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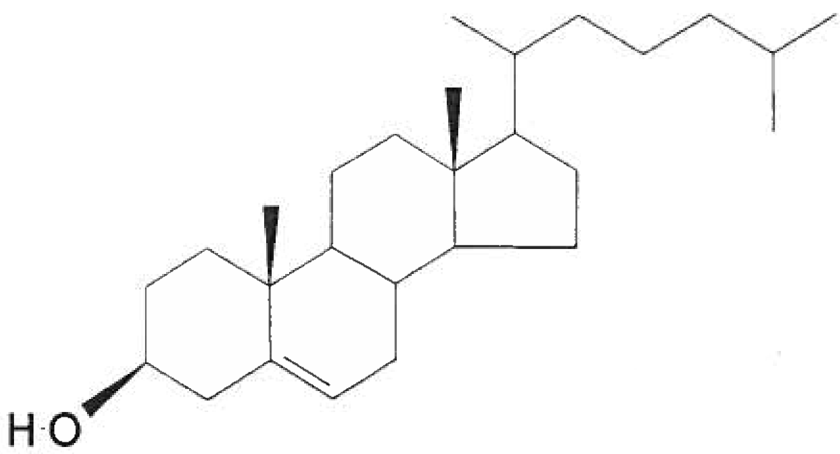

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Review Article

Cholesterol Removal From Animal Food—Can It Be Justified?

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Of the indicators of increased early-onset coronary heart disease, raised serum cholesterol is a risk factor or risk marker, but not the cause. Strategies to reduce morbidity and mortality from coronary heart diseases generally involve reduction of plasma total and low density lipoprotein cholesterol through lowering the intake of total and saturated fat and of dietary cholesterol. Several methods for the reduction of cholesterol in commonly eaten foods have been developed successfully over the past few years with the aim of lowering dietary cholesterol. These approaches are biological (microorganisms, enzymes), chemical (solid-liquid-extraction, extraction with organic solvents, complex formation) or physical processes (distillation, crystallization, supercritical fluid extraction). While the application of these procedures to animal foods, especially to dairy products, is technically feasible, the relatively small contribution of dietary cholesterol to raised plasma cholesterol does not appear to justify the costs involved or to be scientifically justified.

Introduction

Cholesterol is an extremely important animal steroid and is found in all cells and tissues of animals and humans. The average adult human being contains roughly 150 g of cholesterol and synthesizes 700–1500 mg of cholesterol daily to replenish faecal and other losses. Cholesterol has a number of vital functions. In the intestine cholesterol (as bile acids) modulates the absorption of dietary fat. It is a major component of cell membranes and of nerves. Cholesterol is also the precursor for the steroid hormones of the cortex of the suprarenal gland, of male and female sexual hormones, vitamin D and is essential for the growth and development of young mammals (1,2).

While cholesterol is of profound biological importance, between 1950 and 1960, evidence was being gathered to suggest that an excess might be deleterious. A positive correlation was found between increased serum cholesterol concentrations and an increased risk of coronary heart disease (CHD). Animal and human experimentation showed that plasma cholesterol was raised by increased intake of saturated fat and cholesterol. The lipid or cholesterol hypothesis of coronary heart diseases developed from these findings. This hypothesis states that high dietary intakes of cholesterol and/or total or saturated fatty acids coupled with insufficient dietary polyunsaturated or monounsaturated fatty acids can raise total and low density lipoprotein (LDL) cholesterol concentration level. Through the development of atherosclerosis, CHD morbidity and mortality is increased. It should be noted that this is not a trivial problem for public health; in most developed industrialized countries CHD is a major cause of death and disability (see 3–5).

The relationship between raised plasma total and LDL cholesterol and CHD risk is widely accepted. Strategies to lower CHD routinely concentrate on reducing plasma cholesterol concentrations through dietary change (6–10). However, it must be emphasized that such programs do not focus exclusively on plasma lipids but also include risk reduction through control of blood pressure, cessation of smoking, body weight control and increased physical activity (11). As well as targeting at-risk groups, these programs also seek to educate the general population on the importance of a balanced diet. The degree of intervention varies with serum cholesterol concentration. People with concentrations >200 mg/dL (5.2 mmol/L) are considered to be at increased risk and to need attention. For most people with serum cholesterol concentrations of 200–240–250 mg/dL (5.2 to 6.2 or 6.5 mmol/L) dietary advice and reduction of other risk factors is appropriate (6,8,12). Above this higher figure, cholesterol reduction through drug therapy is probably necessary. The basic principles of a dietary treatment are the control of obesity through reduction of energy intake (and exercise) and modification to lower plasma cholesterol (and triacylglycerols, if necessary). The following dietary recommendations are said to be critical (10):

Animal fats should be replaced by plant oils containing polyunsaturated fatty acids;

The intake of dietary cholesterol is to be limited to 300 mg per day maximum;

Preference to be given to foods that are rich in dietary fibre and complex carbohydrates; and,

Try to eat fish regularly.

Medication is only advisable if dietary change alone does not lower the serum cholesterol.

Cholesterol Removal from Foods

The historical importance attached to dietary cholesterol as a possible causative factor in human hypercholesterolemia and so to increased risk of CHD has led to certain foods being regarded as bad and to consumer preference for low-cholesterol and cholesterol-reduced foods. However, the concept that certain foods are free of cholesterol is far too simplistic. Cholesterol is a natural component of many foods but at widely different levels. Plants although low in cholesterol, do contain some and fruits, seeds and leaf lipids contain very small quantities of cholesterol in addition to plant sterols (13,14). Vegetable oils contain mainly phytosterols (avenasterol, campesterol, sitosterol, stigmasterol and others) but cholesterol has been found at concentrations of 5 to 30 mg per kg. Of course, cholesterol is mainly found in animal foods (**Table 1**), principally in the plasma membranes, lipoproteins and fats. The highest cholesterol levels are found in the brain, bone marrow, kidney and liver (15). Cholesterol is present in the milk of all mammals at modest concentrations which vary not only from one mammal to the other, but also within the same species (16). During the commercial processing of milk, the cholesterol travels with the bulk fat to the product; 1 g of milk fat generally contains about 3 mg of cholesterol.

Table 1 Cholesterol content of different foods (15)

Food		Fat content (g/100 g)	Cholesterol content (mg/100 g)
Human milk		4.0	25
Cow's milk		3.8	12.3
Skim milk		0.1	3
Cream		31.7	109
Yoghurt		3.8	12.2
Cheese:	Brie	27.9	100
	Camembert	22.3	62
	Cheddar	32.3	100
	Blue cheese	29.8	88
	Emmental	29.7	92
	Fresh cheese	31.5	103
	Gouda	29.2	114
	Parmesan	25.8	68
	Quark	5.1	17
Egg	whole	14.4	396
	yolk, liquid	31.9	1260
Butter		83.2	240
Tallow		96.5	100
Lard		99.7	86
Veal	muscles only	0.8	70
	liver	4.1	360
	kidney	6.4	380
Beef	muscles only	1.9	60
Pork	muscles only	1.9	65
	liver	5.7	340

Processes for Cholesterol Removal from Food

A natural consequence of the consumer preference for products low in cholesterol has been the development of cholesterol-reduction technology and biological, physical and chemical methods have become available to achieve this aim (**Table 2**).

Table 2 Methods for cholesterol removal from fats (17)

Method	Process	Mechanism
Biological:		
microorganism	*Rhodococcus* *Nocardia*	transformation
enzyme	cholesterol reductase cholesterol oxidase	transformation
Chemical:		
solid-liquid	silica gel	absorption
extraction	activated carbon	adsorption
complex formation	β-cyclodextrine	emulgation
	bile salt	adsorption
Physical:		
distillation	steam distillation short path distillation	fractions
crystallization	fractionation	low and high melting lipids
supercritical fluids	extraction with carbon dioxide	fractions

Biological processes

Enzymes and microorganisms have been used for lowering cholesterol in foods. In nature there are a number of cholesterol-degrading bacterial species, principally *Nocardia* and *Rhodococcus* (18,19). These have been isolated from butter, bacon, pork fat and chicken fat and also from soil (18). In these (and other) bacteria, cholesterol is modified through various enzymes, principally cholesterol oxidase(s) and reductase(s). Cholesterol oxidase has been isolated from *Nocardia erythropolis* (20,21), *Nocardia rhodochrous* (22), *Rhodococcus equi* or *erythropolis* (18,23) and *Streptomyces* sp. (24). These enzymes were shown to be of the intrinsic (membrane-bound) type. Extracellular enzymes have also been described in the broth filtrates of *Streptomyces violascens* (25), *Brevibacterium sterolicum* (26), *Streptoverticillium cholesterolicum* (27) and *Rhodococcus equi* (23,28). The strain *Rhodococcus equi* no. 23 isolated from butter produced larger amounts of extracellular cholesterol oxidase than the strain no. 33, isolated from bacon (23). A culture of suspension of *Nocardia labegensis* appears to have potential for the biological degradation of sterols in animal and vegetable fats (29). Microcapsules containing cholesterol degrading microorganisms may also be used to reduce the cholesterol levels in liquid foods (30).

The use of bacteria to lower cholesterol in foods raises some important issues. Firstly, there is the question of bacterial inoculation of the food which may be of consumer concern. Moreover, some of the species may be pathogenic and, in fact, *Rhodococcus equi* has been identified as an important pathogen (31). Lately it has been suggested to identify the genes for the enzyme cholesterol-reductase and to transfer them by genetic engineering into the lactic acid bacteria to be used as starter cultures for cheese manufacture (32). This

would obviate the problems associated with the use of bacteria not used in conventional food processing.
Equally important is the question of harmful effects of the cholesterol by-products of the bioconversion. When cholesterol is reduced to coprostanol, this does not seem to be a problem as it is absorbed poorly or not at all (33). Cholesterol reductase from *Eubacterium* effects this change. *Rhodococcus* strains catalyse the conversion of cholesterol to 4-cholesten-3-one (18) whose appearance and disappearance differs according to the strain used (34). In the case of oxidized cholesterol, the problem is more serious. Now, much attention has been focused on the role of oxidatively-damaged lipoproteins in human atheroclerosis (35,36). Furthermore, it has been suggested that oxidized cholesterol is itself toxic (37). This problem appears to be potential rather than actual as only 4-cholesten-3-one (23,38), not 7-keto- or 7-β-hydroxycholesterol (39) has been found.

Physical processes

Distillation and crystallization methods. Cholesterol is soluble in steam so treatment of fat with steam stripping technology can remove a large fraction of it. This technique allows reductions of up to 95% of the cholesterol in fish oils and anhydrous butter fat. The procedure is quite simple. The fat is first de-aerated, mixed with steam, heated and then flash vaporized with subsequent cholesterol removal by thin-film stripping with a countercurrent of steam. The product is then cooled and stored in the absence of oxygen (40).
Short-path distillation and melt crystallization are processes to fractionate fat (41,42). The first process consists of evaporation of fat into a substantially gas-free space. In the latter procedure, the fat is liquified and then cooled to different temperatures to crystallize different components which are separated from the liquid phase. Milk fat is essentially a mixture of different triglycerides with varying physical properties (including melting point) so it is possible to fractionate it into fractions with different chemical compositions and also different cholesterol levels (43).

Supercritical fluid extraction. There is a relatively new technique in the food industry (44). In this process, a product is treated with gas (usually carbon dioxide) of high density, low viscosity and reduced surface tension under high pressure and at high temperatures. The technique has been applied for delipidation of protein, de-oiling of lecithin, isolation of important oils, fractionation of butterfat, decaffeination of coffee, oil extraction from oilseeds, removal of bitter aroma compounds from hops and also removal of cholesterol from different foods (45–51). The procedure has the great advantages of low energy costs, higher yields, products which are of superior quality because of the use of lower temperature, the absence of potentially explosive and toxic solvents and that no toxic by-products are formed (45,52).

Chemical processes

Solid–liquid extraction. Cholesterol can be removed from liquid foods by selective solid extraction with adsorbents such as activated charcoal, coated porous glass, ceramic and plastics (53–56). Silica gel may also be used as an adsorbent (57).

Complex formation. β-cyclodextrin, a cyclic oligosaccharide of seven glucose units, forms an insoluble inclusion complex with cholesterol (58). Starch-containing products also can form such complexes (59). All of these inclusion complexes are stable in aqueous solutions which permits cholesterol removal from another (lipid) phase. Incidentally β-cyclodextrin can be fermented by the human colonic flora (60).
It is also possible to minimize cholesterol in anhydrous milk fat with an aqueous solution of bile salts and one or several glyceryl esters. The resulting emulsion is decanted and the aqueous phase (containing the extracted cholesterol) is then separated. After repeated extractions, the cholesterol content can be reduced by up to 97% (61).
Polymer-supported saponins such as digitonin (consisting of the aglycon digitonenin linked to a pentasaccharide) or tomatine (composed of a polycyclic steroidal secondary amine, tomatidine, and a tetrasaccharide) form insoluble cholesterol complexes which can be removed by filtration or centrifugation. The polymers may be regenerated by benzene extraction which restores their original cholesterol binding capacity (62–64).

Cholesterol Removal from Different Foods

The processes described in world patent and scientific literature relate mainly to the removal of cholesterol from fats and oils. The world-wide attempts to produce low-cholesterol products have generated many patents which show collectively that these endeavours are supported by important economic interests (**Table 3**). Most of these patents are concerned with cholesterol removal from milk fat. The preferred methods are complex formation with β-cyclodextrin, distillation and extraction with supercritical carbon dioxide. Some examples of cholesterol removal from animal foods are given below.

Eggs
Egg yolk is high in cholesterol and a number of techniques have been applied to its removal. Bioconversion with *Rhodococcus equi* as well as treatment with extracellular and cell-linked enzymes of strain no. 23 can be used for the removal of cholesterol from egg yolk (19,112,113). Extraction with organic solvents removes all the lipids from egg yolk, including cholesterol as well as the phospholipids (**Table 4**). Following extraction with a mixture of hexane and isopropanol, the crude egg oil which is then degummed, refined and bleached results in a product containing less than 40% of the cholesterol present in the original, i.e. 24 mg/g

Table 3 Patents for cholesterol removal from fats

Process	Fat of: Milk	Egg	Fish	Meat	Other
Biological					
microorganism	Chosson (29) Nippon (65)		Nippon (65)	Nippon (65)	Chosson (29) Nippon (65)
enzyme	Saito (66)	Saito (66)			Saito (66)
Chemical					
solid-liquid extraction:					
– silica gel	Herrmann (57)	Cully (67)	Croda (68)		Croda (68)
– activated charcoal	Keen (53,55) Athnasios (69)		Keen (53) Athnasios (69)		Keen (53,55) Athnasios (69)
complex formation: – β-cyclodextrin	Oakenfull (70) Courregelongue (58,71) Mentink (72–75) Graille (76–79) Bayol (80)	Bayol (80) Cully (81,82)	Mentink (72–75)	Mentink (72–75)	Courregelongue (58,71) Oakenfull (70) Mentink (72,74) Bayol (80) Hohnen (83) Morinaga (84) Roderbourg (85)
– saponin	Richardson (86) Schwartz (87)				
– bile salt	Montet (88)				Lindheimer (61)
other:	Keen (56)			Keen (56)	Keen (56)
– organic solvent	Johnson (89)				Snow (90,91)
– salt or polybase	Roczniak (92) Wrezel (93)		Wrezel (93)	Wrezel (93)	Wrezel (93)
Physical					
distillation:	Mont (94) Tirtiaux (95) Hoche (96,97) Conte (98) Perry (99) Johnson (100) Marschner (101–103) Expanchimie (104)	Marschner (101–103)	Hoche (96)	Hoche (96)	Mont (94) Tirtiaux (95) Hoche (96) Johnson (100) Marschner (101–103)
supercritical gas	McLachlan (105,106) Ryoshoku (107) Cully (67)	McLachlan (106) Cully (67) Klemann (108,109)		McLachlan (106) Klemann (108,109)	McLachlan (106) Klemann (108,109)
other	Herrmann (57)	Bracco (110)			Chapman (111)

Table 4 Cholesterol removal from foods

Product		Condition	Cholesterol concentration (mg/g): Before	After	References
egg yolk oil	E	ethanol/hexane/water 1:2:1:1	35.2	7.5	Tokarska (114)
egg yolk	E	hexane/isopropanol 3:2, separation on silicic acid column	4 fractions A–D	B and D: chol.-free A: 2.39 C: 36.10	Blackwelder (115)
dried egg yolk	S	163 atm/40 °C	18.52	15.54	Froning (116)
		238 atm/45 °C		13.33	
		306 atm/45 °C		6.38	
		374 atm/55 °C		6.34	
trout fish muscle	S	27.6 MPa/50 °C with ethanol	3.1[a]	0.1[a]	Hardardottir (46)
			3.1[a]	<0.01[a]	
ground beef	S	172 bar/40 °C	1.41	1.11	Chao (117)
		172 bar/50 °C	1.33	0.81	
		310 bar/35 °C	1.34	0.99	
		310 bar/50 °C	1.37	0.86	
dehydrated beef	S[b]	0.85 g/cm³, 45 g/g	1.56	0.69	Wehling (118)
		0.90 g/cm³, 30 g/g	1.56	0.49	
		0.90 g/cm³, 45 g/g	1.56	0.19	

E = extraction; S = supercritical carbon dioxide; [a] = g/g dry matter; [b] = extraction temperature at 55 °C; g/cm³ = density of CO_2; and number of grams of CO_2 used per gram of sample.

(119). Supercritical extraction with carbon dioxide has also been used to separate cholesterol from egg yolk (114,116). It is also possible to decrease cholesterol concentrations in egg yolk by feeding pullet or laying hens with a diet supplemented with lovastatin (120,121).

Fish

Supercritical fluid extraction with and without ethanol removes 97 and 99% of the cholesterol, respectively, from fish muscle, but the solubility of muscle proteins is decreased (46) (**Table 4**).

Meat

Extracellular and cell-linked enzymes of the strain *Rhodococcus equi* no. 23 were used to remove cholesterol from pork fat. After 48 h of incubation of the pork fat with extracellular enzymes or with a suspension of bacterial cells, 5 and 13% of the original cholesterol, respectively, were present (122). Continuous extraction with β-cyclodextrin seems to remove up to 80% of the sterols from pork and beef fat (58,71). Cholesterol and fat can be removed from dehydrated beef products and ground beef by extraction with supercritical carbon dioxide due to the physical nature and low moisture contents of these products, but probably not from intact muscle (**Table 4**). Chao *et al.* (117) found that up to 39.8% of the cholesterol and 37.9% of the total lipid could be removed during extraction at 172 bar/50 °C of ground beef. Under other pressure conditions, there was a similar cholesterol reduction, but 71.2% of the lipid was removed. In another report, the levels of cholesterol and total lipid in dehydrated beef powder were both reduced by more than 87% (118). Extraction of edible beef tallow by supercritical CO_2 with pressure of 345 bar at 40 or 50 °C resulted in a 60–70% reduction in cholesterol (123). After taste panel evaluation, the extracted beef powders had slightly better scores for beef flavour and overall acceptability than the control, because compounds responsible for the 'beefy' flavour are apparently not removed (118).

Milk and dairy products

The methods for cholesterol removal from milk fat and butter have been reviewed extensively (17,124–127, 188). As one would expect, most of the available techniques have been tested. Anhydrous milk fat has been incubated at 28 °C for 12 h with *Nocardia* with a reduction of cholesterol content of 86 to 89% (29); in complete contrast only 7% of the cholesterol was removed after 15 h of incubation of cream with an extract of *Rhodococcus equi* (19). In milk treated by cholesterol oxidase, the cholesterol concentration was halved within 10 h at 3 and 7 °C (38) or was reduced by 78% within 3 h at 37 °C (39).

Cholesterol has been removed from milk and dairy products by a β-cyclodextrin-based process and the resulting low-cholesterol butter and cheese appear to be indistinguishable from conventional products (128). Multiple extraction with β-cyclodextrin allows the removal of 41% of the sterols from anhydrous butter (58,71). Pilot experiments describing cholesterol removal from cream and anhydrous butter fat using polymer-supported saponins have been reported recently (64,129).

Milk fat can be fractionated by short-path distillation (43) and crystallization (42,43,130). After short-path distillation, the solid fraction contains cholesterol at a concentration of 0.2 mg/g fat compared with 2.6 mg/g fat in native milk fat or to 16.6 mg/g fat in the liquid fraction (43). After melt crystallization, the concentration of cholesterol in the liquid fraction (3.8 mg/g fat) is nearly twice that in the solid fraction (2.0 mg/g fat). This may be the result of the higher affinity of cholesterol for the triglycerides of the liquid fraction (43).

Milk fat can also be fractionated by supercritical carbon dioxide giving fractions with differing cholesterol levels (43,48,51,131–134). The first experiments in this area were described by Kaufmann *et al.* (48) at a pressure of 200 bar at 80 °C. The cholesterol content in the liquid extract was 5.5 mg/g, in the solid extract 2.3 mg/g and 2.6 mg/g in the original fat. Kankare *et al.* (51) used pressure conditions at 120, 150 and 200 bar at 48 °C and afterwards the cholesterol was concentrated in the first of three extracts. Bradley (131) obtained fractions in which the cholesterol content was reduced by up to 90% of that in the original using increasing pressure and different quantities of carbon dioxide. Shishikura *et al.* (133) decreased the cholesterol content of supercritical carbon dioxide-extracted butter oil to less than one quarter of the original by passing the extract through a silica gel column. Fractions of butteroil obtained at 40 °C, 17.2 and 20.7 MPa had lower cholesterol levels (1.07 and 1.76 mg/g) than those obtained at 10.3 and 13.8 MPa (2.96 and 2.78 mg/g) (134). Chidambara Raj *et al.* (135) have pointed out that with a processing plant capacity 240 or 800 tonnes/year, the fractionation of anhydrous milk fat with supercritical carbon dioxide will add $0.75 or $0.34 per kg of product.

Cholesterol-Reduced Foods—a Perspective

The extensive published literature as well as the numerous patents show that cholesterol removal from food is now possible economically and technically. While all animal foods contain cholesterol and some (e.g. eggs and certain shellfish) contain relatively high concentrations, most of the focus has been on reducing the cholesterol in milk fat. Corman (85) and Entremont (136) have used β-cyclodextrin while Hoche (96,97) employed a physical process to reduce cholesterol-reduced milk fat products, both on a commercial scale (136). In the U.S.A. OmegaSource (137) has modified a cholesterol stripping technique for an industrial pilot-plant. Taste panel evaluations and a preliminary market study showed that American consumers accepted low-fat fluid milks containing cholesterol-reduced milk fat (138) and a butter made with low-cholesterol milk fat (139). Experimental production of

Table 5 Influence of dietary cholesterol on plasma lipid and lipoprotein cholesterol levels in 25 volunteers in a cross over-study (148)

		Saturated fat		Polyunsaturated fat	
Cholesterol intake	unit	low	high	low	high
P/S of dietary fat		0.34	0.26	1.98	1.43
Dietary cholesterol intake	mg/d	272	836	183	815
Absorption	%	60	54	59	57
Plasma lipid: cholesterol total	mg/dL	240	245	227	230
LDL-Cholesterol	mg/dL	144	154	136	142
HDL-Cholesterol	mg/dL	44	44	43	45
Triglyceride	mg/dL	210	205	196	180

cheese from cholesterol-reduced milk fat has also been described (140). It should be noted that legal aspects of such dairy products and foods with a reduced cholesterol content are under discussion and it will be of great interest to see how these products are treated in terms of food labelling (141–143).

However, given the wide interest in cholesterol reduction and the economic considerations of extra processing, we need to examine whether the process as applied to key foods can be scientifically justified.

Cholesterol in the Human Organism

Although the lipid hypothesis of coronary heart diseases has won wide acceptance, it has many limitations and deficiencies which have been discussed in detail by Gurr (5), Stehbens (144) and also recently by Ravnskov (145). The critical question is: does a lower intake of dietary cholesterol contribute significantly to lowering of plasma cholesterol and so to a reduced risk of coronary disease?

Influence of dietary cholesterol on serum cholesterol

Much of the early work on dietary influences on plasma cholesterol was done on model species and systems. One commonly used model was the rabbit which is exquisitely sensitive to dietary cholesterol and shows severe hypercholesterolemia in response to modest supplementation (146). The common marmoset (*Callithrix jacchus*) is another responsive species (147). In marked contrast, humans and other model species (e.g. rats) are far less responsive. The intestinal absorption of dietary cholesterol by humans is far from complete. The rate of absorption rate depends on a number of factors including the total dietary cholesterol load. McNamara *et al.* (148) showed in 75 human volunteers that there was a fractional absorption of about 60% at a daily intake of 200–300 mg of cholesterol. When the load was increased to 800 mg/d the fractional absorption was only 55% (**Table 5**). In these subjects, an increase in dietary cholesterol supply had no substantial effect on the plasma lipids. Indeed, it is now accepted that for most people the influence of dietary cholesterol on plasma cholesterol is really quite small. Hopkins (149) has shown that baseline dietary cholesterol is a major determinant of the response. However, the response is greatly modified by a number of factors. One potential variable is the type of dietary fat, i.e. polyunsaturated or saturated fatty acids, although this does not seem to influence cholesterol absorption significantly. Kestin *et al.* (150) showed in a double blind test on 25 healthy men, who were given an amount of cholesterol equivalent to two egg yolks that there was little or no change in plasma cholesterol. They concluded that limitation of cholesterol intake was unnecessary in the general population, but only to persons who had hypercholesterolemia insensitive to dietary treatment.

Moreover the synthesis of cholesterol by the human organism is under feed-back control (148). Increased dietary cholesterol supply decreases by 20% the rate of endogenous cholesterol synthesis.

The general population responds to increased cholesterol intake through lower intestinal absorption and/or suppression of endogenous synthesis and/or increased sterol excretion and/or increased deposition in the tissues. In most people the compensation is such that the influence of dietary cholesterol is minimal. They can react as compensators (or hyporesponders) to dietary cholesterol. In the study of McNamara *et al.* (148) only 31% of the test subjects showed an increased serum cholesterol level after a higher dietary cholesterol intake. The serum cholesterol of the compensators remained unchanged after the cholesterol intake had changed from low to high (**Table 6**). In this group the cholesterol synthesis in the mononuclear leukocytes clearly decreased by 26%. This indicates that most individuals have a precise feedback control mechanism and are in a position to maintain serum cholesterol at a relatively constant level when the cholesterol supply changes moderately. However, there are non-compensators (or hyperresponders) who exhibit raised cholesterol following a dietary cholesterol load but in any population their numbers are relatively small.

Further arguments against cholesterol removal from animal food

There are several objections to be raised to the second part of the lipid hypothesis, which concern the relationship between serum cholesterol and atherosclerosis as well as coronary heart diseases. The etiology of heart-circulation diseases are multifactorial and comprise various risk factors independent of each other. Based on the Multiple Risk Factor Intervention Trial Study (MRFIT study) including about 13,000 men with high risk (hypercholesterolemia, high blood pressure, smok-

Table 6 Influence of dietary cholesterol on plasma cholesterol levels and sterol synthesis rates at compensators and noncompensators (148)

		Compensators (n = 52)		Noncompensators (n = 23)	
Cholesterol intake	unit	low	high	low	high
Plasma cholesterol	mg/dL	237 ± 45	234 ± 41	214 ± 58	240 ± 63
Increase	%		0		12
Sterol synthesis	pmol/h per 10^6 cells	9.6 ± 2.7	7.1 ± 2.1	9.1 ± 2.7	8.0 ± 1.7
Reduction	%		26		12

ing), the influence of the most important risk factors on mortality from coronary heart diseases has been calculated as follows: smoking 22%, hypertension 20%, hypercholesterolemia 18%, obesity and inactivity 5%. Age, sex and inheritance, which cannot be influenced, are responsible for 35% (151). Among the additional risk factors, more than 200 had already been listed 10 years ago (152).

The statistical correlations, however, do not permit a causal relation between serum cholesterol and coronary heart diseases. Evidence for a direct relationship is derived from trials with animals and humans. For the latter, international comparative studies, population and intervention studies have been consulted. Primary intervention is practiced on individuals who do not yet suffer from coronary heart disease, by influencing their nutritional habits and/or style of life. The secondary intervention takes place after breakout of the disease (153).

Epidemiological studies that compare the populations of different countries only yield statistical data, but they do not reveal causal relations and therefore have a limited informative value. The lipid hypothesis has been criticized repeatedly and the different intervention studies have been evaluated (5,144,145,154–166). Ravnskov (164) has analysed 22 controlled studies, according to which the serum cholesterol level could be reduced by dietary recommendations or lipid lowering medication. The results obtained from the control and the intervention trial groups were submitted to statistical evaluation. It appeared that the serum cholesterol level decreased by 1 to 16%, whereas total mortality and mortality from coronary heart disease underwent no significant changes within all trial groups and subgroups (**Table 7**). After Schmidt (167) non-coronary mortality was significantly increased. Ravnskov (164) concluded that lowering serum cholesterol concentrations does not reduce mortality and is not appropriate to prevent coronary heart disease, so that cholesterol campaigns should be stopped (145). In an editorial of *Circulation*, Hulley *et al.* (163) also suggested a major shift of direction in the cholesterol campaign. Furthermore, prevention trials designed to lower cholesterol in men with high cholesterol concentrations result in increased mortality from other causes (159). Morgan *et al.* (168) showed that low serum cholesterol is associated with depressive symptoms in elderly men.

Hypercholesterolemia is one of the many risk factors of coronary heart diseases, but not its cause. It indicates a change in metabolism. The lipid hypothesis stresses the importance of serum cholesterol, dietary cholesterol and the quality of dietary fat. It must however be considered that the serum cholesterol level can also be influenced by other dietary factors such as soluble dietary fibres, carbohydrates, vegetarian eating habits, obesity, saccharose and vitamin C (169,170).

Conclusions

Although removal of cholesterol is technically feasible, the present study on the influence of dietary cholesterol on serum cholesterol levels concludes that there is no need for the food industry to produce cholesterol-free or cholesterol-reduced foods because the influence of dietary cholesterol on serum total and LDL cholesterol level is relatively weak so that the extra costs to the consumer are unjustified. This agrees with the conclusions of Renner and Gurr (171,172), and also applies

Table 7 Mean weighted odds ratios for three events; all trials and subgroups of trials (164)

	All deaths			Fatal coronary heart disease			Non-fatal coronary heart disease		
	n	Odds ratio[1]		n	Odds ratio[1]		n	Odds ratio[1]	
All trials	24	1.02	0.97–1.07	27	0.94	0.88–1.00	24	0.90	0.84–0.96[a]
Men only	21	1.02	0.97–0.07	22	0.95	0.89–1.02	19	0.90	0.84–0.96[a]
Primary prevention	12	1.02	0.95–1.08	12	0.92	0.83–1.02	11	0.83	0.75–0.92[a]
Secondary prevention	12	1.02	0.95–1.10	15	0.96	0.88–1.04	13	0.96	0.89–1.04
Diet	9	1.00	0.94–1.06	10	0.93	0.93–1.03	9	0.94	0.85–1.04
Drugs	14	1.04	0.97–1.12	16	0.95	0.87–1.03	14	0.87	0.79–0.95[a]

[a] Z score > ±2.53 ($P < 0.01$)

[1] Odds ratios were the ratio between events and non-events in the treatment group and events and non-events in the control group.

to the dairy industry. The price of products thus processed and the image of dairy products, which should be processed as little as possible are further factors to be considered. It is said that consumer perception is marketing reality (173), but not all that is feasible and desirable from the marketing perspective is actually reasonable.

The lipid hypothesis of coronary heart disease itself presents an argument against cholesterol-reduced butter. It requests cholesterol removal from butter; on the other hand, the saturated fatty acids, which remain in the product, are also incriminated (3–5). Cholesterol removal from butter therefore does not satisfy the requirements of the lipid hypothesis and is a doubtful enterprise.

Another item to be taken into account is the mean daily cholesterol intake of the consumers in industrialized countries. In Switzerland, for instance, one person ingests about 450 mg of cholesterol per day. Approximately 21% or 139 mg originate from meat and meat products, 38% or 171 mg from egg and 28% or 120 mg from milk, milk products and butter. About 80 mg are from milk and milk products, 44 mg from butter (174). Cholesterol-free butter therefore would lower the mean daily cholesterol intake by only 10%.

Epidemiological studies such as the Basle study or the Swiss National Research Program 1A have shown that increased milk consumption decreases cholesterol levels (175,176). Thus, in men who drank more than 3 dL of milk per day the serum cholesterol decreased by 5 mg and more per dL in comparison with men who did not consume milk (176). This result has been partly confirmed recently (177–179).

Globally, the campaigns against cholesterol give too much importance to serum cholesterol in atherogenesis. It is true that the serum cholesterol level can be decreased by dietary measures or lipid-reducing medication as is shown by the intervention study, but the incidence of coronary heart diseases remained unchanged and the total mortality increased slightly (145,164). Other risk factors as being overweight, smoking, hypertension and lack of physical exercise are undoubtedly as important as serum cholesterol. A campaign that is based on a unique risk factor will miss its goal. On one hand, it frightens people and provokes hysterical reactions and phobia. On the other hand, it is not very useful since one risk factor contributes little to atherogenesis. An overall appreciation should bear in mind that balanced and moderate nutrition is the most suitable recommendation to maintain health at its best. One single food product does not determine the state of human health nor cause disease. Nutrition as a whole is decisive for human health.

Finally, the lipid hypothesis of coronary heart disease can today be replaced by a more comprehensive hypothesis, the free radical hypothesis, and should be examined from a different viewpoint. Many investigations point out the significance of free radicals and other reactive oxygen species for the etiology of different human diseases (180–182). It is not to be excluded that the disequilibrium of naturally occurring prooxidants such as iron or copper and antioxidants such as the enzymes glutathione peroxidase, superoxide, catalase or vitamin E, C, β-carotene, vitamin B_2 and selenium can become also effective in atherogenesis through the formation of free radicals which induce lipid peroxidation, the formation of oxycholesterols and of oxidized lipoproteins (5,35–37,180,183–187). These modified lipoproteins are then taken up by the scavenger receptors of macrophages to form foam cells. These cells are present in fatty streaks which are a characteristic of the early atherosclerosis lesions (5,35,36). In this context cholesterol removal from animal foods is unlikely to modify oxidant and antioxidant intake or to alter other risk factors.

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