sir Arthur Eddington has remarked that in size man is somewhere about midway between the stellar spaces and the ultimate atoms. *The Lancet* occupies a similar mid-position in the medical universe, though the 237 bound volumes on the library shelf reveal that sometimes it has expanded towards the stars and sometimes shrunk towards the atoms, according to the varying abundance of paper and other literary tools. Now we seem to be in for some lean months. But though from this week we must have fewer pages we shall, with the printer's help, get more on them. By encroaching on our margins, by using slightly closer type, by jealously trimming every white space, we hope to provide our readers with at least an adequate minimal diet. Economy in words will be more desirable than ever... Short papers are more likely to be read and more likely to be published. But papers can be long at 500 words and short at 5000, according to their material. The secret of brevity is repeated revision; the author's best friends are a month's delay and several candid critics.

(6 July 1940)

Editorial

Health Policy on Blood Cholesterol

Time to Change Directions

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and Thomas B. Newman, MD, MPH

A U-shaped association between the level of blood cholesterol and subsequent mortality has been reported in many studies over the past two decades.[1-3] The right-hand limb of the U is the well known higher risk of death from coronary heart disease (CHD) at higher levels of blood cholesterol; this positive association, shown in clinical trials to be causal and reversible, is the cornerstone of U.S. policies directed at lowering high blood cholesterol.[4] The left-hand limb of the U is the higher risk of deaths from non-CHD causes at lower levels of blood cholesterol; the basis for this negative association remains poorly understood, and its implications for health policy have received inadequate attention.[5,6]

This issue of *Circulation* contains a report on the 1990 National Heart, Lung, and Blood Institute Conference on Low Blood Cholesterol: Mortality Associations that presents a statistical overview of available cohort studies. The unprecedented size of the study (68,406 deaths)

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provides a unique opportunity to examine cause-specific mortality at the low end of the cholesterol distribution in both sexes. In the women, moreover, there are unexpected findings pertaining to the right-hand, high cholesterol limb.

Low Blood Cholesterol and Noncardiovascular Deaths

Beginning with the left-hand limb, the study finds a significantly increased risk of noncardiovascular death in both men and women with total cholesterol levels below 160 mg/dl for a surprisingly large and diverse set of causes. In round numbers, such men had a 20% higher age-adjusted rate of cancer deaths than those with cholesterol levels between 160 and 199 as well as a 40% higher rate of noncardiovascular noncancer deaths; the latter included increased rates of injury deaths (by 35%), respiratory system deaths (by 15%), digestive system deaths (by 50%), and "other" causes of death (by 70%). The respiratory and digestive system death rates showed a graded response throughout the cholesterol distribution, which continued to decline with increasing cholesterol levels above 200 mg/dl. Among women, the patterns of the association between low blood cholesterol and increased rates of various causes of noncardiovascular deaths were similar to those in men, except that the excess in cancer mortality was smaller (about 5%).

What is the explanation for the association between low cholesterol and higher risk of death? Among the five possibilities,[6,7] *chance* is extremely unlikely at the probability values reported. *Bias,* or experimental error, is also an unlikely explanation with such hard end points and high-quality studies. Two statistical sources of bias that are undoubtedly present but do not explain the findings are competing mortality and regression dilution bias. Competing mortality—the fact that if low cholesterol is associated with a low rate of cardiovascular deaths, then more people with low cholesterol will be available to die from other causes—can only have a trivial effect when the large majority (in this case 90%) of the cohort is still alive. Regression dilution bias—the underestimate in the strength of an association caused by random error[8]—means that the true associations between low blood cholesterol and mortality in the population are actually even larger than the effect sizes noted in the pooled sample.

The third possibility, *effect-cause,* has been the favorite until now. Many experts have held, for example, that preclinical cancer already present in some individuals at the time of the blood cholesterol measurement may have lowered the cholesterol level.[2,3] Effect-cause, however, is not likely to explain many of the low-cholesterol associations, because the conference report analyses excluded deaths occurring during the first 5 years after the cholesterol measurement, and a recent report on the largest single cohort has shown the excess in many categories of noncardiovascular deaths—cancer of the lung and liver, pulmonary disease, cirrhosis, and suicide—to continue undiminished for 12 years after the cholesterol measurement.[9] Moreover, effect-cause makes little biological sense for the observed excess of acute causes of death such as stroke or trauma.

The two remaining explanations, as noted in the conference report, are *confounding* and *cause-effect.* Obviously, it is vitally important to know which of these is operating, but at present our ability to make this distinction is limited.

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Confounding would be present if the association between the predictor variable of interest (blood cholesterol) and the outcome (mortality) were a result of both factors being related to a third, confounding factor. Alcohol intake, for example, might both lower blood cholesterol level and be a cause (either directly or through lifestyle companions like cigarette smoking and depression) of cancer, pulmonary disease, cirrhosis, and suicide. The conference report examined this possibility by stratification—looking separately at nondrinkers, light drinkers, and moderate-to-heavy drinkers—and found that low blood cholesterol had similar associations with noncardiovascular mortality in all three strata. Similarly, the associations were present after adjusting for age, blood pressure, body mass indext, and cigarette smoking. It remains possible that improved measurements of these potential confounders would produce different results (e.g., through a better ability to separate out the heavy drinkers); that these potential confounders do explain some of the individual cause-specific associations within the overall noncardiovascular mortality outcome that was examined by stratification; or that other confounders, which were not studied, might have been operating (e.g., socioeconomic status). A recent report from the Whitehall Study[10] indicates that lower socioeconomic status and health state at baseline may partly explain the association between low blood cholesterol and respiratory deaths and suggests that better measures of these phenomena might have more completely explained the association. The Whitehall Study was too small to examine most of the cause-specific associations between low blood cholesterol and mortality noted in the conference report.

A second strategy for distinguishing between confounding and cause-effect is to consider other lines of evidence that contribute to causal inference. Randomized blinded trials are the best such evidence because the role of confounders, whether measured or not, is limited to chance maldistributions that are taken into account in the tests of statistical significance. The randomized trials of cholesterol interventions are summarized briefly in the conference report and examined in detail elsewhere.[11–17] Alarmingly, meta-analysis of primary prevention trials reveals higher rates of noncardiovascular deaths in men receiving active treatment to lower their blood cholesterol level; these increases are statistically significant for both injury and cancer mortality. Although this finding is not present in the secondary prevention trials,[13,18] statistically significant results in randomized trials strongly suggest causality.

Interventions that change blood cholesterol from high to moderate levels might well influence disease rates through biological mechanisms that differ from those responsible for the effects of native low blood cholesterol levels in people who have not received an intervention. Therefore, despite the superficial similarities (increased rates of dying from cancer and injuries) between the clinical trial findings and the epidemiological findings, the two sets of results may be unrelated. The clinical trial findings may represent adverse effects of drugs, especially the fibric acid derivatives clofibrate and gemfibrozil, more than effects of dietary intervention.[14] The epidemiological findings of higher death rates from various causes in those with cholesterol levels below 160 mg/dl are probably a mixed bag, due to confounding, partly to effect-cause, and partly to cause-effect through mechanisms to be clarified.[19–22]

High Blood Cholesterol and Cardiovascular Deaths in Women

Before considering policy implications, we will turn to a second major finding in the conference report. The right-hand limb of the cholesterol-total mortality curve is almost flat in women; Figure 1 and Table 3 of the report show that among women high blood cholesterol is not associated with all-cause mortality nor even with cardiovascular mortality.

This surprising observation is explained partly by the fact that cardiovascular deaths are comprised not only of deaths caused by CHD but also of those caused by other vascular diseases, including stroke. Looking just at CHD mortality (in Table 6 of the Jacobs et al conference report and in a meta-analysis by Manolio et al[23] from another recent National Institutes of Health conference) reveals the expected positive association; the risk ratio is almost as large for middle-aged women as it is for middle-aged men. Neither of these reports examines the relation between blood cholesterol and stroke, but other studies have established the existence of a significant negative association between the blood cholesterol level and risk of death from hemorrhagic stroke in men[24,25] and in women.[24] Hemorrhagic stroke, although much less common than CHD in both sexes, makes up a higher proportion of the total cardiovascular deaths in women because of their lower CHD rates.[26]

Therefore, in middle-aged men the negative association between blood cholesterol and hemorrhagic stroke death is numerically less important than the positive association with CHD death.[25] However, in middle-aged women it may be that this negative association (perhaps combined with negative associations for other components of cardiovascular death) has a substantial impact on the association between high blood cholesterol and overall cardiovascular death rates. This has implications for policy decisions on preventing cardiovascular disease in women.

We are coming to realize that the results of cardiovascular research in men, which represents the great majority of the effort thus far, may not apply to women. Although the proportion eventually dying of CHD is similar in the two sexes, the disease occurs 7–10 years later in women than in men. Low high density lipoprotein cholesterol may be a stronger risk factor and high low density lipoprotein (LDL) cholesterol a weaker risk factor in women than in men.[27] The attenuation of the strength of the cholesterol-CHD association in the elderly may be more pronounced in women than in men.[23] Blood cholesterol appears to be a risk factor for CHD recurrence or death among women who already have CHD, as it is in men.[28] However, almost all of the cholesterol-lowering intervention trials have been carried out in men. In women, we are limited to studies of intermediate outcomes, angiographic studies that have shown regression in atherosclerotic lesions after lowering LDL cholesterol levels among women with familial hypercholesterolemia.[29]

Conclusions

In summary, the field of cardiovascular disease epidemiology has recently been enriched by two new

bodies of evidence that have non-CHD deaths as outcomes of interest: one in the arena of observational cohort studies and the other in the arena of randomized clinical trials. Both bodies of evidence are based on meta-analyses that combine eligible studies to produce enough power for examining cause-specific mortality patterns. The findings call into question policies built over the past several decades on evidence that focussed only on CHD as the outcome. We are led to three conclusions: two related to the cohort study findings and one to the clinical trial findings.

First Cohort Study Finding

There is an association between low blood cholesterol and noncardiovascular deaths in men and women. There is no longer any doubt that the 6% of middle-aged adults with cholesterol values below 160 mg/dl are at increased risk of dying from a variety of causes, which includes lung cancer, other noncolon cancers, respiratory disease, digestive disease, trauma, hemorrhagic stroke, and other residual causes. While we await evidence on the causal basis for each of these associations (which probably differs for the different outcomes), it may be time to review national policies aimed at shifting the entire population distribution of blood cholesterol to the left.[5] A cholesterol-lowering diet may not be prudent[30] for those adults whose cholesterol levels place them on the left-hand limb of the total mortality U.

Second Cohort Study Finding

There is no association between high blood cholesterol and cardiovascular deaths in women. In contrast with the evidence for men, there is a surprising absence of association between high blood cholesterol and cardiovascular deaths in women. It appears that this is partly caused by a negative association between blood cholesterol and hemorrhagic stroke deaths, which counterbalances the positive association between blood cholesterol and CHD deaths (which are less numerous in women than in men among the middle-aged). While the causal basis for these phenomena. The sex difference calls into question the general practice of extrapolating to women the findings from epidemiological studies and clinical trials in men. With the exception of those who already have coronary disease or other reasons for being at a comparable very high risk of CHD death, it no longer seems wise to screen for and treat high blood cholesterol in women.

Randomized Trial Finding

Primary prevention trials of cholesterol intervention reveal an increase in non-CHD death rates that is similar in magnitude to the decrease in CHD death rates. It is only in secondary prevention trials of patients at high risk because they already have coronary disease that beneficial effects of cholesterol intervention on mortality have been observed. For primary prevention in patients who do not yet have manifestations of coronary disease (or other reasons for being at a comparable very high risk of CHD death), it now seems unwise to treat high blood cholesterol with drugs.

This last conclusion fits with the Canadian policy of not screening or treating high blood cholesterol in young adults, which is based on the very poor cost-effectiveness of doing so in this low-risk segment of our population.[31,32] It also fits with a growing set of recommendations by other experts.[14,16,33–35]

These three conclusions indicate the need for a change in direction for cholesterol policy. Efforts to identify and treat people with high blood cholesterol have been gaining momentum for several decades and have now reached the point that some experts recommend screening and treatment for blood cholesterol in children. The new evidence on non-CHD causes of death makes it clear that this pediatric policy is unwise[11,36] and indicates that we should draw back from universal screening and treatment of blood cholesterol for primary prevention in adults as well.

This change in direction—limiting cholesterol screening and intervention to the minority in our population for which the benefits clearly predominate over the harms (those with coronary disease or other reasons for being at a comparable very high risk of CHD death)—will not be easy. However, a willingness to be patient while we sort out the causal basis for the increases in non-CHD deaths rests on firm ethical grounds. The overriding ethical obligation is to do no harm. Particularly when considering the long-term use of drugs for people who are in good health, the burden of proof falls on the proponents of the intervention.[14,37,38] We need now to pull back our national policies directed at identifying and treating high blood cholesterol in the primary prevention setting and put on hold well-meant desires to intervene while we await convincing evidence that the net effects will be beneficial.

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KEY WORDS • cholesterol • coronary heart disease • Editorial Comments • mortality

13.

CHOLESTEROL AS RISK FACTOR FOR MORTALITY IN ELDERLY WOMEN

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Summary 92 women aged 60 years and over (mean 82·2, SD 8·6) living in a nursing home and free from overt cancer were followed-up for 5 years. 53 died during this period; necropsy revealed cancer in only 1 patient. Serum total cholesterol at entry ranged from 4·0 to 8·8 mmol/l (mean 6·3, SD 1·1). Cox's proportional hazards analysis showed a J-shaped relation between serum cholesterol and mortality. Mortality was lowest at serum cholesterol 7·0 mmol/l, 5·2 times higher than the minimum at serum cholesterol 4·0 mmol/l, and only 1·8 times higher when cholesterol concentration was 8·8 mmol/l. This relation held true irrespective of age, even when blood pressure, body weight, history of myocardial infarction, creatinine clearance, and plasma proteins were taken into account. The relation between low cholesterol values and increased mortality was independent of the incidence of cancer.

Introduction

TOTAL serum cholesterol values tend to rise during life, but not beyond age 60 in men and 70 in women; concentrations then decrease slightly.[1,2,3] Total serum cholesterol is related positively to the incidence of coronary heart diseases in elderly people.[4,5] Yet an increased mortality rate has been demonstrated in septuagenarian men with low cholesterol values, and has been attributed to the large number of cancers in that group.[6] However, except for prostatic cancer, which seems to increase at an age-dependent rate in elderly men, cancer mortality declines from the age of 70, and is not important enough to account for the association of mortality with low cholesterol values in very old people, especially women.[7,8] We tried to eliminate the role of cancer as a confounding factor in a prospective study designed to assess the relation between mortality and total serum cholesterol in a group of elderly women.

Subjects and Methods

Subjects

The subjects were drawn from 111 women living in a home for elderly people. We excluded smokers, women who had been living in the home for less than 6 months, those known to have cancer, those with an acute illness or liver disease, and those taking drugs to lower serum cholesterol. The remaining 92 women (mean age 82·1 [SD 8·6], range 60–97 years) were followed-up for 5 years. Before entry to the study the following were recorded: a complete medical history, physical findings, electrocardiogram, chest X-ray, and laboratory tests (blood count, erythrocyte sedimentation rate, blood glucose, glycosylated haemoglobin, total cholesterol, proteins, protein electrophoresis, creatinine clearance).

Statistical Methods

For variables that could be quantified the following were calculated—minimum, maximum, first quartile, median, third quartile, mean, standard deviation. Pearson's correlation coefficient was used to measure the linear relation between two quantifiable variables. The Mann-Whitney test was used to compare the means between two groups. We used Cox's proportional hazards technique[9] for assessing the relation between serum total cholesterol level and mortality. We took into account age, body weight, blood pressure, history of myocardial infarction, creatinine clearance, and level of plasma proteins as possible interacting or confounding factors of mortality.

Data Analysis Strategy[10,11]

We started with Cox's model without interaction, using the following variables: cholesterol (C), squared value of cholesterol (C2), age (A), blood pressure (BP), body weight (BW), creatinine

TABLE I—DISTRIBUTION OF VARIABLES

Variable	Minimum	Maximum	First quartile	Median	Third quartile	Mean (SD)
Age (yr)	60·7	97·1	76·7	83·4	87·5	82·2 (8·6)
Diastolic BP (mm Hg)	67	110	76	81	88	82 (8)
Weight (kg)	34	90	45	53	68	57 (14)
Cholesterol (mmol/l)	4·0	8·8	5·5	6·0	7·1	6·3 (1·1)
Creatinine clearance (ml/min)	14	111	32	41	56	45 (21)
Plasma proteins (g/l)	60	80	67	70	73	70 (4)

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TABLE II—CORRELATIONS BETWEEN CHOLESTEROL AND OTHER QUANTIFIABLE VARIABLES

—	Age	Creatinine clearance	Plasma proteins	Body weight	Diastolic BP
Correlation coefficient	−0·29	0·11	0·13	0·04	0·12
95% confidence interval	(−0·46, −0·10)	(−0·10, 0·30)	(−0·07, 0·33)	(−0·18, 0·25)	(−0·09, 0·31)

clearance (CC), history of myocardial infarction (MI), and plasma proteins (P).

In the first step we tested for interactions between A and C, and between A and C2. Then the test was repeated, with A being replaced, in succession, by BP, BW, CC, MI, and P. Since no such interaction was found, it was possible, in the second step to look for confounding factors for cholesterol among A, BP, BW, CC, MI, and P. In the third step, we tested the final relation obtained between cholesterol and mortality.

Finally, we tested the stability of the model by assessing 92 new models with only 91 patients each, according to Storer and Crowley.[12]

Results

Table I shows the distribution of the quantifiable variables at the time of entry into the study. A history of myocardial infarction had been documented in 7 patients, could be excluded in 78, and was uncertain in 7. Table II shows the correlations of cholesterol with the other quantifiable variables. In the patients who had antecedents of myocardial infarction, the mean cholesterol value was 5·9 mmol/l (SD = 1·2). It was 6·3 mmol/l (SD = 1·1) in those without an antecedent of myocardial infarction (Mann-Whitney test: p = 0·28).

During the 5 year follow-up, 53 of the 92 patients died. The causes of death were—hepatic carcinoma undetected at entry to the study, 2%; infection (bronchopneumonia, septicaemia), 38%; vascular diseases (stroke, myocardial infarction, pulmonary embolism, heart failure), 32%; miscellaneous (eg, renal insufficiency, dehydration, non-cancerous intestinal obstruction, trauma, cachexia), 24%; unknown, 4%.

In the patient who died from cancer, initial cholesterol value was 5·9 mmol/l. All 6 patients who died from stroke had initial cholesterol values above the median (6·0 mmol/l).

Cox Analysis

Step 1 showed no significant interaction between cholesterol and the other variables. Step 2 showed that the only confounder for cholesterol was age. The final proportional hazards model is:

$$\lambda(t; A, C) = h(t) \exp(0{\cdot}074\,A - 2{\cdot}543\,C + 0{\cdot}1815\,C2)$$

where $\lambda(t; A, C)$ is the hazard function and represents mortality; h is the variation of mortality during the 5 years when age and cholesterol are fixed; and t is the time since entry to the study. The likelihood ratio test gives a p value of 0·03 for the presence in the model of the variables C and C2. Details about the estimated coefficients are given in table III.

Fig 1 shows how the relative death rate varies with cholesterol when age is fixed. The death rate is lowest with

TABLE III—STATISTICS RELATED TO THE HAZARD FUNCTION

Variable	Estimated coefficient	Standard error	Estimated coefficient/ standard error	Exponential (estimated coefficient)
Age	0·074	0·020	3·72	1·077
Cholesterol	−2·543	1·183	−2·15	0·079
(Cholesterol)²	0·182	0·093	1·95	1·200

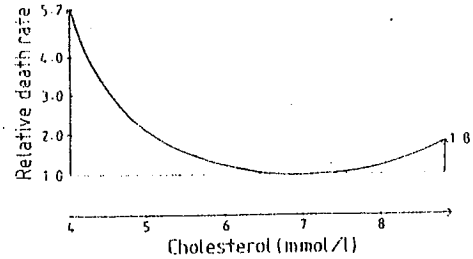

Fig 1—Relative death rate and total cholesterol.

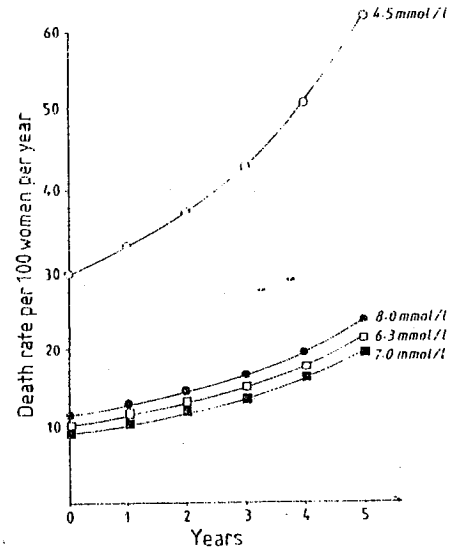

Fig 2—Death rate (hazard function) for women aged 82·2 at entry in the study, according to different levels of cholesterol.

serum cholesterol of 7·0 mmol/l (95% CI 5·4–8·6), 5·2 times (CI 1·1–23·9) greater than the lowest with 4·0 mmol/l, and 1·8 times (CI 0·4–7·7) greater with 8·8 mmol/l.

When we verified the stability of the model (step 4 of the strategy), the cholesterol value associated with the minimum death rate ranged from 6·8 to 7·3. The preceding 5·2 multiplier (associated with cholesterol value of 4·0 mmol/l) ranged from 4·2 to 6·5, and the 1·8 multiplier (associated with cholesterol value of 8·8 mmol/l) ranged from 1·4 to 2·3. We conclude that, despite the small number of subjects, the results obtained with the model are reliable. Fig 2 is an illustration of the final model.

Discussion

Several studies done in younger populations, mostly middle-aged men, have shown an excess of deaths at both extremities of the cholesterol distribution curve.[3,13-18] The mortality peak at the higher end of the curve is widely ascribed to cardiovascular diseases. As in our study, the peak is often more pronounced at the lower end.[13,15,19] The excess of mortality in subjects with low cholesterol is generally attributed to cancer.[3,13,20-23] In the Whitehall study,[18] the inverse association between cancer mortality and cholesterol values was confined to the first 2 years of follow-up, and the authors suggested that this phenomenon resulted from the metabolic consequences of cancer that was present but unsuspected at the time of examination. However, in a 17-year prospective study, Salmond et al[22] found, in New Zealand Maoris aged 25–74, an inverse and non-linear

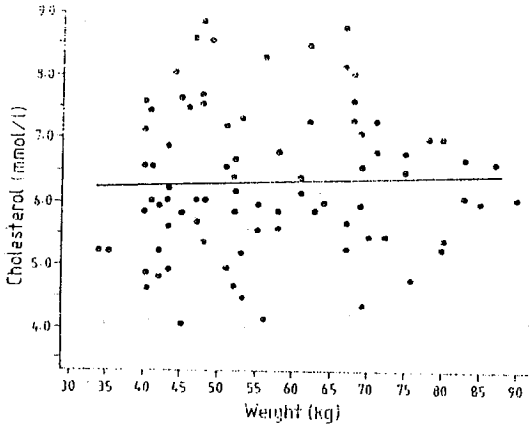

Fig 3—Relation between body weight and cholesterol.

r = 0·04; p = 0·69.

association of cholesterol with total mortality in women. This association remained significant when deaths in the first 5 years of follow-up were excluded, and could not be explained by undetected illness causing low cholesterol concentrations at the time of examination. The results of three Chicago epidemiological studies do not generally support the hypothesis of an inverse association between serum cholesterol and cancer in urban middle-aged white American males and females.[24] In a 7-year follow-up study of men aged 35 to 62 years, Kozarevic et al[25] found an inverse relation between serum total cholesterol and overall mortality without significant association between cholesterol and cancer mortality.

Anderson et al[26] state that, after age 50, there is no increased overall mortality with either high or low cholesterol levels, the association of mortality with low cholesterol being confounded by people whose cholesterol levels are falling, perhaps because of diseases predisposing to death. However, in a 7-year prospective study of 10 000 men aged 40–65, Yaari et al[14] found a J shaped relation between cholesterol and total mortality which persisted after removing data on early mortality (first two years).

In our elderly female population, there was a J-shaped relation between serum cholesterol and overall mortality (fig 1). In a group of elderly men living in a nursing home, Rudman et al[27,28] found, instead of a J-shaped curve, a linear inverse relation between serum total cholesterol and overall mortality, but the follow-up duration was only 14 months. A pattern similar to ours was found in a 10-year follow-up of septuagenarians by Agner and Hansen,[6] but only in men. In the Honolulu heart study[16] the ideal range of cholesterol values corresponding to minimum death risk in men aged 50 to 71 was 200–220 mg/dl (5·16–5·68 mmol/l). The optimum value found in our population (7 mmol/l) is distinctly above that range, and this discrepancy could be explained by differences in age and sex, since our patients were older females.

The raised mortality rate related to low cholesterol values in elderly people is commonly attributed to cancer, as in younger people. From Australian age and cause specific mortality rates Dugdale[29] estimated that lowering the serum cholesterol of the whole population by 10% should lengthen median life by 1 year, but the percentage of deaths from cancer should rise from 26·8 to 29·6. Our results clearly suggest that cancer mortality alone does not account for the excess of deaths in elderly women with low cholesterol. Nevertheless, the mortality peak is much higher in women with lowest initial cholesterol values than in those with highest values. In the group of 11 patients with cholesterol less than 5 mmol/l, 9 died during the study, and in 6 cases the death was due to infection. It is not likely that low cholesterol was only a marker of poor nutritional status, since the relation between cholesterol and mortality was independent of plasma protein level. There was either no correlation between body weight and cholesterol (r = 0·04, fig 3). According to Oliver[30] an increase in plasma cholesterol might be an adaptive process during ageing, necessary to maintaining the physical or chemical characteristics of the cell membrane. If this hypothesis is true, a reduction of cholesterol, either by drugs or by a high intake of polyunsaturated fats, should not be advisable in the elderly, at least when total cholesterol value is not over 7 mmol/l.

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Articles

Cholesterol and all-cause mortality in elderly people from the Honolulu Heart Program: a cohort study

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Summary

Background A generally held belief is that cholesterol concentrations should be kept low to lessen the risk of cardiovascular disease. However, studies of the relation between serum cholesterol and all-cause mortality in elderly people have shown contrasting results. To investigate these discrepancies, we did a longitudinal assessment of changes in both lipid and serum cholesterol concentrations over 20 years, and compared them with mortality.

Methods Lipid and serum cholesterol concentrations were measured in 3572 Japanese/American men (aged 71–93 years) as part of the Honolulu Heart Program. We compared changes in these concentrations over 20 years with all-cause mortality using three different Cox proportional hazards models.

Findings Mean cholesterol fell significantly with increasing age. Age-adjusted mortality rates were 68·3, 48·9, 41·1, and 43·3 for the first to fourth quartiles of cholesterol concentrations, respectively. Relative risks for mortality were 0·72 (95% CI 0·60–0·87), 0·60 (0·49–0·74), and 0·65 (0·53–0·80), in the second, third, and fourth quartiles, respectively, with quartile 1 as reference. A Cox proportional hazard model assessed changes in cholesterol concentrations between examinations three and four. Only the group with low cholesterol concentration at both examinations had a significant association with mortality (risk ratio 1·64, 95% CI 1·13–2·36).

Interpretation We have been unable to explain our results. These data cast doubt on the scientific justification for lowering cholesterol to very low concentrations (<4·65 mmol/L) in elderly people.

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Introduction

High concentration of total serum cholesterol is known to be directly related to mortality in individuals aged younger than 65 years. Previous clinical trials have not had large numbers of patients aged older than 70 years, and researchers have been unable to conclusively show this relation in elderly people.[1,2] Results of several studies have shown an inverse relation, or no relation, between total cholesterol concentration and risk of death in elderly people.[3–6] A U-shaped distribution has also been recorded, in which low concentrations of serum cholesterol in elderly people predict increased mortality.[7] These findings suggest that cholesterol metabolism and homoeostatic mechanisms might differ between older and younger populations.

Corti and colleagues[8] however, suggested that frailty (or disease) in elderly people is more likely to contribute to decreased survival than low cholesterol alone. They took data from the Established Populations for Epidemiologic Studies in the Elderly, in which 4066 patients had serum lipids measured and were followed up for 4 years, and adjusted the analysis for frailty measures (concentrations of albumin and iron in serum). The modified analysis showed that the relation between total cholesterol and coronary heart disease mortality in elderly people was the same as it was for younger and middle-aged individuals. The researchers therefore concluded that the usual statistical adjustments for traditional coronary heart disease risk factors (ie, excluding older persons from cholesterol screening) do not account for possible changes associated with frailty, and are therefore inappropriate.[8]

By contrast, Manolio and colleagues[9] pooled data from several studies and showed that total cholesterol concentration was significantly correlated with fatal coronary heart disease in both men and women across a broad age range and well into older populations (ages 65–100 years). The relative risk of mortality nonetheless lessened with increasing age. Such reductions in risk of mortality in elderly people could be because elderly people generally have a higher attributable risk.[10] Clearly, whether the total concentration of cholesterol in serum has the same relation to mortality in older people as it does in younger people is not conclusive. These differing opinions have direct clinical relevance, since a judgment about total cholesterol and mortality in the elderly age-group should precede screening and attempts to lower serum cholesterol concentrations.

We have therefore assessed changes in various lipid concentrations over about 20 years from 1972 to 1992 and correlated them with all-cause mortality in a large cohort of Japanese/American men who were followed up in the Honolulu Heart Program. Such longitudinal data for serum cholesterol concentration are not available from cross-sectional studies or from shorter follow-up times.

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Methods

Study population

The Honolulu Heart Program is a longitudinal epidemiological study of cardiovascular disease which began with 8006 Japanese/American men, living on the island of Oahu, Hawaii in 1965. The men were born between 1900 and 1919 (age 45–68 years at the time of the first examination in 1965–68). Details of the selection process for the cohort have been published.[11] The entire cohort has undergone six examinations so far. This report is based on the fourth examination of the cohort which was done in 1991–93, and the ascertainment of mortality which was done between the fourth examination and Dec 31, 1996. At the fourth examination, 3741 men aged 71–93 years were assessed (80% of survivors). The study was approved by the institutional review board of Kuakini Medical Center. Procedures were done in accordance with institutional guidelines, and written informed consent was obtained from all participants.

Data collection

The fourth examination included gathering of demographic information, medical and psychosocial questionnaires, cognitive function testing, fasting blood tests, a 2-h glucose tolerance test, blood pressure, anthropometry, spirometry, and an electrocardiogram. All these variables were assessed with standard methods, as used in previous examinations.[12,13] We measured fasting cholesterol and high-density lipoprotein (HDL) cholesterol in 3572 patients. Morbidity and mortality has been assessed since the beginning of the study by monitoring hospital discharge records and death certificates. Data collection is believed to be complete for all-cause mortality. Attrition in this cohort is very small—at the fourth examination only five men were lost to follow-up.

Measurement of variables

At both the third and fourth examinations, procedures for taking and preparing blood specimens for laboratory analysis were standardised by guidelines of the lipid standardisation laboratory of the US Centers for Disease Control and Prevention. Lipid concentrations were measured in San Francisco as part of the Cooperative Lipoprotein Phenotyping Study in the third examination, and at the University of Vermont in the fourth. Comparability of these two examinations was not assessed.

For all examinations, blood specimens were taken by venepuncture after an overnight fast of at least 12 h. Specimens were then put in edetic acid vacutainer tubes and placed in an ice bath. Plasma was separated in a refrigerated centrifuge at 4°C within 1–2 h after collection. Separated plasma was thoroughly mixed, transferred into cryovials, and frozen for later measurement of total cholesterol concentration.[14]

Covariates were selected because of their potential relation with either serum cholesterol or mortality. Body-mass index was defined as weight (kg) divided by height (m) squared. Physical activity index was calculated by multiplication of the approximate oxygen consumption of five different levels of activity with the reported usual numbers of hours a day engaged in that activity.[15,16] Hypertension was defined as systolic blood pressure 140 mm Hg or greater, or diastolic blood pressure 90 mm Hg or greater, or if the patient was taking antihypertensive drugs. Diabetes mellitus was defined by history (as diagnosed by doctor), taking medications (insulin or oral hypoglycaemics), fasting glucose of 7·0 mmol/L or greater, or 2-h post-load glucose of 11·1 mmol/L or greater.

Since low concentrations of cholesterol might be associated with physical frailty, some frailty measures were used as covariates. Details on measurement of forced expiratory volume and hand-grip strength have been published.[17,18] Physical function was measured by self-report of ability to undertake 17 activities of daily living.[19] If participants had difficulty with even one activity of daily living, their physical function was judged poor.

The third Honolulu Heart Program examination was held in 1971–74, about 20 years before the fourth examination. Longitudinal data for serum cholesterol and weight were available for 3398 participants from both the third and the fourth examinations. Weight change was defined as weight loss of 10% or greater.

Data analysis

Participants were divided into quartiles of serum cholesterol concentration (2·09–4·32, 4·33–4·86, 4·87–5·43, 5·44–9·88 mmol/L). Means of variables were compared with linear regression. Age-adjusted mortality rates were calculated, according to quartiles of cholesterol. Mortality rates were also calculated excluding deaths from the first year of follow-up, since patients who are very ill and close to death might have a very low cholesterol concentration because of chronic disease.

We assessed the association between concentrations of cholesterol in serum and mortality with three separate Cox proportional hazards models, using the first quartile as the reference group. The first model adjusted for age and cardiovascular risk factors (body-mass index, physical activity index, pack-years smoking, alcohol intake, HDL cholesterol, chronic hypertension and diabetes, and serum fibrinogen). The second model added frailty measures (haemoglobin, forced expiratory volume, hand-grip strength, weight loss 10% or greater between the third and fourth examinations, and poor physical function). A third model included the above variables and cirrhosis, coronary heart disease, stroke, and cancer at the fourth examination.

We assessed the effect of change in cholesterol between the third and fourth examinations on mortality after the fourth examination. On the basis of tertiles of cholesterol at each of these examinations, we created nine groups: low/low, low/intermediate, low/high, intermediate/low, intermediate/intermediate, intermediate/high, high/low, high/intermediate, and high/high. The third Cox proportional hazards model was repeated with these nine groups entered in the same model, and the

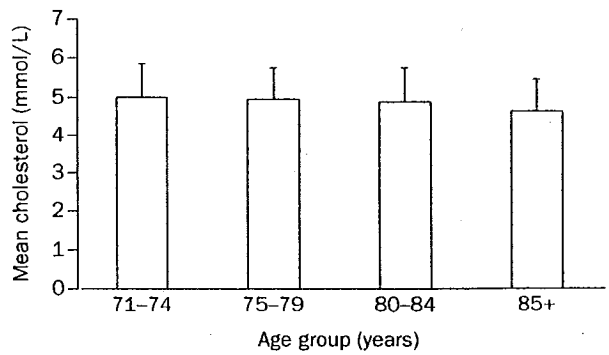

Figure 1: **Mean concentrations of serum cholesterol**
Serum cholesterol concentrations by 5-year age groups (n=3572). Test for trend p<0·0001.

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	Quartiles of serum cholesterol				
	1 (n=904)	2 (n=858)	3 (n=902)	4 (n=908)	p-value*
Cholesterol concentration (mean [SE], mmol/L)	3·85 (0·01)	4·61 (0·01)	5·15 (0·01)	5·99 (0·01)	
Age (mean [SD], years)	78·6 (5·0)	77·9 (4·7)	77·4 (4·4)	76·9 (4·1)	<0·0001
Body-mass index (mean [SE], kg/m²)	23·4 (0·11)	23·3 (0·11)	23·6 (0·10)	23·6 (0·10)	0·034
HDL (mean [SE], mmol/L)	1·21 (0·01)	1·32 (0·01)	1·35 (0·01)	1·39 (0·01)	<0·0001
Hypertension	624 (69%)	618 (72%)	677 (75%)	708 (78%)	<0·0001
Fibrinogen (mean [SE], mg/L)	303·2 (2·13)	300·4 (2·17)	306·0 (2·12)	317·3 (2·12)	<0·0001
Haemoglobin (mean [SE], g/dL)	14·5 (0·05)	14·8 (0·05)	15·0 (0·05)	15·1 (0·05)	<0·0001
Grip strength (mean [SE], kg)	29·3 (0·20)	30·4 (0·21)	30·7 (0·20)	30·6 (0·20)	<0·0001
Weight loss ≥10%†	248 (30%)	160 (20%)	173 (20%)	149 (17%)	<0·0001
Poor physical function†	430 (48%)	309 (36%)	352 (39%)	336 (37%)	<0·0001
Examination 3 cholesterol (mean [SE], mmol/L)	5·11 (0·03)	5·45 (0·03)	5·74 (0·03)	6·11 (0·03)	<0·0001

All means are adjusted for age. *Test for trend. †Data not available for all patients.

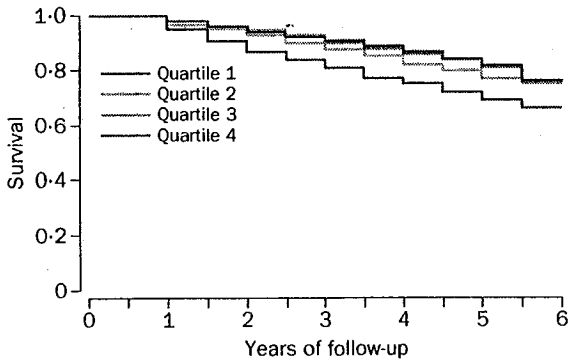
Table 1: **Population characteristics based on quartiles of cholesterol concentrations**

intermediate/intermediate group as reference. All statistical analyses were done with SAS software, version 8.0.

Results

Mean cholesterol concentration fell significantly with increasing age—from 5·00 mmol/L in those aged 71–74, to 4·93 mmol/L in those aged 75–79, to 4·85 mmol/L in those aged 80–84, and 4·61 mmol/L in those aged older than 85 years (test for trend $p<0·0001$) (figure 1). Mortality rates were significantly higher in the 20% non-respondents than in those who participated in the fourth examination. The rates for non-respondents were high even when compared with those in the lowest quartile of cholesterol, suggesting that non-respondents had more serious illness, and therefore did not participate in the examination.

For increasing quartiles of cholesterol concentration, there were significant positive associations with body mass index, HDL cholesterol, hypertension, fibrinogen, haemoglobin, hand-grip strength, and cholesterol concentration at the third examination (table 1). There were significant negative relations with age, weight loss of 10% or greater between the third and fourth examinations, and poor physical function. There were no significant associations with physical activity index, smoking, alcohol intake, diabetes, forced expiratory volume after 1 s (FEV_1), cirrhosis, congestive heart disease, stroke, and cancer.



Quartile 1	1	0·95	0·91	0·87	0·84	0·81	0·77	0·75	0·72	0·69	0·66	0·66
Quartile 2	1	0·97	0·95	0·93	0·90	0·88	0·85	0·82	0·80	0·77	0·76	0·76
Quartile 3	1	0·98	0·96	0·95	0·93	0·90	0·88	0·86	0·84	0·81	0·75	0·75
Quartile 4	1	0·98	0·96	0·94	0·92	0·91	0·89	0·87	0·84	0·82	0·76	0·76

Figure 2: **Probability of mortality by quartiles of serum cholesterol**

Kaplan-Meier survival curves for 5-year all-cause mortality in association with quartiles of serum cholesterol at examination 4 (1991–93). Wilcoxon Log-Rank test $p<0·0001$.

	Quartiles of serum cholesterol			
	1	2	3	4
All deaths				
Participants	904	858	902	908
Deaths	259	173	147	148
Mortality rate*				
Unadjusted	72·3	48·2	37·9	37·9
Age-adjusted	68·3	48·9	41·1	43·4
Age-adjusted relative risk for mortality (95% CI)	1	0·72† (0·60–0·87)	0·60† (0·49–0·74)	0·65† (0·53–0·80)
Deaths excluding first year				
Deaths	215	147	127	132
Age-adjusted Mortality rate*	56·8	41·3	35·2	38·4
Age-adjusted relative risk ratio for mortality (95% CI)	1	0·73‡ (0·59–0·90)	0·62† (0·50–0·77)	0·69§ (0·55–0·86)

*p<0·0001 people per year. †p=0·0012. ‡p=0·0010. §p=<0·0010.

Table 2: **Mortality rates by quartiles of cholesterol**

Follow-up was defined as the time between measurement of cholesterol in serum at the fourth examination (1991–93), and Dec 31, 1996. There were 727 deaths in the group over this time period (table 2). Table 2 shows age-adjusted mortality rates and relative risk for mortality. The results did not change significantly when deaths from the first year of follow-up were excluded (table 2). Kaplan-Meier survival curves showed lowest survival rates for those with the lowest serum cholesterol concentrations (Wilcoxon Log-Rank test $p<0·0001$) (figure 2).

Three separate Cox proportional hazards models were analysed with total mortality as the endpoint, and the first quartile of serum cholesterol as reference (table 3). In the first model (adjusted for age and cardiovascular risk factors) significant associations with mortality were seen in the third and fourth quartiles of cholesterol, compared with the first quartile. These relations were not apparent in the second model, in which frailty measures were added as covariates. No significant associations were seen between serum cholesterol and mortality in model 3, in which chronic diseases were added to the model. We

Cox model	n	Quartiles of serum cholesterol 1	2	3	4
1	3163	1	0·82 (0·65–1·03)	0·65 (0·51–0·83)*	0·73 (0·57–0·93)†
2	2864	1	0·90 (0·70–1·17)	0·77 (0·59–1·00)	0·86 (0·66–1·12)
3	2853	1	0·93 (0·71–1·20)	0·77 (0·59–1·01)	0·88 (0·67–1·15)

*p=0·0005; †p=0·0103.

Table 3: **Cox proportional hazard models for association between quartiles of serum cholesterol and all-cause mortality**

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	Examination 4		
	Low (2·40–4·50 mmol/L)	Intermediate (4·51–5·25 mmol/L)	High (5·26–9·88 mmol/L)
Examination 3			
Low (2·04–5·12 mmol/L)	1·64* (1·13–2·36)	1·39 (0·91–2·12)	1·05 (0·61–1·81)
Intermediate (5·13–5·95 mmol/L)	1·22 (0·80–1·87)	1	1·25 (0·81–1·93)
High (5·96–10·34 mmol/L)	1·58 (0·93–2·69)	1·33 (0·87–2·03)	1·38 (0·94–2·02)

The nine groups were based on tertiles of cholesterol at examinations three and four (low/low, n=609; low/intermediate 364; low/high, 173; intermediate/low, 337; intermediate/intermediate, 415; intermediate/high, 373; high/low, 174; high/intermediate, 362; high/high, 591). Cox proportional hazards analysis was done with the intermediate/intermediate group as reference.*p=0·0089.

Table 4: **Relative risk for mortality based on change in cholesterol between examinations three and four**

repeated model 2 several times, with each frailty measured individually. Each of these frailty measures by themselves did not change the significant association between cholesterol and mortality. However, when all were put into the model at the same time, the significance of the association between cholesterol and mortality was lost.

We divided patients into two groups—those with coronary heart disease risk factors (smoking, hypertension, diabetes mellitus, or coronary heart disease) and those without. Cox model 1 was repeated for these two groups separately. Mortality was lower in the higher cholesterol quartiles for both subgroups. Compared with patients in the first quartile, those in the fourth quartile had a relative risk of 0·75 (p=0·038) in those with risk factors, and of 0·56 (p=0·023) in those that did not.

The third Cox proportional hazards model was repeated with the nine groups detailed in the methods section entered in the same model as dummy variables, with the intermediate/intermediate group as reference (table 4). Only the low/low group had a significant association with mortality.

Discussion

Our data accord with previous findings of increased mortality in elderly people with low serum cholesterol, and show that long-term persistence of low cholesterol concentration actually increases risk of death. Thus, the earlier that patients start to have lower cholesterol concentrations, the greater the risk of death. Cholesterol metabolism and homoeostatic mechanisms might differ in the very old (>75 years), and little information is available about cholesterol-mortality relations in this age group.

The reasons for these results are not clear. Perhaps they indicate a selective mortality; those individuals who are susceptible to biological effects of high serum cholesterol die before they reach age 75 years. The individuals who are left would be a select group with lower cholesterol and whose genetic makeup or other factors protect them from the effects of higher cholesterol concentrations. To some degree Honolulu Heart Program data support this hypothesis—there are few individuals with truly high concentrations of cholesterol remaining in this population. Previous data on concentration of cholesterol from this population show that the distribution of cholesterol has shifted towards the left as the cohort ages.

Is frailty a mortality trait? Frailty measures correlated with low serum cholesterol at the fourth examination, but are unlikely to explain the adverse effects of cholesterol in the low/low group (table 4). Since we have no data correlating frailty measures from the third examination, it seems implausible to posit that there was a group of patients in examination 3 with low cholesterol who also had increased frailty measures over this 20-year period. Nonetheless we cannot rule out this possibility.

The most striking findings were related to changes in cholesterol between examination three (1971–74) and examination four (1991–93). There are few studies that have cholesterol concentrations from the same patients at both middle age and old age. Although our results lend support to previous findings that low serum cholesterol imparts a poor outlook when compared with higher concentrations of cholesterol in elderly people, our data also suggest that those individuals with a low serum cholesterol maintained over a 20-year period will have the worst outlook for all-cause mortality. Iribarren and colleagues[20] suggested that a decline in serum cholesterol might occur over a decade before diagnosis of disease, and such long-term morbidity could be attributable to chronic subclinical infections with hepatitis B, or to chronic respiratory disease resulting in repeated respiratory infections. These disorders could increase concentration of proinflammatory cytokines that cause hypocholesterolaemia.[21] Our present analysis suggests that this hypothesis is implausible and is unlikely to account for the adverse effects of low cholesterol over 20 years.

Is this low/low effect unique to individuals of Japanese ethnic extraction? There is no evidence to support such a contention. Risk factors for atherosclerosis in Japanese are much the same as those for whites.[12] Did these individuals follow a very atypical, very low-fat, low-cholesterol diet, or were they taking powerful lipid-lowering agents? In a separate analysis of the small group of patients who took lipid-lowering drugs, there was no evidence of significant mortality differences and therefore no need to exclude these patients from the analysis. Although there are several prospective clinical trials showing that reduction of serum cholesterol is beneficial, their relevance to elderly people is not straightforward.[1,2] Patients in such studies are self-selected for their interest in participating and there are insufficient numbers aged older than 70 years to allow meaningful conclusions. The absence of comparability studies of laboratory analyses for 20 years might restrict interpretation of variations in cholesterol concentrations. However, this seems unlikely.

Clinically, two issues emerge. First, is there a difference in biological effect from a permanent, untreated, intrinsically low concentration of cholesterol when compared with the effect in those who have a dietary or pharmacologically induced reduction of cholesterol? As far as we are aware, this issue has not been addressed scientifically. Second, in view of our data, and those of others, is there scientific justification for attempts to lower cholesterol to concentrations below 4·65 mmol/L in elderly people? We believe that until more information about these complex relations is available, prudence dictates a more conservative approach in this age group.

Contributors

Irwin Schatz, Kamal Masaki, and J David Curb designed this study. Irwin Schatz and Kamal Masaki wrote the paper and all authors contributed to the final version. Katsuhiko Yano and Beatriz Rodriguez did data analysis, and Randi Chen did biostatistical analyses.

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Adipose tissue fatty acids and risk of myocardial infarction —a case-control study

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Objectives: To study the association between content in adipose tissue of very long-chain n-3 fatty acids, *trans* fatty acids, linoleic acid and α-linolenic acid and risk of a first myocardial infarction.
Design and subjects: A case-control design among 100 patients and 98 population controls both men and postmenopausal women, age 45–75 y. Adipose tissue fatty acids were determined by gas–liquid chromatography. Intake data were obtained through interview using a validated food frequency questionnaire.
Results: Dietary intake and adipose tissue content of the fatty acids studied correlated significantly. Adipose tissue contents of eicosapentaenoic acid (20:5n-3), docosapentaenoic acid (22:5n-3) and docosahexaenoic acid (22:6n-3) were significantly lower while those of *trans* fatty acids, linoleic and α-linolenic acid were significantly higher in patients than in controls. Age and sex adjusted odds ratios (OR) were significantly reduced with increasing quintiles of very long-chain n-3 fatty acids, thus the OR in the fifth compared to the first quintile was 0.23 (95% CI 0.08–0.70). After further adjustment for waist-to-hip ratio, smoking, family history of CHD and content of *trans* fatty acids, the OR in the highest quintile was 0.17 (95% CI 0.04–0.76) and the *P* for trend 0.016. Age and sex adjusted OR was increased in the fifth compared to the first quintile of *trans* fatty acids (OR 2.81, 95% CI 1.16–6.84), linoleic acid (OR 2.10, 95% CI 0.87–5.07) and α-linolenic acid (OR 1.96, 95% CI 0.83–4.61), and *P* for trend was 0.002, 0.005 and 0.020, respectively. The trends remained significant after adjustment for waist-to-hip ratio, smoking, and family history of coronary heart disease. *Trans* fatty acids, linoleic acid and α-linolenic acid in adipose tissue were strongly correlated, indicating a common source, most likely margarine. When each of these fatty acid species were adjusted for the two others the trends were no longer significant.
Conclusion: Intake of very long-chain n-3 fatty acids as reflected in adipose tissue content is inversely associated with risk of myocardial infarction. *Trans* fatty acids, linoleic and α-linolenic acid were intercorrelated and associated with increased risk. It is suggested that the increased risk may be connected to *trans* fatty acids or to some other factor associated with margarine consumption.
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Descriptors: *Trans* fatty acids; linoleic acid; α-linolenic acid; omega-3 fatty acids; coronary heart disease; biological markers
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Introduction

Increased plasma levels of low-density lipoprotein (LDL)-cholesterol is the dominant risk factor for coronary heart disease (CHD) and recently published secondary (The Scandinavian Simvastatin Survival Study Group, 1994) and primary preventive trials (Shepherd *et al*, 1995) have shown that reduction of plasma cholesterol by HMG-CoA reductase inhibitors is associated with a significant reduction in mortality from CHD. LDL-cholesterol is strictly correlated to total cholesterol and at the population level total serum cholesterol is primarily determined by the content of saturated fatty acids in the diet (Keys, 1980). In the 25 y follow-up of the Seven Countries Study, dietary intake of saturated (and *trans*) fatty acids was found to be associated with increased CHD mortality (Kromhout *et al*, 1995a). On the other hand it has become clear that differences in mean cholesterol levels among populations cannot totally explain the differences in CHD mortality. In the 25 y follow up of the Seven Countries Study a three fold difference in CHD mortality was found between different populations at the same cholesterol level after adjusting for age, cigarette smoking and systolic blood pressure (Verschuren *et al*, 1995). It thus appears that the expression of risk may be modulated by a number of other dietary factors than saturated fatty acids, some being potentiating and others protective. A number of factors have been discussed in this context, eg fruit and vegetables, fiber,

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Contributors: JI Pedersen initiated and coordinated the study and was responsible for finalizing the paper. J Ringstad was responsible for the clinical part of the study and wrote, together with JIP, the first draft of the paper. K Almendingen was responsible for detailing of the protocol, for the dietary assessment and took an active part in the inclusion process. TS Haugen was responsible for the analytical procedures and I Stensvold for the statistical treatment. DS Thelle was responsible for the epidemiological aspects of the study and participated in designing the study and in the analysis of the data. All investigators contributed to the writing of the manuscript.
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antioxidants, very long-chain n-3 (omega-3) fatty acids, *trans* fatty acids, alcohol and coffee. In particular, in high risk populations such factors may be decisive in determining those who get a myocardial infarction and those who do not. Such factors may influence risk through mechanisms such as aggregation of platelets, fibrinolysis, LDL oxidation and cardiac arrhythmia. Among fatty acids, α-linolenic acid and very-long-chain n-3 fatty acids in particular have been proposed to favorably influence risk through mechanisms other than through effects on serum lipids (de Deckere *et al*, 1998).

The measurement error of methods used to obtain information on dietary intake is generally too large to give precise information on the risk associated with particular nutrients. Use of biomarkers with long half-life may reduce the differential information bias, which is often a problem in case–control studies. For this reason biological markers for intake are preferable to dietary measurements in such studies. The long-term intake of fatty acids not formed in the body is reflected in the content of these acids in adipose tissue (Beynen *et al*, 1980). For assessing risk associated with intake of such fatty acids, the composition of adipose tissue fatty acids may thus be used. In this case–control study we have correlated the risk of having a first myocardial infarction in a moderately high-risk population to the content of very long-chain n-3 fatty acids, trans fatty acids, linoleic acid, and α-linolenic acid in subcutaneous fat.

Methods

Design and subjects

A total of 112 cases with a first acute myocardial infarction and 107 controls were recruited during 1996 according to the same eligibility criteria. Eligible subjects were native men and postmenopausal women, aged between 45 and 75, without previously reported myocardial infarction or other serious disease (cancer, diabetes, alcohol or drug abuse, major psychiatric disease) which might affect their dietary patterns. Subjects with weight changes more than 5 kg during the last year, or using hypolipemic drugs, were excluded from the study. Informed consent was obtained in accordance with the ethical standards set by the committees on human experimentation. Of those asked to participate 5% refused.

Incident cases were diagnosed (typical history, ECG and enzyme changes) with a first myocardial infarction (MI; ICD 9-code 410), and admitted within 24 h of manifesting symptoms to the coronary care units of Østfold Central Hospital, Fredrikstad/Sarpsborg and Ullevål Hospital, Oslo. Two of the patients had angina prior to the infarction, but no dietary changes had been made.

Controls were recruited from the population in the catchment areas. They were selected in order to correspond to cases in terms of sex, age (5 y interval) and geographical location. As it was thought that low response rates from population-based samples would compromise the internal validity of the study, controls were recruited among friends and acquaintances of the cases, state and municipal employees and people attending recreational centers for elderly and retired. Thus, controls were drawn to represent the exposure levels in the populations giving rise to cases. An effort was made to recruit the control subjects within 2 months of the MI episode of the corresponding cases in order to avoid seasonal variations in the dietary pattern.

Samples

Subcutaneous adipose tissue was taken from the buttock by needle aspiration as described by Beynen and Katan (1985). In cases the adipose samples were taken within 4 days of admission to hospital. The samples were immediately frozen on dry ice and stored at −70°C until analyzed. Needle biopsies were obtained from 112 cases and 107 controls. No fat aspirate or a sample too small for analysis was obtained from 12 cases and nine controls, leaving a total of 100 cases and 98 controls from whom satisfactory results for fatty acid composition of adipose tissue were obtained. Fasting blood samples were drawn and stored at −70°C until analyzed in a central laboratory. For most cases samples were obtained within 24 h of onset of symptoms, although in some of the cases admitted to the Ullevål Hospital the fasting blood samples were drawn within 3 days of onset of symptoms.

Anthropometric measures (weight, height and waist and hip circumferences) were taken directly from all subjects after a detailed interview on cardiovascular disease risk factors and dietary habits.

Fatty acids in adipose tissue biopsies

Fatty acids were determined by gas–liquid chromatography. Extraction and direct methanolysis were modified from Viga and Grahl-Nielsen (1990). The adipose tissue (approximately 20 mg) was transferred to thick-walled glass tubes. Anhydrous 3 M HCL in methanol, 0.7 ml, was added and the tubes were flushed with nitrogen to remove oxygen. The tubes were securely closed with screw caps and incubated at 100°C for 2.5 h. After cooling 1 ml 6% $NaHCO_3$ was added and the methyl esters were extracted with hexane. A small amount of Bondesil-NH_2 (Varian AB, Solna, Sweden) was added to remove trace amounts of unesterified free fatty acids and unhydrolyzed mono- and diglycerides. The samples were vortexed and the solvent transferred to another tube, taken to dryness under nitrogen and redissolved in 2 ml of hexane. One microliter was injected into a Shimadzu GC-17A gas chromatograph equipped with an autoinjector AOC 17 and a flame ionization detector (Shimadzu Corp. Kyoto, Japan). A fused silica capillary column length 100 m, 0.25 mm i.d. was used (SP™–2560, Supelco Inc., Bellefonte, PA, USA). The temperature program was as follows: the starting temperature of 130°C was held for 3 min, then raised at 5°C/min up to 180°C and kept at this temperature for 30 min and then raised at 6°C/min up to 193°C, where it was held for 85 min. Helium was used as the carrier gas with flow 1.3 ml/min. Column pressure was 250 kPa and splitless injection was used. Identification of the different peaks was done by comparing the retention times to commercial standards and the percentage distribution was calculated using the Class GC-10 software (Shimadzu Corp., Kyoto, Japan). The intra-assay coefficient of variation was found to vary between 2.1 and 4.5% for different fatty acids.

Dietary assessment

The habitual diet of the participants was assessed using a standardized and validated food frequency questionnaire developed at the Institute for Nutrition Research, University of Oslo (Nes *et al*, 1992). The questionnaires were filled in during a 90 min interview with the participants. Total energy and fatty acid composition of the diet were calculated using the official Norwegian food composition

able. For calculation of trans fatty acids, results from the recent *TRANS*FAIR study were made available (van Poppel on behalf of the *TRANS*FAIR Study Group, 1998). In this collaborative European study the fatty acid compositions of 95 different Norwegian food items were analyzed with special emphasis on the content of different *trans* fatty acid isomers.

Power calculation

It was estimated that a minimum number of 60 participants in each group would be necessary to show differences in *trans* fatty acids between the groups similar to those in the Norwegian part of the EURAMIC study (Aro *et al*, 1995) at a $P < 0.05$ level and power at 80%. It was decided that 100 patients and a corresponding number of control subjects should be recruited in order to secure a sufficient number of successful fat biopsies as well as dietary data.

Statistical methods

Summary statistics for all the measured variables were calculated for cases and controls. Differences in mean values or proportions between case and control groups were tested by Student's *t*-test or the chi-square statistics. Linear trend across quintiles among the control subjects was estimated by weighted linear regression. The mean values were introduced as the dependent variable and the quintiles as independent variables with values of 1–5.

Odds ratios (OR, 95% confidence intervals, and two-tailed *P*-values) of first myocardial infarction were estimated for the quintiles of the chosen fatty acid relative to the lowest quintile level by unconditional logistic regression. Data analysis was performed using the statistical package SPSS 7.0 (SPSS Inc., Chicago, IL). The distribution of total *trans* fatty acids, linoleic acid, α-linolenic acid and very long-chain n-3 fatty acids among control subjects was used to compute cut-off points for quintiles of exposure to these categories of fatty acids. The model included the following risk factors: age, sex, waist-to-hip ratio, current smoking and family history of coronary heart disease. The fatty acid was entered as a continuous variable along with other confounders, when testing for trend. Pearson's correlation coefficients were used to evaluate correlations between intake of fatty acids and content in adipose tissue. *P*-values ≤ 0.05 were considered significant.

Results

The prevalence of risk factors in MI cases and control subjects is shown in Table 1. Waist-to-hip ratio, total cholesterol, HDL cholesterol, triglycerides, smoking, family history of CHD and length of education differed significantly between cases and controls. The lower serum total cholesterol in cases is explained by the lowering of serum lipids after an acute MI (Ahnve *et al*, 1989; Aro *et al*, 1995). The much larger difference between cases and controls in HDL cholesterol (31%) than in total cholesterol (8%), however, probably reflects a significantly lower HDL cholesterol in the cases. The larger waist-to-hip ratio in cases was significant both in men and women.

Adipose tissue fatty acids in patients and controls

With the methods used, about 40 different fatty acids could be identified in the adipose tissue fat extracts (Table 2). The identified fatty acids made up about 99% of the total area of

Table 1 Comparison of cardiovascular risk factors between cases and controls

	Cases (n = 100)[a]		*Controls (n = 98)*[a]		
Risk factor	*Mean (%)*	*s.d.*	*Mean (%)*	*s.d.*	*P-Value*
Age (y)[b]	62.4	8.1	62.4	7.7	0.98
Women included (%)	28.0		27.6		0.94
Body mass index (kg/m^2)	26.5	3.8	26.4	3.6	0.88
Waist-to-hip ratio	0.96	0.08	0.91	0.08	< 0.001
Total cholesterol (mmol/l)	5.7	1.0	6.2	1.2	0.004
HDL-cholesterol (mmol/l)	0.9	0.3	1.3	0.4	< 0.001
Triglycerides (mmol/l)	1.8	0.7	1.4	0.6	< 0.001
Smoking (percentage current smokers)	59.6		20.4		< 0.001
History of antihypertensive drugs (%)	23.0		19.4		0.60
Family history of CHD[c] (%)	62.0		42.9		0.01
Length of education (y)	10.2	3.3	13.4	3.9	< 0.001
Married or cohabitant (%)	72.7		74.5		

[a]For some of the variables the number is smaller (for blood lipids: 90 cases and 93 controls).
[b]Age range: men—cases, 46–75, controls, 48–75; women—cases, 50–75, controls, 51–75.
[c]Coronary heart disease in first degree relatives.

Table 2 Fatty acid composition of adipose tissue in cases and controls (g/100 g, mean and s.d.)

	Cases (n = 100)		*Controls (n = 98)*		*t-test,*
Fatty acids	*Mean*	*s.d.*	*Mean*	*s.d.*	*P-value*
C12:0	0.41	0.22	0.46	0.21	0.08
C14:0	3.47	0.87	3.72	0.85	0.04
C14:1*t*	0.10	0.03	0.12	0.03	0.001
C14:1*c*	0.51	0.20	0.59	0.18	0.003
C15:0	0.37	0.09	0.41	0.09	0.01
C16:0	21.49	2.15	21.44	2.12	0.88
Sum C16:1*t*	0.67	0.22	0.64	0.16	0.30
Sum C16:1*c*	7.63	1.84	8.10	1.56	0.05
C17:0	0.26	0.05	0.27	0.05	0.05
Sum C17:1*t*	0.06	0.02	0.06	0.02	0.59
C17:1*c*	0.35	0.06	0.37	0.05	0.004
C18:0	3.25	0.82	3.17	0.76	0.50
Sum C18:1*t*	3.00	1.06	2.55	0.71	< 0.001
Sum C18:1*c*	40.49	2.44	41.01	2.03	0.11
C19:0	0.10	0.05	0.09	0.04	0.44
Sum C18:2*t*/*c*	0.68	0.19	0.68	0.18	0.96
C18:2n-6*c*,*c*	12.23	2.78	11.23	2.14	0.01
C20:0	0.16	0.07	0.14	0.05	0.03
C18:3n-6	0.03	0.02	0.03	0.02	0.79
Sum C20:1*t*	0.26	0.14	0.22	0.10	0.04
C20:1n-11*c*	0.32	0.12	0.34	0.11	0.13
C20:1n-9*c*	0.67	0.18	0.70	0.17	0.32
C18:3n-3	0.76	0.18	0.71	0.15	0.02
C20:1n-5	0.10	0.05	0.11	0.04	0.32
C18:4n-3	0.28	0.08	0.27	0.07	0.74
C20:2	0.14	0.04	0.13	0.03	0.02
C22:0	0.02	0.02	0.02	0.02	0.06
C20:3n-6	0.13	0.06	0.13	0.05	0.39
Sum C22:1*t*	0.01	0.02	0.01	0.01	0.01
C22:1n-11	0.07	0.07	0.07	0.05	0.97
C20:3n-3 + C22:1n-9	0.05	0.05	0.05	0.06	1.00
C20:4n-6	0.26	0.18	0.25	0.19	0.79
C20:4n-3	0.05	0.03	0.06	0.03	0.10
C20:5n-3	0.09	0.06	0.12	0.08	0.001
C21:5n-3?	0.13	0.12	0.10	0.03	0.04
C22:4n-6	0.04	0.04	0.04	0.03	0.84
C22:5n-3	0.19	0.09	0.23	0.10	0.001
C22:6n-3	0.24	0.15	0.32	0.20	0.002
Sum	99.08	0.25	98.98	0.48	

c = cis; *t* = *trans*; *t*/*c* = mixture of *trans*, *trans*; *trans*, *cis*; and *cis*, *trans* isomers. Sum denotes sum of all isomers within the respective groups.

the chromatograms. Several fatty acids differed in percentage content (g/100 g) between cases and controls. Only those fatty acids that are of exogenous, or almost exclusively of exogenous origin, and where a significant correlation was found between dietary intake and adipose tissue content will be considered. A highly significant correlation ($P < 0.01$) between dietary intake and adipose tissue content was found for linoleic acid ($r = 0.492$), α-linolenic acid ($r = 0.437$), very long-chain n-3 fatty acids ($r = 0.573$) and *trans* fatty acids ($r = 0.295$). Linoleic acid, α-linolenic acid, 18:1 *trans* fatty acids, 20:1 *trans* fatty acids as well as total *trans* fatty acids were all significantly higher in adipose tissue of the MI cases than of the controls. Eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) were all significantly lower in MI cases than in the controls. *Trans* fatty acids, linoleic acid and α-linolenic acid were positively correlated with each other (Table 3) but not with total very long-chain n-3 fatty acids. Total very long-chain n-3 fatty acids were slightly inversely correlated with 18:1 trans fatty acids (Table 3). None of the fatty acids were correlated with total energy intake.

Risk of MI and adipose tissue fatty acids

The OR adjusted for sex and age was significantly reduced with increasing quintiles of very long-chain n-3 fatty acids (P for trend 0.001; Table 4). The trend remained significant after further adjustment for waist-to-hip ratio, smoking and family history for CHD (P for trend 0.01. After adjusting for *trans* fatty acids the OR remained significantly below one in the highest quintile (OR = 0.17, 95% CI = 0.04–0.76, P for trend = 0.02). Even if high density lipoprotein (HDL)-cholesterol was not related to adipose tissue fatty acids it was considered a potential confounder. After addition of HDL-cholesterol to the model the OR was significantly reduced below one also in the fourth quartile (OR = 0.25, 95% CI = 0.02–0.43). Education was correlated to smoking and control for length of education may therefore result in overadjustment. However, after addition of length of education to the model, the OR in the highest quintile remained significantly below one (OR = 0.14, 95% CI = 0.03–0.67).

The odds ratio adjusted for sex and age was significantly increased in the fifth quintile of *trans* fatty acid content (Table 4). Further adjustment for waist-to-hip ratio, smoking and family history of CHD widened the confidence interval, but the trend was still significant (P for trend = 0.03).

Except for the lower odds ratios in the second and third quintiles, similar trends as for *trans* fatty acids were seen also for linoleic and α-linolenic acid (Table 4). Since the adipose tissue content of these three fatty acid species was highly intercorrelated, the odds ratios for each fatty acid were further adjusted for the two others. After adjustment for linoleic acid or α-linolenic acid the trend in increased risk for increasing content of *trans* fatty acids was no longer significant. After adjustment for *trans* fatty acids the trend for increased risk in the fifth quintile of linoleic acid was no longer significant (P for trend = 0.08) and the OR drops below one after adjustment for α-linolenic acid (P for trend = 0.67). When the OR in quintiles of α-linolenic acid are adjusted for *trans* fatty acids or for

Table 3 Age- and sex-adjusted levels of different fatty acids by quintiles among controls

Fatty acids	*Q1*	*Q2*	*Q3*	*Q4*	*Q5*	*Trend P-value*
			Quintiles of total VLCn-3 fatty acids			
Total VLCn-3	0.28	0.52	0.68	0.85	1.32	—
Linoleic (18:2n-6)	11.60	11.68	11.00	10.63	11.26	0.25
Total *trans*	4.63	4.08	4.13	3.72	3.96	0.10
18:1*trans* isomers	2.88	2.65	2.51	2.28	2.44	0.05
Oleic (18:1 *cis*)	41.17	41.04	40.63	41.53	40.69	0.77
α-linolenic (18:3n-3)	0.70	0.74	0.71	0.67	0.73	0.90
Total energy intake (kJ)	11165	8426	9361	10027	10022	0.88
			Quintiles of total trans fatty acids			
Total *trans*	2.87	3.55	4.08	4.56	5.45	—
18:1 *trans* isomers	1.71	2.17	2.51	2.82	3.56	0.002
Oleic (18:1 *cis*)	42.60	41.47	40.87	40.05	40.10	0.01
Linoleic (18:2n-6)	9.88	10.86	11.32	12.12	11.94	0.02
α-linolenic (18:3n-3)	0.61	0.65	0.73	0.77	0.78	0.01
VLCn-3	0.66	0.87	0.77	0.77	0.57	0.52
Total energy intake (kJ)	9125	9737	9936	10192	9904	0.11
			Quintiles of linoleic acid			
Linoleic (18:2n-6)	8.54	9.91	11.10	12.24	14.38	—
Total *trans*	3.81	3.79	4.11	4.09	4.74	0.05
18:1*trans* isomers	2.33	2.22	2.61	2.57	3.05	0.05
Oleic (18:1 *cis*)	41.76	41.24	40.71	40.99	40.37	0.04
α-linolenic (18:3n-3)	0.55	0.63	0.70	0.77	0.90	0.001
VLCn-3	0.75	0.65	0.76	0.83	0.66	0.98
Total energy intake (kJ)	10131	9498	8371	10711	10250	0.68
			Quintiles of α-linolenic acid			
α-linolenic (18:3n-3)	0.52	0.62	0.68	0.78	0.94	—
Linoleic (18:2n-6)	9.08	10.16	11.03	11.83	14.10	0.01
Total *trans*	3.82	3.87	3.86	4.13	4.86	0.07
18:1*trans* isomers	2.30	2.34	2.37	2.60	3.15	0.05
Oleic (18:1 *cis*)	41.86	41.60	41.27	40.62	39.69	0.01
VLCn-3	0.62	0.72	0.76	0.84	0.71	0.29
Total energy intake (kJ)	8918	9910	9589	10547	9921	0.19

VLCn-3 (very long-chain n-3 fatty acids) = sum of 20:4n-3, 20:5n-3, 22:5n-3 and 22:6n-3.
Total *trans* = sum of all identified *trans* fatty acids.

Table 4 Odds ratio of first myocardial infarction in quintiles of different fatty acids

	Q1	*Q2*	*Q3*	*Q4*	*Q5*	*P for trend*
			Quintiles of very long-chain n-3 fatty acids			
Cut-off values (%)[a]	−0.43	> 0.43	> 0.61	> 0.73	> 0.99	
No cases/controls	26/19	30/20	24/20	13/20	7/19	
OR adjusted[b]	1.00	1.02	0.80	0.43	0.23	0.001
(95% CI)		(0.44–2.36)	(0.34–1.89)	(0.17–1.12)	(0.08–0.70)	
OR adjusted[c]	1.00	1.83	1.37	0.57	0.13	0.01
(95% CI)		(0.66–5.09)	(0.46–4.11)	(0.18–1.83)	(0.03–0.56)	
OR adjusted[d]	1.00	1.97	1.72	0.73	0.18	0.02
(95% CI)		(0.70–5.56)	(0.55–5.38)	(0.22–2.45)	(0.04–0.80)	
			Quintiles of total trans fatty acids			
Cut-off values (%)[a]	−3.35	> 3.35	> 3.81	> 4.37	> 4.75	
No cases/controls	15/19	16/20	17/20	11/20	41/19	
OR adjusted[b]	1.00	1.02	1.06	0.70	2.81	0.002
(95% CI)		(0.39–2.62)	(0.41–2.75)	(0.25–1.95)	(1.16–6.84)	
OR adjusted[c]	1.00	1.19	1.15	0.80	2.25	0.03
(95% CI)		(0.38–3.75)	(0.36–3.73)	(0.22–2.82)	(0.78–6.48)	
OR adjusted[e]	1.00	1.08	0.92	0.46	1.23	0.32
(95% CI)		(0.33–3.47)	(0.27–3.07)	(0.12–1.81)	(0.37–4.09)	
OR adjusted[f]	1.00	1.04	1.07	0.54	1.49	0.19
(95% CI)		(0.32–3.37)	(0.33–3.48)	(0.14–2.07)	(0.47–4.69)	
			Quintiles of linoleic acid			
Cut-off values (%)[a]	−9.40	> 9.40	> 10.51	> 11.63	> 13.56	
No cases/controls	17/19	10/20	16/20	23/20	34/19	
OR adjusted[b]	1.00	0.57	0.90	1.34	2.10	0.01
(95% CI)		(0.21–1.56)	(0.36–2.28)	(0.53–4.20)	(0.87–5.07)	
OR adjusted[c]	1.00	0.57	0.90	1.39	2.13	0.02
(95% CI)		(0.17–1.88)	(0.29–2.81)	(0.46–4.23)	(0.74–6.13)	
OR adjusted[d]	1.00	0.52	0.75	1.19	1.54	0.08
(95% CI)		(0.16–1.71)	(0.24–2.35)	(0.38–3.69)	(0.50–4.79)	
OR adjusted[e]	1.00	0.39	0.53	0.64	0.60	0.67
(95% CI)		(0.11–1.34)	(0.15–1.83)	(0.15–2.63)	(0.11–3.38)	
			Quintiles of α-linolenic acid			
Cut-off values (%)[a]	−0.59	> 0.59	> 0.65	> 0.73	> 0.83	
No cases/controls	21/19	8/20	14/20	19/20	38/19	
OR adjusted[b]	1.00	0.36	0.64	0.88	1.96	0.02
(95% CI)		(0.13–1.02)	(0.25–1.62)	(0.36–2.15)	(0.83–4.61)	
OR adjusted[c]	1.00	0.44	0.91	1.05	2.95	0.01
(95% CI)		(0.13–1.50)	(0.29–2.89)	(0.35–3.22)	(1.00–8.71)	
OR adjusted[d]	1.00	0.45	0.84	0.89	2.32	0.08
(95% CI)		(0.13–1.51)	(0.27–2.65)	(0.27–2.91)	(0.70–7.65)	
OR adjusted[f]	1.00	0.43	0.79	0.91	2.18	0.32
(95% CI)		(0.12–1.52)	(0.22–2.87)	(0.24–3.54)	(0.36–13.06)	

[a]Cut-off values for quintiles in the control group.
[b]Adjusted for sex and age.
[c]Further adjusted for waist-to-hip ratio (continuous variable), smoking (yes/no) and family history of CHD (yes/no).
[d]Adjusted for sex, age, waist-to-hip ratio, smoking, family CHD-history and total *trans* fatty acids.
[e]Adjustedfor sex, age, waist–hip ratio, smoking, family CHD-history and α-linolenic acid.
[f]Adjusted for sex, age, waist–hip ratio, smoking, family CHD-history and α-linoleic acid.
Very long-chain n-3 fatty acids and total *trans* fatty acids as given in Table 3.

linoleic acid the OR of the fifth quintile are slightly reduced and the trends are no longer significant (P for trend = 0.08 and 0.32, respectively). In all three cases, a model that included the two other fatty acids at the same time did not provide any extra information (not shown).

Discussion

The myocardial infarction patients in this retrospective study had higher levels of *trans* fatty acids, α-linolenic and linoleic acid, and lower levels of very long-chain n-3 fatty acids in their subcutaneous fat tissue than healthy control subjects of similar age and sex. The subcutaneous fatty acid composition reflects the long term dietary intake over periods of years prior to the sampling (Katan *et al*, 1997). It is therefore most unlikely that the reported associations should be due to changes in dietary habits due to the cardiac event, especially as all the patients except two were completely unaware of having coronary heart disease prior to the episode which led to inclusion in the study. The dietary habits recorded among patients and controls showed a fat intake pattern which was highly compatible with the fatty acid composition of the subcutaneous fat tissue, and highly significant correlations between dietary linoleic, α-linolenic, *trans* and very long-chain n-3 fatty acids and the content in adipose tissue were found. Furthermore, the control subjects had a higher intake of very long-chain n-3 fatty acids (mainly as fish and cod liver oil) as well a lower intake of margarine high in *trans* fatty acids, linoleic and α-linolenic acid. The strong intercorrelations between these last three fatty acids (Table 3) are a clear indication of a common source, most probably margarine.

The control subjects were chosen among friends and relatives of the patients, as well as other available persons from the same area, but had only to be free of known diseases and be of the same age and sex. They had on average 3 y more education than the patients, and only 20%

smokers. A previous study of a random sample of the Norwegian population showed that in an age group comparable to ours 29% of the men were smokers and the mean length of education was 10.4 y (Johansson *et al*, 1997). Our control subjects thus had a slightly lower number of smokers and more education than expected. Smoking and waist-to-hip ratio were adjusted for, but not other health related factors associated with education. To control for education together with smoking may be considered unreasonable as the association between smoking and education is quite strong. Adjustment for length of education in addition to smoking did not alter the odds ratio in the highest quintile of VLC n-3 fatty acids but this does not completely exclude the possibility that part of the associations found may be related to other factors associated with education. The difference in educational length between the cases and the controls in our study reflects the striking social gradient in risk for coronary heart disease which has been observed in Norway as well as other parts of Europe (Jenum *et al*, 1998; Lynch *et al*, 1996). No morbidity data are available in Norway, but a recent analysis of the use of health services, risk factor distribution and mortality rates suggests that there is a considerably lower incidence of coronary heart disease in the more educated population segments (Jenum *et al*, 1998). Thus, any sampling of patients with myocardial infarction is likely to result in a population with less education than a healthy control group. There is also a strong social gradient with regard to food habits corresponding to what was observed in the present study (Johansson *et al*, 1999).

The most marked finding in this study was the significant reduction in odds ratio associated with a high content of VLC n-3 fatty acids in adipose tissue. Epidemiological studies on the associations between intake of VLC n-3 fatty acids or fish consumption have given inconsistent results. Several prospective observational studies (Kromhout *et al*, 1985, 1995b; Daviglus *et al*, 1997) and a randomized intervention study (Burr *et al*, 1989) have shown that low intake of VLCn-3 fatty acids or fish compared to no intake is associated with reduced mortality of CHD. Several other prospective studies have not shown any protective effects of fish consumption (Ascherio *et al*, 1995; Morris *et al*, 1995; Pietinen *et al*, 1997). The lack of consistency may reflect the large measurement error inherent in dietary studies. Somewhat more consistent results have been obtained when the content of VLCn-3 fatty acids in blood or tissue samples has been used as markers for intake of these fatty acids. Eicosapentaenoic acid in serum phospholipids was found to be significantly lower in male subjects who sustained a myocardial infarction compared to controls (Miettinen *et al*, 1982). Wood *et al* (1987) found lower content of docosahexaenoic acid in adipose tissue of MI patients than in that of controls and also an inverse relation between platelet membrane eicosapentaenoic acid and risk of angina pectoris. In more recent case–control studies serum phospholipid VLCn-3 fatty acids were inversely related to risk of myocardial infarction (Simon *et al*, 1995) and red cell membrane VLCn-3 fatty acids inversely related to risk of primary cardiac arrest (Siscovick *et al*, 1995). In an autopsy study an inverse relation was found between degree of coronary atherosclerosis and content of docosahexaenoic acid in adipose tissue (Seidelin *et al*, 1992). Only in one prospective study has no association been found between biological markers for intake of VLCn-3 fatty acids (content in serum cholesterol esters and in phospholipids) and incidence of myocardial infarction (Guallar *et al*, 1995). In the recent European multicenter case–control (EURAMIC) study, also no overall association was found between adipose tissue content of DHA and risk of myocardial infarction (Guallar *et al*, 1999). In the Norwegian population of that study, however, an inverse association was found. The main difference between that study and the one reported here is the higher level of VLCn-3 fatty acids in the present study. Only DHA (22:6n-3) was used as indicator of intake in the EURAMIC study. In our study DHA made up about half the total amount of very-long-chain n-3 fatty acids. When this is taken into consideration it is seen from the cut-off values given that the highest quintile in the EURAMIC study corresponds closely to the third quintile in this study.

Very high correlations between intake of VLCn-3 fatty acids and the content in adipose tissue have been found (Tjønneland *et al*, 1993). Adipose tissue VLCn-3 fatty acids probably give a better estimate of the long-term exposition to these dietary components than a single food frequency questionnaire, which is the method generally employed to obtain information on food intake in epidemiological studies. Errors due to recall bias, underreporting, recent changes in dietary habits etc may thus be minimized. On this basis we tend to conclude that, in the relatively high-risk population of this study (cf mean serum cholesterol in controls, Table 1), intake of VLC-n-3 fatty acids reduces the risk of myocardial infarction. There are many plausible mechanisms for a cardioprotective effect of VLCn-3 fatty acids: these fatty acids may reduce serum triglycerides (Harris, 1997), reduce blood pressure (Bønaa *et al*, 1990), reduce platelet aggregability (Von Schacky & Weber, 1985) and may also have an antiarrythmic effect (Siscovick *et al*, 1995; Leaf & Kang, 1996).

The upper quintile of *trans* fatty acids was associated with increased risk of MI. This is in line with three prospective studies (Pietinen *et al*, 1997; Willett *et al*, 1993; Hu *et al*, 1997) and one case–control study (Ascherio *et al*, 1994) where a positive association between intake of *trans* fatty acids and risk of myocardial infarction has been found. In studies where the amount of *trans* fatty acids in adipose tissue or serum lipid fractions have been used as markers of intake, the results are inconsistent. A positive association between amount of *trans* fatty acids and coronary heart disease has been found in some studies (Thomas *et al*, 1983; Siguel & Lerman, 1993) but not in others (Roberts *et al*, 1995; van de Vijver *et al*, 1996). In the large European multicenter (EURAMIC) study no overall association was found between adipose tissue *trans* fatty acid content and risk of MI (Aro *et al*, 1995). It should be noticed, however, that both in the Finnish and the Norwegian study populations significant positive associations were found with OR of 5 in the highest quartile compared with the lowest. Also, after exclusion of the cases (24% of the total number) and controls from the two Spanish centers, all in the first quartile of the distribution, the odds ratio for MI in the third quartile was significantly higher than in the lowest. Even if the results of this study suggest that the intake of *trans* fatty acids may not be a dominating risk factor, it cannot be excluded that it may be a contributing factor at a high level of intake. It is now well documented that *trans* fatty acids increase LDL cholesterol, decrease HDL cholesterol and increase lipoprotein (a) (Lichtenstein, 1998), all factors associated with increased risk of coronary heart disease.

Increased odds ratio was also found in the highest quintile of intake of both linoleic and α-linolenic acid. The amounts of these three groups of fatty acids were significantly correlated (Table 3), indicating a main common source—in the dietary survey found to be margarine (unpublished). It is thus possible that a confounding factor related to margarine intake may underlie the associations with *trans* fatty acids as well as with linoleic and α-linolenic acid. Polyunsaturated fatty acids due to their cholesterol-lowering effect are generally considered to be beneficial in relation to CHD. This view is supported by epidemiological data (Hu *et al*, 1997) and at the population level the decrease in CHD mortality in North America, Australia and Europe has occurred concomitant with an increased consumption of vegetable oils at the expense of animal fat. It cannot be excluded, however, that very high intakes of these two polyunsaturated fatty acids may be associated with increased risk, as has been found in some studies (Blankenhorn *et al*, 1990; Hodgson *et al*, 1993). Even if they lower serum cholesterol, they are easily oxidized and may thus promote oxidative modification of low density lipoproteins implicated in the genesis of atherosclerosis (Witztum & Steinberg, 1991). Results from the large health professionals follow-up study (Ascherio *et al*, 1996) and from the Nurses Health Study (Hu *et al*, 1997) indicate that intake of α-linolenic acid is inversely related to risk of myocardial infarction. The median intakes of α-linolenic acid in the fifth quintile of these studies were 1.5 and 1.36 g/day, respectively—far below a median intake of 1.7 g/day for the entire patient group in our study (unpublished). In one secondary intervention study a Mediterranean-like α-linolenic acid-rich diet was found to be efficient in preventing recurrent myocardial infarction (de Lorgeril *et al*, 1994). Again it should be noted that the intake of α-linolenic acid in the Norwegian population is probably higher than in the Mediterranean region due to the high consumption of soybean oil-based margarine. This is reflected in the high proportion of α-linolenic acid in adipose tissue reported here. In the EURAMIC study the content of α-linolenic acid in adipose tissue of the Norwegian subjects was higher than in any other of the European centers, eg three times higher than in the subjects of the two Spanish centers (Bakker *et al*, 1997). It was not reported if the inreased risk associated with *trans* fatty acids in the Finnish and Norwegian study populations in the EURAMIC study (Aro *et al*, 1995) remained after adjustment for linoleic or α-linolenic acid. It can be speculated that, at such high levels, any beneficial effects of α-linolenic acid (reduction in serum cholesterol, precursor to long-chain n-3 fatty acids) may be outweighted by some unfavorable effects, eg its proneness to oxidation.

The possibility that the intake of *trans* fatty acids from partially hydrogenated fish oils might carry an increased risk of coronary heart disease was raised in the 1970s, when it was observed in an English autopsy study that patients who died from cardiovascular disease were found to have a higher level of C16:1*trans* fatty acids (and also C18:1*trans* fatty acids) in their fat tissue than patients dying from other causes (Thomas, 1992). C16:1*trans* is typical of partially hydrogenated fish oils in addition to very long chain *trans* fatty acids like C20:1*trans* and C22:1*trans*. These very long-chain trans fatty acids were not determined in the EURAMIC study and it cannot be excluded that the calculated relative risks associated with *trans* fatty acids might have been underestimated, in particular in the Norwegian and Dutch populations where partially hydrogenated fish oil has been an important ingredient in margarine production. In a previous study we have shown that partially hydrogenated fish oil has at least as unfavorable an effect on blood lipids as butterfat, raising LDL cholesterol and Lp(a) and reducing HDL cholesterol (Almendingen *et al*, 1995). In this study no difference was found in content of C16:1*trans* between cases and controls. C20:1*trans* and C22:1*trans* were higher in cases than in controls but the contents were very low. The lower than expected content of these *trans* fatty acids may be related to the fact that margarine industry in recent years has reduced the use of partially hydrogenated fish oil in margarine. Whether the calculations of odds ratios were based on only the dominating C18:1*trans* isomers or sum of *trans* fatty acids, the results were the same. Thus, the relatively small amount of very long-chain *trans* fatty acids does not appear to add appreciably to the risk.

In conclusion, the results from this case–control study are consistent with a decreased risk of myocardial infarction with increasing content of very long-chain n-3 fatty acids in adipose tissue. The content of *trans* fatty acids, linoleic and α-linolenic acid was associated with increased risk. These three fatty acid species are intercorrelated because of a common main source, probably margarine. The increased risk may be connected to *trans* fatty acids, although it cannot be totally excluded that also very high intake of linoleic and/or α-linolenic acid may be related to increased risk, nor can some other confounding factor associated with margarine consumption be excluded.

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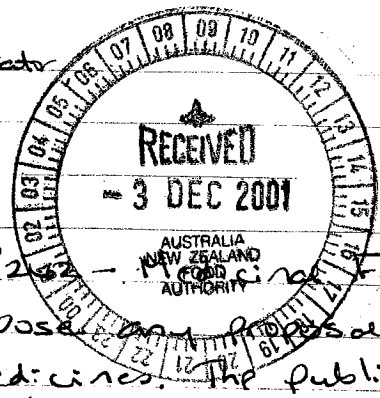

Proposal P252 - Novel Foods.

I oppose any proposal which will permit the promotion of foods as medicines. The public is already being exploited by untrue or half true published claims for benefits of various dietary manipulations and any relaxation of current regulations will make a bad situation worse.

For example, many promotions promise that polyunsaturated oil use will reduce the risk of heart disease because of cholesterol lowering properties. The enclosed copy from The B.M.J. shows that this claim is untrue but "truth can go out the window" if some see a commercial advantage is telling only half the truth. Also, claims for health benefits from increased soy consumption are often exaggerated or just plain wrong. Only if a food is shown to be medically beneficial in the long term by way of tested dietary hypothesis/clinical trials, and only if the patient is monitored by a health professional should foods be used or prescribed as medicines.

Yours sincerely,

ENTERED IN DATABASE
already entered on 10/12

Valerie James

Enclosed (1.) Rose et al. "Corn oil in the Treatment of Ischemic Heart Disease" The British Medical Journal, 12 June 1965 pp. 1531 - 1533.

(2.) Lee, "Don't go Overboard with the Soy Foods" Medical Letter, Nov. 2001.

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Corn Oil in Treatment of Ischaemic Heart Disease

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Brit. med. J., 1965, **1**, 1531–1533

It has been shown that ischaemic heart disease tends to be associated with elevated levels of serum cholesterol, both in populations (Keys *et al.*, 1958) and in individuals (Kannel *et al.*, 1961). There is also evidence that population levels of serum cholesterol are sometimes correlated with (among other characteristics) a high intake of animal fats and a relatively low intake of unsaturated vegetable oils (Bronte-Stewart *et al.*, 1955). Attempts to demonstrate such a correlation in individuals between customary diet and serum-cholesterol level have been unsuccessful (Morris *et al.*, 1963); but the level may be altered by changes in fat intake (Ahrens *et al.*, 1955; Gordon and Brock, 1958; and Pilkington *et al.*, 1960).

From this it has seemed worth investigating the effects on ischaemic heart disease of reducing the level of serum cholesterol. Adequately controlled therapeutic trials have been reported for cholesterol-lowering hormones (Stamler *et al.*, 1960; Oliver and Boyd, 1961), with negative results. Nevertheless it is desirable to test the dietary hypothesis more directly. The results of prophylactic trials of this kind are unlikely to be available for some time. In the meantime it is of clinical interest to know whether patients with established ischaemic heart disease can be benefited by dietary manipulation. A simple reduction of fat intake has failed to show any benefit (Ball *et al.*, 1964); but no adequately controlled "double-blind" trial of an unsaturated oil has yet been reported.

Aims

Our purpose was to study the effects of prescribing a vegetable oil and a restricted fat diet to patients with ischaemic heart disease. The primary interest was in an unsaturated oil with cholesterol-lowering effect. But large doses of any oil may have secondary effects on diet and nutrition, so that differences between an unsaturated-oil group and a control group might be due to these secondary effects rather than to unsaturated fatty acids as such. It could, for example, be relevant that mortality from heart disease is low in Italy and Greece, whose inhabitants consume much olive oil; this oil has no major effect on serum-cholesterol level, its main fatty acid (oleic acid) being only moderately unsaturated. The trial was therefore designed to study effects not only of a more highly unsaturated oil (corn oil) but also of olive oil. It seemed likely that if any differences emerged between the olive-oil and corn-oil groups these would reflect the specific effects of polyunsaturated fatty acids.

Methods

Patients were accepted for the trial who met the following criteria: (1) *Either* electrocardiographic evidence of infarction (abnormal Q/QS waves, or typical serial ST/T changes) *or* clinical history of angina of effort, meeting World Health Organization precise criteria (Rose, 1962) with or without changes in the resting electrocardiogram, but without valvular disease, anaemia, or syphilis. (2) Age under 70 years. (3) Absence of heart failure, and also of any non-cardiac disease likely to threaten life within two years. (4) Absence of personal or geographical factors likely to interfere with clinic attendance or the taking of oil.

When a new patient was accepted for the trial a sealed envelope was opened containing the allocation instructions. In the case of patients allocated to an oil group the instructions referred only to a code number. Thus the physicians in charge knew which patients were receiving oil, but they did not know until the end of the trial the kind of oil that they were receiving.

All patients received conventional treatments, at the discretion of the physicians. At the time when the trial started long-term anticoagulant therapy was seldom used. Later it became more popular, especially for patients suffering reinfarction. To avoid confusion by possible interactions between treatments a few patients already receiving this treatment were excluded from entry to the trial; and in addition the occurrence of infarction after entry was taken as an end-point, the patient being then withdrawn from the trial.

Patients in both oil groups were instructed to avoid fried foods, fatty meat, sausages, pastry, ice-cream, cheese, cakes (except plain sponge), etc. Milk, eggs, and butter were restricted. An oil supplement of 80 g./day was prescribed, to be taken in three equal doses at meal-times. The general nature and purpose of treatment were explained, together with the fact that different patients were receiving different kinds of oil. No advice on dietary fat was give to control patients.

All patients attended a special follow-up clinic, initially at monthly intervals, and later every two months. Assessment was by standardized history, physical examination, and electrocardiography. The electrocardiograms were assessed without knowledge of the patient's treatment group. The trial was planned to cover three years' observation of each patient; but by the end of two years only one-half of the patients remained in the trial, the rest being dead, removed for reinfarction, or lost to follow-up. Consequently the results for only the first two years will be reported here.

Fears have recently been expressed, both within the profession and outside it, that clinical trials may sometimes operate against the best interests of the patients. We would like to

TABLE I.—*Characteristics of Patients at Entry to Trial in the Three Treatment Groups*

	Treatment Group		
	Control	Olive Oil	Corn Oil
Total No. of patients	26	26	28
Mean age at entry (years)	58·8	55·0	52·6
" body weight (kg.)	71·8	71·4	75·9
" serum cholesterol (mg./100 ml.)	253	262	263
History of angina only	5	4	4
Resting E.C.G. normal	0	3	2
" " abnormal	5	1	2
History of infarction	21	22	24
1 infarct only	17	17	20
2 or more infarcts	4	5	4
Resting E.C.G. normal	1	4	3
" " abnormal	20	18	21
Diastolic B.P. ≤90 mm.	9	14	13
" " >90 mm.	12	8	11
No exertional dyspnoea	9	7	15
Exertional dyspnoea	12	15	9
No heart failure	14	15	19
Heart failure*	7	7	5

* Jugular venous congestion *or* oedema *or* basal fine rales.

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record our own experience of the reverse—namely, that the necessity in this trial for careful supervision and continuity of care resulted in unusually good doctor–patient relations and patient morale.

Eighty patients entered the trial. Thirty-one were known to have had an infarction within the previous month; in the remainder the disease was more stable. Some of the characteristics of patients in the three groups at the time of entering the trial are shown in Table I. There are no significant differences between the groups with regard to any of the characteristics listed (P in each case >0.05), and it seems likely that at the start of the trial the prognosis for each of the three groups was approximately similar.

Results

The prescribed dose of oil was 80 g./day. But many found the treatment burdensome: distaste, nausea, and diarrhoea were the commonest complaints. An attempt was made to assess how much oil each patient actually took, based on the number of cans issued, the amounts left in the returned cans, and the patients' own statements. The results are indicated in Table II: they are, of course, maximum estimates. Patients in all three groups tended on average to lose a little weight as time went on. Unexpectedly, the average loss was greatest in the corn-oil group.

TABLE II.—*Estimated Amounts of Oil Consumed by Patients at Different Stages of the Trial*

Period	Olive Oil		Corn Oil	
	No. of Patients	g./day (Average)	No. of Patients	g./day (Average)
0–6 months	24	73	28	74
6–12 ,, ...	19	52	22	64
12–18 ,, ...	16	47	19	62
18–24 ,, ...	13	51	13	51
Mean	—	58	—	64

Dietary assessments were performed on those patients still in the trial during the second year of follow-up, using a self-administered questionary (Keen and Rose, 1958). This method is appropriate for comparing the relative intakes of different groups, although the absolute levels may be misjudged. Table III shows the estimated averages. It is interesting to note the spontaneous adjustment of appetite in the two oil groups, whose mean daily calorie intakes were very similar to those of the control group.

TABLE III.—*Dietary Assessment During Second Year in Trial*

Group	No. of Patients	Estimated Mean Daily Intake					
		Carbo-Hydrate	Protein	Fat	Calories	Calories from Oil	Total Calories
Control ..	16	249	64	70	1,933	—	1,933
Olive oil ..	12	216	49	45	1,505	c. 540	c. 2,045
Corn oil ..	15	189	57	50	1,475	c. 595	c. 2,070

Serum-cholesterol values were measured at each visit by the method of Zak, Zlatkis, and Boyle (Henly, 1957). This method probably gives readings that are 18 mg./100 ml. too high (G. R. Cooper, personal communication, 1964). Because of the large variation in absolute levels between patients the results (Table IV) have been related to the change observed in each patient from his or her own initial level. The mean values for control and olive-oil patients showed no significant change, although the administration of olive oil seems to have increased the variability of readings. The depressions seen in the corn-oil group are highly significant, except in the final period. (The latter seems to be a chance phenomenon, since among those who continued taking oil for a third year the level fell again.) The depressions represent the average over all patients, including some who probably took little or no corn oil.

TABLE IV.—*Changes in Serum-cholesterol Levels at Different Periods of the Trial, With Their Standard Errors and Significance Levels*

Period (Months)	Control		Olive Oil		Corn Oil	
	Mean and S.E. (mg./100 ml.)	P	Mean and S.E. (mg./100 ml.)	P	Mean and S.E. (mg./100 ml.)	P
0–6 ..	+4·4 (±7·2)	>0·5	+3·5 (±9·2)	>0·7	−25·0 (±8·8)	<0·01
6–12 ..	+0·3 (±9·2)	>0·8	+12·0 (±17·5)	>0·4	−30·8 (±10·5)	<0·01
12–18 ..	−7·9 (±9·4)	>0·4	+4·0 (±20·2)	>0·6	−30·3 (±9·9)	<0·01
18–24 ..	−2·8 (±12·1)	>0·8	−0·9 (±10·2)	>0·8	−19·9 (±13·5)	<0·2

Progress was measured according to five criteria. (1) *Sudden death.* (2) *Fatal infarction.* (3) *Non-fatal infarction,* diagnosed on the basis of a suggestive history, together with either S.G.O.T. level exceeding 40 units/ml. *or* electrocardiographic (E.C.G.) evidence of new Q wave or typical ST elevation. (4) *Non-fatal probable infarction,* defined as central chest pain accompanied by E.C.G. evidence of new ST depression (Minnesota Code criteria IV 1–3, see Blackburn *et al.,* 1960) or T-wave flattening or inversion (Minnesota Code criteria V 1–3). (5) *Other significant cardiac pain,* defined as central chest pain lasting one hour or more, unaccompanied by raised S.G.O.T. or by the E.C.G. changes specified in (3) or (4), *or* sudden worsening of angina of effort.

The first four of these categories are classed as "major cardiac events," and patients developing them were withdrawn from the trial. Patients developing "other significant cardiac pain" stayed in the trial.

Four patients were removed from the trial for other reasons. Two developed non-cardiac thromboembolism and were given anticoagulant therapy. The other two were removed because of diabetes mellitus. One of them already had mild diabetes, but glycosuria increased considerably soon after he started oil. Oil was stopped and glycosuria disappeared. Oil was restarted, but was stopped a month later because heavy glycosuria recurred. The other patient, not a previously recognized diabetic, developed glycosuria with a diabetic glucose-tolerance test a few weeks after starting oil. In addition to these withdrawals six patients were lost to follow-up.

The trial has been analysed in six-month periods (Table V). The state of the groups at the end of each period has been given

TABLE V.—*Progress of Patients*

	0–6 Months			6–12 Months			12–18 Months			18–24 Months		
	Control	Olive	Corn	Control	Olive	Corn	Control	Olive	Corn	Control	Olive	Corn
Major cardiac events:												
Sudden death	—	1	2	—	—	—	1	1	1	—	—	—
Fatal infarction	—	—	1	—	1	1	—	—	—	—	—	—
Definite infarction, non-fatal	2	2	1	—	—	—	1	1	2	—	1	—
Probable infarction, non-fatal	1	1	—	—	—	—	1	—	4	—	1	—
Total	3	4	4	0	1	1	3	2	7	0	2	0
"Other significant cardiac pain"	3	1	2	1	—	1	1	1	—	—	—	—
Removed from trial for other complications	—	—	—	—	1*	1‡	—	1†	1†	—	—	—
Lost to follow-up	—	1	—	1	—	1	1	1	—	—	1	—
Proportion in trial and free of major cardiac events, as percentage of those not removed from trial for other complications nor lost to follow-up	88	84	86	88	79	81	75	68	52	75	57	52

* Gangrene. † Diabetes mellitus. ‡ Pulmonary embolism.

as the number of patients still in the trial and free of any "major cardiac event," expressed as a percentage of the total number in the trial at that point—that is, the number of starters less those removed for non-cardiac complications or lost to follow-up.

At two years the proportion of patients remaining free of major cardiac events is greater for the control group (75%) than for the two oil groups (olive oil 57%, corn oil 52%). The likelihood that the difference between the control and corn-oil groups was due to chance is 0.05–0.1 (S.E. of difference, ±13%). Among those patients who were followed for a third year no new trend emerged.

Conclusion

The aim of the trial was not to duplicate the many carefully regulated laboratory studies of the effects of unsaturated oils on serum cholesterol. Rather was it aimed to study the feasibility and possible value of such regimes in a typical group of hospital coronary patients. Among such persons, and especially where treatment is prolonged, it is inevitable that some will comply incompletely or not at all. We estimated that initially the patients in the two oil groups took on average about 90% of the prescribed 80 g./day; but after a year the estimated average intake fell to around 60% of the ideal. As a result the differences in serum-cholesterol levels between the two oil groups are less than would be predicted theoretically, although still highly significant. Such limitations may be unavoidable in a clinical trial of a rather unpleasant regime.

The patients receiving the key treatment (corn oil) fared worse than those in the other two groups: two years from the start of treatment infarction or death had occurred in one-quarter more of the corn-oil than of the control group. This difference closely approaches the conventional significance level ($0.1 > P > 0.05$). The probability that a true difference of the same magnitude but in the other direction may have been missed by chance is less than 1 in 1,000. It is concluded that under the circumstances of this trial corn oil cannot be recommended as a treatment of ischaemic heart disease. It is most unlikely to be beneficial, and it is possibly harmful.

Summary

Eighty patients with ischaemic heart disease were allocated randomly to three treatment groups. The first was a control group. The second received a supplement of olive oil with restriction of animal fat. The third received corn oil with restriction of animal fat. The serum-cholesterol levels fell in the corn-oil group, but by the end of two years the proportions of patients remaining alive and free of reinfarction (fatal or non-fatal) were 75%, 57%, and 52% in the three groups respectively. The likelihood that the worse experience of the patients treated with corn oil was due to chance alone was 0.05–0.1. The likelihood that the trial failed by chance to detect a true and important benefit from corn oil was extremely remote. It is concluded that under the circumstances of this trial corn oil cannot be recommended in the treatment of ischaemic heart disease.

We are grateful to Professor W. S. Peart for advice and encouragement, to Paddington General Hospital for clinic facilities, and to physicians of St. Mary's and Paddington General Hospitals for referral of patients.

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Carcinoid Syndrome Associated with Oat-cell Carcinoma of Bronchus

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Brit. med. J., 1965, 1, 1533–1535

The remarkable potential of carcinomas of the bronchus to produce endocrine disorders, such as Cushing's syndrome (Brown, 1928), hyponatraemia (Winkler and Crankshaw, 1938), gynaecomastia (Hardy, 1960), and hypercalcaemia (Connor *et al.*, 1956), is now well recognized. The most recent addition to this group of disorders is the carcinoid syndrome, and this is known to be associated with 5-hydroxytryptamine (5-H.T.) excretion (Williams and Azzopardi, 1960) and also with 5-hydroxytryptophan (5-H.T.P.) secretion (Gowenlock *et al.*, 1960). However, 5-H.T. and its metabolites are probably not the true mediators of carcinoid flushes, and the release of a vasoactive peptide from the tumour has recently been suggested as a possible (Oates *et al.*, 1964).

We recently had the opportunity to study 5-H.T. metabolism in two patients in a patient with many features of the carcinoid syndrome who was ultimately shown to have an oat-cell carcinoma of the bronchus. As this association has only rarely been recognized and our ignorance of the mechanism responsible for the flushing in the carcinoid syndrome continues, it was thought that our investigations of a single patient would be of sufficient interest to justify a case report.

Case Report

A bus-conductor aged 43 was admitted to hospital with a history of upper abdominal discomfort, nausea, anorexia, and loss of weight for the previous three months; during the month preceding admis-

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Don't Go Overboard With the Soy Foods

interview

Researcher of the Month

David Zava, Ph.D.

David Zava, Ph.D. is a biochemist and an experienced breast cancer researcher who has spent decades looking at breast cancer tissue under the microscope. He helped Aeron Laboratories develop their saliva hormone assay testing procedures and is now working as a consultant. He is also the recent co-author, with Daniel Herman, of a book, *A Woman's Right to Know: The Breast Cancer Prevention Report* due out next fall. Over the past few years, Dr. Zava has contributed much to Dr. Lee's knowledge and understanding of progesterone, estrogen and breast cancer, and we're always intrigued to know what's on his mind. This month he wanted to talk about the dangers of eating too much soy, which has been touted recently as a virtual miracle food for menopausal and hormonal problems.

JLML: Dr. Zava, why are you warning women away from too much soy when everything we're reading and hearing encourages women to eat soy foods as much as possible for menopausal symptoms and to prevent breast cancer?

DZ: People do fine with average soy consumption, eating it here and there, but the way Americans are jumping into it, I think we're going to have problems. I reviewed the literature on soy very carefully when I was researching my book, and really got down to the nitty gritty, and what I found is that there are an enormous number of toxins *(antinutrients)* in soy. What I mean by this is that there are plant chemicals produced naturally in the soybean that, if not removed first by soaking, slow cooking and fermentation can cause serious health problems if you eat too much of them.

JLML: What about using soy foods to treat menopausal symptoms?

DZ: Soy foods can be important as one part of a balanced nutrition and lifestyle approach to menopausal symptoms but they aren't a solution by themselves. Even high consumption of soy isolates that are loaded with isoflavones only suppresses menopause symptoms minimally for most women. The recent Bowman-Gray School of Medicine study tested the ability of soy protein isolates to relieve menopausal symptoms. The

positive side of the study was that the intensity of hot flashes was reduced; however there was no statistical difference in the number of hot flashes these women were having.

With natural progesterone and/or estrogen replacement therapy women often get complete relief of menopausal symptoms. Because the natural hormones are safe and effective when used properly, I think the push to use soy is the wrong approach, and is seriously compromising the health and quality of life of millions of women.

JLML: What effects do the antinutrients in soy have on the body?

DZ: In studying the literature on soy I found there are about five types of plant chemicals in the soybean that can be toxic to humans if they are not removed by special processing. Over thousands of years of experimentation the Asians learned how to remove the toxins from soybeans and reap the remarkably rich nutrients from the bean. The primary toxins in soybeans include allergens, phytates, protease inhibitors, genistein, and goitrogens. The allergens can cause very pronounced allergic reactions in some individuals. This probably accounts for some 10 to 20 percent of the population in the Western world.

The phytates can be a problem because they tightly bind up essential minerals, particularly zinc, preventing them from being absorbed into the body. Some studies have indicated that excessive phytate consumption in children can cause physical stunting because of its zinc-depleting capacity. Phytates are not a problem if adequate animal protein is included in the diet, so I would expect phytates would be more of a problem for vegans. Zinc is needed for over 50 enzyme reactions in the body, including many of those necessary for brain functions.

The third antinutrient in soybeans is a phytochemical that inhibits the enzymes that digest protein into the simple building blocks, amino acids. These are called Bowman-Birk or protease inhibitors.

JLML: Aren't protease inhibitors what AIDS patients are taking now?

DZ: Yes, and we've learned the hard way from them that using protease inhibitors inhibits pancreatic enzymes. The last thing in the world you need when you have a wasting disease is something that's going to prevent you from digesting food. Even a healthy person doesn't want their digestion compromised.

JLML: And if you are estrogen dominant or taking birth control pills, you already have a problem with high copper and low zinc levels, and this will only make it worse. Dr. Ellen Grant, a hormone researcher from England, believes that this is the imbalance that causes the mood swings and irritability in PMS and in menopausal women.

DZ: That's right. But again, if you eat animal protein with legumes the phytates are not a problem.

Genistein is the next on the list of antinutrients. When I first began to study soy's anticancer properties I thought genistein was the answer. My first publication reflected my sentiments. As I "read on" in the scientific literature, I became less and less convinced that genistein was beneficial. Its structure is similar to that of estradiol, and it fits nicely into the estrogen receptor and turns the receptor on much like estradiol. However, genistein has many other actions in the human body. It inhibits a number of different enzymes, some of which are responsible for synthesizing estrogens, including those that convert androgens to estrone (aromatase) and estrone to estradiol (17-HSD). It also is a potent inhibitor of tyrosine kinases, enzymes that shuttle high-energy phosphate molecules in the cells for the purpose of driving cellular processes like cell proliferation. Cancer cells tend to over-express tyrosine kinases, so genistein has been shown to be quite useful in blocking cancer cell proliferation. However, this can be a double edged sword because normal cells also need some tyrosine kinase activity. This includes hair follicles, memory neurons in the brain, and so forth.

A third thing very high levels of genistein does is block glucose transport into cells by inhibiting an enzyme called GLUT-1. This is one of the major glucose transporters that sits on the outside of brain cells, red blood cells and in many other places, and shuttles glucose into the cells. Because the brain is very dependent on glucose for its energy source I would be concerned that too much genistein over a prolonged period of time eventually would be toxic to the brain.

I don't think soy in the form consumed by Asians over thousands of years is any problem as regards brain function. In fact, it may be beneficial. What concerns me, however, is that we just don't have any information on what long term impact excessive amounts of genistein in processed soyfoods, poweders or in pill form will have on brain function, since very high genistein inhibits at least three of the metabolic pathways needed to maintain normal brain function.

The fifth antinutrient in soybeans is called a goitrogen. This is a chemical that latches on to iodine, preventing it from absorbing into the body from the gastrointestinal tract. Iodine is needed to make thyroid hormone. Low thyroid function has been associated with poor brain development. Anyone who has been deficient in thyroid hormone understands quite well what impact this can have on normal brain function, especially at a time in life as we grow older and "fuzzy thinking" creeps into our vocabulary.

JLML: How should we weigh the protective effects of genistein against breast cancer that we read and hear so much about in the popular media?

DZ: Surprisingly there's actually very little evidence in the literature either from epidemiological studies or experimental animal studies that prove soy protects against breast cancer. That's not to say it doesn't have other beneficial properties, such as heart-protective benefits, but only that it doesn't appear to protect the breast from cancer. Many people have made the assumption that genistein, which is present in very high levels in soybeans, acts like tamoxifen and protects against breast cancer. What I have found in my research is that genistein acts like a pro-estrogen, not an

anti-estrogen like tamoxifen. The suggestion by the popular press that genistein at the concentrations found in foods inhibits the growth of breast cancer cells is just plain wrong and not based on scientific fact. Several abstracts submitted to the Second conference on soy held in Brussels Belgium reported that soy protein isolates increased the proliferation of normal breast cells in intact humans. This is entirely consistent with what I found working with human breast cancer cells in test tubes using levels of genistein commonly found in the blood of individuals consuming a lot of soy foods . Others have found similar results, that is genisein is a pro-estrogen, in human breast cancer cells. So given the lack of evidence that soy foods protect against breast cancer, I find it amazing that there is so much emphasis on it for breast cancer prevention.

JLML: So this is a food that is a medicine in moderation, and a potential poison in excess. What guidelines would you give both women and men for eating soy foods?

DZ: If you are not allegic to soyfoods, eat them primarly as fermented soy products, which includes miso and tempeh, because many of the antinutrients have been reduced in this form. If you eat soy in in the form of tofu, soy milk, or powders for example, eat it with a balance of mineral-rich sea vegetables such as kombu and nori, and animal protein, preferably fish, to counter the higher content of antinutrients such as phytates and digestive enzyme inhibitors.

JLML: Thank you Dr. Zava, this is important information for women to have. Is there anything else you would like to tell our readers?

DZ: Yes. As part of my research I am looking for women who have been diagnosed with breast cancer while they were using progesterone. They can contact me by mail in care of the John Lee Medical Letter at P.O. Box 3527, Santa Barbara, CA 93130-3527, or via e-mail at info@salivatest.com.

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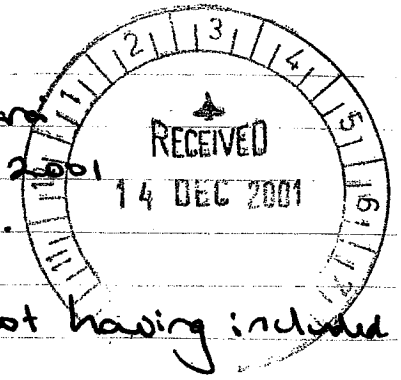


Re: Additional information for P242, A417.

Dear Administrator,

Please accept my apologies for not having included this information in my earlier submissions.

(1) Re P242: It would be unwise to allow Medicinal claims for any food. The current Medicines Act should be vigorously enforced instead. You will see from the enclosure (from packaging) that food marketers are unlikely to tell the customer all that they need to know.

(2) Re 417: Note that, as it has been repeatedly demonstrated scientifically, plant sterols alter hepatic lipid synthesis of enzyme activities. Some of these alterations are the same as, or similar to, that of the cholesterol lowering "resin" cholestyramine, so far, no exhaustive nor long term studies have been done and so purchasers will be taking place in an experiment with unknown outcomes. The New England Journal of Medicine published a statement by Rifkind that "the effect on mortality of reducing cholesterol may be delayed and that a trial of five to seven years is not long enough" (NEJM 1987, 317, 1279) The Health and Disability Act ensures that consumers must be told when they are taking part in an experiment the health outcomes of which are unknown, and will remain unknown until studies/clinical trials of more than five to seven years are completed and evaluated.

Yours sincerely,

[redacted]

This is misleading: The information is very incomplete. Regulation by phytosterols of cholesterol metabolism key enzymes, means that basic body processes are altered.

pro-activ

Flora has created a dietary breakthrough with Flora pro-activ, a spread enriched with natural plant sterols.
The selected plant sterols in Flora pro-activ reduce cholesterol uptake into the bloodstream. A low level of cholesterol in the bloodstream is an important factor in helping to maintain a healthy heart.

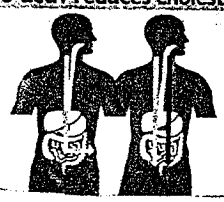
What are plant sterols and how do they work?

Plant sterols are natural ingredients found in small amounts in all plant foods and have always been a part of our diet. **Flora pro-activ** is enriched with these natural ingredients derived from vegetable oils such as sunflower and canola oils.

Plant sterols compete with cholesterol and actively reduce cholesterol absorption from the intestine. Instead of entering the bloodstream, most of the cholesterol together with the plant sterols passes straight through the body.

How Flora pro-activ reduces cholesterol uptake

Without Flora pro-activ cholesterol enters the bloodstream



With Flora pro-activ cholesterol passes out through the body

How much do I need to use?

We recommend around 25grams a day which is about the same amoun you would normally spread on 3-4 slices of bread a day.
Incorporating **Flora pro-activ** is as easy as replacing your current brand of spread with **Flora pro-activ.**

What will happen if I stop using Flora pro-activ?

If you stop eating a plant sterol enriched spread, your body will again ab cholesterol from the intestine, so you need to ensure you keep eating **Flora pro-activ.**

How should I use Flora pro-activ?

Flora pro-activ has the great taste of **Flora** and can be used on toast a sandwiches and melted on vegetables. **Flora pro-activ** is lower in fat t other margarines and spreads on the market and for this reason is not suitable for frying. Use **Flora pro-activ** of course in cooking and baking your favourite foods.

5

This paper shows that the cholesterol lowering effect (total serum cholesterol levels) will not be the result of reduced cholesterol intake from the food we eat. It will be caused by alterations in enzyme metabolic reactions.

Occasional Survey

DIETARY FATS AND CORONARY HEART DISEASE: UNFINISHED BUSINESS

E. H. AHRENS

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Summary In the scientific and lay press, dietary recommendations that are aimed at prevention of coronary heart disease in the general public are appearing more and more frequently. The dietary pattern that is now most widely advocated is a low-fat, low-cholesterol diet with a polyunsaturated/saturated ratio of 1. The argument for such a dietary change is supported mainly by extrapolations from epidemiological data and from animal experimentation. Reasons are given for concluding that the recommendations are unwise, impractical, and unlikely to lead to a reduced incidence of arteriosclerotic disease. Since complacency is equally inappropriate, a few practical questions are outlined that should be settled before the public is assured that a low-fat diet will lead to a reduced risk of coronary heart disease.

INTRODUCTION

THE debate over the optimal quality and quantity of fat in the diet of the Western world has become more and more heated in the past 2 years, but hardly more illuminating. The issue has long since moved out of the scientific press and into the public arena: in the U.S.A. it seems to have turned into a political skirmish between politicians, the Department of Agriculture, and the National Institutes of Health (NIH),[1,2] in which the wages of war include territorial claims, the profitability of agricultural and food processing interests, and the balance of international trade.

THE VARIOUS SIDES OF THE DEBATE

On the scientists' side the debate is waged by proponents of several points of view: those who think the "diet-heart question" is utter nonsense, totally without scientific basis;[3,4] others who promote a low-fat, low-cholesterol diet for the general public;[5] those who favour a national diet low in cholesterol and saturated fats but moderately high in polyunsaturated fats;[6] some who conclude that refined sugar is mainly to blame for the high prevalence of coronary heart disease (CHD) in Western countries,[7] and others who point to suboptimal intakes of fibre.[8] There is also a minority group, to which I belong, that believes that the time is not yet ripe for drawing up national guidelines and dietary recommendations for the general public, but wishes to see the public fully informed on the progress made as well as the questions remaining.

REASONS FOR THE DEBATE

The debate is fired by the sheer magnitude of the numbers of CHD deaths; by the advances in understanding of fat and sterol metabolism gained over the past 30 years; by the belief that almost everyone in affluent societies is hyperlipidæmic; and by a medical ethos that is uneasy with inaction.

REPORTS AND RECOMMENDATIONS

If we knew precisely which diet can prevent or allay the development of CHD, there would be no debate. The fact that the available evidence is soft ensures that opinions are divided. The fact that the argument has recently become less objective seems to me to be proof-positive that the experts do not yet know—for sure—what to advise or when to advise it. This is not to say that panels of informed and conscientious medical scientists have not tried to reach valid conclusions: indeed, there have been more than fifteen reports over the past 10 years emanating from official and quasi-official organisations in Britain, Germany, Holland, Scandinavia, Australia, New Zealand, Canada, and the United States. Committees convened there have almost uniformly warned against the increasing obesity in affluent societies; some have concluded that our Western diets should be lower in cholesterol and saturated fats, while others haves opted for increased intakes of polyunsaturated fats.

The McGovern Report

In the U.S.A. the most recent and highly publicised recommendations to the public were the six "dietary goals" set forth by a Senate Select Committee chaired by Senator McGovern. The first report (February, 1977) implicitly promised that a national shift to a diet lower in saturated fat and cholesterol, lower in refined sugar and higher in starches, and very much lower in salt would surely lead to a reduced incidence of CHD, stroke, hypertension, diabetes, and cancer.[5] A revision undertaken in reply to widespread objections (December, 1977), stated more conservatively that such a diet would decrease the *probability* of premature incidence of disease.[9] No one really doubts that the McGovern diet can be translated into a feasible diet that the public can learn to like, but at least one commentator[10] has calculated that U.S. agriculture does not have the physical means to provide it to the entire nation.

A Report of the American Society of Clinical Nutrition

In the McGovern report and other official statements the scientific basis for the recommendations set forth was neither documented nor critically analysed. Rather, these documents presented a codification of opinions and beliefs. The weakness of this approach has recently been redressed by the American Society for Clinical Nutrition. In 1978 its directors called together a panel of 9 medical scientists with wide experience in clinical medicine, human metabolism and nutrition, epidemiology, and animal experimentation. The panellists were intentionally selected as representatives of a spectrum of viewpoints, often with opposing biases. They were asked to weigh the quality of all published scientific evidence relating to six dietary issues—dietary fat, cholesterol, carbohydrates, alcohol, excess calories, and salt. Each of 6 panellists was persuaded to assemble an annotated review on one of the six issues; 24 outside advisers assisted in this labour. The assembled panel debated the

reviews for completeness and balance. Then it measured the quality and strength of the evidence against eleven criteria: the consistency of the epidemiological data *among* and *within* population groups: the strength, independence, and temporal relationships of the epidemiological data; the effects of new exposure to or removal of the putative factors in already affected as well as in apparently unaffected individuals; necropsy data; the results of animal experiments; and finally the plausibility of the postulated biological mechanisms. Six brief Consensus Statements were fashioned out of these measurements.

These Consensus Statements and the supporting reviews have been published as a supplement to the December, 1979, issue of the *American Journal of Clinical Nutrition*. An introduction[11] describes the panel's attempt to put numbers to the strength of the evidence on each of the several dietary issues. What is truly novel is that it also measures the cohesiveness of the views of the 9 panellists on each of the issues—a factor that has rarely been quantified and almost never displayed in previous reports on diet and health. These measures of cohesiveness were made after the panellists had reviewed, debated, and dissected the evidence on each of the issues over a period of 15 months, and only after a consensus on each of the issues had been reached.

In regard to dietary fats, cholesterol, and arteriosclerotic disease, the panel concluded that the association of these two factors to arteriosclerotic disease *among* population groups was strong, but *within* population groups weak; that the strength and independence of the association were weakened by the confounding effects of a number of genetic, environmental, and socioeconomic factors; that proof from necropsy evidence was either lacking or confounded by other factors; that the effects of changing the intakes of saturated fats and cholesterol both in primary and in secondary prevention trials offered suggestive but not definitive proof of the association; that animal experimentation had succeeded in producing models of arteriosclerotic disease in some but not all species, with wide differences in responsiveness to the intake of cholesterol and saturated fat even within species; and that the underlying biological explanations were not well understood.

The panel as a whole voted that the evidence relating the intake of saturated fat and dietary cholesterol to atherogenesis in man was convincing; further, they agreed that a reduction of saturated fats and cholesterol would lead to reduced levels of plasma cholesterol. On the other hand the evidence on disease prevention by diet modification was considered unconvincing. Thus, the panel could not guarantee that lipid-lowering by dietary means would necessarily lead to a reduced incidence of new events of CHD. Although these conclusions represented a consensus, the spread of opinion (or lack of cohesiveness) of the 9 panellists' individual views on these issues was considerable—far greater, for instance, than on salt and hypertension.

THE LIPID HYPOTHESIS

In a recent talk in Houston, Texas, before the 5th International Symposium on Atherosclerosis, Dr R. I. Levy, director of the National Heart, Lung and Blood Institute of the NIH expressed his view that a diet lower in cholesterol and saturated fat (a "prudent diet") could be expected to lead to a reduction of CHD. The basis for this view is the as yet unproven hypothesis (the lipid hypothesis) that a reduction of the level of plasma cholesterol by whatever means (dietary, drug, or otherwise) will lead to a reduced incidence of CHD.

I have previously described my reasons for concluding that this hypothesis is a valid working theory.[12] However, it has only once been properly tested. In a large clinical trial of the lipid-lowering drug clofibrate,[13] involving 15 000 hypercholesterolæmic but otherwise well males in a 6-year experiment in Edinburgh, Prague, and Budapest, there was a highly significant decrease in new events of non-fatal myocardial infarction in the drug-treated group. There was, however, no reduction in total mortality: indeed, a disturbing increase in incidence of pathology of the intestinal tract was noted. All questions of drug toxicity aside, it was clearly shown that the greater the reduction of plasma cholesterol, the greater the reduction of new events of non-fatal myocardial infarction.

MY RESISTANCE TO GENERAL RECOMMENDATIONS

In view of these results, why oppose the issuance of dietary recommendations to the general public at this time? Let it be clear at the outset that my laboratory will consider itself bountifully rewarded for its efforts if a reduction of plasma lipids, achieved by dietary means, can be shown clearly to reduce the incidence of CHD: we published the first clear proof that plasma lipids can be predictably altered by changes in the quality of dietary fat 25 years ago,[14,15] and since then we have been, amongst others, in the forefront of a parade of research that has added substantially to our present understanding of disordered lipid metabolism in man. Thus, we are intellectually and emotionally involved in the outcome of any proper test of the lipid hypothesis. However, if the public's diet is going to be decided by popularity polls and with diminishing regard for the scientific evidence, I fear that future generations will be left in ignorance of the real merits, as well as the possible faults, in *any* given dietary regimen aimed at prevention of CHD.

My reasons for resisting the current advocacy of a low-fat national diet are four in number.

1. There has been no previous test of the "prudent diet". The only dietary trials carried out with proper controls and with minimal confounding biases—namely, the 12-year Finnish trial in two mental institutions[16] and the 8-year Veterans Administration Hospital trial in Los Angeles[17]—compared two diets with moderately high fat intakes (35–40% of total calories) in which the degree of unsaturation of the two fat mixtures varied as widely as was feasible under the test conditions. As noted above, these primary-intervention trials resulted in suggestive but not conclusive evidence that the incidence of new events of cardiovascular disease was reduced on the more highly unsaturated fat regimen. In neither experiment was total mortality reduced.

On the other hand, a trial of the low-fat dietary regimen recommended by the McGovern Committee[9] and the American Heart Association[18] has never been carried out. It seems that the proponents of this dietary change are willing to advocate an untested diet to the nation on the basis of suggestive evidence obtained in tests of a *different* diet. This illogic is presumably justified by the belief that benefits will be obtained, vis-a-vis CHD prevention, by *any* diet that causes a reduction in plasma lipid levels.

2. The "prudent diet" will have only a small effect on plasma lipid levels. How great a reduction in lipids can be anticipated if the nation is persuaded to adopt the low-fat dietary guidelines? To approach an answer to this question, let us use the most recent data on food consumption in the U.S.A.[19] and calculate the effect of reducing the nation's fat intake from 44% to 30% of a mean daily food intake of 2500 kcal/day, raising the polyunsaturated/saturated (P/S) ratio of the fat from 0·44 to 1·0, and lowering the cholesterol intake from 500 to 300 mg per day. The Keys formula[20] predicts a mean decrease in plasma cholesterol from 220 to 194 mg/dl*, a 12% decrease. But 100% compliance with the dietary guidelines will certainly not be attained, any more than it has been in the anti-cigarette campaign waged by the Government over the past 15 years. Even 50% compliance seems unlikely to me; this translates to a mean decrease in plasma cholesterol of 6%—that is, from 220 to 207 mg/dl.

Is this small decrease worth the enormous effort required to achieve it? The answer is uncertain, for the risk/benefit ratio depends on facts we simply do not have. Thus, I believe the burden of proof rests on those who claim that real benefits will be derived from this minor change in our national dietary pattern.

3. Any one diet produces different results in different people. The argument for a one-diet approach assumes that all members of the population react similarly to diet changes, like pure-bred laboratory animals. This assumption is simply incorrect; the more closely we look, the more often we identify the wide individual differences that clearly reflect our heterogeneous genetic endowment.

We have amply documented these differences in our studies of human responses to changing intakes of fat and cholesterol. This laboratory has had more than 25 years' experience in more than 800 inpatients, each fed for at least 12 weeks on orally administered formulas in which wide variations in carbohydrate/fat calories, saturated/unsaturated fat quality, and cholesterol/plant-sterol intakes have been tested in our studies of the key factors in cholesterol homœostasis—absorption, transport, synthesis and its feedback control, conversion to bile acids, and tissue storage. I need no further evidence to convince me, first, that I cannot predict in advance how and by how much any given individual will respond to a given dietary challenge.

Let me cite examples that bear directly on the question of changes in cholesterol intake. 4 patients were put on diets in which the cholesterol content was increased two to four times over that accepted as the mean daily intake of 500 mg in U.S.A. adults today. The first patient experienced a continuous rise in plasma cholesterol levels from 250 to 450 mg/dl over a 5-week period; the other 3 showed only trivial changes in plasma levels. However, one of them demonstrated complete compensation for absorbed cholesterol by an increase in neutral sterol excretion; the second also compensated fully by a reduction in endogenous cholesterol synthesis that matched the amount of cholesterol absorbed, milligram for milligram; and the third showed no change in excretion or synthesis, but reacted by retaining one-third of the daily cholesterol intake in tissue stores. We do not know to this day what proportions of the general population fall into these four response-groups.

Let me also cite examples that bear on the question of exchanges of dietary fats differing markedly in degree of unsaturation, such as corn oil and lard. In such experiments, carefully monitored on a metabolic ward, we measured the plasma levels of cholesterol that were attained after 6–8 weeks' ingestion of each dietary mixture, always at eucaloric intake levels and at fat intakes of 40% of total calories. If the corn oil period is taken as the baseline in each patient, the substitution of lard (P/S=0·43) for corn oil (P/S=8) in 13 patients caused plasma cholesterol levels to rise 5, 15, 16, 17, 18, 19, 20, 23, 27, 28, 32, 33, and 39%; coconut oil (P/S=0·03) exchanged for corn oil in 6 patients caused rises in plasma levels of 5, 34, 37, 40, 60, and 77%; butter (P/S=0·06) exchanged for corn oil in 9 patients caused increases of 18, 30, 30, 32, 35, 36, 53, 60, and 84%. We could not have predicted the extent of these variations, nor can we today identify who will respond how.

It is clear from these data that individuals vary widely in their responsiveness to changes in quality of fat and in cholesterol intake. Equally convincing are the variations we have seen in substitution of carbohydrate for fat calories.

On the basis of these studies I can confidently predict that adoption of the "prudent diet" will cause various reactions in different segments of the population. No one can say today what proportion of the general population will experience a decline in plasma cholesterol levels, nor of what degree; how many will increase cholesterol synthesis to match a decrement in cholesterol absorption, nor to what degree; how many will experience a flux of cholesterol into or out of tissue stores, nor to what degree; and how many will be totally unaffected by the change.

Moreover, it is absolutely certain that no one can reliably predict whether a change in dietary regimens will have any effect whatsoever on the incidence of new events of CHD, nor in whom. All of these caveats bring me to my fourth reason for objecting to general recommendations at this time.

4. Crucial questions remain to be resolved. Any broad-scale change in the dietary patterns of the Western world should follow and not precede a resolution of the many unsolved problems that bear directly on our understanding of lipid and lipoprotein metabolism in man, and their relationships to atherogenesis. A Working Group Report from the National Heart, Lung and Blood Institute of the NIH[21] urged in 1977 that the first priority of effort should be directed at the prevention and control of arteriosclerotic disease. But is it not obvious that the success of prevention depends on the thoroughness of our understanding of the root causes of disease? Lacking that understanding, are we in danger of launching an all-out war against the wrong foe?

Hyperlipidæmia is only one of the risk factors predisposing to CHD, and the genesis of hyperlipidæmia in more than 95% of the general population is simply not understood: according to Goldstein et al.,[27] less than 1% of the hyperlipidæmia in the U.S.A. is monogenic in origin.

Thus, the uncertainties are numerous. But let me identify just three of the immediate problems that demand solution before any dietary guidelines are issued to the general public.

The choice between a low-fat diet with P/S=1 and a moderate-fat diet withP/S>2.—Diets taken at eucaloric levels will affect the fatty-acid composition of the tissues in predictable ways: the lower the fat intake and the higher the carbohydrate intake, the more saturated the body's fatty acids. By contrast, the higher the intake of polyunsaturated fats, the more unsaturated the fatty-acid composition of the lipids in plasma and in all formed elements of the blood; in adipose tissue and other

* Conversion factor, mg/dl to mmol/l=0·026.

stored lipids; and in all membrane lipids. Given these facts, on what basis do we choose to produce tissues rich in saturated fatty acids ("hard fats") when the choice is available of creating tissue lipids that are highly unsaturated ("soft fats")? Scientists are only now beginning to test whether the fatty-acid composition of plasma lipoproteins affects the transport of cholesterol into and away from the bulk tissues (adipose tissue, muscle, and connective tissue), into or away from arterial wall tissues, endocrine organs, and the central nervous system. Does the "softness" of fat in cell membranes affect the immunoresponsiveness of lymphocytes? What about the permeability of cell membranes to solutes, anæsthetic agents, drugs, foreign bodies, bacteria, viruses, metastatic tumour cells? Is the ageing of cells affected by their "softness"? What about the thrombotic potential of the blood in "hard fat" versus "soft fat" patients: is the balance between aggregating and antiaggregating prostaglandins and their oxidation products altered? Why is the incidence of spontaneous mammary tumours in undernourished C_3H mice so markedly reduced, and the rate restored by sustituting fat for carbohydrates in the same subcaloric diet? Are cell receptors modified by the degree of saturation of cell membrane lipids?

Even if low-fat diets and highly unsaturated-fat diets cause the same amount of cholesterol-lowering in the plasma of most of the general population (which we do not know), how can we make the choice now, in the face of the unanswered questions in the paragraph above, to request the general public to adhere to a low-fat diet?

HDL/LDL ratios and dietary fat quality.—The recent resurgence of interest in the ratio of high-density to low-density lipoproteins (HDL/LDL) as predictors of CHD risk is only now leading investigators to define the effects of diet on this ratio in plasma lipoproteins. There is only one published report[23] on this question today, but this deficiency will doubtless be remedied within the next few years.

The VLDL and chylomicron cascade.—Similarly, there are no published data on the question whether the rates of conversion of very-low-density to low-density lipoproteins (VLDL to LDL) and of chylomicrons to remnants is affected by the fatty-acid composition of the structural elements of these lipoproteins. Nevertheless, the catabolism of VLDL, LDL, and chylomicrons appears to be critical in control of their circulating levels; indeed, some workers consider them to be the key determinants in the process of atherogenesis.

CONCLUSIONS

In view of these many considerations and uncertainties, I feel it is irresponsible to make the dietary recommendations that are being so widely proposed to the general public at this time. Let no one conclude that I am complacent about the diet of the Western world, or about our sedentary way of life. I have no interest in preserving the status quo. Indeed, I can forsee both economic and health benefits in adopting a style of living that emphasises the output side of our energy balance while simultaneously reducing food intakes, both of which can be expected to diminish the incidence and impact of hypertension and diabetes; the incidence of CHD itself may decrease as the population adopts a lifestyle characterised by reduced cigarette-smoking and reduced total body-weight.

I am truly sympathetic to the need for speed in settling the many unresolved questions that bear on the diet-heart proposition, for I believe that their solution will point the way to a series of rational dietary approaches to the prevention of CHD, a disorder that seems to have many causes, hence many solutions. Hyperlipidæmia is certainly associated with and may even be one of those causes, but it is important to recognise that hyperlipidæmia also has many causes and hence many solutions. I believe it is anything but a service to the public to postulate *one* dietary solution for hyperlipidæmia, no matter how well-meaning one is in advocating it. Let us address the unanswered questions and demand the means to solve them quickly.

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Christmas Quiz

ANYONE READ THE LANCET?

How well have you read *The Lancet* in the past year?

1. Why are hobbits extinct in England now?
2. What was Brunel doing upside down?
3. Where did A. C. Dornhorst publish his memorable chesty phrase?
4. What replaced weaver's bottom?
5. Where did *male oscuro* strike?
6. Who carried an alarming iron load?
7. Fleeting intercostal pain has an eponym. What is it?
8. With the prize in mind, whose initials were J.B.P.A. de M. de L.?
9. What smiled in response to viral exposure?
10. Areca nuts, burnt seashells . . . what is missing in the recipe?
11. When was V day?
12. *Cholo coquero* is not a Mexican chicken dish in chocolate sauce, but what is it?
13. Where would you find the "county of the three impasses"?
14. Cystisine is associated with what sort of poisoning?
15. What was Moses' cure for asthma?

A prize, The Illustrated Origin of Species, *Richard Leakey's presentation of Darwin's book, will be awarded to the three most nearly correct entries. Entries should be sent to the London office of* The Lancet *and will be held until Monday, Feb. 4, to allow overseas readers to enter.*

Original Paper

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Effects of Dietary Phytosterols on Liver Lipids and Lipid Metabolism Enzymes

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Phytosterols
Lipid metabolism
Fatty acids
Cholesterol
Rat

Abstract
The effects of dietary phytosterols on lipid metabolism have been assessed through determination of liver lipids (sterols and fatty acids) and lipid metabolism enzymes (acetyl-CoA carboxylase, malic enzyme, glucose-6-phosphate dehydrogenase) in rats fed 12 or 24 mg cholesterol a day and 0–96 mg phytosterols. The results indicate that, provided the dietary phytosterol to cholesterol ratio is at least 1 and in the presence of a dietary cholesterol excess, phytosterols do exert a regulatory role through decreases of both acetyl-CoA carboxylase and malic enzyme activities. A ratio of 2 enhances this effect. At the same time, liver fatty acids and cholesterol contents significantly decrease.

Introduction

Phytosterols have long been known to be hypocholesterolemic in most species of animals [1–8]. Numerous hypotheses deal with this effect. It is likely that, among complex phenomena, the inhibition of cholesterol absorption by phytosterols is most important [8–10]. In previous papers [8, 11], we reported the effects of dietary phytosterols on plasma lipids of Wistar rats and suggested with others [12] the effects of phytosterols on cholesterol synthesis. A previous study by Gerson et al. [13] using ^{14}C-labeled molecules indicates that intraperitoneal injection of β-sitosterol increases the rate of biosynthesis of cholesterol and lipids in different tissues and their oxidative degradation. To further document this point, we have focused on liver lipids and lipid metabolism enzymes.

Materials and Methods

Animals and Diets

After a 7-day adaptation period, male adult Wistar rats weighing 215 ± 12 g were randomly assigned to eight dietary groups (12 rats/group). They were all fed the basal diet, supplemented or not with cholesterol or

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Table 1. Composition of the diets (wt %; unless otherwise indicated)

	Group						
	1	2	3	4	5	6	7
Casein	16	16	16	16	16	16	16
Cornstarch	68	68	68	68	68	68	68
Butter	8	8	8	8	8	8	8
Cellulose	4	4	4	4	4	4	4
Mineral mix	3	3	3	3	3	3	3
Vitamin mix	1	1	1	1	1	1	1
Cholesterol, mg/day	12	12	12	12	24	24	24
Phytosterols, mg/day	–	12	24	48	–	24	48
Ratio	0	1	2	4	0	1	2

maize phytosterols (β-sitosterol: 72.5%; campesterol: 20.5%; stigmasterol: 7%) (table 1). Animals were individually housed in metabolic cages. They were given water ad libitum, and 22 g diet/day. The phytosterol dose was mixed in to each ration [11]. The experiment lasted 4 weeks, including the adaptation week. Rats received phytosterols during 3 weeks.

Sample Analysis

Liver was sampled post-mortem, washed in cold saline and immediately frozen. Enzymatic activities were determined on microsomes purified from 1 g liver homogenized in 10 ml 0.25 *M* sucrose and centrifuged [14]. Acetyl-CoA carboxylase activity was determined according to Chang et al. [15] modified by Chakrabarty and Leveille [16]. Malic enzyme activity was measured according to Hsu and Lardy [17]. Glucose 6-phosphate dehydrogenase activity was determined according to Ficht et al. [18].

Lipid composition was studied after Folch extraction [19]. Sterols were prepurified from the lipid extract by saponification with 5% KOH in methanol and hexane extraction [20]. Sterols were analyzed as trimethylsilyl derivatives by capillary gas chromatography (OV 1701, 25 m × 0.32 mm i.d.). Peaks were identified by comparison with commercial standards, and quantified using cholestane as internal standard. Fatty acids were analyzed as methyl esters [21] by capillary gas chromatography (Supelcowax 10M, 25 m × 0.32 mm i.d.). Peaks were identified by comparison with purified standards and quantified using heptadecanoate as internal standard.

Results

Animals

As described elsewhere [11], food consumption was identical in all groups (mean consumption: 21.4 ± 0.1 g/day); feeding the high-cholesterol diet induced a significant increase in animal body weight, which was reduced by phytosterol supplementation. Liver weight was significantly enhanced in group 2 (11.1 ± 0.3 g) and reduced in group 8 (9.7 ± 1.1 g), as compared with other groups (mean: 10.6 ± 0.2 g).

Enzyme Activities (table 2)

Feeding sitosterol induced no change in enzyme activities of rats fed the lower cholesterol dose, except an enhancement of acetyl-CoA carboxylase activity in rats fed the highest phytosterol dose (group 4: $p < 0.05$, vs. group 1).

Without added phytosterols, the higher cholesterol dose (24 mg/day) tended to increase, although not significantly, the enzyme activities (group 5 vs. group 1). For this higher dose, feeding phytosterols decreased both acetyl-CoA carboxylase and malic enzyme activities (groups 6–8 vs. group 5). Due to a large standard deviation of the results in group 5, the observed decrease of glucose 6-phosphate dehydrogenase activity is not significant. Nevertheless, it is noticeable that, for the same phytosterols to cholesterol ratios, animals fed the highest cholesterol dose present significantly lower activities of this enzyme (groups 6 vs. 2, 7 vs. 3, 8 vs. 4; $p < 0.01$).

Table 2. Enzyme activities

Group	Acetyl-CoA carboxylase	Malic enzyme	Glucose-6-phosphate dehydrogenase
1	538±109	0.39±0.16	1.33±0.20
2	536±43	0.39±0.07	1.32±0.20
3	522±154	0.30±0.06	1.26±0.15
4	664±123*	0.32±0.06	1.48±0.26
5	741±199	0.44±0.13	2.39±1.23
6	235±59**	0.16±0.04*	0.46±0.19
7	225±92***	0.16±0.04*	0.57±0.19
8	233±99***	0.16±0.03*	0.62±0.15

Values are means ± SEM of 12 rats.

Acetyl-CoA carboxylase activity is expressed as μmol HCO_3^- incorporated in malonyl CoA/min/μg protein. Malic enzyme and glucose-6-phosphate dehydrogenase activities are expressed as μmol NADPH/min/μg protein. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, versus the group receiving the same cholesterol supplementation without phytosterols (Student's t test).

Table 3. Liver fatty acids and sterol contents

Group	Fatty acids g/100 g	Cholesterol mg/100 g	Campesterol mg/100 g	β-Sitosterol mg/100 g
1	3.1±0.3	216.4±1.5	0.81±0.04	2.95±0.20
2	3.5±0.3	218.7±3.1	1.86±0.17***	3.96±0.23*
3	3.6±0.3	210.1±2.8*	1.96±0.15***	3.74±0.16***
4	3.0±0.2	199.9±1.9***	2.19±0.16***	3.88±0.24***
5	7.8±2.8	301.0±13.3	0.87±0.07	2.92±0.06
6	2.8±0.3***	239.4±9.0***	4.68±0.51***	8.84±0.25***
7	2.7±0.3***	209.4±6.1***	5.03±0.50***	10.90±0.27***
8	3.0±0.3***	203.4±7.7***	8.00±0.66	12.86±0.75***

Values (wet weight) are means ± SEM of 12 rats. * $p < 0.05$; *** $p < 0.001$, versus the group receiving the same cholesterol supplementation without phytosterols (Student's t test).

Sterol Analysis

For both cholesterol doses, feeding phytosterols induces an increase of liver phytosterols (table 3). Stigmasterol was not detected. In the same way, feeding the highest cholesterol dose induces an increase of liver cholesterol (group 5 vs. group 1).

In the presence of 12 mg cholesterol, feeding phytosterols reduces the cholesterol liver concentration, most efficiently for the highest phytosterol/cholesterol ratio (7.6% decrease; $p < 0.001$). In the presence of the highest cholesterol dose, the phytosterol-associated reduction of liver cholesterol is more important ($p < 0.001$ for any group), the highest phytosterol/cholesterol ratio being the most efficient (32.4% decrease). Despite the difference of dietary cholesterol overload, both groups 4 and 8 (phytosterol/cholesterol ratios: 4) present the same liver cholesterol concentration.

Fatty Acid Analysis

The highest cholesterol dose (24 mg, group 5) induces a 2.5-fold increase of liver total fatty acids (table 3). Feeding phytosterols reduces this concentration to the usual level, whatever the phytosterol/cholesterol ratio. The fatty acid composition is not significantly modified. No trienoic acid was detected. Arachidonic acid represents 17.4 ± 2.6 wt% of total liver fatty acids and is not affected by the diet.

Discussion

The results we present here clearly demonstrate the direct effect of phytosterols feeding on hepatic lipid synthesis enzyme activities. Although animals were fed butter as the sole source of fat, providing 0.7% of the total energy as polyenoic fatty acids, no deficiency symptom has been detected. In particular, the arachidonic acid level remained normal in the liver, and no eicosatrienoic acid could be detected. Hence, the effects we describe here are actually linked to the dietary treatment and not to a presumed essential fatty acid deficiency.

The observed variations of both malic enzyme and acetyl-CoA carboxylase activities are associated with the variations of liver fatty acid content. In a previous paper [11], we have described the effects of phytosterols on plasma triglyceride concentration. The enhancement of acetyl-CoA carboxylase activity observed in group 4 is associated with an increase of plasma triglyceride concentration. On the other hand, when associated with the highest cholesterol dose, phytosterols decrease malic enzyme and acetyl-CoA carboxylase activities and exert a hypotriglyceridemic effect.

The decrease of liver fatty acids content reflects a decrease of liver lipid content, which has already been described after intraperitoneal injection of β-sitosterol [13].

The modifications of liver phytosterol concentrations are well known and only reflect the absorption of dietary phytosterols. The changes of liver cholesterol concentration may have two different origins. First, phytosterols play an inhibitory role in cholesterol digestive absorption in rats [9], in rabbits [22], in chicken [23, 24] and in man [25–27]. Second, phytosterols act on rate-limiting enzymes of sterols metabolism, HMG-CoA-reductase and cholesterol-7-α-hydroxylase. Nevertheless, this last point is still controversial: depending on the administration mode and dose, phytosterols either activate [28] or inhibit [12] or have no influence [10] on α-hydroxylase activity. In any case, phytosterols activate HMG-CoA-reductase activity. From these studies and from ours, it is likely that the administration mode is essential. When given in the diet, phytosterols do reduce plasma and hepatic cholesterol, provided the phytosterol/cholesterol ratio is at least 2 [11]. The mechanisms which are implicated in this phenomenon are both the reduction of cholesterol absorption [8] and the regulation of cholesterol metabolism key enzymes. It must be pointed out that phytosterols are more efficient when the cholesterol overload is important and that a minimal phytosterol/cholesterol ratio of 2 is required. Such a ratio might be obtained by supplementing the diet with phytosterols. This supplementation may be achieved in several ways, mainly through butter and margarines, as suggested by Pollak [29].

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