

CLINICAL RESEARCH

Effect of dietary cholesterol on plasma cholesterol concentration in subjects following reduced fat, high fibre diet

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Abstract

One hundred and sixty eight subjects participated in a randomised crossover study to determine whether halving or doubling the present dietary cholesterol intake from eggs had any influence on blood cholesterol concentration in people following current dietary recommendations. During the first eight weeks all participants were advised to follow a reduced fat diet (26% total energy for hyperlipidaemic patients, 35% total energy for normolipidaemic volunteers) with an increased ratio of polyunsaturated to saturated fatty acids. This background diet was continued throughout the 16 week experimental period, during which participants ate either two or seven eggs a week. A small but significant increase in total cholesterol was seen after four weeks in the group eating seven eggs a week compared with that in the group eating two eggs a week, but this was no longer apparent after eight weeks.

Previous studies suggesting that dietary cholesterol has a greater effect on the serum cholesterol concentration either have been carried out against a background of a higher fat intake or have contrasted extreme cholesterol intakes. A further reduction in dietary cholesterol seems to be unnecessary in those people who have already reduced their intake of saturated fat and increased the ratio of polyunsaturated to saturated fatty acids and fibre rich carbohydrate.

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Introduction

The results of several investigations have suggested that dietary cholesterol has an important influence on plasma cholesterol concentration,¹⁻³ to an extent that can be predicted with the Keys formula.⁴ Two research groups have found a few subjects whose plasma cholesterol concentration is particularly sensitive to increases in dietary cholesterol.⁵⁻⁷ Some authorities advise a specific reduction in dietary cholesterol because of the suggestion that the mean plasma cholesterol concentration of the population would be decreased by greatly reducing the dietary intake of the few people who are hyperresponders.⁸⁻⁹

Several features of previous studies, however, limit the generalisations that can be made from their results. Most have been short,¹⁻¹⁰ and several have included small numbers of participants.²⁻¹⁰⁻¹¹ Other investigations have contrasted quantities of cholesterol that are unlikely to be consumed in the long term.³⁻¹²⁻¹³ Finally, the background diet has been high in saturated fat, in contrast with the diet low in saturated fat that is currently recommended.¹⁴⁻¹⁵

Eggs are a major source of dietary cholesterol. We therefore examined the effects of halving and almost doubling the present national average intake of four eggs a week¹⁶ in a large group of volunteers who were following a low fat, high fibre diet to help to determine whether a reduction in dietary cholesterol should be included in dietary recommendations.

Subjects and methods

SUBJECTS

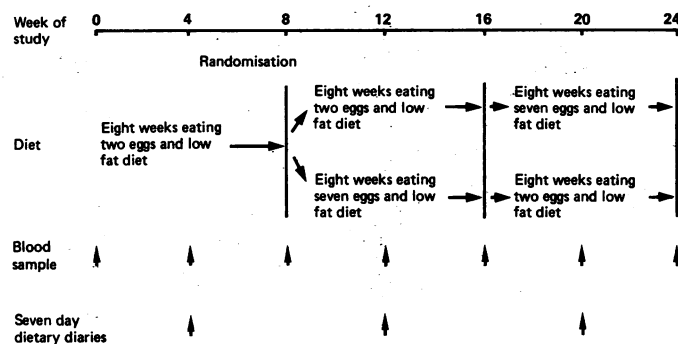
Two groups of participants were studied. One hundred and ninety four healthy volunteers were recruited by an advertisement in a local newspaper. Thirty five of these respondents were not included in the study because they had an intercurrent illness, were found to be hyperlipidaemic, or felt unable to participate once the details of the study had been explained. Twenty four other participants withdrew during the study because they felt unable to stick to the dietary protocol, and their data were excluded. One hundred and thirty five volunteers completed the study (27 men, 108 women).

Thirty three patients (20 men, 13 women) who had hyperlipidaemia and who attended the lipid clinic at John Radcliffe Hospital were also studied.

The diagnoses were: familial hypercholesterolaemia (five men, seven women), hypertriglyceridaemia (four men, one woman), familial combined hyperlipidaemia (three men), and common hypercholesterolaemia (eight men, five women). All had relatively stable plasma lipid concentrations before the study, and drugs for treating hyperlipidaemia were unchanged during the study. The participants gave informed consent, and the study design was approved by the central Oxford research ethics committee.

EXPERIMENTAL DESIGN AND DIETS

The figure shows the experimental design. The study lasted for 24 weeks and comprised an eight week run in period followed by two further eight week experimental periods. During the run in period diets were individually prescribed according to participants' energy requirements. The aim during



Design of experiment.

this period was to achieve a stable body weight on a low fat, high fibre diet that would be the background diet for the entire study. Table I shows the distribution of various nutrients in a 1500 kcal diet, the most commonly prescribed energy level. Individual energy requirements ranged from 1000 kcal to 3000 kcal, and nutrients were adjusted accordingly. The diets for the hyperlipidaemic patients were lower in total fat content and had a higher ratio of polyunsaturated fatty acids to saturated fatty acids than those recommended for the healthy volunteers, which were based on the recommendations of the panel of the Committee on the Medical Aspects of Food Policy¹⁷ but had a slightly higher ratio of polyunsaturated to saturated fatty acids.

After the run in period, during which all participants ate up to two eggs a week, subjects were randomised into two groups. The first group continued to eat two eggs a week, and the second increased its egg consumption to seven eggs a week. In the experimental diet one egg was substituted for one ounce of medium fat meat, or equivalent protein exchange, to keep the diet isocaloric and saturated fat intake constant. After eight weeks the two groups crossed over. The background diet remained unchanged throughout the 16 week experimental period.

Dietary advice was reinforced at regular intervals throughout the study. Each participant completed three dietary diaries, one beginning at week 4, one at week 12, and one at week 20—that is, halfway through each dietary period. These were used as one method of assessing dietary compliance. Analysis of these dietary diaries showed that the volunteers followed closely the dietary advice. In particular, participants found that modifying their egg intake was easy, and compliance with this aspect of the advice was excellent.

The mean age of all 168 participants was 45 (range 22 to 69). The mean (SD) body mass index at the beginning of the study was 24.8 (3.2) but decreased to 23.9 (3.1) (range 18.2-34.7) at the end of the run in period ($p < 0.05$). The body weights did not change during the experimental period.

LABORATORY MEASUREMENTS

Blood samples from fasted subjects were taken at recruitment and at every four weeks throughout the study. The total cholesterol concentration and the cholesterol concentration in the lipoprotein subfractions were measured with a Technicon AutoAnalyzer II. The total cholesterol concentration was measured enzymatically in diluted plasma with kits obtained from Boehringer.¹⁸⁻²⁰ Very low density lipoprotein cholesterol and low density lipoprotein cholesterol were precipitated with the heparin manganese method, leaving high density lipoprotein cholesterol in solution to be measured.²¹ In a second aliquot very low density lipoprotein cholesterol was precipitated with sodium dodecyl sulphate, leaving high density lipoprotein cholesterol and low density lipoprotein cholesterol in solution.²² The quantities of cholesterol in the various subfractions were then calculated by subtraction. The plasma triglyceride concentration was measured with an enzymatic method²³ with a CoBas-Bio centrifugal analyser manufactured by Roche Diagnostics.

In addition to the dietary diaries another indication of compliance with the diet modified in fat was obtained by measuring the fatty acid composition of serum triglyceride²⁴ at the beginning of weeks 0, 12, and 24. The triglyceride fraction was identified by thin layer chromatography, and the methyl esters of the fatty acids were measured by gas liquid chromatography. There was a sustained increase in the proportion of linoleic acid (C18:2), indicating compliance with the background dietary advice. Persuading the subjects to change their usual diet to one that was low in saturated fat and with a high ratio of polyunsaturated to saturated fatty acids was undoubtedly the most difficult aspect of the dietary experiment.

STATISTICAL METHODS

The significance of the differences in mean total cholesterol concentration and low density lipoprotein cholesterol concentration between the participants when they were eating two eggs and when they were eating seven eggs was assessed by Student's *t* test. Logarithmically transformed data were used. The mean differences after four and eight weeks of eating two or seven eggs and the 95% confidence intervals were also calculated.²⁵

Two approaches were used to determine whether there were any participants whose plasma cholesterol concentration was particularly sensitive to increases in dietary cholesterol. Firstly, the histograms of individual differences at the end of the periods of eating two eggs a week and seven eggs a week were examined for skewness, and, secondly, the prevalence of hyperresponders and hyporesponders was compared as suggested by Brongseest-Schoute *et al.*,⁵ Katan and Beynen,⁶ and Oh and Miller.⁷ In our calculations cholesterol concentrations that were more than two SD on either side of the mean were regarded as a hyporesponse or hyperresponse.

Results

Table II shows the mean total cholesterol concentrations and low density lipoprotein cholesterol concentrations and table III the mean differences and 95% confidence intervals. After four and eight weeks there were no significant differences in the mean concentrations between the two egg and seven egg periods for patients attending the lipid clinic. This was true for the whole group of patients and for the patients who had familial hypercholesterolaemia, who were examined separately because of the possibility that their response might differ from that of the other patients. In the group of healthy volunteers there were significant differences in the total cholesterol concentrations at the end of four weeks. Both the total cholesterol and low density lipoprotein cholesterol concentrations of the combined patients and subjects also differed significantly at the end of the four weeks. At the end of eight weeks, however, there were no significant differences in total cholesterol and low density lipoprotein cholesterol concentrations in the patients from the lipid clinic, in the healthy volunteers, or in both groups combined, and the variance about the mean was similar. There were no significant differences in high density lipoprotein cholesterol or plasma triglyceride concentrations at four or eight weeks.

TABLE I—Composition of 1500 kcal diet indicating difference in cholesterol intake during the two experimental periods

	Energy intake (kcal)	% Energy			Polyunsaturated: saturated fatty acids	Cholesterol (mg/day)		Fibre (g/day)
		Protein	Carbohydrate	Total fat		Seven eggs/week	Two eggs/week	
Hyperlipidaemic patients	1500	18	56	26	0.8	308	120	41
Healthy volunteers	1500	15	50	35	0.6	350	150	33

TABLE II—Mean concentrations* of total and low density lipoprotein cholesterol during study

	Mean (SEM) total cholesterol concentration (mmol/l)			Mean (SEM) low density lipoprotein cholesterol concentration (mmol/l)		
	Treated hyperlipidaemic patients (n=33)	Normolipidaemic subjects (n=135)	Total group (n=168)	Treated hyperlipidaemic patients (n=33)	Normolipidaemic subjects (n=135)	Total group (n=168)
Initial pretreatment	6.83 (0.03)	5.45 (0.02)	5.70 (0.02)	4.69 (0.04)	3.35 (0.03)	3.57 (0.02)
After four weeks:						
Of eating seven eggs	6.78 (0.03)	5.32 (0.02)†	5.57 (0.02)†	4.68 (0.05)	3.20 (0.03)	3.43 (0.03)†
Of eating two eggs	6.72 (0.05)	5.17 (0.02)†	5.43 (0.02)†	4.59 (0.05)	3.07 (0.03)	3.31 (0.03)†
After eight weeks:						
Of eating seven eggs	6.66 (0.03)	5.33 (0.02)	5.57 (0.02)	4.68 (0.05)	3.15 (0.05)	3.41 (0.03)
Of eating two eggs	6.52 (0.04)	5.23 (0.02)	5.46 (0.02)	4.63 (0.05)	3.03 (0.03)	3.30 (0.03)

*Mean concentrations of log transformed data.
†p<0.05, Student's paired t test.

TABLE III—Mean difference (95% confidence interval) in total and low density lipoprotein cholesterol concentration between patients eating two eggs a week and those eating seven eggs after four and eight weeks

	Mean difference in total cholesterol concentration (mmol/l)			Mean difference in low density lipoprotein cholesterol concentration (mmol/l)		
	Treated hyperlipidaemic patients (n=33)	Normolipidaemic subjects (n=135)	Total group (n=168)	Treated hyperlipidaemic patients (n=33)	Normolipidaemic subjects (n=135)	Total group (n=168)
After four weeks	0.05 (-0.25 to 0.36)	0.15 (0.04 to 0.26)	0.13 (0.03 to 0.23)	0.06 (-0.18 to 0.31)	0.12 *	0.11 *
After eight weeks	0.13 (-0.11 to 0.37)	0.12 (0 to 0.24)	0.12 (0 to 0.23)	0.06 (-0.16 to 0.27)	0.13 (0 to 0.26)	0.12 (0 to 0.23)

* Confidence intervals not calculated because of skewed distribution.

TABLE IV—Prevalence of changes in low density lipoprotein cholesterol concentration after four weeks

Change (mmol/l):	-1.3	-1.2	-1.1	-1.0	-0.9	-0.8	-0.7	-0.6	-0.5	-0.4	-0.3	-0.2	-0.1	0	+0.1	+0.2
Prevalence:	2	2	4	4	3	5	1	9	3	10	6	3	13	9	10	10
Change (mmol/l):	+0.3	+0.4	+0.5	+0.6	+0.7	+0.8	+0.9	+1.0	+1.1	+1.4	+1.5	+1.6	+1.7	+2.2	+2.7	
Prevalence:	12	9	8	1	6	12	3	5	4	1	1	2	1	1	1	

The total cholesterol concentration of individual participants varied in response to the ingestion of seven eggs a week compared with two eggs a week by -21% to +44% after four weeks and by -23% to +49% after eight weeks. Further analysis, however, produced no evidence for a group of hyperresponders at the end of eight weeks of eating seven eggs. Histograms of the differences in total cholesterol and low density lipoprotein cholesterol concentrations of participants after eating two and seven eggs a week were plotted. The differences in total cholesterol concentration were not significantly skewed in any group at any time. At the end of four weeks the differences in low density lipoprotein cholesterol concentrations were significantly skewed in the normolipidaemic subjects (coefficient of skewness $g_1=2.38$) and in the whole group ($g_1=2.11$, table IV), but after eight weeks this skewness was no longer apparent in either the normolipidaemic subjects or the whole group. The differences in the hyperlipidaemic subjects were not significantly skewed at any time.

Discussion

A reduction in the total and saturated fat intake reduces the plasma concentration of total cholesterol and low density lipoprotein cholesterol and forms the cornerstone of national dietary recommendations aimed at lowering the risk of coronary heart disease. Most dietary recommendations also include advice to increase the ratio of polyunsaturated to saturated fatty acids to reduce further the total cholesterol and low density lipoprotein cholesterol concentrations.^{26 27} These changes may also modify haemostatic variables known to be associated with coronary heart disease.²⁸

The relation between dietary cholesterol and total plasma cholesterol concentration is less clear. Reducing saturated fat in the diet usually reduces cholesterol intake, but advice concerning a

further reduction in dietary cholesterol is a particularly controversial feature of current dietary recommendations. The suggestion that dietary cholesterol should be restricted is based on two premises. The first, based on the Keys formula⁴ and several other feeding experiments, is that decreasing dietary cholesterol will decrease serum cholesterol concentration. The second is that there is a small population of hyperresponders whose serum cholesterol concentration rises significantly with small increases in cholesterol from food. There are, however, difficulties associated both with the interpretation of the formula and feeding studies and with the definition of hyperresponse.

The Keys formula⁴ is a mathematical method of predicting change in plasma cholesterol concentration as a result of dietary change. With this formula the predicted increase in total cholesterol concentration in patients eating seven eggs a week compared with two is 0.2 mmol/l. After eight weeks we found a mean difference of 0.1 mmol/l (95% confidence interval 0 to 0.23).

Where this formula has been used to predict change, modifying the intake of dietary cholesterol has usually resulted in a greater change of both predicted and observed cholesterol concentrations. Earlier investigations, however, did not include studies of the effects of dietary cholesterol taken as part of a diet in which several other features were capable of lowering cholesterol concentration (low saturated fat intake, increased ratio of polyunsaturated to saturated fatty acids, high fibre intake). Most have been carried out in subjects consuming a background diet with an appreciably higher fat content than that currently recommended, and extreme quantities of dietary cholesterol have been contrasted. The present study was designed to investigate the effects on plasma cholesterol concentrations of a contrast between people eating two and seven eggs a week and otherwise following a diet low in saturated fat, with

a modest increase in the ratio of polyunsaturated to saturated fatty acids, and high in dietary fibre. The contrast was intended to provide the basis for practical recommendations and entailed comparing the results of halving and almost doubling the present national average egg intake. The findings suggest that if the current nutritional guidelines are followed there is negligible additional benefit to be gained from restricting the intake of eggs and little disadvantage in a modest increase.

The results of this investigation also underline the importance of longer term studies. After four weeks we noted a small but significant difference in the total plasma cholesterol concentration in the healthy volunteers and in the total cholesterol and low density lipoprotein cholesterol concentrations in the whole group; these differences were no longer evident after eight weeks of follow up. Short term changes, reflecting a transient response with insufficient time for metabolic adaptation, have occurred after other dietary changes—for example, temporary hypertriglyceridaemia after a change to a high carbohydrate diet²⁹—and should not form the basis of dietary recommendations. The duration of the study may also explain why we have been unable to define clearly a population of hyperresponders. The skewed distribution of differences in total cholesterol and low density lipoprotein cholesterol concentrations between the two egg and seven egg periods, seen after four weeks but not after eight weeks, suggests that hyperresponse may be temporary. Most studies have not attempted to define hyper-response, so it is difficult to compare data.

Our data do not exclude the possibility that dietary cholesterol may have a greater influence on the serum cholesterol concentration when fed as part of a diet high in saturated fat or when more extreme intakes are compared; nor do they exclude the occurrence of hyper-response under such circumstances. Dietary cholesterol in substantial quantities may influence the composition of the intermediate density lipoproteins with atherogenic potential. Connor *et al* have suggested a cholesterol/saturated fat index for calculating the hypercholesterolaemic-atherogenic potential of food.³⁰ Our findings suggest that the cholesterol/saturated fat index may not be helpful in determining foods with a high atherogenic potential when they are taken as part of a low fat diet: two eggs have a cholesterol/saturated fat index of 29 whereas 100 g of high fat meat has a value of 18. Calculation of the cholesterol/saturated fat index is based largely on the cholesterol content of the food. In the present recommendations, in which cholesterol intake has already been reduced by decreasing saturated fat intake, there seems little to be gained by further emphasising reductions in the intake of foods high in cholesterol such as eggs.

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100 YEARS AGO

Most people who have been to the "Wild West" show at Earl's Court must have been struck by the enormous vocal power displayed by Mr. Frank Richmond, the "Orator," who explains the action of the realistic drama so vividly presented by Buffalo Bill's cowboys and redskins. The voice of this modern Stentor is a physiological curiosity in its way, for he has often made himself distinctly heard by an audience of thirty thousand persons in the open air. This throws Mr. Gladstone's famous "record" at Blackheath in 1874 altogether into the shade. Some idea of the vast amount of work which the "Orator" gets out of his vocal organs may be formed from the fact that his running commentary on the show contains more words than the part of Hamlet, which, as is well known, taxes the powers of the best trained actors. This severe effort the "Orator" makes—and makes successfully—twice a day for months together, under much less favourable acoustic conditions than players even in the largest theatres. A few physical details respecting such a vocal athlete may, therefore, be interesting to some of our readers. For these we are indebted to the courtesy of Dr. Robert C. Myles, of New

York, whose examination, it may be added, was confirmed by Sir Morton Mackenzie. The vocal cords are of ordinary length, and not much above the average in breadth, but the vocal processes at once strike the observer by their extraordinary development. They project inwards towards the middle line like two large spurs when the glottis is open. The great leverage thus given to the laryngeal muscles allows them to act to the best advantage with a minimum of effort. The larynx itself is of a large size, and the pharynx is exceptionally roomy and well developed, whilst the mucous membrane covering it is remarkably free from granulations and roughness of any kind. The "Orator's" vital capacity is not above the ordinary standard, but what breath-power he has he utilises to the utmost with the art of a trained elocutionist. Mr. Richmond, we believe, was on the stage before he occupied his present position, and the secret of his remarkable delivery lies more in the perfection with which he had learned to use his natural advantages than in any notable peculiarity of physical conformation. (*British Medical Journal* 1887;ii:732.)