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Clinical Nutrition and Metabolism Group Symposium on 'Nutrition and antioxidants'

Antioxidants and lipoprotein metabolism

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Lipoprotein metabolism plays a significant role in the pathogenesis of atherosclerosis and risk of vascular disease. Elevated levels of LDL and low levels of HDL are linked to the risk of cardiovascular disease. It is now widely accepted that oxidative modification of LDL affects the metabolism of lipoproteins, leading to their increased accumulation in arterial intima. The present paper will examine some of the accumulating evidence from studies showing relationships between dietary intake of antioxidant nutrients, i.e. vitamins E and C, the carotenoids and the flavonoids, and their influence on lipoprotein metabolism. In particular, the present paper will review the available literature on the affect of antioxidant supplementation on lipoprotein oxidation, and hence metabolism of LDL in human subjects.

Lipoproteins: Antioxidants: Vitamins: Flavonoids

Lipoproteins are lipid–protein emulsions synthesized in the body to solubilize and transport fat and fat-soluble components such as triacylglycerols, cholesterol and some fat-soluble micronutrients in the blood. They are assembled in the liver, with the exception of chylomicrons and small amounts of HDL which are synthesized in intestines (Zubay, 1993). Lipids and lipid-soluble nutrients obtained from the diet are assembled in the intestine to form chylomicrons. These lipoproteins enter the bloodstream via the lymphatic system and supply the dietary fat primarily in the form of triacylglycerols to tissues. The remaining chylomicron remnants are taken up by the liver (Fig. 1). The half-life for clearance of chylomicrons and remnants from plasma of human subjects is 4–5 min.

Lipoproteins assembled in the liver contain dietary lipids supplied by chylomicron remnants and lipids obtained from *de novo* synthesis. These lipoproteins, the VLDL, are secreted into the bloodstream. HDL which are synthesized and secreted by the liver interact with VLDL particles and chylomicrons in the bloodstream and promote their maturation and function by the transfer of proteins and cholesteryl esters. The main biological function of VLDL is to supply fatty acids to peripheral tissues. Lipoprotein lipase (*EC* 2.3.1.43) on the surface of vascular endothelial cells hydrolyses VLDL-triacylglycerol to free fatty acids which can

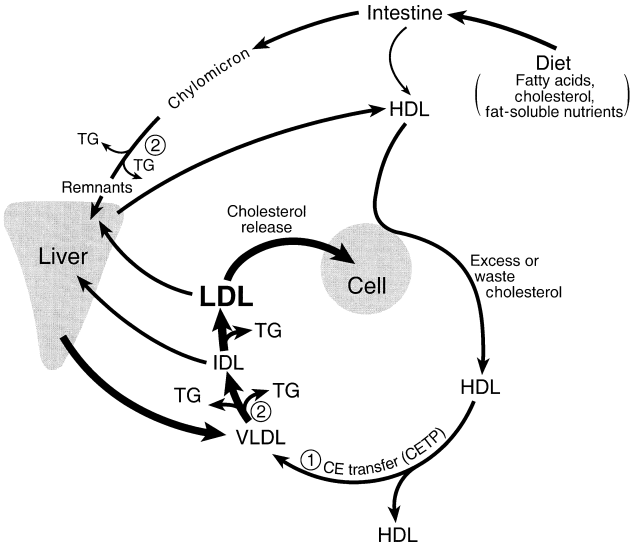

Fig. 1. Pathways for the synthesis and catabolism of lipoproteins. CETP, Cholesteryl ester transfer protein; CE, cholesteryl ester; TG, triacylglycerols. (1), Transfer of cholesteryl esters from HDL to VLDL; (2) release of TG by lipoprotein lipase (*EC* 3.1.1.34) from chylomicrons and VLDL into the tissues.

Abbreviations: apo, apolipoprotein; CETP, cholesteryl ester transfer protein.

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then be taken up by tissue cells. With increasing hydrolysis VLDL loses most of its triacylglycerol and progressively changes into IDL and finally to the cholesterol-rich LDL (Fig. 1). VLDL and IDL have a short half-life and are removed from the circulation within hours. LDL have a relatively longer half-life and circulate in blood for about 2–3 d before they are cleared from the circulation by the liver. HDL has the longest lifespan in the circulation, about 5–6 d (Zubay, 1993). LDL are a major carrier of cholesterol to tissues and HDL particles remove the excess or unused cholesterol from cells. Cholesterol, after being taken up by HDL, is converted to cholesteryl esters by the enzyme lecithin-cholesterol acyltransferase (*EC* 2.3.1.43). Some HDL are taken up by the liver directly. The remainder of the cholesteryl esters are transferred to VLDL through the action of cholesteryl ester transfer protein (CETP). The cholesteryl esters are either delivered to extrahepatic tissues by LDL or subsequently taken up by the liver as part of IDL or LDL (Zubay, 1993).

The delivery of cholesterol by LDL to various tissues in the body can take place via several routes. The most common route is the uptake by native or classical receptors on cells. These receptors recognize apolipoprotein (apo) B100- and apoE-containing lipoprotein particles, which include apoE-containing IDL and chylomicron remnants, and apoB100-containing LDL particles. However, since levels of apoB100-containing LDL particles are much higher than those of apoE-containing chylomicron remnants, it is mainly apoB100-containing LDL particles that are taken up by the receptors (Mackness & Durrington, 1995). On entry into cells LDL is metabolized in the lysosomes or microsomes. ApoB components of LDL are hydrolysed to the constituent amino acids, and cholesteryl esters are hydrolysed to free cholesterol. Cholesterol which is released is either incorporated into cell membranes, metabolized to steroid hormones in the adrenal glands, to bile acids in the liver, to vitamin D in skin, or re-esterified and stored as cholesteryl esters. Excess or unused cholesterol is removed by the HDL particles. An increase in cholesterol in cells inhibits the synthesis of LDL receptors, and hence reduces the cellular uptake of cholesterol (Zubay, 1993; Mackness & Durrington, 1995).

However, there are two other known routes by which LDL can enter cells, and neither pathway is down-regulated by the cholesterol content of the cell (Sparrow *et al*. 1989; Mackness & Durrington, 1995). One route is a non-receptor pathway, and this pathway is of significance only at high blood concentrations of LDL. The second pathway is the scavenger receptor pathway, which is believed to play a key role in the build up of cholesterol in arterial intima and can be affected by the antioxidants. Scavenger receptors do not recognize the native LDL but rather engulf modified LDL particles. One such modification is caused by the oxidation of LDL particles. Although the exact mechanism by which the oxidation of LDL takes place *in vivo* is not clear, it is widely accepted that oxidatively-modified LDL is metabolized in a manner which leads to build up of cholesterol in arterial intima, and plays an important role in the pathogenesis of atherosclerosis.

LDL oxidation can be initiated *in vitro* by incubation with cells which include activated polymorphonuclear leukocytes, macrophages, endothelial cells, smooth muscle cells and lymphocytes (for references, see Rice-Evans & Bruckdorfer, 1995). In cell-free systems, a variety of pro-oxidants have been identified which include the enzyme lipoxygenase (*EC* 1.13.11.12), haem, Cu ions, γ-irradiation, oxygen-derived species such as HOC1, peroxynitrite (ONOO) and sulphydryl compounds (Rice-Evans & Bruckdorfer, 1995). Other factors which can affect the oxidative modification of LDL *in vivo* are the residence time of LDL in plasma (Schwenke, 1995; Salter *et al*. 1998), the enzyme paraoxonase (*EC* 3.1.1.2) associated with HDL (Mackness & Durrington, 1991), antioxidant protection by HDL (Singh *et al*. 1997), density of LDL (Chait *et al*. 1992), fatty acid unsaturation (Reaven *et al*. 1993; Visioli *et al*. 1995) and the antioxidant content of LDL (Tertov *et al*. 1998). Antioxidants, especially vitamin E, have been shown to inhibit oxidative modification of LDL in both *in vitro* and *in vivo* studies (Esterbauer *et al*. 1991; Belcher *et al*. 1993; Suzukawa *et al*. 1995). These studies demonstrate that LDL resists oxidation until it is depleted of its antioxidant content. The time period of LDL resistance to oxidation is termed the lag phase (Fig. 2; lipid oxidation is monitored at 234 nm to measure conjugated dienes, the lipid oxidation products)

Vitamin E, LDL oxidation and lipoprotein metabolism

Vitamin E or α-tocopherol is a lipid-soluble nutrient obtained from a diet rich in vegetable oils, animal foods, cereals and fruit and vegetables. It is a major antioxidant component of LDL (Table 1) and its importance in inhibiting LDL oxidation was recognized after the initial reports by

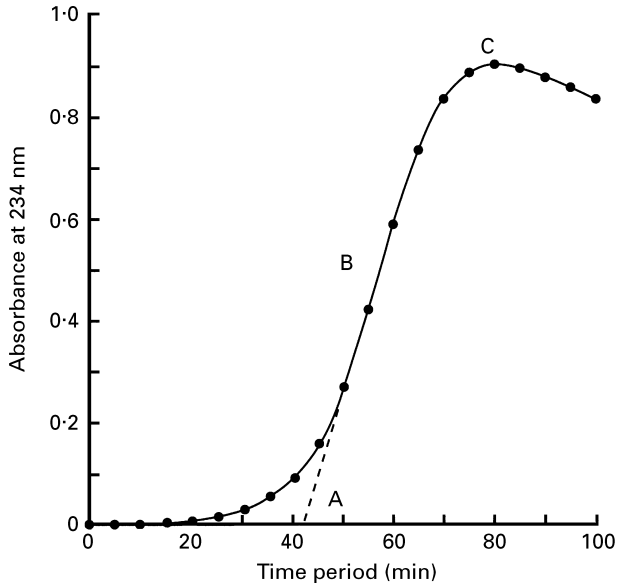

Fig. 2. Kinetics of LDL oxidation catalysed by copper *ex vivo*. Reaction curve showing the kinetics of copper-initiated oxidation of LDL (measured as absorbance at 234 nm due to the appearance of lipid oxidation products, the conjugated dienes). A is the lag phase and is equal to the time interval of resistance of LDL to oxidation. A is calculated from the intersection of a line drawn by extrapolation of the linear phase (B) through the horizontal axis. B is the propagation phase and C is the termination phase when there is no further increase in absorbance at 234 nm.

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Table 1. Lipid-soluble antioxidants in LDL particle
(Mean values and ranges)

	mmol/mol LDL				mol/mol apoB	
Antioxidant	Mean	Range*	Mean	Range†	Mean	Range†
α-Tocopherol	6·4	2·9–14·9	7·68	2·87–14·4	16·1	6·57–25·14
γ-Tocopherol	0·51	0·18–1·26	0·35	0·05–1·13	0·74	0·19–1·98
α-Carotene	0·12	0·02–0·52	0·06	0·01–0·22	0·13	0·03–0·52
β-Carotene	0·29	0·03–1·87	0·27	0·07–0·69	0·59	0·15–1·68
Lutein+zeaxanthin	0·04	0·01–0·16	0·07	0·03–0·38	0·15	0·07–0·36
Lycopene	0·16	0·03–0·70	0·47	0·08–1·54	1·02	0·32–2·84
Cryptoxanthin	0·14	0·03–0·70	0·11	0·02–0·38	0·24	0·05–0·91
Ubiquinol-10	0·10	0·03–0·35				

apoB, apolipoprotein B.
* Previously published data from Esterbauer *et al.* (1991).
† Analysis done in the authors' laboratory.

Esterbauer *et al*. (1989). This group reported that *in vitro* LDL oxidation is prevented until LDL is depleted of its antioxidant nutrients such as tocopherols and carotenoids. Subsequently, other antioxidant components of LDL, e.g. ubiquinones (Stocker *et al*. 1991) and flavonoids (de Whally *et al*. 1990), have also been reported to be important in delaying the oxidation of LDL.

Earlier work by Esterbauer's laboratory (Dieber-Rothender *et al*. 1991; Ziozenkova *et al*. 1996) showed that in unsupplemented samples there was no correlation between the α-tocopherol content of LDL and its resistance to oxidation, i.e. the lag phase. Subsequently, several studies have reported a lack of correlation between lag phase and plasma vitamin E concentrations in unsupplemented samples except for one study (Porkkala-Sarataho *et al*. 1996). The latter workers reported a positive correlation between the oxidation resistance of LDL and VLDL, and plasma vitamin E. However, there is a general consensus that a significant correlation between LDL oxidation and α-tocopherol emerges when LDL is loaded with α-tocopherol by oral ingestion (Princen *et al*. 1992; Reaven *et al*. 1993; Jialal *et al*. 1995; Suzukawa *et al*. 1995).

A few studies (Stocker *et al*. 1991; Bowry *et al*. 1992, 1993) have shown a pro-oxidant effect of vitamin E on LDL oxidation, since there was an increase in LDL oxidation after supplemention. They proposed that peroxidation is propagated within lipoprotein particles by reaction of the tocopheroxyl radical with polyunsaturated fatty acid moieties in the lipid, and that this reaction can be inhibited by the presence of antioxidants such as ubiquinones and ascorbic acid which can regenerate α-tocopherol (Bowry *et al*. 1993; Thomas *et al*. 1996). We have recently found that in Irish subjects (age 25–45 years) supplemention with 100 mg vitamin E/d for 1 month produced a small but non-significant decrease in LDL-cholesterol, a significant ($P = 0{\cdot}014$) increase in apoB levels, and a significant ($P = 0{\cdot}029$) increase in the susceptibility of LDL to oxidation (Table 2; Chopra *et al*. 1997). In the same study, the same supplement, when given to volunteers of the same age-group in other European centres, produced an antioxidant effect on lipoprotein oxidation. (All samples were analysed centrally.) Whether vitamin E supplements exert an anti-oxidant or a pro-oxidant effect appears to be influenced, therefore, by factors in blood about which we still have insufficient information. In our study (Chopra *et al*. 1997),

Table 2. Effect of vitamin E supplementation in healthy non-smoking subjects in Northern Ireland*
(Mean values with their standard errors)

	Total cholesterol (mmol/l)		ApoB (μmol/l)		Lag phase (min)	
	Mean	SE	Mean	SE	Mean	SE
Baseline	2·92	0·14	0·635	0·03	56	1·02
Supplementation (100 mg/d for 4 weeks)	2·77	0·15	0·747	0·03	51	1·06
Statistical significance of difference (paired *t* test)	NS		$P = 0{\cdot}014$		$P = 0{\cdot}029$	

* Data from Northern Ireland arm (*n* 63) of AIR2-CT93-0888 multicentre (five European centres) study.

we also found a significant reduction in γ-tocopherol and carotenoid levels of LDL. Whether this reduction in carotenoid levels could have accounted for the reduction in the lag phase is not known. There was no correlation between the carotenoid levels and the lag phase, but in most *in vivo* supplementation studies with carotenoids there is usually no increase in the lag phase (Gaziano *et al*. 1995; Chopra *et al*. 1996).

In *in vivo* studies with vitamin E where both *ex vivo* LDL oxidizability and serum lipid levels were measured, no effect of vitamin E supplementation on plasma or serum cholesterol levels was observed (Table 3). However, these studies were done in normocholesterolaemic subjects (Esterbauer *et al*. 1991; Jialal *et al*. 1995; Suzukawa *et al*. 1995). In hypercholesterolaemic patients, vitamin E supplementation at 500 mg/d for 3 months was shown to significantly increase the apoA and HDL levels ($P < 0{\cdot}05$), reduce total cholesterol : HDL by 23% and reduce apoA : apoB by 17·9% (Cloarec *et al*. 1987). Hypercholesterolaemia is believed to increase the vitamin E levels in blood as a high-cholesterol diet is associated with increased plasma vitamin E, and a low-cholesterol diet with reduced vitamin E levels (Bitman *et al*. 1976). However, recently it was reported that tocopherol : cholesterol is the same in low- and high-cholesterol individuals (Muldon *et al*. 1996). Thus, how vitamin E affects lipoprotein metabolism is not clear at present.

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Table 3. Supplementation studies with vitamin E

Reference	Subjects	Dose and duration of vitamin E supplementation	Effect
Esterbauer *et al*. (1991)	Normal-lipid human subjects	100–1200 mg/d for 3 weeks	Resistance of LDL to oxidation ↑ No effect on cholesterol
Suzukawa *et al.* (1995)	Normal-lipid human subjects	100 mg/d for 1 week + additional 3 weeks with 300 mg/d	LDL lag phase ↑, lipid peroxidation rate ↓ No effect on TC and TG
Jialal *et al.* (1995)	Normal-lipid human subjects	60–1200 mg/d for 8 weeks	LDL oxidation ↓ No effect on cholesterol and TG
Cloarec *et al.* (1987)	Hyperlipidaemic human subjects	500 mg DL-α-tocopheryl acetate/d for 12 weeks	TC : HDL ↓, apoB ↓ and apoA ↑
Oriani *et al.* (1997)	Normal weanling rabbits	60–260 mg/kg diet (6 week), for 20 weeks vitamin E-deficient or 60 mg vitamin E/kg	TC and TG ↓ in vitamin E-treated animals TC ↑ and HDL ↓ in vitamin E-deficient animals
Shen *et al.* (1996)	Hamsters	HC and SF diet plus 100 mg/kg body wt per d vitamin E for 8 weeks	HDL ↑ Total and LDL-cholesterol ↓ Plasma CETP activity ↓
Liu *et al.* (1997)	Rabbits	Cholesterol feeding plus 5–45 mg vitamin E/kg body wt per d for 14 weeks	No effect on cholesterol No effect on CETP

TC, total cholesterol; TG, triacylglycerols; HC, high-cholesterol; SF, saturated fat; CETP, cholesteryl ester transfer protein; apo, apolipoprotein; ↑, increase; ↓, decrease.

Several animal studies have also shown that vitamin E supplementation affects lipoprotein metabolism by reducing serum triacylglycerols (Oriani *et al*. 1997) and total cholesterol, and increasing HDL-cholesterol levels. In streptozotocin-diabetic rats vitamin E supplementation prevented accumulation of lipid peroxides and maintained normal triacylglycerol levels (Karasu *et al*. 1997). However, in rabbits administration of cholesterol with vitamin E protected against oxidative damage but had no effect on the lipid levels (Prasad & Kalra, 1993). In another study vitamin E administration (100 mg/kg body weight per d for 8 weeks) with saturated fat and a cholesterol-rich diet to rats was reported to suppress cholesteryl ester transfer from HDL by suppressing the CETP activity in the plasma and adipose tissue of lipid-fed rats (Shen *et al*. 1996). An increased concentration of CETP in plasma is associated with reduced HDL-cholesterol and, therefore, it is possible that the increase in HDL-cholesterol observed in human vitamin E supplementation studies is due to the effect on CETP activity. However, recently it has been shown in rabbits that cholesterol feeding together with varying doses of vitamin E (5, 15, 30 and 45 mg/kg body weight per d for 14 weeks) had no effect on the CETP activity of the plasma (Liu *et al*. 1997). Metabolism of lipoproteins in human subjects is more closely related to that of rabbits, hamsters and pigs than that of rats; therefore, whether hypocholesterolaemic effects of vitamin E in human subjects occurs via its effect on CETP is controversial at present.

The effect of vitamin E supplementation on lipoprotein metabolism, therefore, is not fully understood at present, and warrants further carefully planned studies to look at the effect on lipoprotein oxidation and the key enzymes of cholesterol and lipoprotein metabolism, i.e. hydroxymethylglutaryl-CoA reductase (*EC* 1.1.1.88), lipoprotein lipase and tissue and plasma CETP activity.

Vitamin C, LDL oxidation and lipoprotein metabolism

Ascorbate supplementation has been shown to reduce lipoprotein oxidation in genetically-scorbutic rats (Kimura *et al*. 1992), and in non-smoking (Rifici & Khachadurian, 1993) and smoking (Fuller *et al*. 1996) human subjects. Vitamin C is a water-soluble antioxidant nutrient and, therefore, can only protect LDL from modification by extracellular oxygen-derived species such as superoxide ions, H_2O_2 and HOCl. However, an important mechanism through which it can protect oxidation of lipids in LDL is through its ability to regenerate α-tocopherol from the α-tocopheroxyl radical (Packer *et al*. 1979), the latter being produced by interaction of α-tocopherol with the lipid-derived radical species. A combination of vitamin C and E has been reported to be more effective in inhibiting oxidative modification of LDL than vitamin E alone by some workers (Rifici & Khachadurian, 1993) and not by others (Jialal & Grundy, 1993). However, the treatment did not lower levels of undesirable cholesterol, i.e. LDL-cholesterol.

Hypolipidaemic effects of vitamin C which may not be related to its antioxidant action have been reported (Simon & Hudes, 1998). In animal studies vitamin C has been shown to increase the metabolism of cholesterol to bile acids in the liver (Ginter, 1975), and in guinea-pigs sodium ascorbate intakes of up to 5 mg/d have been shown to significantly ($P = 0{\cdot}025$) reduce plasma cholesterol and increase bile acid formation (Hornig & Weiser, 1976). An intake above 5 mg/d did not show any additional benefit. Increased conversion of cholesterol to bile acids would lead to a reduction in cholesterol levels in liver cells and, hence, increased expression of LDL receptors on liver cells, leading to an increased removal of cholesterol from the circulation. In cholesterol-fed rabbits ascorbic acid prevents a rise in serum cholesterol levels (McConnell & Sokoloff, 1964; Sokoloff *et al*. 1967).

In human intervention studies, a reduction in total cholesterol and LDL-cholesterol in hyperlipidaemic subjects has been reported by some researchers (Ginter *et al*. 1970; Paolisso *et al*. 1995) but not by others (Harats *et al*. 1998). Similarly, reports from human observational studies are controversial, a significant negative correlation between ascorbic acid status and total serum cholesterol has been reported by some researchers (Cerna & Ginter, 1978; Greco & LaRocca, 1982) but not by others (Ness *et al*. 1996). Most studies, however, have shown a negative association

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between triacylglycerols and serum vitamin C and a positive association between HDL and serum vitamin C levels. The association between vitamin C and lipids has been discussed in several papers (Ness *et al*. 1996; Simon & Hudes, 1998), including a review by Simon (1992), and it has been suggested that associations between vitamin C and serum lipids, including HDL-cholesterol, seem to be more prominent in hyperlipidaemic subjects.

Various mechanisms have been proposed to explain the association between vitamin C and lipoprotein metabolism. These mechanisms include increased conversion of cholesterol to bile acids in the liver (Ginter, 1975; Holloway & Rivers, 1981), an increased number of LDL receptors in liver cells (Aulinskas *et al*. 1983) and an increase in HDL-cholesterol via protection of HDL from oxidative damage (Rifici & Khachadurian, 1996), through regulation of lipoprotein lipase activity (Kotze, 1975; for review, see Lynch *et al*. 1996) and induction of cytochrome P_{450} activity (Mori *et al*. 1997). For detailed references, see reviews by Jacques *et al*. (1994), Jacques (1995) and Ness *et al*. (1996).

Although the majority of studies suggest that ascorbic acid can affect lipoprotein metabolism via both its hypolipidaemic and antioxidant effects, discrepancies exist in the literature which require clarification, and hence a need for additional well-designed trials.

Carotenoids, LDL oxidation and lipoprotein metabolism

Carotenoids are coloured pigments found in red, green, yellow and orange fruits and vegetables. Results from *in vivo* supplementation studies with carotenoids have not been encouraging. Most studies fail to show protection of LDL after *in vivo* supplementation with carotenoids (Gaziano *et al*. 1995; Nenster *et al*. 1995; Chopra *et al*. 1996). Levy *et al*. (1995) reported that the *trans*-isomer of β-carotene is more effective in protecting LDL than the *cis*-isomer. We have found that *in vivo* supplementation with lutein (a carotenoid found mainly in green leafy vegetables) at a dose of 30 mg/d in sunflower oil had no effect on Cu-initiated oxidation of LDL *ex vivo* (Table 4). However, when a similar dose of lutein was given in olive oil there was a significant increase in the lag phase ($P < 0{\cdot}01$). The placebo group given only olive oil also showed an increase in the lag phase, but this increase was not significant.

A few studies have looked at the effect of carotenoid supplementation on serum triacylglycerol and cholesterol levels. β-Carotene supplementation produced no change in serum triacylglycerol or in total, LDL- and HDL-cholesterol in patients with skin cancer (Nierenberg *et al*. 1991), hypercholesterolaemic post-menopausal women (Nester *et al*. 1995), healthy older women (Ribayamercado *et al*. 1995), healthy smokers (Van Poppel *et al*. 1994) and patients with protoporphyria (Poh-Fitzpatrick & Palmer, 1984). Recently, it has been reported that the ingestion of lycopene (a carotenoid found in tomato products and watermelon (*Citrullus lanatus*)) in various foods, i.e. as spaghetti sauce or tomato juice or capsules for 1 week, increased the resistance of LDL to oxidation (Agarwal & Rao, 1998) but had no effect on total serum cholesterol and lipoprotein-cholesterol levels.

The present evidence suggests, therefore, that although some studies show that carotenoids may protect LDL from oxidation, the effects are usually small and they do not show any effect on lipoprotein metabolism.

Fruits and vegetables, LDL oxidation and lipoprotein metabolism

Diets rich in fruits and vegetables have been shown to increase the resistance of LDL to oxidation (Abbey *et al*. 1995; Harats *et al*. 1998). Recently, we have shown that increased consumption of a carotenoid-rich (i.e. fruit and vegetable) diet (providing approximately 30 mg carotenoids/d) for 2 weeks increases the resistance of LDL to oxidation in smokers and non-smokers (Chopra *et al*. 1996; Hinninger *et al*. 1997). No correlation was observed between the resistance of LDL to oxidation and plasma carotenoid levels. Also, supplementation had no effect on total cholesterol or lipoprotein-cholesterol levels, plasma triacylglycerols or apoA1 or apoB levels. Supplementation of diets with fruits and vegetables in combination with a 'prudent diet' for 2 weeks has been reported to reduce total cholesterol by 6·5%, LDL-cholesterol by 7·3% and serum triacylglycerols by 7% in patients with coronary artery disease (Singh *et al*. 1992*b*). The same authors have also shown that in patients with essential hypertension who ate guava fruit (*Psidicon guajava*) serum cholesterol was reduced by 9·9% and triacylglycerol levels by 7·7%, while HDL-cholesterol was increased by 8·0% (Singh *et al*. 1992).

Table 4. Effect of oral supplementation of human subjects with 30 mg lutein/d in either olive oil or sunflower oil on LDL oxidation *ex vivo* (Mean values and standard deviations for six subjects per treatment)

	Lutein concentration (mol/mol LDL)				Lag phase (min)			
	Baseline		After 2 weeks		Baseline		After 2 weeks	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Olive oil:								
Placebo group	0·066	0·03	0·097*	0·03	47	10	54	10
Supplemented group	0·070	0·03	0·381**	0·11	41	4	50**	3
Sunflower oil:								
Placebo group	0·049	0·02	0·053	0·02	47	7	45	3
Supplemented group	0·056	0·02	0·317**	0·15	44	7	41	4

Mean values were significantly different from baseline values (paired *t* test): * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

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Fruits and vegetables are a rich source of fibre, and dietary fibre is known to lower blood cholesterol levels (Coats, 1998). Thus, the reduction in lipid levels observed in dietary intervention trials with fruits and vegetables may be related to factors other than their antioxidant nutrient components.

Flavonoids, LDL oxidation and lipoprotein metabolism

The 'French Paradox' has initiated a tremendous interest in the flavonoid components in the diet, especially in wine, but also the antioxidant and hypolipidaemic effects of flavonoids obtained from tea and onions. To date, however, studies on wine have produced inconsistent results. Several reports have shown an increase in the resistance of LDL to oxidation following wine supplements (Frankel *et al*. 1993; Furham *et al*. 1995; Carbonneau *et al*. 1997; Nigdikar *et al*. 1998), while other reports fail to show an effect (Sharpe *et al*. 1995; de Rijke *et al*. 1996). The lack of effect might be related to the different compositions of red wines, as it is still not fully known which component(s) of the red wine may be responsible for the effects.

Controversial reports on the effect of tea flavonoids on the resistance of LDL to oxidation in human subjects have been reported. Ishikawa *et al*. (1997) reported an increase in the resistance of LDL to oxidation, but Princen *et al*. (1998) showed that both green and black tea consumption had no effect on LDL oxidation and lipid levels in smoking women. In animals tea consumption, especially green tea, has been reported to reduce lipid peroxides, increase the resistance of LDL to oxidation, reduce total cholesterol and triacylglycerol levels, and increase the HDL-cholesterol levels in both normal and cholesterol-fed hamsters (Vinson *et al*. 1998). Feeding rats with the flavonoids quercetin and hesperidin at 50 mg/kg body weight per d together with a hypercholesterolaemic diet for 3 weeks maintained the normal cholesterol : phospholipid compared with the elevated value observed in rats receiving hypercholesterolaemic diet (Rathi *et al*. 1984). Tea polyphenols given with a diet containing cholesterol have been shown to reduce serum cholesterol and increase faecal elimination of cholesterol and lipids (Muramatsu *et al*. 1986; Yugarani *et al*. 1992). It has been suggested that flavonoids exert a hypocholesterolaemic effect through their stabilizing effect on lysosomes (Monforte *et al*. 1995).

In summary, present evidence suggests that antioxidant nutrients such as vitamins E, C and flavonoids may have a role in lipoprotein metabolism, which may or may not be related to their antioxidant action. Most studies suggest that the hypolipidaemic effect of these nutrients is only effective in hyperlipidaemic subjects. Hypercholesterolaemia is associated with increased oxidant stress (Prasad & Kalra, 1993), therefore the possibility that the hypolipidaemic effect of antioxidant nutrients is related to their antioxidant action cannot be dismissed.

Thus, except for the carotenoids, the findings of antioxidant intervention trials in human subjects support the hypothesis that dietary antioxidants exert a protective action on lipoproteins. Whether antioxidant nutrients affect lipoprotein metabolism through their antioxidant activity or by some other mechanism is not clear at present, and requires further investigation. Animal studies are contradictory, but this situation is not surprising as lipoprotein metabolism follows different pathways in different species. Thus, there is still a need for more carefully-planned human studies to fill the gaps in our knowledge of the effects of antioxidants on lipoprotein metabolism.

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References

Abbey M, Noakes M & Nestel PJ (1995) Dietary supplementation with orange and carrot juice in cigarette smokers lowers oxidation-products in copper oxidized low density lipoproteins. *Journal of the American Dietetic Association* **95**, 671–675.

Agarwal S & Rao V (1998) Tomato lycopene and low density lipoprotein oxidation: A human dietary intervention study. *Lipids* **33**, 981–984.

Aulinskas TH, Van der Westhuyzen DR & Coetzee GA (1983) Ascorbate increases the number of low density lipoprotein receptors in cultured arterial smooth muscle cells. *Atherosclerosis* **47**, 159–171.

Belcher JD, Balla J, Balla G, Jacobs DR Jr, Gross M, Jacob HS & Vercellotti GM (1993) Vitamin E, LDL and endothelium. Brief oral vitamin supplementation prevents oxidised LDL mediated vascular injury in vitro. *Arteriosclerosis and Thrombosis* **13**, 1779–1789.

Bitman J, Weyant J, Wood DL & Wrenn TR (1976) Vitamin E, cholesterol and lipids during atherogenesis in rabbits. *Lipids* **11**, 449–461.

Bowry VW, Ingold KU & Stocker R (1992) Vitamin-E in human low density lipoprotein – When and how this antioxidant becomes a prooxidant. *Biochemical Journal* **288**, 341–344.

Bowry VW & Stocker R (1993) Tocopherol mediated peroxidation – The prooxidant effect of vitamin E on the radical initiated oxidation of human low density lipoprotein. *Journal of the American Chemical Society* **115**, 6029–6044.

Carbonneau M-A, Leger CL, Monnier L, Bonnet C, Michel F, Fouret G, Dedieu F & Descomps B (1997) Supplementation with wine phenolic compounds increases the antioxidant capacity of plasma and vitamin E of low-density lipoprotein without changing the lipoprotein Cu^{2+}-oxidisability: Possible explanation by phenolic location. *European Journal of Clinical Nutrition* **51**, 682–690.

Cerna O & Ginter E (1978) Blood lipids and vitamin C status. *Lancet* **i**, 1055–1056.

Chait A, Ronald MD & Tribble DL (1992) Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, Pattern B. *American Journal of Medicine* **94**, 350–356.

Chopra M, McLoone U, O'Neill M & Thurnham DI (1997) Effect of oral supplementation with vitamin E on carotene levels in different lipoprotein fractions in humans. *Proceedings of The Nutrition Society* **56**, 100A.

Chopra M, McLoone U, O'Neill M, Williams N & Thurnham DI (1996) Fruit and vegetable supplementation – effect on ex vivo LDL oxidation in humans. In *Natural Antioxidants and Food Quality in Atherosclerosis and Cancer Prevention*, pp. 150–155 [JT Kumpulainen and JT Salonen, editors]. Cambridge: The Royal Society of Chemistry.

Cloarec MJ, Perdriset GM, Lamberdiere FA, Colasbelcour JF, Sauzieres JP, Neufeld HN & Goldbourt U (1987) Alpha tocopherol – Effect on plasma lipoproteins in hypercholesterolemic patients. *Israel Journal of Medical Sciences* **23**, 869–872.

Coats AJS (1998) The potential role of soluble fibre in the treatment of hypercholesterolaemia. *Postgraduate Medical Journal* **74**, 391–394.

de Rijke YB, Demacker PNM, Assen NA, Sloots LM, Katan MB & Stalenhoef AFH (1996) Red wine consumption does not affect oxidizability of low density lipoproteins in human volunteers. *American Journal of Clinical Nutrition* **63**, 329–334.

de Whally CV, Rankin SM, Hoult JRS, Jessup W & Leake DS (1990). Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. *Biochemical Pharmacology* **39**, 1743–1750.

Dieber-Rothender M, Puhl H, Waeg G, Streigl G & Esterbauer H (1991) Effect of oral supplementation with D-α-tocopherol on the vitamin E content of human low density lipoproteins and resistance to oxidation. *Journal of Lipid Research* **32**, 1325–1332.

Esterbauer H, Puhl H, Dieber-Rothender M, Waeg G & Rabl H (1991) Effect of antioxidants on oxidative modification of LDL. *Annals of Medicine* **23**, 573–581.

Esterbauer H, Striegl G, Puhl H & Dieber-Rothender M (1989) Continuous monitoring of in vitro oxidation of human low density lipoproteins. *Free Radical Research Communications* **6**, 67–75.

Frankel EN, Waterhouse AL & Kinsella JE (1993) Inhibition of human LDL oxidation by reservatrol. *Lancet* **341**, 1103–1104.

Fuller CJ, Grundy SM, Norkus EP & Jialal I (1996) Effect of ascorbate supplementation on low density lipoprotein oxidation in smokers. *Atherosclerosis* **119**, 139–150.

Furham B, Lavy A & Aviram M (1995) Consumption of red wine with meals reduces the susceptibility of human plasma and low-density lipoprotein to lipid peroxidation. *American Journal of Clinical Nutrition* **61**, 549–554.

Gaziano MJ, Hatta A, Flynn M, Johnson EJ, Krinsky NI, Ridker PM, Hennekens CH & Frei B (1995). Supplementation with β-carotene in vivo and in vitro does not inhibit low density lipoprotein oxidation. *Atherosclerosis* **112**, 187–195.

Ginter E (1975) Ascorbic acid in cholesterol and bile acid metabolism. *Annals of the New York Academy of Sciences* **258**, 410–421.

Ginter E, Kajaba I & Nizner O (1970) The effect of ascorbic acid on cholesterolaemia in healthy subjects with seasonal deficiency of vitamin C. *Nutrition Metabolism* **12**, 76–86.

Greco AM & LaRocca L (1982) Correlation between chronic hypovitaminosis in old age and plasma levels of cholesterol and triglycerides. *International Journal of Vitamin and Nutrition Research* **23**, Suppl., 129–136.

Harats D, Chevion S, Nahir M, Norman Y, Sagee O & Berry EM (1998) Citrus fruit supplementation reduces lipoprotein oxidation in young men ingesting a diet high in saturated fat: presumptive evidence for interaction between vitamins C and E in vivo. *American Journal of Clinical Nutrition* **67**, 240–245.

Hinninger I, Chopra M, Thurnham DI, Laporte F, Richard M-J, Favier A & Roussel A-M (1997) Effect of increased fruit and vegetable intake on the susceptibility of lipoprotein to oxidation in smokers. *European Journal of Clinical Nutrition* **51**, 601–606.

Holloway DE & Rivers JM (1981) Influence of chronic ascorbic acid deficiency and excessive ascorbic acid intake on bile acid metabolism and bile composition in the guinea pig. *Journal of Nutrition* **111**, 412–424.

Hornig D & Weiser H (1976) Ascorbic acid and cholesterol: effect of graded oral intakes on cholesterol conversion to bile acids in guinea pig. *Experientia* **32**, 687–689.

Howard PA & Meyers DG (1995) Effect of vitamin C on plasma lipids. *Annals of Pharmacotherapy* **29**, 1129–1136.

Ishikawa T, Suzukawa M, Ito T, Yoshida H, Ayaori M, Nishiwaki M, Yonemura A, Hara Y & Nakamura H (1997) Effect of tea flavonoid supplementation on the susceptibility of low-density lipoprotein oxidative modification. *American Journal of Clinical Nutrition* **66**, 261–266.

Jacques PF (1995) Effect of vitamin C supplementation on lipoprotein cholesterol, apolipoprotein and triglyceride concentration. *Annals of Epidemiology* **5**, 52–59.

Jacques PF, Sulsky SI, Perrone GA & Schaefer EJ (1994) Ascorbic acid and plasma lipids. *Epidemiology* **5**, 19–26.

Jialal I, Fuller CJ & Huet BA (1995) The effect of α-tocopherol supplementation on LDL oxidation. A dose response study. *Arteriosclerosis, Thrombosis and Vascular Biology* **15**, 190–198.

Jialal I & Grundy SM (1993) Effect of combined supplementation with α-tocopherol, ascorbate and β-carotene on low density lipoprotein oxidation. *Circulation* **88**, 2780–2786.

Karasu C, Ozansoy G, Bozkurt O, Erdogan D & Omerogl S (1997) Antioxidant and triglyceride lowering effects of vitamin E associated with the prevention of abnormalities in the reactivity and morphology of aorta from streptozotocin-diabetic rats. *Metabolism* **46**, 872–879.

Kimura H, Yamada Y, Morita Y, Ikeda H & Matsuo T (1992) Dietary ascorbic acid depresses plasma and low density lipoprotein lipid peroxidation in genetically scorbutic rats. *Journal of Nutrition* **122**, 1904–1909.

Kotze JP (1975) The effect of vitamin C on lipid metabolism. *South African Medical Journal* **49**, 1651–1654.

Levy Y, Ben-Amotz A & Aviram M (1995) Effect of dietary supplementation of different β-carotene isomers on lipoprotein oxidative modification. *Journal of Nutritional and Environmental Medicine* **5**, 13–22.

Liu XQ, Buchanan W, Mathews AJ, Chung BH & Bagdade JD (1997) Lack of effect of vitamin E on cholesterol ester transfer and lipoprotein composition in cholesterol fed rabbits. *Comparative Biochemistry and Physiology* **117B**, 553–559.

Lynch SM, Gaziano JM & Frei B (1996) Ascorbic acid and atherosclerotic cardiovascular disease. *Sub-cellular Biochemistry* **25**, 331–367.

McConnell B & Sokoloff B (1964) The effect of ascorbic acid on the blood cholesterol and clearing factor levels: experimental and clinical study. In *Proceedings of 6th International Congress on Nutrition*, pp. 123–125. Edinburgh: E & S Livingstone Ltd.

Mackness MI & Durrington PN (1991) HDL, its enzymes and its potential to influence lipid peroxidation. *Atherosclerosis* **115**, 243–253.

Mackness MI & Durrington PN (1995) Lipid transport and lipoprotein metabolism. In *Oxidative Stress, Lipoproteins and Cardiovascular Disease*, pp. 33–35 [C Rice-Evans and KR Bruckdorfer, editors]. London: Portland Press.

Monforte MT, Trovato A, Kiriavainen S, Forestieri AM, Galati EM & Curtor BC (1995) Biological effects of hesperidin, a citrus flavonoid. Hypolipidemic activity on experimental hypercholesterolemia in rats. *Farmaco* **50**, 595–599.

Mori T, Itoh S, Ohgiya S, Ishizaki K & Kamataki T (1997) Regulation of CYP1A and CYP3A mRNAs by ascorbic acid in guinea pigs. *Archives of Biochemistry and Biophysics* **348**, 268–277.

Muldon MK, Kritchevsky SB, Evans RW & Kagan VE (1996) Serum total antioxidant activity in relative hypo- and hypercholesterolaemia. *Free Radical Research* **25**, 239–245.

Muramatsu K, Fukuyo M & Hara Y (1986) Effect of green tea catechins on plasma cholesterol level in cholesterol fed rats. *Journal of Nutritional Science and Vitaminology* **32**, 613–622.

Nenster MS, Volden V, Berg T, Drevon A, Ose L & Tonstad S (1995) No effect of β-carotene supplementation on the suscepti-

https://doi.org/10.1017/S0029665199000865 Published online by Cambridge University Press

bility of low density lipoprotein to in vitro oxidation among hypercholesterolaemic postmenopausal women. *Scandinavian Journal of Clinical and Laboratory Investigation* **55**, 477–485.

Ness AR, Khaw KT, Bingham S & Day NE (1996) Vitamin C status and serum lipids. *European Journal of Clinical Nutrition* **50**, 724–729.

Nierenberg DW, Bayrd GT & Stukel TA (1995) Lack of effect of chronic adminstration of oral β-carotene on serum cholesterol and triglyceride concentratons. *American Journal of Clinical Nutrition* **53**, 652–654.

Nigdikar SV, Williams N, Griffin BA & Howard AN (1998) Consumption of red wine polyphenols reduces the susceptibility of low-density lipoproteins to oxidation in vivo. *American Journal of Clinical Nutrition* **68**, 258–265.

Oriani G, Salvatori G, Maiorano G, Belisario MA, Pastinese A, Manchisi A & Pizzuti G (1997) Vitamin E nutrition status and serum lipid pattern in normal rabbits. *Journal of Animal Science* **75**, 402–408.

Packer JE, Slater TF & Wilson RL (1979) Direct observations of a free radical interactions between vitamin E and C. *Nature* **278**, 737–738.

Paolisso G, Balbi V & Volke C (1995) Metabolic benefits deriving from chronic vitamin C supplementation in aged non-insulin dependent diabetics. *Journal of the American College of Nutrition* **14**, 387–392.

Poh-Fitzpatrick MB & Palmer RH (1984) Elevated plasma triglyceride levels are associated with human protoporphyria. *Journal of Laboratory and Clinical Medicine* **164**, 257–263.

Porkkala-Sarataho E, Nyyssonen K & Salonen JT (1996) Increased oxidation resistance of atherogenic plasma lipoproteins at high vitamin E levels in non-vitamin E supplemented men. *Atherosclerosis* **124**, 83–94.

Prasad K & Kalra J (1993) Oxygen free radicals and hypercholesterolemic atherosclerosis – Effect of vitamin E. *American Heart Journal* **125**, 958–973.

Princen HMG, van Duyvenvoorde W, Buytenhek R, Blonk C, Tijburg LBM, Languis JAE, Meinders AE & Pijl H (1998) No effect of consumption of green and black tea on plasma lipid, and antioxidant levels and on LDL oxidation in smokers. *Arteriosclerosis, Thrombosis and Vascular Biology* **18**, 833–841.

Princen HMG, van Poppel G, Vogelezang C, Buytenhek R & Kok FJ (1992) Supplementation with vitamin E but not β-carotene in vivo protects low density lipoprotein from lipid peroxidation in vitro. Effect of cigarette smoking. *Arteriosclerosis and Thrombosis* **12**, 554–562.

Rathi AB, Nath N & Chari SN (1984) Action of vitamin P like compounds on lysosomal fragility in hypercholesterolaemia: implication of ascorbic acid and its metabolites. *Acta Vitaminologica et Enzymologica* **6**, 97–102.

Reaven P, Parthasarthy S, Grasse BJ, Miller E, Steinberg D & Witztum JL (1993) Effects of oleate-rich and linoleate-rich diets on the susceptibility of low density lipoproteins to modification in mildly hypercholesterolemic subjects. *Journal of Clinical Investigation* **91**, 668–676.

Reaven PD, Khow A, Beltz WF, Parthasarthy S & Witzum JL (1993) Effect of dietary antioxidant combinations in humans. Protection of LDL by vitamin E but not by β-carotene. *Arteriosclerosis and Thrombosis* **13**, 590–600.

Ribayamercado JD, Ordovas JM & Russell RM (1995) Effect of β-carotene supplementation on the concentration and distribution of carotenoids, vitamin E, vitamin A and cholesterol in plasma lipoprotein and non-lipoprotein fractions in healthy older women. *Journal of the American College of Nutrition* **14**, 614–620.

Rice-Evans C & Bruckdorfer KR (editors) (1995) *Oxidative Stress, Lipoprotein and Cardiovascular Dysfunction.* London: Portland Press Ltd.

Rifici VA & Khachadurian AK (1993) Dietary supplementation with vitamin C and E inhibits in vitro oxidation of lipoproteins. *Journal of the American College of Nutrition* **12**, 631–637.

Rifici VA & Khachadurian AK (1996) Effect of dietary vitamin C and E supplementation on the copper mediated oxidation of HDL and on HDL mediated cholesterol efflux. *Atherosclerosis* **127**, 19–26.

Salter AM, Mangiapane EH, Bennett AJ, Bruce JS, Billett MA, Anderton KL, Marenah CB, Lawson N & White DA (1998). The effect of different dietary fatty acids on lipoprotein metabolism: concentration-dependent effects of diets enriched in oleic, myristic, palmitic and stearic acid. *British Journal of Nutrition* **79**, 195–202.

Schwenke DC (1995) Selective increase in cholesterol at atherosclerosis susceptible aortic sites after short term cholesterol feeding. *Arteriosclerosis, Thrombosis and Vascular Biology* **15**, 1928–1937.

Sharpe PC, McGrath LT, McClean E, Young IS & Archbold GPR (1995) Effect of red wine consumption on lipoprotein (a) and other risk factors for atherosclerosis. *Quarterly Journal of Medicine* ***88***, 101–108.

Shen GX, Noval C & Angel A (1996) Effect of dietary vitamin E supplements on cholesteryl ester transfer activity in hamster adipose tissue. *Atherosclerosis* **124**, 211–219.

Simon JA (1992) Vitamin C and cardiovascular disease: A review. *Journal of the American College of Nutrition* **11**, 107–125.

Simon JA & Hudes ES (1998) Relation of serum ascorbic acid to serum lipids and lipoproteins in US adults. *Journal of the American College of Nutrition* **17**, 250–255.

Singh K, Chander R & Kapoor NK (1997) High density lipoprotein subclasses inhibit low density lipoprotein oxidation. *Indian Journal of Biochemistry and Biophysics* **43**, 313–318.

Singh RB, Rastogi SS, Niaz MA, Ghosh S, Singh R & Gupta S (1992*b*) Effect of fat modified and fruit- and vegetable-enriched diet on blood lipids in the Indian Diet and Heart Study. *American Journal of Cardiology* **70**, 869–874.

Singh RB, Rastogi SS, Singh R, Ghosh S & Niaz MA (1992*a*) Effects of guava intake on serum total and high density lipoprotein cholesterol levels and on systemic blood pressure. *American Journal of Cardiology* **70**, 1287–1291.

Sokoloff B, Hori M, Saelhof C, McConnell B & Imai T (1967) Effect of ascorbic acid on certain blood fat metabolism factors in animals and man. *Journal of Nutrition* **91**, 110–118.

Sparrow CP, Parthasarthy S & Steinberg D (1989) A macrophage receptor that recognises oxidized low density lipoproteins but not acetylated low density lipoprotein. *Journal of Biological Chemistry* **264**, 2599–2604.

Stocker R, Bowry VW & Frei B (1991) Ubiquinol-10 protects human low density lipoproteins more efficiently against lipid peroxidation than does α-tocopherol. *Proceedings of the National Academy of Sciences USA* **88**, 1646–1650.

Suzukawa M, Ishikawa T, Yoshida H & Nakamura H (1995) Effect of in vivo supplementation with low dose vitamin E on susceptibility of low density lipoprotein and high density lipoprotein to oxidative modification. *Journal of the American College of Nutrition* **14**, 46–52.

Tertov VV, Sobenin IA, Kaplun VV & Orekhov AN (1998) Antioxidant content in low density lipoprotein and lipoprotein oxidation in vivo and in vitro. *Free Radical Research* **29**, 165–173.

Thomas SR, Neuzil J & Stocker R (1996) Cosupplementation with coenzyme Q prevents the prooxidant effect of α-tocopherol and increases the resistance of LDL to transition metal-dependent oxidation initiation. *Atherosclerosis, Thrombosis and Vascular Biology* **16**, 687–696.

Van Poppel G, Hospers J, Buytenhek R & Princen HMG (1994) No effect of β-carotene supplementation on plasma lipoproteins in

healthy smokers. *American Journal of Clinical Nutrition* **60**, 730–734.
Vinson JA & Dabbagh YA (1998) Effect of green and black tea supplementation on lipids, lipid oxidation and fibrinogen in the hamster: mechanisms for the epidemiological benefits of tea drinking. *FEBS Letters* **433**, 44–46.
Visioli F, Bellomo G, Montedoro G & Galli C (1995) Low density lipoprotein oxidation is inhibited in vitro by olive oil constituents. *Atherosclerosis* **117**, 25–32.
Yugarani T, Tan BKH, The M & Das NP (1992) Effects of polyphenolic natural products on the lipid profiles of rats fed high fat diets. *Lipids* **27**, 181–186.
Ziozenkova O, Winklhofer Roob BM, Puhl H, Roob JM & Esterbauer H (1996) Lack of correlation between the alpha-tocopherol content of plasma and LDL, but high correlations for gamma-tocopherol and carotenoids. *Journal of Lipid Research* **37**, 1936–1946.
Zubay G (editor) (1993) Metabolism of cholesterol. In *Biochemistry*, pp. 635–658. Oxford: W. C. Brown Publishers.

https://doi.org/10.1017/S0029665199000865 Published online by Cambridge University Press