

## Functional foods for dyslipidaemia and cardiovascular risk prevention

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A food can be regarded as 'functional' if it can demonstrate a beneficial efficacy on one or more target functions in the body in a convincing way. Beyond adequate nutritional qualities, functional foods should either improve the state of health and wellbeing and/or reduce the risk of disease. Functional foods that are marketed with claims of heart disease reduction focus primarily on the major risk factors, i.e. cholesterol, diabetes and hypertension. Some of the most innovative products are designed to be enriched with 'protective' ingredients, believed to reduce risk. They may contain, for example, soluble fibre (from oat and psyllium), useful both for lowering cholesterol and blood pressure, or fructans, effective in diabetes. Phytosterols and stanols lower LDL-cholesterol in a dose-dependent manner. Soya protein is more hypocholesterolaemic in subjects with very high initial cholesterol and recent data indicate also favourable activities in the metabolic syndrome. *n*-3 Fatty acids appear to exert significant hypotriacylglycerolaemic effects, possibly partly responsible for their preventive activity. Dark chocolate is gaining much attention for its multifunctional activities, useful both for the prevention of dyslipidaemia as well as hypertension. Finally, consensus opinions about tea and coffee have not emerged yet, and the benefits of vitamin E, garlic, fenugreek and policosanols in the management of dyslipidaemia and prevention of arterial disease are still controversial.

**CVD prevention: Diabetes: Diet: Functional foods: Hypercholesterolaemia: Nutraceuticals**

### Introduction

CVD, a major cause of death in Western populations and a constantly growing cause of morbidity and mortality worldwide<sup>(1)</sup>, can be prevented by lifestyle changes, one of which is diet. Lifestyle changes including diet may reduce the risk for premature CHD by 82 %<sup>(2)</sup>, whereas nutritional practices alone may reduce the risk by 60 %<sup>(3)</sup>. Epidemiological and clinical studies indicate that the risk of CHD and hypertension is reduced by a diet rich in fruits, vegetables, unrefined grains, fish and low-fat dairy products, and low in saturated fats and Na. Individuals who adopt this diet benefit by way of a much lower risk of heart disease<sup>(4,5)</sup>. Such diets require, however, major changes in habits and frequently do not fit with what Westerners eat today. Being aware of this fact, the food industry has started to market products labelled as 'functional foods'. Since this term has not yet reached a standard nutritional meaning, regulatory agencies and professional associations of nutrition scientists still use different definitions. International Life Sciences Institute (ILSI) Europe, an industry-sponsored forum in

which representatives from industry, academia and government address nutritional issues, proposed a definition which briefly runs (<http://europe.ilsa.org/NR/rdonlyres/95A18EA7-2EE8-401B-A27E-0950EC0AA965/0/SynthesisFinalDocument22jn09sv.pdf>): 'The term functional foods refers to foods that are consumed as part of a normal food pattern and that have beneficial effects on body functions that go beyond adequate nutritional effects and that are relevant to an improved state of health and well-being and/or a reduction of the risk (not prevention) of disease.'

Functional foods marketed with the claim of reduction of heart disease risk focus primarily on any risk factor, i.e. blood cholesterol, diabetes and hypertension. In the past, this was achieved mainly by a reduced content of a specific food component known to increase risk, i.e. Na or saturated fats. More recently, products have been designed to be enriched with 'protective' ingredients believed to reduce risk, mainly by acting on serum lipids. The most common 'protective' ingredients are fibres, long-chain *n*-3 fatty acids, phytosterols and sterols as well as nutrients based on or enriched with vegetable proteins, mainly soya.

**Abbreviations:** ALA,  $\alpha$ -linolenic acid; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; LDL-R, LDL receptor; SREBP, sterol regulatory element-binding protein.

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### Carbohydrates and fibres

Increasing carbohydrate consumption can exert direct effects on the lipoprotein profile. Raising dietary carbohydrates, while decreasing fat consumption, decreases cholesterolaemia, LDL-cholesterol (LDL-C) and TAG, but it may also lower HDL-cholesterol (HDL-C) concentrations<sup>(6)</sup>.

Fasting and postprandial TAG responses may depend upon the fibre content of the diet. High-carbohydrate–low-fibre diets increase fasting serum TAG<sup>(7)</sup>, whereas high-carbohydrate–high-fibre diets reduce TAG<sup>(8,9)</sup>. Short-term (3–4 weeks) intake of a high-carbohydrate–low-fibre diet may increase fasting TAG significantly by > 50 %, but over a similar time period consumption of a high-carbohydrate–high-fibre diet may decrease serum TAG by 10 % or more<sup>(9)</sup>. Long-term intake of a moderately high-carbohydrate–high-fibre diet significantly decreases serum cholesterol and LDL-C without significantly altering HDL-C or TAG<sup>(10)</sup>.

Diets with a lower glycaemic index are associated with significantly higher HDL-C values *v.* diets with a higher glycaemic index<sup>(11,12)</sup>. The comparison of a high-fibre Mediterranean diet with a low-carbohydrate diet for weight management<sup>(13)</sup> led to similar weight losses, but the high-fibre diet showed more favourable effects on lipids (LDL-C reduction –5.6 % with the high-fibre *v.* –3.0 % with the low-carbohydrate diet). Very recently a low-glycaemic index diet, i.e. emphasising low-glycaemic index breads, cereals, pasta, parboiled rice, beans, peas and nuts, was compared with a high-cereal–low-fibre diet with a similar energy intake. These diets were given for 6 months to 210 participants with type 2 diabetes. In addition to a more marked improvement in HbA<sub>1c</sub> levels, the low-glycaemic index diet increased HDL-C by 17 mg/l, compared with a reduction of 2 mg/l in the high-cereal–low-fibre diet<sup>(14)</sup>.

Dietary fibres are recommended as a safe and practical approach for cholesterol reduction. This activity is limited to soluble fibres, whereas insoluble fibres (cellulose + lignin) do not<sup>(9)</sup>. Soluble fibres known to lower LDL-C<sup>(15)</sup> include pectins from apples and citrus fruits,  $\beta$ -glucan from oats and barley, and fibres from flaxseed and psyllium. A recent paper<sup>(16)</sup> suggests also a potential application in this field of lupin fibre. The cholesterol-lowering effects of soluble fibres are in the order of 5–10 %. The mechanisms of these effects are not fully understood in all cases. An important one is the binding of bile acids, but inhibition of cholesterol synthesis by SCFA, produced by fermentation in the colon, is also hypothesised<sup>(17)</sup>.

Studies with soluble fibres, way back from early reports on oat bran<sup>(18)</sup> and psyllium<sup>(19)</sup>, clearly established their hypocholesterolaemic efficacy. This was well confirmed in two meta-analyses<sup>(20,21)</sup> and led to health claims for foods or supplements containing these ingredients<sup>(22)</sup>. Psyllium is the most effective soluble fibre for reducing cholesterolaemia<sup>(23)</sup>: it leads to LDL-C reductions of about 7 % after intakes of 10 g/d (divided into two portions)<sup>(20)</sup>, sustained for up to 24 weeks of follow up<sup>(24)</sup>. Daily intake of psyllium is also associated with insignificant decreases of HDL-C (–1.3 %) and insignificant increases in fasting TAG (+0.4 %)<sup>(25)</sup>.

Soluble oat fibre is mainly composed of  $\beta$ -glucan. In hypercholesterolaemic individuals the consumption of 4–6 g of this functional ingredient may significantly lower total cholesterol and LDL-C in the range of 8–12 % (Table 1)<sup>(26–28)</sup>.  $\beta$ -Glucan from oat incorporated into a fruit drink was shown to lower total cholesterol and LDL-C by, respectively, 4.8 and 7.7 % (both  $P < 0.01$ ) also in normolipidaemics<sup>(29)</sup>. This was associated with a significant reduction of both cholesterol and bile acid absorption and partially also with an impaired synthesis.

Another convenient source of  $\beta$ -glucan is barley. Confirmation of the effects of concentrated barley  $\beta$ -glucan on blood lipids was obtained in a 10-week controlled study<sup>(30)</sup>, where concentrated barley  $\beta$ -glucan was given to hypercholesterolaemic individuals at two different doses (3 and 5 g/d). Both high-molecular-weight and low-molecular-weight barley  $\beta$ -glucans proved to be effective in LDL-C lowering, mean reductions being 9 % for the 3 g and 13–15 % for the 5 g dose. Target levels of LDL-C values were obtained in some of these individuals, thus indicating a definite potential clinical benefit of barley  $\beta$ -glucan.

There is also a special interest today in fructans, i.e. oligo- and poly-fructosyl polymers, which provide an example of mixed activities of fibre and digestible polysaccharides. Inulin, a poly-fructosyl polymer, does not have hypocholesterolaemic effects but may decrease serum TAG<sup>(31)</sup>. Inulin-type fructans are natural components of the daily diet (asparagus, garlic, onions, etc). Once ingested, they are largely fermented by the colonic bacterial strains such as bifidobacteria, leading to important intestinal systemic effects. Oligofructose, a short-chain fructan obtained from chicory root inulin, protects rats against liver TAG accumulation induced by fructose and, to lesser extent, against steatosis occurring in obese rat strains<sup>(32)</sup>. Fructan linkages may be hydrolysed under mild acidic conditions, but a larger amount of fructan oligomers are absorbed intact across the intestinal mucosa and recovered almost entirely in urine. Oligofructoses can reduce postprandial glycaemia and insulinaemia in rats as well as lower TAG in blood and liver in rats and hamsters<sup>(33)</sup>. These products, in particular inulin-type fructans extracted from chicory roots, may raise the production of incretins by endocrine intestinal mucosal cells. It is noticeable that as a result these products can decrease mean daily energy intake and increase the caecal pool of glucagon-like peptide-1 and peptide YY. Increase of these peptides is of particular significance today, when antagonists of glucagon-like peptide-1 hydrolysis have become available as antidiabetic agents (sitagliptin and vildagliptin)<sup>(34)</sup>. In a pilot study<sup>(35)</sup> 16 g oligofructose per d in patients with non-alcoholic steatohepatitis significantly reduced liver enzymes, glucose and insulin levels with a modest effect on triacylglycerolaemia, thus indicating that possibly this type of dietary component could be of benefit in diabetes management. Although some concern has been expressed based on the observation of increased colonic adenoma progression in a mouse model after inulin feeding<sup>(36)</sup>, the clinical impact of this finding remains elusive and should not deter from the present-day preventive value of prebiotics.

Table 1. Clinical studies on hypercholesterolaemic subjects fed oat bran or  $\beta$ -glucan: serum lipid responses

References	Oat	Amount (g/d)	Number of subjects	Duration (d)	Total cholesterol		LDL-cholesterol		HDL-cholesterol		TAG	
					Baseline value (mmol/l)	Change (%)	Baseline value (mmol/l)	Change (%)	Baseline value (mmol/l)	Change (%)	Baseline value (mmol/l)	Change (%)
Uusitupa <i>et al.</i> (1992) <sup>(230)</sup>	Bran	10.3	20	28	7.40	-6.2	5.36	-8.2	1.33	+1.5	1.58	-1.9
Amundsen <i>et al.</i> (2003) <sup>(231)</sup>	Bran concentrate	5.1	16	21	7.34	-5.4	5.11	-7.5	1.53	-3.2	1.68	+10.6
Gerhardt & Gallo (1998) <sup>(28)</sup>	Bran	3.3	13	42	7.20	-12.6	5.02	-13.0	1.30	-1.4	2.04	-19.9
Davidson <i>et al.</i> (1991) <sup>(26)</sup>	Meal	3.6	20	42	6.79	-7.7	4.79	-10.7	1.34	+0.6	1.42	+0.9
Davidson <i>et al.</i> (1991) <sup>(26)</sup>	Bran	4.0	20	42	6.79	-10.1	4.79	-16.5	1.34	+2.3	1.42	+14.0
Braaten <i>et al.</i> (1994) <sup>(232)</sup>	Gum	2.9	19	28	6.77	-9.2	4.62	-10.0	1.29	-1.5	1.86	-15.1
Anderson <i>et al.</i> (1990) <sup>(233)</sup>	Bran concentrate	3.5	12	14	6.71	-5.4	4.68	-8.5	0.99	-3.3	2.28	+8.7
Onning <i>et al.</i> (1999) <sup>(234)</sup>	Bran, flakes	3.8	52	35	6.58	-5.6	4.34	-5.8	1.39	-9.0	1.85	-12.2
Törönen <i>et al.</i> (1992) <sup>(235)</sup>	Bran concentrate	11.2	13	28	6.42	-4.9	4.27	-3.4	1.44	-1.0	1.92	-13.9
Lovegrove <i>et al.</i> (2000) <sup>(236)</sup>	Bran concentrate	3.0	31	28	6.50	0	4.40	0	1.40	0	1.40	0
Kestin <i>et al.</i> (1990) <sup>(237)</sup>	Bran	5.8	24	28	6.39	-5.6	4.54	-6.6	1.09	0	1.66	-6.2
Van Horn <i>et al.</i> (1991) <sup>(238)</sup>	Instant	2.2	42	28	6.39	-3.2	4.61	-2.6	1.26	-1.7	1.12	-15.1
Anderson <i>et al.</i> (1991) <sup>(27)</sup>	Bran	13.4	10	21	6.26	-8.4	3.18	-6.6	0.77	-4.3	2.16	-0.2
Queenan <i>et al.</i> (2007) <sup>(239)</sup>	Bran concentrate	12	35	21	6.20	-3.3	4.1	-6.3	1.4	0	1.9	0
Kerckhoffs <i>et al.</i> (2003) <sup>(240)</sup>	Bran concentrate	5.9	25	56	6.04	-2.7	4.11	-3.0	1.41	-4.2	1.12	+2.7
Reyna-Villasmil <i>et al.</i> (2007) <sup>(241)</sup>	Fibre	6	38	56	6.03	-3.2	4.36	-11.5	1.09	+27	1.28	-2.4
Poulter <i>et al.</i> (1994) <sup>(242)</sup>	Bran	2.2	59	28	6.01	-2.3	3.78	-4.6	1.55	-2.7	1.50	+13.0
Turnbull & Leeds (1987) <sup>(243)</sup>	Roller	5.4	17	28	6.00	-6.7	4.20	-16.3	1.30	+16.7	1.10	+8.2
Whyte <i>et al.</i> (1992) <sup>(244)</sup>	Bran	10.3	23	28	5.89	-4.1	4.11	-5.6	1.32	-2.5	1.24	+2.9

### *n*-3 Fatty acids

Long-chain PUFA of the *n*-3 series, for example, EPA (20:5*n*-3) and DHA (22:6*n*-3), are provided in the diet almost exclusively by animal foods (especially fish). In addition, the shorter-chain metabolic precursor of the *n*-3 long-chain PUFA, i.e. the 18-C  $\alpha$ -linolenic acid (ALA; 18:3*n*-3), is produced and found in various plant foods (for example, spinach, purslane etc), in certain seeds (for example, nuts and flaxseeds) and oils derived from them (for example, linseed, rapeseed, soya, etc). The amounts of ALA in most vegetable fats and oils are, however, much lower than those of the corresponding *n*-6 fatty acid, linoleic acid.

Some general features of the *n*-3 fatty acids are relevant for understanding their unique functions in the body:

- (1) They cannot be synthesised *de novo* in higher organisms, and even the conversion of ingested ALA, the 18-C precursor, to the long-chain derivatives, especially DHA, is very inefficient in humans, being, however, greater in women than in men, whereas linoleic acid is efficiently converted to arachidonic acid (20:4*n*-6). Therefore, levels of *n*-3 fatty acids in the body depend exclusively upon intakes.
- (2) In the body, they are almost exclusively incorporated into structural lipids (mainly glycerophospholipids) and therefore even relatively small intakes result in relevant changes in the proportion of these fatty acids in pools mainly located in biological membranes.
- (3) Together with arachidonic acid, the *n*-3 long-chain PUFA play a major role in the modulation of membrane properties (for example, structure and related features, such as 'fluidity') and functions (ion channels, receptor responses, signalling pathways, involving also lipid mediators, such as the eicosanoids and other products of phospholipase activities).
- (4) Their requirements may be met with difficulty even with *ad hoc* dietary strategies, since their presence in the typical diet of most Western countries is very limited.

A list of the main actions and mechanism of *n*-3 fatty acids on the cardiovascular system is provided in Table 2.

After the early observation that populations with a high fish consumption have a low incidence of CVD, for example, in the case of Greenland Eskimos<sup>(37,38)</sup>, interest in the cardiovascular effects of this rather unique type of marine fat (mostly fish fat), particularly abundant in cold water fish, has grown consistently. Although fish *per se* contains various nutrients with potentially favourable effects on health (Se, P, iodine, Fe and Cu, low Na, and selected essential amino acids, for example, methionine, cysteine, lysine and vitamins A, B and D), attention has been immediately focused on the *n*-3 fatty acids in fish and fish-derived products (fish oils). As a consequence, over the years products enriched in *n*-3 long-chain PUFA (fish oil capsules) or purified preparations as TAG or ethyl esters have been used for the prevention and treatment of disease. Although, as predictable, bioavailability of the fatty acids is appreciably higher when ingested through fish<sup>(39,40)</sup>, the advantages of using *ad hoc* standardised preparations for long-lasting treatments are obvious.



**Table 2.** Main actions and mechanisms of the effects of long-chain *n*-3 fatty acids (EPA and DHA) on the cardiovascular system

Target	Effects	Mechanisms and comments
Functional effect		
Platelets	Anti-thrombotic	Reduced or modified production of pro-aggregatory eicosanoids (TxA <sub>2</sub> )
Inflammatory cells	Anti-inflammatory	Reduced production of pro-inflammatory eicosanoids (LTB <sub>4</sub> , LTC <sub>4</sub> ) derived from arachidonic acid Production of anti-inflammatory mediators (resolvins from EPA and docosanoids from DHA)
Heart	Anti-arrhythmic	Reduced production of cytokines and adhesion proteins
Blood pressure	Reduction (few mmHg)	Modulation of ion channels in cardiomyocytes (Ca <sup>2+</sup> and Na <sup>+</sup> ) Enhanced endothelial production of NO and reduced vascular resistance due to increased fluidity of erythrocyte membranes
Atherosclerotic lesions	Enhanced plaque stability	Incorporation of the highly unsaturated <i>n</i> -3 fatty acids into plaque lipid pools
Metabolic effects		
Serum TAG	Reduction	Enhanced fatty acid oxidation, reduced TAG synthesis
HDL <sub>2</sub> -cholesterol	Elevation	Modulation of CETP
LDL-cholesterol	Tendency to an increase	Increase of LDL particles size but not number (i.e. lower number of less atherogenic particles)
Total cholesterol	Variable effects	Increment or reduction depending upon intakes of total fats and <i>n</i> -3 fatty acids

TxA<sub>2</sub>, thromboxane A<sub>2</sub>; LTB<sub>4</sub> leukotriene B<sub>4</sub>; LTC<sub>4</sub> leukotriene C<sub>4</sub>; CETP, cholesteryl ester transfer protein.

Numerous studies, carried out in the past, have been based on both fish consumption and supplement intakes and have included secondary-prevention (supplements or dietary advice), cohort, and primary-prevention studies, most of them being randomised controlled trials. Outcomes were also diversified: all-cause mortality, cardiac death, sudden death, myocardial infarction and stroke. A general consensus, with only a few exceptions<sup>(41)</sup>, is that the administration of *n*-3 long-chain PUFA has favourable effects on the cardiovascular system.

In addition to the functional mechanisms underlying the cardiovascular preventive activity, i.e. anti-thrombotic (reduced platelet aggregability), anti-inflammatory, modulation of endothelial function, anti-arrhythmic and plaque-stabilising effects, *n*-3 fatty acids exert major metabolic activities. Primary among these is the reduction of serum TAG. Whereas the favourable cardiovascular effects of *n*-3 fatty acids are generally obtained with relatively low doses of the ethyl esters, i.e. about 1 g/d, more significant triacylglycerolaemic effects may require higher doses.

The recent recommendation of the Nutrition Committee of the American Heart Association specifies that patients without documented CHD should eat oily fish twice weekly; patients with documented CHD should consume about 1 g EPA + DHA daily, preferably from oily fish, although supplements may be used; individuals with hypertriacylglycerolaemia may be advised to ingest 2 to 4 g EPA + DHA daily from capsules<sup>(42)</sup>.

A meta-analysis<sup>(43)</sup> of sixty-five studies reports that fish oil supplements produce these mean changes in serum lipoproteins: total cholesterol, unchanged; LDL-C, +7.5%; HDL-C, +2%; TAG, -27.5%. Studies<sup>(43-45)</sup> using DHA supplements, 0.7 to 1.5 g/d, report similar changes: total cholesterol, +7.0%; LDL-C, +10.3%; HDL-C, +3.3%; TAG, -23.4%. An overview<sup>(46)</sup> of twelve clinical studies on Omacor<sup>®</sup>, a concentrate of *n*-3 (4 g/d), indicates significant lowering of plasma TAG (from -19 to -47%), the best results being obtained in subjects with fasting serum TAG > 5.65 mmol/l. Small increases of LDL-C are, however, associated with this treatment.

The intake of fish oil supplements has the largest impact on individuals with hypertriacylglycerolaemia. For individuals with serum TAG values < 5.6 mmol/l, use of 1–2 g *n*-3 ethyl ester per d is likely to induce lesser changes in total cholesterol and HDL-C, 7–10% increases in LDL-C and 15–25% reductions in fasting serum TAG. A recent American Dietetic Association commentary suggests, however, that consumption of fish may be better suited for the achievement of *n*-3 long-chain PUFA therapeutic intakes<sup>(47)</sup>.

A concern on the possibility that prolonged intake of *n*-3 fatty acids may lead to an increased risk of diabetes in hyperlipidaemic individuals was raised by a number of investigators<sup>(48)</sup>. A long-term study on 1000 Italian patients followed for up to 1 year, however, failed to detect any significant changes in glycaemic regulation in *n*-3-treated patients<sup>(49)</sup>. Indeed, there was clear evidence of a progressive improvement of HDL-C levels and a trend toward a reduction of LDL-C levels at the end of the study<sup>(50)</sup>.

*n*-3 PUFA from fish oil are involved in a number of nuclear receptor-regulated mechanisms, some of which, for example, liver X-receptor, PPAR, sterol regulatory element-binding protein (SREBP) and nuclear factor 4-α, may regulate tissue lipid metabolism and consequently the associated metabolic and lipoprotein disorders<sup>(51)</sup>. On the basis of *in vitro* studies, the activation of PPARα has been proposed to be the most significant. Other mechanisms, i.e. activation of the liver X-receptor, SREBP-1 and nuclear factor 4-α, may participate in the lipid-lowering mechanism at steps subsequent to PPARα activation<sup>(48)</sup>. By all of these mechanisms, *n*-3 fatty acids may not only affect lipidaemia, improving triacylglycerolaemia and also potentially HDL-cholesterolaemia<sup>(52)</sup>, but may also improve other atherosclerosis risk factors, in particular hypertension<sup>(53)</sup>.

Very recently support has been given to the possibility of an additive CHD-protective effect of *n*-3 fatty acids given with statins. The Japan EPA Lipid Intervention Study (JELIS)<sup>(54)</sup> randomised 18 645 secondary prevention patients to 1.8 g EPA per d together with ongoing statin treatment. The authors reported a 19% relative reduction in

major cardiovascular events without any changes in total mortality. The low cost of this therapeutic choice and its excellent tolerability suggests this as a potential standard treatment in low-risk populations.

The concomitance of low amounts of *n*-3 fatty acids in our average diet and need for prolonged administration for prevention and treatments has led to the development of selected preparations (enriched foods or special formulations). These should combine acceptability and adequate bioavailability of their relatively low contents of *n*-3 fatty acids. These fatty acids are therefore being incorporated into a number of commercially available, natural foods that, due to rather unique structural features, appear to be particularly suited as efficient fatty acid vehicles. This is the case of *n*-3 fatty acids incorporated into cows' milk, i.e. the only food specifically produced in nature with the aim to be consumed by rapidly growing organisms requiring high fat intakes. In milk, fat is highly dispersed into microglobules that greatly facilitate fat digestion and absorption. The administration of milk enriched with *n*-3 fatty acids, providing about 300 mg of these fatty acids per d (i.e. about 120–140 mg EPA/d and 180–200 mg DHA/d in both of studies), to healthy subjects resulted indeed in plasma *n*-3 fatty acid elevation and in the reduction of serum TAG<sup>(55,56)</sup>.

Formulation of other types of food should, however, define the optimal chemical structure (ethyl esters, TAG or phospholipids) and the best formulation for the incorporation into foods, including the presence of adequate amounts of antioxidants in order to prevent their degradation by auto-oxidation. A cautionary note was recently published on the possibility that *n*-3 intake from fish may be associated with increased intakes of methylmercury and other contaminants, also including dioxins and polychlorinated biphenyls<sup>(57)</sup>. While the authors point out that low-level methylmercury may adversely affect early neural development, the potential toxicity of commercial preparations appears to be very low.

While research interests in the effects of *n*-3 fatty acids have been focused mainly on the long-chain compounds, the role of *n*-3 ALA, typically present in certain plants and plant-derived products, for example, flaxseed oil, walnut oil, zero-erucic rapeseed oil, should also be considered. Although this fatty acid, precursor to EPA and DHA, can be converted to some extent into EPA<sup>(58)</sup>, the overall conversion to DHA is rather inefficient<sup>(59)</sup>.

ALA has been studied in a limited number of investigations: so far, some epidemiological studies<sup>(60,61)</sup> and a few mechanistic ones<sup>(62,63)</sup> suggest a potential cardiovascular protection by this unsaturated fatty acid. The opinions, however, on this topic are still divided. The UK Food Standards Agency workshop considered that the evidence was still equivocal<sup>(64)</sup>, whereas a recent review<sup>(65)</sup> has concluded that, although ALA supplementation may cause small decreases in fibrinogen concentrations and fasting plasma glucose, most cardiovascular risk markers do not appear to be affected. Thus, dietary supplementation with ALA to reduce CVD cannot be directly recommended, except as being part of a healthy diet. However, the essentially inexhaustible supply of this fatty acid obtainable from plant sources suggests that the research should be continued<sup>(66)</sup>.

## Plant sterols and stanols

All plants produce sterol compounds, also termed phytosterols, chemically related to cholesterol. The average consumption from foods in adults is about 160–350 mg/d, i.e. intakes that do not appreciably alter cholesterol metabolism, whereas an increased intake of phytosterols reduces the intestinal absorption of dietary and biliary cholesterol. This has been the basis for the development of phytosterol-enriched functional foods. The mechanism of reduced cholesterol absorption is believed by some authors to occur by interference with the Niemann-Pick C1-like 1 (NPC1L1) transporter<sup>(67)</sup>, but contrasting findings have been provided<sup>(68)</sup>. The most likely mechanism is micellar competition with cholesterol within the small intestine. The final outcomes are alterations in serum lipoproteins with significant decreases in LDL-C concentrations<sup>(69–76)</sup>.

The major plant sterols ( $\beta$ -sitosterol and campesterol) can be saturated using commercial processes to form stanols (sitostanol and campestanol). Sterols or stanols can be incorporated into functional foods, such as reduced-fat spreads (for example, margarines), salad dressings, yogurts, bars, juices and other beverages<sup>(77)</sup>. Esterification of sterols and stanols with fatty acids to form sterol and stanol esters makes them more fat soluble and allows a more effective incorporation into fat-containing foods<sup>(69,78)</sup>. Recently, capsule and tablet formulations of phytosterols have been made available, apparently with similar hypolipidaemic efficacy<sup>(77)</sup>. It is suggested by some authors, but not by others, that plant sterols may, in the long run, suppress bile acid synthesis, particularly in concomitantly statin-treated patients, thus reducing their cholesterol-lowering potential<sup>(79)</sup>.

Phytosterols and phytostanols have similar hypocholesterolaemic efficacy, whereas possible differences in fat-soluble vitamin absorption are disputed<sup>(80,81)</sup>; effectiveness on serum LDL-C levels is similar even when used in functional foods with a low fat content<sup>(82,83)</sup>. There is also controversy as to whether either sterols or stanols provide a better option for cardiovascular prevention<sup>(84)</sup>. Comparative studies have, however, failed to detect any difference in the cholesterol-lowering effects of sterol- *v.* stanol-enriched margarines at 2 g daily doses<sup>(84,85)</sup>. There is also the possibility of esterifying fatty acids with plant sterols, thus achieving improved activity on the LDL-C:HDL-C ratio<sup>(86)</sup>.

Relevant clinical studies on phytosterols and stanols are listed in Table 3. Very recently, in a meta-analysis<sup>(87)</sup> on eighty-four trials including 141 trial arms, a pooled reduction of LDL-C of 0.34 mmol/l, i.e. 8.8 %, for a mean daily dose of 2.15 g phytosterols was established. The authors indicate maximal LDL-C reduction (i.e. about 13 %) with daily doses of 3–4 g, whereas higher doses, i.e. up to 8–9 g/d, do not appear to provide a more effective reduction. The effect is significantly correlated with baseline LDL-C concentrations, explaining 16 % of the variation in residuals ( $r = 0.4$ ;  $P < 0.0001$ ). Food format seems to influence the response: at high daily doses, the maximal estimated LDL-C-lowering effect of solid foods was 5.2 % larger than that of liquid foods<sup>(87)</sup>; however, at low daily doses, the dose–response curve is steeper for liquid *v.* solid foods. A recent meta-analysis has shown that

**Table 3.** Clinical trials investigating the cholesterol-lowering effects of plant sterols and stanols

References	Sterol/stanol	Amount (g/d)	Subject number	Duration (d)	Total cholesterol			LDL-cholesterol			HDL-cholesterol			TAG		
					Baseline value (mmol/l)	Change (%)	Baseline value (mmol/l)	Baseline value (mmol/l)	Change (%)	Baseline value (mmol/l)	Baseline value (mmol/l)	Change (%)	Baseline value (mmol/l)	Change (%)	Baseline value (mmol/l)	Change (%)
Gylling & Miettinen (1996) <sup>(245)</sup>	Sitostanol ester	3	7	28	6.64	-11.00	4.20	4.20	-14.00	1.17	1.17	ns	2.43	ns	2.43	ns
Miettinen <i>et al.</i> (1995) <sup>(69)</sup>	Sitostanol ester	2.6	102	365	6.08	-10.25	4.16	4.16	-16.35	1.38	1.38	ns	1.22	ns	1.22	-1.64
Vanhanen <i>et al.</i> (1993) <sup>(246)</sup>	Sitostanol ester	3.4	34	42	5.84	-6.33	3.74	3.74	-8.82	0.37	0.37	ns	1.63	ns	1.63	-4.29
Hallikainen <i>et al.</i> (2000) <sup>(247)</sup>	Phytosterols	2.04	35	28	6.24	-9.29	4.43	4.43	-0.54	1.60	1.60	ns	1.11	ns	1.11	-2.70
Amundsen <i>et al.</i> (2002) <sup>(248)</sup>	Phytosterols	1.5	38	112	7.38	-7.18	5.76	5.76	-10.07	1.23	1.23	ns	0.84	ns	0.84	ns
Neil <i>et al.</i> (2001) <sup>(74)</sup>	Phytosterols	2.5	29	56	7.28	-6.31	5.08	5.08	-10.04	1.49	1.49	2.68	1.34	-6.94	1.34	-6.94
Hallikainen <i>et al.</i> (2000) <sup>(247)</sup>	Phytosterols	2.04	35	28	6.24	-7.37	4.43	4.43	-10.16	1.60	1.60	ns	1.11	-4.59	1.11	-4.59
Mussner <i>et al.</i> (2002) <sup>(249)</sup>	Phytosterols	1.82	63	21	6.06	-3.86	3.95	3.95	-6.58	1.51	1.51	3.45	1.26	-4.35	1.26	-4.35
Hendriks <i>et al.</i> (1999) <sup>(250)</sup>	Phytosterols	3.24	80	25	5.16	-6.80	3.05	3.05	-9.90	1.64	1.64	-1.50	1.13	-4.80	1.13	-4.80
Vanstone <i>et al.</i> (2002) <sup>(80)</sup>	Mixture of sterols and stanols	1.8	15	21	6.40	-11.6	4.18	4.18	-13.8	1.20	1.20	-3.30	2.21	-3.10	2.21	-3.10

phytosterols dispersed in orange juice are less effective than when they are given in milk or yoghurt<sup>(88)</sup>.

In addition the relative LDL-C-lowering effect is more pronounced when phytosterols are consumed in multiple daily intakes *v.* single daily intakes (mean of -8.91 *v.* -6.14 % at similar daily doses). Age appears to influence the effect: a highly significant LDL-C-reducing effect (-14 %) was reported in prepubertal children with familial hypercholesterolaemia without, however, any improvement in vascular endothelial function<sup>(89)</sup>.

Both the Nutrition Committee of the American Heart Association<sup>(42)</sup> and the Adult Treatment Panel III of the National Cholesterol Education Program<sup>(90)</sup> recommend the use of plant sterols and stanols to decrease serum LDL-C levels. There are, however, concerns about margarines containing plant sterols and stanols, related to the energy intake associated with consuming > 2 g daily and whether consumers consistently achieve these goals<sup>(91)</sup>.

In an effort to improve the LDL-C lowering by plant sterols, combination therapy with fibres has been attempted. Cookies with 7.6–8 g psyllium per d and 2.6 g daily of plant sterols allow an LDL-C reduction of 9 %, again with a modest rise of triacylglycerolaemia; there is also evidence of an increased LDL receptor (LDL-R)-mediated uptake<sup>(92)</sup>. The possibility of administering plant stanol esters in a water-soluble form was investigated<sup>(93)</sup> by testing the combination of soluble  $\beta$ -glucan from oat with plant stanol esters, i.e. 5 g with or without 1.5 g plant stanols. The combination further reduced LDL-C by 9.6 %, compared with control muesli, and by 4.4 % compared with  $\beta$ -glucan muesli, confirming an additive activity of the two agents. Similarly, a water-soluble formulation of plant sterol esters in orange juice significantly reduced both total (-5 %) and LDL-cholesterolaemia (-9.4 %;  $P < 0.001$ ) compared with baseline and placebo beverages<sup>(94)</sup>. Interestingly, a significant reduction of C-reactive protein concentrations (-12 %) was observed with the sterol beverage.

The lipid-lowering effect of phytosterols can be synergistic and complementary to those of *n*-3 fatty acid: in particular, the combination of phytosterols with long-chain *n*-3 fatty acid reduces plasma cholesterol by 13.3 %, i.e. more effectively than long-chain *n*-3 fatty acid alone<sup>(95)</sup>.

Both in the USA and in Europe phytosterols are officially functional ingredients. The American Food and Drug Administration approved the health claims for plant sterol and stanol esters and the prevention of risk of CHD in 2000 (<http://www.fda.gov/Food/Labeling/Nutrition/LabelClaims/HealthClaimsMeetingSignificantScientificAgreementSSA/ucm074747.htm>); the European Food Safety Authority approved a similar claim in 2008 ([www.efsa.europa.eu/cs/BlobServer/Scientific\\_Opinion/nda\\_op\\_ej825\\_art\\_14\\_0038\\_plant\\_stanol\\_ester\\_en.pdf?ssbinary=true](http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/nda_op_ej825_art_14_0038_plant_stanol_ester_en.pdf?ssbinary=true)).

## Nuts

Nuts, generally perceived as being unhealthy because of their high fat content, have instead recently shown potential health benefits, since a number of epidemiological and intervention studies indicated favourable plasma lipid responses after their consumption<sup>(96,97)</sup>. A recent review<sup>(98)</sup>



considered all clinical studies based on nuts and concluded that the consumption of approximately 50–100 g (i.e. approximately 1.5–3.5 servings) of nuts at least five times per week, as part of a heart-healthy diet with a 35 % total fat content (high in MUFA and/or PUFA), may decrease total cholesterol between 2 and 16 % and LDL-C between 2 and 19 % in normo- and hyperlipidaemic individuals. Prospective data from the Physicians' Health Study<sup>(99)</sup> indicated a strikingly reduced risk of sudden cardiac death associated with nut consumption.

Among tree nuts, walnut may be the most desirable, because of its peculiar composition, such as having a low lysine:arginine ratio, high levels of arginine, folate, fibre, tannins and polyphenols, and being very rich in ALA. Consumption of just four walnuts per d may provide an optimal daily intake of this essential fatty acid. Feldman reviewed<sup>(100)</sup> the effects of walnut intake on the main biomarkers of atherosclerotic disease or disease outcome in six controlled intervention studies in primarily healthy, normo- or hyperlipidaemic subjects. In hyperlipidaemics a 4–5 % decrease of total cholesterol follows the consumption of about 50–55 g walnuts per d.

Hazelnut is another shell-nut that has attracted interest for its fatty acid composition (very similar to olive oil), richness of fat-soluble vitamins, essential minerals (Se), antioxidants and phenolics (caffeic acid), soluble fibre and phytochemicals<sup>(101)</sup>. In a recent controlled investigation<sup>(102)</sup> on fifteen hypercholesterolaemic men given a hazelnut-enriched diet (40 g/d, i.e. 11.6 % of total energy), there was no change in body weight, but clear reductions of VLDL-cholesterol, TAG and apo B (respectively –29.5, –31.8 and –9.2 %) as well as small reductions in total cholesterol and LDL-C (–5.2; –3.3 %) with increased HDL-C (+12.6 %), all statistically significant. With dietary guidance many individuals may be able to incorporate the 240 kcal (1004 kJ) from hazelnuts into their diet, in order to achieve favourable changes in lipid parameters without weight gain. Other beneficial nuts are almonds, also useful for controlling postprandial glycaemia<sup>(103)</sup>, and macadamias<sup>(104)</sup>.

These and other data induced the Food and Drug Administration<sup>(105)</sup> to authorise a health claim about the relationship between the consumption of nuts and reduced risk of CHD. The new Healthy Eating Pyramid as provided by Willett<sup>(106)</sup> indicates that one to three servings of nuts and legumes should be incorporated into the diet each day for lifelong health.

## Proteins

### *Soya proteins*

Soya protein consumption has been shown to successfully reduce cholesterolaemia in experimental animals, when elevated by dietary means<sup>(107,108)</sup>, as well as in humans with cholesterol elevations of genetic or non-genetic origin<sup>(109–113)</sup>. In addition, prospective observational studies, initially in vegetarians<sup>(114)</sup>, then in Chinese women<sup>(115)</sup> and more recently in a large population in Japan<sup>(116)</sup>, have shown a reduction of total cholesterol and LDL-C as well as of ischaemic and cerebrovascular events with a daily soya protein intake of more than 6 g, compared with less than

0.5 g/d. More recently the 20-year follow-up of the Nurses' Health Study also indicated a significant correlation between vegetable protein intake and reduced cardiovascular risk<sup>(117)</sup>.

In the earliest studies in the 1970s, a soya protein preparation, given to hospitalised hypercholesterolaemic patients, was found to be highly effective for cholesterol lowering and well tolerated. In this controlled cross-over investigation in twenty patients who underwent a 6-week study<sup>(109)</sup>, there was a 20–22 % reduction in total cholesterol level and a 22–25 % reduction in LDL-C, without significant changes of triacylglycerolaemia. The reduction in plasma cholesterol was inversely related to the baseline cholesterolaemia and not modified by the addition of dietary cholesterol<sup>(109)</sup>.

The large number of ensuing clinical studies are summarised in a meta-analysis<sup>(118)</sup> of thirty-eight studies up to 1995, in both hypercholesterolaemic and normolipidaemic individuals. This meta-analysis confirmed that serum LDL-C concentrations are modified, dependent on baseline cholesterolaemia, from a minimum of –7.7 % in subjects with total cholesterol in the normal range (<2000 mg/l), up to –24 % in clear-cut hypercholesterolaemics. The results of this meta-analysis were criticised recently, since more recent studies appeared not to confirm the very powerful cholesterol-reducing effect of soya proteins<sup>(119)</sup>. However, when the initial cholesterolaemia is taken into account, it appears quite clearly that even the most recent studies are in line with the Anderson meta-analysis<sup>(120)</sup>, since both in old and more recent studies, ranges of cholesterol responses of the groups of patients belonging to the same quartile are essentially identical. Old studies were based on severely hypercholesterolaemic individuals, whereas patients with cholesterolaemia in the very high range (>3350 mg/l) were not selected for treatment in recent studies. The major influence of baseline cholesterolaemia on the final effect of soya proteins was highlighted in an editorial<sup>(121)</sup>. A recent systematic meta-analysis of available randomised controlled studies mainly in subjects with moderate hypercholesterolaemia confirmed that the inclusion in the diet of a modest amount of soya protein (25 g) produces a highly significant reduction of total cholesterol and LDL-C, equivalent to about 6 % LDL-C reduction<sup>(122)</sup>.

The cholesterol-reducing effect of soya proteins, potentially leading to a reduced cardiovascular risk, became the basis for the US Food and Drug Administration approval of the health claim for the role of soya protein consumption in coronary disease risk reduction<sup>(123)</sup>.

Rodent and *in vitro* studies have attempted to establish a link between the hypocholesterolaemic effects of soya and the activation/depression of liver LDL-R<sup>(124,125)</sup>. Animals on cholesterol or cholic acid dietary regimens with casein have a dramatic down-regulation of liver LDL-R and this effect is reversed in the presence of soya proteins. Two studies have addressed the potential of soya protein preparations to increase LDL-R expression in human subjects. In the first<sup>(126)</sup>, familial hypercholesterolaemic patients were treated with animal protein or textured soya protein (with the addition of cholesterol to balance the two diets). Both plasma lipids and LDL degradation by circulating

lympho-monocytes (used as mirror images of hepatocytes), were monitored. After the animal protein diet there were minimal changes in LDL-C levels or LDL-R activity, whereas during the soya protein diet, in addition to a marked LDL-C reduction, an increase of about 8-fold in LDL degradation was observed. This study clearly suggests that some soya protein components are able to up-regulate LDL-R-mediated LDL degradation, as was recently confirmed in individuals with lesser cholesterol elevations<sup>(127)</sup>.

A full understanding of the bioactive components in soya has become vital for the selection of the most appropriate forms of soya for treating hypercholesterolaemia, since the selection of the most efficacious products for treatment can only be made once the active component/s and their mechanism of action have been fully elucidated.

Initially isoflavones, i.e. the oestrogen-like components typically found predominantly in soya and only in very limited amounts in other grain legumes, were considered to have a main role in cholesterol reduction, based primarily on studies in monkeys<sup>(128)</sup>. A number of more recent reports have, however, definitely concluded that they make only a marginal contribution to the hypocholesterolaemic action in respect to the protein itself<sup>(119,129,130)</sup>. To confirm this, the soya products used in the first clinical studies were practically free of isoflavones as a consequence of processing<sup>(131)</sup>.

Proteomics may be a powerful tool to achieve the objective of sorting out the bioactive components of the protein<sup>(132)</sup>. The major storage proteins of soya are 7S and 11S globulins. From early studies the 7S globulin appeared to be primarily responsible for the hypocholesterolaemic effects of soya protein preparations, whereas the 11S component appeared essentially inactive<sup>(133,134)</sup>. Very recently a hypocholesterolaemic protein subcomponent has been suggested, i.e. by showing that the isolated 7S globulin  $\alpha'$  subunit given to cholesterol-fed rats leads to a strong up-regulation of liver LDL-R activity as well as to dramatic plasma cholesterol and TAG reductions<sup>(135)</sup>.

Since proteins are hydrolysed in the gastrointestinal tract, it is quite likely that the hypocholesterolaemic soya components are peptides with less than fifteen amino acids, considering their potential to be absorbed. Many animal-feeding studies and two clinical trials<sup>(136,137)</sup> have reported LDL-lowering effects of soya peptide preparations. In particular, Cho *et al.* disclosed<sup>(138)</sup> that the octa-peptide FVVNATSN, when added to HepG2 cells, leads to a significant LDL-R activation (about 2.5-fold). Preliminary findings seem to suggest that this subcomponent is absorbed intact.

Another interesting activity of these proteins, initially shown by Deibert *et al.*<sup>(139)</sup>, and then by Kohno *et al.*<sup>(140)</sup> is that daily administration of four candies containing in total 5 g of 7S globulin to moderately overweight Japanese individuals with hypertriglycerolaemia can reduce TAG by 14 % and raise HDL-C by 5 %, while exerting a remarkable 6 % reduction in the mean visceral fat area (– 10 % in individuals with visceral fat area greater than 100 cm<sup>2</sup>). As foreseen, in neither of these studies<sup>(139,140)</sup> in individuals with essentially normal cholesterolaemia was there evidence of any significant LDL-C reduction. Finally, beneficial effects of a soya protein-based diet were also

recently described in Iranian women with the 'metabolic syndrome'<sup>(141)</sup>; these women had also reduced inflammatory markers after soya, as well as reduced insulin resistance<sup>(142)</sup>. The remarkable effectiveness of this very simple regimen thus reinforces the recommendation to increase the intake of soya proteins for cardiovascular protection.

#### Other vegetable proteins

Lupin seed is also considered very useful by the food industry, since it contains up to 35–40 % protein. Two main species are cultivated, white lupin (*Lupinus albus*) and narrow-leaf lupin (*L. angustifolius*). Whole seeds of narrow-leaf lupin showed a remarkable cholesterol-lowering effect in pigs fed a cholesterol-rich diet, compared with casein<sup>(143)</sup>. The results of this study are probably due to the combined effects of several bioactive components, such as protein, soluble and insoluble fibres, phytosterols and possibly others. Other studies were focused only on the proteins. White lupin protein, evaluated in a rat model of hypercholesterolaemia<sup>(144)</sup>, indicated a substantial reduction of cholesterolaemia, with moderate changes of TAG and no effect on glucose. The decrease in plasma TAG concentrations appears to depend on a down-regulation of the liver SREBP-1c<sup>(145)</sup>, a transcription factor that regulates the expression of lipogenic enzymes. White lupin protein was also evaluated in a rabbit model of focal soft plaque generated in the common carotid arteries<sup>(146)</sup>. In this model, carotid lesions are mostly constituted by extracellular lipids and macrophages, thus reflecting the main feature of the human arterial plaques, defined as unstable, frequently associated to acute ischaemic events<sup>(147)</sup>. Dietary treatment with lupin protein significantly reduced atherosclerosis development *v.* casein. White lupin protein isolate was also shown to significantly lower blood pressure in spontaneous diabetic and hypertensive rats<sup>(148)</sup>. This effect is possibly related to the high content of arginine, which may also lead to an increased NO production, and was confirmed in a preliminary clinical study<sup>(149)</sup>, based on a lupin beverage (daily lupin protein intake = 36 g), indicating both reduced blood pressure as well as a clear cholesterol reduction in the hypercholesterolaemic subjects. A recent paper<sup>(150)</sup> has shown that lupin kernel flour added to bread has also a positive effect on blood pressure: both the fibre and the protein may be responsible.

Other legumes investigated in the rat model of hypercholesterolaemia are pea<sup>(151,152)</sup>, chickpea<sup>(153)</sup> and faba bean<sup>(154)</sup>: all induce a significant decrease of plasma LDL-C. A comparative study of diets containing four different legumes – baked beans, marrowfat peas, lentils and butter beans<sup>(155)</sup> – showed effectiveness of them all, but baked beans and butter beans were the most potent. The hypocholesterolaemic activity was confirmed also in the pig model of cholesterolaemia: a study compared bean, pea, lentil and butter bean<sup>(156)</sup>, while another investigated only pea<sup>(157)</sup>. There are also a few clinical studies available: most, including two by one of us, were based on common beans<sup>(158–161)</sup> or mixed beans<sup>(162)</sup>, in some cases together with other bioactive components such as oat<sup>(163)</sup>; one was based instead on broad bean<sup>(164)</sup>. As already observed in the



case of soya, the hypocholesterolaemic effect was evident ( $-7$  to  $-9\%$  total cholesterol) when the patients had an initial cholesterolaemia  $> 2400$  mg/l. In addition, very recently Winham & Hutchins<sup>(165)</sup> reported that baked bean consumption in moderately hypercholesterolaemic adults (mean LDL-C 1380 mg/l) was associated with a reduction of LDL-cholesterolaemia ( $-5.4\%$ ) with no changes in HDL-C levels.

#### Fish protein

Fish proteins have also attracted interest. Animal studies with fish proteins *v.* casein have suggested a potential hypocholesterolaemic activity<sup>(166,167)</sup>. Similarly to soya, fish proteins increased liver LDL-R and SREBP-2 mRNA concentrations and significantly reduced cholesterolaemia. Different from vegetable proteins, however, an HDL-C reduction was noted, albeit with an increased mRNA expression for apo AI. At present, however, little evidence has come from studies in human subjects, where, at best, fish intake has been linked to a hypotriacylglycerolaemic activity<sup>(50)</sup>. A recent study<sup>(168)</sup>, however, has shown that a 6-week diet based on herrings, in comparison with a reference diet, significantly increases HDL-C and decreases TAG without any adverse effect on *in vivo* oxidation or serum antioxidants. Another potentially beneficial activity of fish proteins is on blood pressure, since angiotensin-converting enzyme (ACE) inhibitory peptides, well described mainly in milk and some plant proteins<sup>(169)</sup>, have been also detected in fish<sup>(170)</sup> and could contribute to a reduced cardiovascular risk.

#### Dark chocolate

Cocoa is a flavonoid-rich food that has been recently investigated for its possible role in the prevention of CVD<sup>(171)</sup>. Cocoa flavonoids, catechins and procyanidins, can markedly decrease oxidant-induced peroxynitrite production *in vitro* and increase the NO synthase expression and NO-dependent vasodilation in rabbit aortic rings<sup>(172)</sup>. In healthy adults, drinking flavonoid-rich cocoa may improve NO-dependent vasorelaxation and flow-mediated dilation in the brachial arteries<sup>(173)</sup>. A controlled investigation in healthy volunteers showed that ingestion of flavanol-rich cocoa is associated with acute elevations of circulating NO species, enhanced flow-mediated response of conduit arteries and augmented microcirculation<sup>(174)</sup>. These findings suggested evaluation of dark chocolate, particularly rich in flavanols, *v.* flavanol-free white chocolate in patients with essential hypertension<sup>(175)</sup>. Administration of dark chocolate in essential hypertensives reduced ambulatory blood pressure by  $-11.9$  (SD 7.7) mmHg (24 h systolic) and  $-8.5$  (SD 5.0) mmHg (24 h diastolic), whereas 90 g white chocolate had no effects.

More recently both normocholesterolaemic and hypercholesterolaemic human subjects received graded doses of cocoa powder (from 13 to 26 g/d) high in polyphenols, or a placebo (low-polyphenol cocoa). There was a clear reduction of cholesterolaemia at both doses of the high-polyphenol cocoa as well as a significant rise of HDL-cholesterolaemia (in addition to a marked reduction of

circulating oxidised LDL) *v.* no activity for the placebo cocoa. These changes were highly significant in individuals with LDL-C  $\geq 3.23$  mmol/l (1300 mg/l)<sup>(176)</sup>. These findings confirm the earlier observation in the study in hypertensives where, after dark chocolate, there was an improvement of the homeostasis model assessment (HOMA) index and a small, statistically significant, reduction of serum LDL-C (from 3.4 to 3.0 mmol/l;  $P < 0.05$ )<sup>(175)</sup>.

#### Other functional ingredients

##### Red-yeast rice

An extract of the Chinese red-yeast rice, *Xuezhikang*, contains a family of naturally occurring statins and is used as a supplement for CVD prevention in many countries. Two high-quality trials<sup>(177,178)</sup> and two other studies<sup>(179,180)</sup> have examined the effects of Chinese red-yeast rice. Doses of 1.2 to 2.4 g/d reduced total cholesterol ( $-19.7\%$ ), reduced LDL-C ( $-19.8\%$ ), increased HDL-C ( $+9.6\%$ ) and decreased TAG ( $-17.6\%$ ). In addition, Zhao *et al.*<sup>(181)</sup> recently reported a favourable outcome in the controlled China Coronary Secondary Prevention Study where a 1.20 g daily dose of *Xuezhikang* (comparable with 10 mg lovastatin) reduced CHD events by 50.8% over 4 years in type 2 diabetics.

In a very recent randomised trial<sup>(182)</sup>, LDL-C level was significantly lower in the red-yeast rice group than in the placebo group at both week 12 ( $P < 0.001$ ) and week 24 ( $P = 0.011$ ). Significant treatment effects were also observed for total cholesterol at week 12 ( $P < 0.001$ ) and week 24 ( $P = 0.016$ ). Levels of HDL-C, TAG, liver enzyme, creatine phosphokinase, body weight, and angina scores did not differ significantly between groups.

Occurrence of adverse events after *Xuezhikang* treatment is low, but appears to be of the same type as with statins, i.e. muscle complaints and others. It is mandatory that patients be aware of the possibility that side effects may occur due to the presence of a pharmacologically active molecule. Finally, it is important to observe that the commercialisation of products containing more than a trace of lovastatin is forbidden in the USA.

##### Guggul gum

Guggul gum or guggulipid, a resin from the mukul myrrh tree (*Commiphora mukul*), used for many medicinal purposes for millennia, has been tested for hypocholesterolaemic effects in at least four clinical trials<sup>(179)</sup>. Only two adequate randomised controlled trials are available<sup>(183,184)</sup>, with inconsistent findings. One additional controlled study<sup>(185)</sup> was suitable for analysis. A total of 127 subjects used a variety of different extracts, with doses ranging from 50 mg/d to 6 g/d: mean results after treatments from 3 to 8 weeks indicate a reduction of total cholesterol of 2.7%, LDL-C of 2.0%, HDL-C of 1.0% and TAG of 10.1%. Rashes were noted in 15% of the high-dose guggulipid-treated subjects, 3% of the low-dose guggulipid group and none of the placebo group. Otherwise, guggulipid was well tolerated.

*Fenugreek*

Seeds or leaves from fenugreek (*Trigonella foenum-graecum*) have also a long-standing recognition for diabetes and other metabolic diseases. Data from at least six clinical trials are available, but only one study<sup>(186)</sup> appears to be a high-quality randomised controlled trial. It showed reduction of total cholesterol by 13.4 %, of LDL-C by 15.1 % and of HDL-C by 2.7 %. Additional randomised controlled trials are necessary to establish the safety and hypocholesterolaemic efficacy of well-characterised preparations of fenugreek products.

*Evening primrose oil*

Evening primrose oil, rich in  $\gamma$ -linolenic acid, has been studied in rodents but limited data are available for human subjects<sup>(187,188)</sup>; these data do not support a hypocholesterolaemic efficacy.

*Artichoke*

Similarly, artichoke has been examined in several clinical trials<sup>(189,190)</sup>, but the data are insufficient to make an assessment or recommendation at present.

*Cinnamon*

Cinnamon, a popular flavouring agent, has insulin-enhancing activity, antioxidant properties and decreases serum lipid values in diabetic individuals<sup>(191,192)</sup>.

While these food ingredients are safe and widely used, their efficacy for favourably altering serum lipoprotein values require further controlled trials.

**Controversial topics***Vitamin E*

Whereas extensive epidemiological data and observational studies had indicated that vitamin E and other antioxidant supplements would have CVD-protective effects, disappointing results have been provided by recent randomised intervention trials<sup>(193,194)</sup>. The Nutrition Committee of the American Heart Association no longer recommends vitamin E supplementation<sup>(42)</sup>. Vitamin E is, however, a generic term for eight naturally occurring lipophilic compounds, called tocopherols and tocotrienols. While tocopherols appear only to possess antioxidant properties, tocotrienol intake has been associated with anti-thrombotic, antiproliferative and hypocholesterolaemic effects<sup>(195)</sup>. An elegant development in the understanding of the mode of action of tocotrienols has come from recent *in vitro* work indicating enhanced Insig-dependent ubiquitination and degradation of 3-hydroxy-3-methyl-glutaryl (HMG)-CoA reductase by  $\alpha$ - and  $\gamma$ - (not by  $\alpha$ -) tocotrienols<sup>(196)</sup>. Enhanced degradation of HMG-CoA reductase can well explain the *in vivo* observation of increased LDL-R and HMG-CoA reductase mRNA following rice bran oil with a high content of  $\gamma$ -tocotrienol<sup>(197)</sup>. While these findings are potentially worthy of more in-depth evaluation, studies in human subjects with tocotrienols have provided conflicting results<sup>(195,198,199)</sup>.

*Garlic*

Garlic has been used as a food and medicine for centuries and many studies have examined its hypocholesterolaemic effects. From the extensive data available it seems that a daily consumption of one-half to one clove of garlic may decrease total cholesterol by 5–9 %; consumption of garlic extracts and dried garlic powder were similarly suggested to reduce cholesterolaemia by 2–6 %<sup>(200–203)</sup>. In a recent trial<sup>(204)</sup>, patients with moderate hypercholesterolaemia (LDL-C 1300–1900 mg/l) were randomised to: blended raw garlic (4 g), or an equivalent amount of powdered garlic supplement, garlic extract supplement or placebo over 6 months. Modest negative effects on LDL-C concentrations, i.e. ranging from + 32 mg/l to + 2 mg/l (placebo had the best effect) were observed.

A recent meta-analysis<sup>(205)</sup> in this journal, including twenty-nine trials, was conducted in order to determine the impact of garlic on total cholesterol, TAG, as well as LDL-C and HDL-C. Garlic was found to significantly reduce total cholesterol (– 0.19; 95 % CI – 0.33, – 0.06 mmol/l) and TAG (– 0.11; 95 % CI – 0.19, – 0.06 mmol/l), but exhibited no significant effect on LDL-C or HDL-C. Future studies should be conducted evaluating the impact of adjunctive garlic therapy with fibrates or statins on TAG concentrations.

*Coffee*

Coffee consumption may possibly reduce the risk of myocardial infarction, but data are as yet inconclusive<sup>(206,207)</sup>. Very recent findings indicate that in individuals aged  $\geq 65$  years high intakes of caffeinated beverages reduce relative CVD risk and heart disease mortality close to 50 % in those taking four or more cups per d<sup>(208)</sup>. A dose–response decrease and a similar protective effect were found for a daily caffeine intake of some milligrams; similar results were observed also in patients with type 2 diabetes<sup>(209)</sup>.

Moderate or high intakes of unfiltered or instant coffee appear to be associated with serum LDL-C values about 5 % higher *v.* baseline<sup>(207,210,211)</sup>. The active constituents of coffee apparently responsible for this effect are diterpenes, such as kahweol and cafestol; however, a major part of these diterpenes is retained by the paper filters used in making filtered coffee<sup>(211)</sup>. Cornelis and El-Sohemy tried to unravel the complex genetic factors favouring CHD or protecting from it<sup>(212,213)</sup>. Genetic susceptibility, in fact, may play a significant role: individuals with the apo E3/E3 genotype tend to have greater elevations in LDL-C in response to cafestol *v.* carriers of the other genotypes and, in addition, this may be the case of individuals with the apo A-I 83 CC genotype<sup>(212)</sup>.

*Tea*

Green tea consumption appears to protect from CVD<sup>(3,214–216)</sup>, but results are again not consistent. A recent meta-analysis reported that the incidence of myocardial infarction was 11 % lower among individuals who consumed three cups of tea daily compared with non-users, but this reduction was not statistically significant and

there was large variability across studies<sup>(217)</sup>. While moderately high levels of tea consumption (more than four cups per d) may reduce CVD risk, the effects of tea consumption on serum lipoproteins are not clearly established<sup>(218,219)</sup>. One small, placebo-controlled study reported a 3.8 % reduction in serum total cholesterol and a 7.5 % reduction in LDL-C after consumption of five servings of black tea daily by mildly hypercholesterolaemic adults; fasting serum TAG levels were slightly higher and HDL-C levels were unchanged<sup>(220)</sup>.

Very recent work has focused on the possible activity of green tea and its constituent epigallocatechin gallate on insulin secretion and the obesity-related insulin resistance syndrome. An enhanced insulin sensitivity has been observed in human subjects and laboratory animals. In diabetics Nagao *et al.*<sup>(221)</sup> noted enhanced adiponectin levels and reduced waist circumference after a continuous ingestion of a catechin-rich green tea for 12 weeks; this was accompanied by reduced HbA<sub>1c</sub> in patients treated with insulinotropic agents. A mechanistic study<sup>(222)</sup> in an obese insulin-resistant dog model showed, instead, a rise of the insulin sensitivity index by 60 % after green tea and a decrease of the TAG concentrations by 50 %, associated with an enhanced expression of PPAR $\gamma$ , GLUT4, LPL and adiponectin in the adipose tissue and PPAR $\alpha$  and LPL in skeletal muscle. The potential therapeutic role of green tea in diabetes and hyperlipidaemias thus needs further evaluation.

#### Policosanols

Policosanols extracted from the Cuban sugar cane wax have been reported to substantially decrease serum LDL-C concentrations<sup>(223)</sup>. Carefully controlled clinical trials from other countries have not confirmed these observations<sup>(224–227)</sup> and the effects of these compounds on serum lipids should be considered as unsubstantiated.

#### Conclusion

Diet and lifestyle goals for CVD prevention have received significant support from newer dietary recommendations, now with the potential to reduce serum total cholesterol and LDL-C by 30 % or more, as well shown by the group of Jenkins with their 'portfolio diet', including fibres, plant proteins, nuts and phytosterols<sup>(228)</sup>. While an increase of carbohydrate consumption may lower cholesterol and LDL-C values, but also HDL concentrations, vegetable proteins can selectively reduce LDL-C in experimental animals and in humans with cholesterol elevations of genetic or non-genetic origin. Reduced fat intake is a hallmark in the prevention of arterial disease: recent studies underline the role of a high-monounsaturated (40 % of fat) diet that shows essentially no difference in benefit *v.* a standard low-fat (20 %) diet. These dietary approaches can also lead to a reduced blood pressure.

Numerous functional ingredients, for example, plant sterols and stanols, have a direct cholesterol-lowering activity, by mechanisms that may require further investigation. Novel polyphenols, for example, from dark chocolate, provide significant benefit on blood pressure as

well as on the risk of thrombosis and certainly deserve deeper investigation.

Concerning the overall effects of *n*-3 fatty acids from fish or fish oil supplements, there is a general agreement, on the basis of outcomes in primary- and secondary-prevention studies, that increased consumption significantly reduces the rates of cardiac and especially of sudden death<sup>(229)</sup>.

Novel dietary approaches to cardiovascular prevention are thus of major significance in clinical research and practice. The apparent growing dislike of patients, particularly in primary prevention, for chronic drug intake will certainly lead to a wider use of these products in the cardiovascular field.

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