The role of high-density lipoprotein and lipid-soluble antioxidant vitamins in inhibiting low-density lipoprotein oxidation

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1. The oxidation of low-density lipoprotein (LDL) is believed to play a central role in atherogenesis. We have compared the effect of antioxidant vitamins and high-density lipoprotein (HDL) on the Cu^{2+} -catalysed oxidation of LDL. 2. Antioxidant vitamin supplementation significantly reduced conjugated diene formation but did not affect the formation of lipid peroxides. 3. Conversely, HDL did not affect conjugated diene formation but inhibited the formation of lipid peroxides by up to 90%. 4. The inhibition by HDL of lipid peroxide formation in oxidized LDL

was dependent on the concentration of HDL and was not due to HDL chelating Cu^{2+} . 5. Large interindividual variations in the inhibition of lipid peroxide formation by autologous HDL were evident, which were related to the rate of lipid peroxide generation in the LDL. 6. We conclude that HDL is a powerful antioxidant or more probably inhibitor of LDL oxidation *in vitro* and may play an important role *in vivo* in preventing atherosclerosis by inhibiting LDL oxidation in the artery wall.

INTRODUCTION

High concentrations of serum low-density lipoprotein (LDL) are directly related to the likelihood of developing atherosclerosis [1]. One current theory to explain the development of the foam-cell-laden fatty streaks in the arterial wall, which are believed to initiate atherosclerosis, proposes that oxidative modification of LDL is critical. The unsaturated fatty acids of the phospholipid molecules in the surface coat of LDL entering the subintimal space are oxidized by the action of lipoxygenase (or some other peroxidase activity). Several cell types have been shown to oxidize LDL *in vitro* including endothelial cells, smooth muscle cells and macrophages [2–5], all of which are present in the artery wall. Monocyte-derived macrophages present in the subintimal space express a receptor that recognizes the modified apolipoprotein B (apo-B) of oxidized LDL [6]. The macrophage receptor (the acetyl-LDL or scavenger receptor) is not subject to down-regulation, and continuing uptake of oxidized LDL by the macrophages leads to the formation of lipid-laden foam cells. Secretion of monocyte chemotactic protein-1 (MCP-1) and macrophage colony stimulating factor by the foam cells [7] may cause further recruitment and retention of lipid-laden macrophages, which aggregate to form the fatty streak. Thus oxidized LDL is believed to play a central role in the events that initiate atherosclerosis.

Several studies have indicated an increase in the products of lipid peroxidation in the plasma of patients with coronary artery disease [8,9] and diabetes mellitus [10,11]. However, these findings are difficult to interpret as there may be several sources of lipid peroxidation products other than LDL (including platelet arachidonic acid metabolism), and therefore the increase in peroxidation products does not necessarily reflect an increase in circulating oxidized LDL. LDL isolated from the plasma of patients with diabetes and coronary heart disease is, however, more susceptible to oxidation *in vitro* than LDL from normal

subjects [12,13], and, if this is the case *in vivo*, it may be responsible for their increased incidence of premature atherosclerosis.

Interest in high-density lipoprotein (HDL) as a potentially protective factor against atherosclerosis was reawakened in 1975 by the publication of Miller and Miller [14]. Many epidemiological studies have shown a strong inverse relationship between the serum concentration of HDL and the incidence of coronary and cerebral atherosclerosis [15]. Serum HDL concentration exerts its effect independently of other coronary risk factors, and in women and men from middle-life onwards is a stronger determinant of risk than LDL [16,17]. The mechanism whereby HDL provides this protection is not well understood. It has been postulated that it is the role of HDL in reverse cholesterol transport that provides this protection [18]. Recent experimental evidence, however, has suggested another possibility because it appears that HDL can also protect LDL against oxidation. Incubating LDL in the presence of HDL during Cu^{2+} and Fe^{2+} oxidation prevents the formation of thiobarbituric acid reactive substances (TBARS) and lipid peroxides [19,20], prevents changes to the electrophoretic mobility typical of oxidized LDL and prevents macrophage degradation [21]. HDL also prevents the induction of the synthesis and secretion of MCP-1, the cytotoxicity of oxidized LDL to endothelial and smooth muscle cells [22–24], and the cytotoxicity of surface remnants of triacylglycerol-rich lipoproteins to these cells [25]. Most interest in factors that might potentially protect LDL against oxidation has not, however, focused on the role of HDL but rather on the effect of lipid-soluble antioxidant vitamins (incorporated into LDL both *in vitro* and by dietary supplementation). These studies have mainly concentrated on measuring the formation of conjugated dienes [26–28], the initial stage in the oxidation of polyunsaturated fatty acids, rather than actual lipid peroxides.

In this report we describe the comparative effects of vitamin supplementation and HDL on the generation of conjugated

Abbreviations used: LDL, low-density lipoprotein; HDL, high-density lipoprotein; MCP-1, monocyte chemotactic protein-1; TBARS, thiobarbituric acid reactive substances; CHOD-PAP, cholesterol oxidase-*p*-aminophenazone; GPO-PAP, glycerol phosphate oxidase-*p*-aminophenazone; apo-B, apolipoprotein B.

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dienes and lipid peroxides during the Cu^{2+} -catalysed oxidation of LDL.

MATERIALS AND METHODS

Subjects

Blood was withdrawn by venipuncture from 16 volunteers (seven male, nine female, age 24–45 years) before and 20 days after vitamin supplementation with selenium–ACE tablets (Wassen, Hemel Hempstead, Herts., U.K.). The daily vitamin supplementation was 200 mg of selenium, 18 mg of β -carotene, 180 mg of vitamin C and 74 mg of vitamin E (as α -tocopherol). Serum was prepared by centrifugation at 2000 *g* for 15 min at 4 °C and used immediately to prepare lipoprotein subfractions. A small portion was stored at –20 °C for lipid and apolipoprotein analysis.

Materials

Cholesterol oxidase-*p*-aminophenazone (CHOD-PAP) and glycerol phosphate oxidase-*p*-aminophenazone (GPO-PAP) enzymic reagents for the measurement of cholesterol and triacylglycerol were purchased from Biostat Ltd., Stockport, Cheshire, U.K., and Merck CHOD-iodide reagent was obtained from BDH, Poole, Dorset, U.K. All other reagents were from Sigma (London) Chemical Co., Poole, Dorset, U.K. and were of analytical reagent grade.

Preparation of lipoproteins

LDL (*d* 1.019–1.063 g/ml) and HDL (*d* 1.063–1.21 g/ml) were prepared by sequential ultracentrifugation using a Beckman L8-55M ultracentrifuge fitted with a Beckman 50.4 Ti Rotor (Beckman Instruments Ltd., Palo Alto, CA, U.S.A.) using conditions that prevent lipoprotein modification [29].

Measurement of LDL oxidation

Conjugated diene production

LDL protein (0.75 mg) was incubated in oxygen-saturated EDTA-free PBS (0.05 M, pH 7.2) with 1.66 μM CuSO_4 [26] in the presence or absence of 0.75 mg of autologous HDL protein in a total volume of 3 ml. The generation of conjugated dienes was determined by the increase in A_{234} using a continuously recording spectrophotometer (Beckman DU-68). The lag time was determined as the intercept of extrapolations of the parts of the curve representing the lag and propagation phases.

Generation of lipid peroxides

The generation of lipid peroxides was determined as described [30]. LDL protein (1.5 mg), HDL protein (1.5 mg) or LDL (1.5 mg) plus HDL (1.5 mg) from the same donor were incubated in oxygen-saturated EDTA-free PBS containing 5 μM CuSO_4 in a total volume of 1.0 ml. Blanks consisted of lipoprotein in PBS without CuSO_4 . Incubation periods varied from 0 to 24 h at 37 °C after which oxidation was terminated by the addition of 24 μM EDTA and 20 μM butylated hydroxytoluene (final concentration). Lipid peroxides present in the lipoproteins were determined by mixing 0.1 ml of the reaction mixture with 1.0 ml of CHOD-iodide reagent followed by incubation for 30 min at room temperature in the dark. After this period, the A_{385}

of the solution was determined. The concentration of lipid peroxides was calculated using a molar absorption coefficient of $2.46 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ [30].

Effect of HDL concentration on lipid peroxide generation in LDL

LDL (1.5 mg of protein) was incubated with 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.5 and 2.0 mg of HDL protein from the same donor, in oxygen-saturated EDTA-free PBS containing 5 μM CuSO_4 in a final volume of 1 ml for 6 h at 37 °C. After this time, oxidation was terminated and the concentration of lipid peroxides determined as described above. Blanks consisted of the lipoprotein mixture without Cu^{2+} and HDL (at the various concentrations) incubated for the same period in the presence of 5 μM CuSO_4 .

Lipid peroxide generation in HDL in the presence of LDL

HDL (1.5 mg) in the presence or absence of 1.5 mg of LDL was incubated in oxygen-saturated EDTA-free PBS containing 5 μM CuSO_4 in a total volume of 1 ml. After a 5 h incubation at 37 °C, apo-B-containing lipoproteins were precipitated with 3.2% (w/v) (final concentration) poly(ethylene glycol) [31]. Poly(ethylene glycol) was also added to the incubations containing only HDL as a control. Lipid peroxides in the HDL-containing supernatant were determined as described previously.

Association of Cu^{2+} with lipoproteins

HDL protein (1.5 mg), LDL (1.5 mg) or HDL (1.5 mg) plus LDL (1.5 mg) was added to PBS containing 5 μM CuSO_4 . The mixture was then centrifuged in Amicon Centriflow Cones with a molecular-mass cut-off of 25000 Da to remove lipoproteins. The Cu^{2+} concentration in the lipoprotein-free solution was determined by atomic absorption spectroscopy (AA/AE spectrophotometer 357, Instrument Laboratory, Warrington, Lancs., U.K.).

Other analytical procedures

Total serum cholesterol and triacylglycerols were measured using the CHOD-PAP and GPO-PAP enzymic kit methods respectively. Serum HDL cholesterol was determined after the precipitation of apo B-containing lipoproteins with heparin/ Mn^{2+} [32]. Serum apo-B was measured by a rate immunonephelometric technique [33], using the Beckman Array according to the manufacturer's instructions, and reagents and antibodies supplied by the manufacturer.

Statistical analysis

Comparisons between results were made by analysis of variance and differences were sought by Student's unpaired *t* test. Triacylglycerol data were converted into a Gaussian distribution by logarithmic transformation before statistical analysis.

Presentation of results

The generation of lipid peroxides within HDL in the presence of LDL (50.8 ± 5.2 nmol of lipid peroxide/mg of HDL protein) was not significantly different from the generation within HDL incubated separately [56.2 ± 3.9 nmol of lipid peroxide/mg of HDL protein (*n* = 6)], therefore the concentration of LDL lipid peroxides in the LDL/HDL mixture was calculated as the difference between the lipid peroxides generated in the mixture and those generated in the HDL alone.

RESULTS

Lipids and lipoproteins

The serum lipid and lipoprotein concentrations of the subjects studied were unaffected by dietary supplementation with selenium-ACE tablets (Table 1).

Table 1 Serum lipid and lipoprotein parameters of the 16 individuals studied

Results for cholesterol and apo-B are means $\pm$ S.D. and those for triacylglycerol are geometric means ($+1$ S.D., -1 S.D.).

	Total serum		Serum HDL cholesterol (mmol/l)	Serum apo-B (mg/dl)
	Cholesterol (mmol/l)	Triacylglycerol (mmol/l)		
Before vitamin supplementation	5.23 $\pm$ 1.74	1.25 (+2.66, -0.59)	1.62 $\pm$ 0.45	96.1 $\pm$ 27.3
After vitamin supplementation	5.84 $\pm$ 0.91	1.43 (+3.31, -0.62)	1.41 $\pm$ 0.45	91.5 $\pm$ 25.4

Figure 1 Examples of conjugated diene generation in 0.75 mg of LDL incubated with 1.66 μ M CuSO_4

(a) 'Normal' pattern of conjugated diene production showing (i) lag phase, (ii) propagation phase and (iii) decomposition phase. (b) A rapid conjugated diene generator with a short lag phase, found in three of the 16 individuals investigated. Experimental procedures are described in the Materials and methods section.

Table 2 Oxidation of LDL by CuSO_4 before and after supplementation with antioxidant vitamins

For conjugated diene formation, 0.75 mg of LDL was incubated with 1.66 μ M CuSO_4 , and, for lipid peroxide generation, 1.5 mg of LDL or 1.5 mg of LDL plus 1.5 mg of HDL was incubated with 5 μ M CuSO_4 for 8 h. Results are means $\pm$ S.E.M. for 16 determinations. *Significantly different from values obtained before vitamin supplementation, $P < 0.001$; †significantly different from LDL value, $P < 0.001$. The LDL + HDL value was 52% of the LDL value before vitamin supplementation and the percentage after vitamin supplementation was 45.5.

	Duration of conjugated diene lag phase (min)	Lipid peroxides generated (nmol/mg of LDL)	
		LDL	LDL + HDL
Before vitamin supplementation	63.3 $\pm$ 9.2	128.7 $\pm$ 15.3	67.1 $\pm$ 13.1†
After vitamin supplementation	132 $\pm$ 39*	136.2 $\pm$ 14.3	61.9 $\pm$ 8.4†

Figure 2 Effect of antioxidant vitamins on the generation of lipid peroxides in LDL

Experimental conditions are described in the Materials and methods section; 1.5 mg of LDL protein was incubated with 5 μ M CuSO_4 for the time periods indicated. Points are means $\pm$ S.D. for 12 determinations. ●, Before supplementation; ○, after supplementation.

Effect of lipid-soluble vitamin supplementation on conjugated diene and lipid peroxide generation in LDL incubated with Cu^{2+}

Two patterns of conjugated diene production were observed upon incubation of isolated LDL with Cu^{2+} . In the majority of individuals, conjugated diene generation followed the pattern illustrated in Figure 1(a) which can be regarded as the normal pattern of generation. However, in three of the 16 cases studied, there was a very short lag period of 10 min or less (Figure 1b) which may indicate that the LDL from these individuals was either deficient in antioxidants or was in some other way, as yet unexplained, more susceptible to oxidation, compared with the majority of individuals studied, as vitamin supplementation did not have a greater effect on the lag phase of the fast oxidizers.

Supplementation of the dietary intake of antioxidant vitamins with selenium-ACE tablets doubled the lag phase from 63.3 $\pm$ 9.2 min to 132 $\pm$ 39 min ($P < 0.001$) (Table 2). Vitamin supplementation did not affect the amount of lipid peroxide generated within LDL at any of the time points tested in direct comparison with the inhibition of conjugated diene production

Figure 3 Lipid peroxides generated in lipoprotein fractions incubated with Cu^{2+}

Experimental procedures are described in the Materials and methods section; 1.5 mg of LDL, HDL or 1.5 mg LDL plus 1.5 mg HDL were incubated with $5 \mu\text{M}$ CuSO_4 for the time periods indicated. Points are means $\pm$ S.E.M. of 16 determinations. ●, LDL; ▲, HDL; ○, LDL + HDL.

(Figure 2). No difference was found before and after vitamin supplementation in the generation of lipid peroxides in LDL during the first hour of incubation with Cu^{2+} . However, the concentration of lipid peroxides generated during the first 40 min is at the lower limit of sensitivity of the assay (10–30 nmol/mg of lipoprotein [34]) and at this lower limit of sensitivity the coefficient of variation of the assay is 14%. It is therefore not possible to discount differences in lipid peroxide generation in LDL before and after vitamin supplementation during the first hour of incubation with Cu^{2+} , although we have found no evidence of this. Co-incubating HDL with LDL in the presence of Cu^{2+} had no effect on the generation of conjugated dienes (results not shown).

Effect of HDL on conjugated diene and lipid peroxide generation in LDL incubated with Cu^{2+}

The generation of lipid peroxides in LDL, HDL or LDL plus HDL combined upon incubation with Cu^{2+} is shown in Figure 3. Lipid peroxides were generated continuously in LDL up to 24 h, whereas, in HDL, lipid peroxide generation was far less than in LDL and reached a maximum between 2 and 8 h before declining. Incubating LDL in the presence of HDL with Cu^{2+} causes a significant reduction in lipid peroxide generation between 2 and 8 h ($P < 0.05$), indicating that HDL can protect LDL against Cu^{2+} -catalysed oxidation.

The amount of protection against LDL lipid peroxide generation given by autologous HDL showed large interindividual variations. Two patterns of inhibition were evident. First, in individuals with LDL that was resistant to oxidation, the HDL

Figure 4 Lipid peroxide generation and HDL protection against lipid peroxide generation in LDL in two individuals

(a) A slow oxidizer with relatively poor protection from autologous HDL against LDL lipid peroxide generation. The difference between the 8 h points is 182% (LDL compared with LDL + HDL). (b) A rapid oxidizer with relatively good protection from autologous HDL against LDL lipid peroxide generation. The difference between the 8 h points is 773% (LDL compared with LDL + HDL). Experimental procedures are described in the Materials and methods section; 1.5 mg of LDL was incubated with $5 \mu\text{M}$ CuSO_4 in the presence and absence of HDL for the time periods indicated. Points are the means of duplicate determinations. ●, LDL; ○, LDL + HDL.

was relatively poor at preventing lipid peroxide generation (Figure 4a) and secondly, in individuals with LDL that was susceptible to oxidation, HDL protection was marked (Figure 4b). The inhibition of lipid peroxide generation in LDL by HDL was significantly correlated with the degree of lipid peroxide generation in the LDL ($r = 0.765$, $P < 0.001$) but not with the lag phase in conjugated diene generation. This effect may be caused by a defect in the HDL or may provide evidence for an enzymic mechanism of protection, requiring the generation of substrate(s) at sufficient concentration for the enzyme to catalyse the reaction efficiently, a concentration that is only attained in rapidly oxidizing LDL.

Incubating LDL with Cu^{2+} for 8 h in the presence or absence of HDL resulted in a 48% decrease in LDL lipid peroxides (Table 2); this protection was not significantly altered by the antioxidant vitamin supplementation.

The protection offered by HDL against LDL oxidation was further investigated in a subset of seven individuals. Each individual LDL (1.5 mg) was incubated with autologous HDL at various concentrations between 0 and 2 mg of HDL protein, and the generation of lipid peroxides was analysed. As shown in Figure 5, inhibition of lipid peroxide generation is linear up to

Figure 5 Effect of HDL concentration on lipid peroxide generation in LDL

Experimental procedures are described in the Materials and methods section; 1.5 mg of LDL was incubated with various concentrations of HDL in the presence of $5 \mu\text{M}$ CuSO_4 for 6 h. Points are means $\pm$ S.E.M. for seven determinations.

1.2 mg of HDL protein under these conditions, and at 2 mg of HDL protein protection is 90 %, indicating that HDL is a very powerful inhibitor of LDL oxidation.

Association of Cu^{2+} with lipoproteins

The amount of free Cu^{2+} in solution decreases from $4.56 \pm 0.14 \mu\text{M}$ ($n = 5$) in the absence of lipoproteins to $3.17 \pm 0.61 \mu\text{M}$ ($P > 0.05$, $n = 6$) in the presence of HDL, to $1.15 \pm 0.08 \mu\text{M}$ in the presence of LDL ($P > 0.001$, $n = 6$) and $1.21 \pm 0.27 \mu\text{M}$ in the presence of HDL plus LDL ($P < 0.001$, $n = 4$). There was no significant difference between the amount of Cu^{2+} bound by LDL alone and LDL plus HDL. No difference was found in the amount of lipid peroxides generated within either LDL or HDL when Cu^{2+} concentrations were varied between 3 and $10 \mu\text{M}$ (results not shown).

DISCUSSION

The results presented here provide further evidence that supplementing the normal dietary intake of lipid-soluble antioxidant vitamins can protect isolated LDL against Cu^{2+} -catalysed oxidation. Several previous studies have indicated that increasing the dietary intake of these vitamins, or supplementing LDL *in vitro* with antioxidants such as tocopherol, protects LDL against conjugated diene generation under oxidizing conditions [26–28]. A note of caution is, however, engendered by our findings regarding suggestions that increasing antioxidant vitamin intake in order to make LDL in the artery wall more resistant to oxidation may be a useful means of reducing the risk of accelerated atherosclerosis. This is because vitamin supplements, which in our experiments produced significant decreases in the rate of conjugated diene formation, had no effect on the subsequent generation of lipid peroxides. There are no previous reports of the effect of vitamins on lipid peroxides. Our results do not discount the possibility that vitamin supplementation affects lipid peroxide generation in the first hour of incubation. Conjugated diene formation is measured during the early phases of the oxidation with Cu^{2+} whereas lipid peroxide formation

occurs over a longer time course. It seems therefore that natural antioxidants such as ubiquinol-10, β -carotene and vitamin E are rapidly exhausted and they may offer little long-term protection [34]. This is important because the retention of LDL in the arterial wall is likely to be for many hours or even days, particularly in the case of apo-(a)-containing LDL [35]. In our experiments, vitamin C present in the supplement would not be expected to affect LDL oxidation *in vitro* because it would be removed during the preparation of LDL. However, *in vivo*, vitamin C will be more important in regenerating tocopherol.

HDL has been shown to prevent many of the changes associated with the oxidation of LDL. HDL can prevent TBARS and lipid peroxide generation during Fe^{2+} - or Cu^{2+} -catalysed LDL oxidation [19,20], changes in the electrophoretic mobility of oxidized LDL and macrophage degradation via the scavenger receptor pathway [21], oxidized-LDL-induced MCP-1 production by macrophages [22] and the cytotoxicity of oxidized LDL to endothelial and smooth muscle cells in culture [23]. This, however, is the first detailed analysis of the effect of HDL on chemical parameters of LDL oxidation using lipoproteins isolated from individuals.

In our experiments, HDL was a very powerful inhibitor of lipid peroxide generation during the Cu^{2+} -catalysed oxidation of LDL. The inhibition was dependent on HDL concentration and reached a maximum of 90 %. There were, however, large differences between individuals in the extent of inhibition by autologous HDL, and a major factor determining this was the rate of lipid peroxide generation of the LDL (Figure 3). HDL had no effect on conjugated diene production, indicating that it acted later in the peroxidation cascade.

It has been suggested that HDL prevents LDL oxidation by chelating the Cu^{2+} or Fe^{2+} ions used as oxidants [36]. An HDL species has been isolated from human plasma by antibody affinity chromatography, which has both transferrin and caeruloplasmin associated. This chelates both Fe^{2+} and Cu^{2+} [36]. However, this particular HDL species was not present in HDL isolated by ultracentrifugation, the method used in this study. It is unlikely for a number of reasons that HDL inhibits LDL oxidation by a physical process such as chelating metal ions or by providing antioxidants for LDL.

HDL has no effect on the Cu^{2+} -catalysed generation of conjugated dienes, but does prevent lipid peroxide generation in LDL, and increasing antioxidant vitamin intake does not affect lipid peroxide generation. HDL inhibits LDL oxidation by endothelial and smooth muscle cells and prevents cytotoxicity after LDL oxidation [24]. The results presented in this paper also suggest a non-physical mechanism of inhibiting LDL oxidation. In our experiments, for example, HDL inhibited lipid peroxide generation in a concentration-dependent manner. Even at the lowest concentration investigated (0.2 mg/ml HDL protein), there was significant inhibition of oxidation. Even at concentrations of HDL of 1.5 mg/ml, the amount of free Cu^{2+} in solution decreased minimally and no difference was found in the amount of lipid peroxides generated within either LDL or HDL when the Cu^{2+} concentration was varied between 3 and $10 \mu\text{M}$ (results not shown). Furthermore, if HDL were acting in our experiments by chelating Cu^{2+} , it would be expected to inhibit both conjugated diene and lipid peroxide generation, which was not the case, indicating a biochemical mechanism involving a specific part of the pathway leading to lipid peroxide formation. Therefore HDL does not appear to be capable of chelating sufficient Cu^{2+} to prevent oxidation. Additional evidence for this view comes from reports that HDL also inhibits LDL oxidation in systems not using redox metals to catalyse oxidation but rather endothelial and smooth muscle cells [24].

Several lines of evidence suggest that HDL acts enzymically to prevent LDL oxidation. The large interindividual differences in the inhibition of lipid peroxide generation found even when the ratio of HDL to LDL is kept constant appear to be dependent on certain properties of the LDL. HDL is 7 times more efficient at inhibiting lipid peroxide generation in LDL that rapidly oxidizes than it is with LDL that oxidizes more slowly. This would indicate that the action of HDL is critically dependent on the concentration of a substrate generated during oxidation. HDL did not prevent conjugated diene formation, indicating that this substrate is generated later in the peroxidation cascade. Several enzymes are associated with HDL, including lecithin-cholesterol acyltransferase, paraoxonase, phospholipase and protease. Preliminary work from this laboratory has suggested that paraoxonase (aryldialkylphosphohydrolase; EC 3.1.8.1) is the activity responsible for the inhibition of lipid peroxide generation by HDL [37]. The exact mechanism operating to prevent the generation of lipid endoperoxides within LDL during oxidation is not known and is the subject of further research in our laboratory.

The inverse relationship between serum HDL concentration and susceptibility to the development of coronary heart disease has been known for many years. HDL protects against this disease even when LDL concentrations are high [38]. The exact mechanism whereby HDL provides this protection is still a matter of considerable debate. Evidence indicates that it plays a central role in reverse cholesterol transport, thereby preventing a build-up of excess cholesterol in the peripheral tissues [18]. However, it is becoming increasingly clear that HDL is a powerful inhibitor of LDL oxidation. HDL is present in the subintimal space of the artery at a concentration 20 times greater than that of LDL, and is therefore ideally positioned to inhibit LDL oxidation in the artery wall and thus to prevent the processes that initiate atherogenesis. It should also not be overlooked that HDL is the dominant lipoprotein species throughout the tissue fluid and that it might thus have a more ubiquitous role in protecting tissues against cytotoxic lipid peroxidation products other than LDL, which have been implicated in a wide variety of diseases [39,40].

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