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Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does α-tocopherol

(atherosclerosis/lipid hydroperoxide/antioxidant/ascorbate/polymorphonuclear leukocyte)

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ABSTRACT **The temporal disappearance of natural antioxidants associated with human low density lipoprotein (LDL) in relation to the appearance of various classes of lipid hydroperoxides was investigated under three types of oxidizing conditions. Freshly isolated LDL from plasma of healthy subjects was free of detectable amounts of lipid hydroperoxides as measured by HPLC postcolumn chemiluminescence detection. Exposure of such LDL to a mild, constant flux of aqueous peroxyl radicals led to rapid and complete oxidation of ubiquinol-10, followed by slower partial depletion of lycopene, β-carotene, and α-tocopherol. After an initial lag period of complete inhibition of detectable lipid peroxidation, formation of hydroperoxides of cholesterol esters, triglycerides, and phospholipids was observed. The onset of detectable lipid peroxidation corresponded closely with the completion of ubiquinol-10 consumption. However, small amounts of ascorbate, present as a contaminant in the LDL preparation, rather than ubiquinol-10 itself were responsible for the initial lag period. Thus, complete consumption of ubiquinol-10 was preceded by that of ascorbate, and exposure of ascorbate-free LDL to aqueous peroxyl radicals resulted in immediate formation of detectable amounts of lipid hydroperoxides. The rate of radical-mediated formation of lipid hydroperoxides in ascorbate-free LDL was low as long as ubiquinol-10 was present, but increased rapidly after its consumption, even though more than 80% and 95% of endogenous carotenoids and α-tocopherol, respectively, were still present. Qualitatively similar results were obtained when peroxyl radicals were generated within LDL or when the lipoprotein was exposed to oxidants produced by activated human polymorphonuclear leukocytes. LDL oxidation was reduced significantly by supplementing the lipoprotein preparation with physiological amounts of either ascorbate or ubiquinol-10. Our data show that ubiquinol-10 is much more efficient in inhibiting LDL oxidation than either lycopene, β-carotene, or α-tocopherol.**

Free radical-mediated lipid peroxidation may be of critical importance in various degenerative diseases, including atherosclerosis (1). Biochemical and clinical studies have suggested that oxidatively modified low density lipoprotein (LDL) is atherogenic (reviewed in refs. 2 and 3). Recent epidemiological studies indicate that low levels of the natural plasma antioxidants vitamins E and C may contribute to high incidences of ischemic heart disease (4, 5), suggesting that the prevention of LDL oxidation by antioxidants could diminish the risk of developing atherosclerosis.

Ascorbate (the reduced form of vitamin C) and, in a site-specific manner, bilirubin are more effective in inhibiting aqueous radical-mediated lipid peroxidation in human plasma than all of the other endogenous antioxidants, including α-tocopherol (the most reactive form of vitamin E) (6–9). α-Tocopherol is generally regarded as the only significant lipid-soluble antioxidant present in human blood (10) and, on a molar basis, it represents by far the major antioxidant in LDL (11). *In vitro* studies on Cu^{2+}-mediated oxidation of LDL have shown that α-tocopherol was always consumed before γ-tocopherol and carotenoids, and that rapid LDL oxidation occurred only after complete consumption of all these antioxidants (12, 13). However, endogenous levels of α-tocopherol in LDL correlate poorly with the lipoprotein's resistance to oxidation (11, 13, 14), and the authors suggest that such resistance may depend on more than one variable and/or that additional, presently unrecognized antioxidants are present in the lipoprotein. Although known for more than 20 years to protect biological membranes against oxidation (15), ubiquinol-10 (the reduced form of coenzyme Q_{10}) has recaptured interest as a natural, lipid-soluble antioxidant (16, 17). Ubiquinol-10 is consumed before α-tocopherol when plasma is exposed to oxidants produced by activated human polymorphonuclear leukocytes (PMN) (18), suggesting that ubiquinol-10 is a highly reactive antioxidant.

We have attempted to determine the relative importance of various physiological, LDL-associated antioxidants in preventing peroxidation of the lipoprotein under different types of oxidative stress. Special emphasis has been placed both on rapid isolation of LDL and analysis of lipid-soluble antioxidants, combined with a highly sensitive and selective method for the detection of lipid hydroperoxides (19). This is important because we anticipate that the most efficient antioxidants in LDL are particularly prone to autoxidation and therefore may be "lost" during lengthy isolation procedures. We show that ubiquinol-10 is an important natural antioxidant associated with human LDL that protects the lipoprotein from peroxidative damage more effectively than either α-tocopherol or carotenoids.

MATERIALS AND METHODS

Apart from the following ones, the chemicals used were the same as described (7, 19). Ubiquinone-10 (coenzyme Q_{10}), β-carotene (type IV), retinol palmitate, and ascorbate oxidase (EC 1.10.3.3) were purchased from Sigma. Ascorbic acid (Goldmark) was obtained from Aldrich; *d*-α-tocopherol, from Kodak; and dodecyltriethylammonium phosphate, from Regis (Morton Grove, IL). (all-*E*)-Lycopene and α-carotene were generously given to us by H. Keller (Hoffmann–La Roche, Basel). Lipid hydroperoxide standards were prepared as described (19). Ubiquinol-10 was made from ubiquinone-10 by dithionite reduction (20) and used within 24 hr. Organic solvents of HPLC quality (Mallinckrodt) and "nanopure" water were used for all experiments. All aqueous solutions and

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Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane) hydrochloride; AMVN, 2,2′-azobis(2,4-dimethylvaleronitrile); LDL, low density lipoprotein; PMA, phorbol 12-myristate 13-acetate; PMN, polymorphonuclear leukocytes.

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buffers were treated with Chelex-100 (Bio-Rad) to remove contaminating transition metals. Other chemicals were from Sigma or Merck and of the highest quality available.

Plasma was prepared from freshly obtained heparinized blood of nonfasted, healthy, and normolipidemic male subjects (25–34 years old); LDL was isolated by a 30-min centrifugation (Beckman TL-100 tabletop ultracentrifuge) as described in "Procedure 7" (21) and was used immediately for experiments. For some experiments, plasma was incubated for 10 min at 25°C in the presence of ascorbate oxidase (1 unit/ml) prior to isolation of LDL. Preliminary experiments showed that at this concentration ascorbate oxidase depleted plasma ascorbate completely within 5 min.

Oxidation of LDL was carried out in a shaking water bath at 37°C under air. For chemical oxidation, 2.4 ml of freshly prepared LDL (1.2–2.4 mg of protein per ml) was kept for 3 min at 37°C and then either 24 μl of 100 mM 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH, dissolved in 0.154 M NaCl) or 12 μl of 100 mM 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN, in methanol) was added. AAPH and AMVN are water- and lipid-soluble azo compounds that thermally decompose to produce peroxyl radicals at constant rates within the water- and lipid-phase, respectively (22, 23). For some experiments 12 μl of freshly prepared 1 mM ubiquinol-10 (in ethanol) was added to the LDL solution immediately prior to initiation of oxidation with AAPH. At various time points, 200-μl aliquots were withdrawn, added to cold methanol (10 vol), and extracted by vigorous shaking with hexane (50 vol). For cellular oxidation of LDL, human PMN were isolated freshly from heparinized blood (24), washed twice, and resuspended in 100 mM phosphate-buffered saline containing 5 mM glucose (pH 7.4). LDL (4.2 ml of 0.3–0.6 mg of protein per ml) was then added to 6.8 ml of PMN to give a final concentration of 5×10^6 cells per ml. After 5 min at 37°C, the LDL/PMN mixture was divided into a 1-ml control sample to which 1 μl of dimethyl sulfoxide was added and a 10-ml sample to which 10 μl of phorbol 12-myristate 13-acetate (PMA, 1 mg/ml of dimethyl sulfoxide) was added. For some experiments, an aliquot of a freshly prepared ascorbate solution was added to the LDL/PMN mixture prior to the addition of PMA (final ascorbate concentration, 50 μM). At the time points indicated, a 500-μl aliquot of the reaction mixture was removed and centrifuged immediately for 2 min at 10,000 × *g* to remove cells; 400 μl of the supernatant was extracted with 5 vol of cold methanol and 12.5 vol of hexane.

After centrifugation of the biphasic extract, the aqueous methanol phase was filtered (0.2 μm; Gelman) and analyzed immediately for ascorbate by HPLC as described (8). Urate was determined (25) within 2 weeks of storage of samples at −20°C. For lipid-soluble antioxidants, the hexane layer was removed, taken to dryness, resuspended in ethanol, and analyzed immediately for α-tocopherol and ubiquinol-10 by HPLC with electrochemical detection with an eluent composition of methanol/reagent alcohol of 22.5/77.5 (vol/vol) and an applied potential of +0.6 V (20). This allowed simultaneous detection of tocopherols and ubiquinols as well as the carotenoids retinol palmitate, lycopene, α-carotene, and β-carotene.

Lipid hydroperoxides were determined directly by HPLC postcolumn chemiluminescence detection (19, 26) with an ultra-sensitive CLD-100 chemiluminescence detector (Tohoku Electronic, Sendai, Japan) equipped with a circulating cooling system set at 4°C. Phospholipid hydroperoxides present in the aqueous methanol phase were analyzed as described (26), while unoxidized cholesterol and hydroperoxides of cholesterol esters and triglycerides were separated on an LC-18 column (5 cm × 4.6 mm; 5-μm particle size) with methanol (1 ml/min) as the mobile phase. HPLC analytical and guard columns were from Supelco. Because of the extremely high sensitivity of the detector, the concentration of microperoxidase was reduced by a factor of 25 to 1 μg/ml of postcolumn chemiluminescence reagent. The detection limit for methyl linoleate hydroperoxide was ≈40 fmol—i.e., about 25 times more sensitive than previously described (26). The different classes of lipid hydroperoxides were quantitated by assuming the same relative sensitivity in the assay as methyl linoleate hydroperoxide (19). The amounts of cholesterol ester- and triglyceride hydroperoxides were standardized against endogenous cholesterol present in the organic phase.

RESULTS

To assess partially the quality of the LDL preparations used in this study, LDL was analyzed by both sodium dodecyl sulfate/polyacrylamide gel electrophoresis and size-exclusion HPLC (TSK-G4000 WS column, 60 cm × 7.5 mm; Pharmacia) (27). These analyses showed that apolipoprotein B-100 represented more than 97% of the total protein present, with albumin as the main proteinaceous contaminant. In addition, LDL contained small amounts of the water-soluble antioxidants urate and ascorbate, amounting to 9.0 ± 2.3% (mean ± SD, $n = 13$) and 2.1 ± 0.8% ($n = 3$) respectively, of that originally present in the corresponding plasma samples; bilirubin was not detected. Analysis of both organic and aqueous methanol extracts showed that LDL isolated and used within ≈70 min of blood collection was free of detectable amounts of lipid hydroperoxides (Fig. 1).

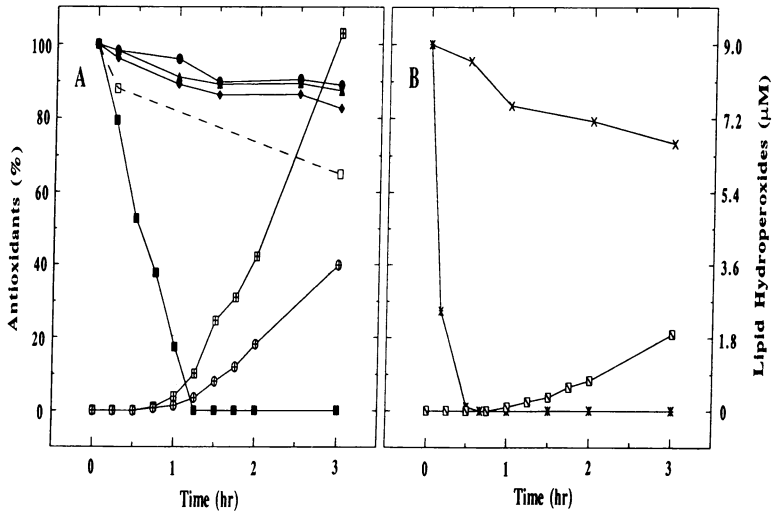

FIG. 1. Antioxidant defenses and lipid peroxidation in freshly isolated human LDL exposed to the water-soluble radical generator AAPH. LDL was isolated from plasma and incubated under air at 37°C in the presence (solid lines) or absence (broken line) of 1 mM AAPH. Aliquots were extracted, and the hexane (*A*) and the aqueous methanol (*B*) phases were analyzed for antioxidants and lipid hydroperoxides. Levels of the antioxidants ubiquinol-10 (■, □; initial concentration, 1.26 μM), lycopene (◆; 1.06 μM), β-carotene (▲; 0.98 μM), α-tocopherol (●; 36.0 μM), ascorbate (*, 1.15 μM), and urate (X, 26.6 μM) are given as the percentage of the initial concentrations (left ordinate). Levels of cholesterol ester hydroperoxides (□), triglyceride hydroperoxides (⊕), and phospholipid hydroperoxides (□) are given in μM (right ordinate). Note that hydroperoxides could not be detected within the first 30 min. One experiment typical of nine experiments for the hexane phase and three experiments for the aqueous methanol phase are shown.

Exposure of LDL to aqueous peroxyl radicals produced by AAPH at the low rate of 60 pmol/ml per min {calculated by using $R_i(aq) = 1.0 \times 10^{-6}$ [AAPH] as described for aqueous, protein-containing solutions (28)}, caused immediate depletion of endogenous ubiquinol-10 which was complete within 75 min (Fig. 1*A*). Consumption of ubiquinol-10 was primarily the result of radical-mediated oxidation: in the absence of AAPH, only 15–20% was lost within 75 min because of autoxidation. The peroxyl radical-induced concurrent consumption of α-tocopherol, lycopene, and β-carotene as shown in Fig. 1*A* and α-carotene and retinol palmitate (not shown) was very slow; even after 4 hr of exposure, 80–90% of these lipid-soluble antioxidants were still present. During the first 30 min of the experiment, the antioxidant defenses present in the LDL preparation completely protected all lipids from detectable peroxidation. After this lag period, hydroperoxides of cholesterol esters and triglycerides appeared simultaneously at comparatively low rates while ubiquinol-10 was present. Subsequent to complete consumption of ubiquinol-10, rates of peroxidation were markedly higher, despite the presence of physiological levels of endogenous α-tocopherol and carotenoids. In the absence of AAPH, no lipid hydroperoxides were detected in LDL incubated at 37°C for 4 hr under air. When LDL was supplemented with 5 μM ubiquinol-10 prior to exposure to AAPH, formation of LDL lipid hydroperoxides was reduced substantially (not shown).

Ascorbate is the only endogenous antioxidant in plasma that can completely protect all plasma lipids from aqueous peroxyl-mediated detectable oxidation (7, 8). As it was present at ≈1 μM in the LDL preparation (see above), we investigated the relationship between aqueous peroxyl radical-induced depletion of ascorbate and the onset of LDL lipid oxidation. Analysis of the aqueous methanol phase (Fig. 1*B*) showed that exposure of LDL to AAPH resulted in consumption of 99% of ascorbate within 30 min. Complete consumption of ascorbate coincided with onset of detectable hydroperoxide formation of phospholipids (Fig. 1*B*), cholesterol esters, and triglycerides (Fig. 1*A*), suggesting that ascorbate was effectively protecting LDL lipids from peroxidation. Urate was consumed linearly over the duration of the experiment (Fig. 1*B*).

To separate antioxidant protection of LDL by ascorbate from that provided by ubiquinol-10, fresh plasma was treated with ascorbate oxidase to remove endogenous ascorbate prior to isolation of the lipoprotein and its exposure to AAPH. Fig. 2 shows that removal of ascorbate did not change the sequence of endogenous antioxidant consumption when LDL was exposed to AAPH (compare with Fig. 1). (Quantitative differences between Figs. 1 and 2 most probably are due to use of different LDL preparations.) However, no lag period of detectable lipid peroxidation was observed in the absence of ascorbate (Fig. 2). Immediately after initiation of the reaction, hydroperoxides of cholesterol esters, triglycerides, and phospholipids were formed at an initial rate of ≈13 pmol/ml per min. The corresponding rate was ≈450 pmol/ml per min immediately after complete consumption of ubiquinol-10 (i.e., between 2 and 3 hr). Thus, the rate of lipid peroxidation increased ≈35-fold within the first 3 hr, when at least 70% of the endogenous levels of lycopene, β-carotene, and α-tocopherol were still present.

The relative importance of endogenous antioxidants in inhibiting LDL lipid peroxidation under two additional types of radical-generating conditions was examined. When the lipid-soluble radical initiator AMVN was added to LDL, ubiquinol-10 was again the first lipid-soluble antioxidant to be consumed (Fig. 3). As was the case with aqueous peroxyl radicals, in the presence of AMVN, lipid peroxidation in LDL was inhibited markedly while ubiquinol-10 was present and proceeded more rapidly subsequent to its complete

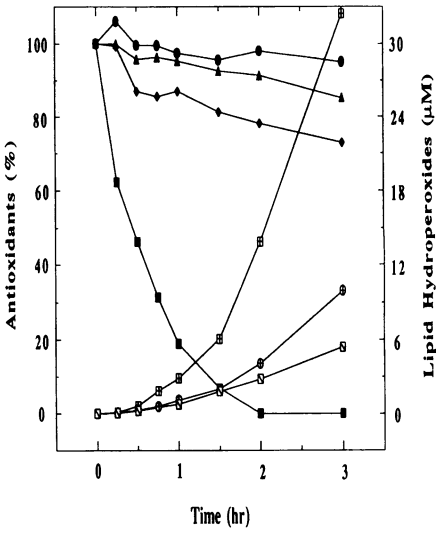

FIG. 2. Antioxidant defenses and lipid peroxidation in ascorbate-free human LDL exposed to the water-soluble radical generator AAPH. LDL, isolated from plasma previously treated with ascorbate oxidase (1 unit/ml) to deplete it of ascorbate but not other antioxidants, was incubated under air at 37°C in the presence of 1 mM AAPH. Aliquots were extracted and the hexane and aqueous methanol phases were analyzed for antioxidants and lipid hydroperoxides. Levels of the antioxidants ubiquinol-10 (initial concentration, 0.85 μM), lycopene (0.36 μM), β-carotene (0.50 μM), and α-tocopherol (39.9 μM) are given as the percentage of the initial concentrations (left ordinate). Levels of the lipid hydroperoxides are given in μM (right ordinate). Note that hydroperoxides were detected from the beginning of the experiment. Symbols are the same as in Fig. 1. One experiment typical of three is shown.

consumption, despite the presence of all other lipid-soluble antioxidants. Hydroperoxides were not formed in the absence of AMVN. Water-soluble urate was not consumed,

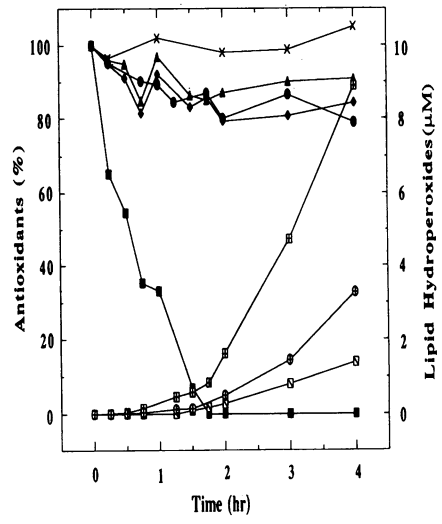

FIG. 3. Antioxidant defenses and lipid peroxidation in human LDL exposed to lipid-soluble radical generator AMVN. LDL was isolated from plasma and incubated under air at 37°C in the presence of 0.5 mM AMVN. Aliquots were extracted and the hexane or aqueous methanol phases were analyzed for antioxidants and lipid hydroperoxides. The levels of the antioxidants ubiquinol-10 (initial concentration, 0.92 μM), lycopene (0.39 μM), β-carotene (0.51 μM), α-tocopherol (24.4 μM), and urate (29.0 μM) are given as the percentage of the initial concentrations (left ordinate). The levels of the lipid hydroperoxides are given in μM (right ordinate). Symbols are the same as in Fig. 1. One experiment typical of three is shown for the hexane and aqueous methanol phases.

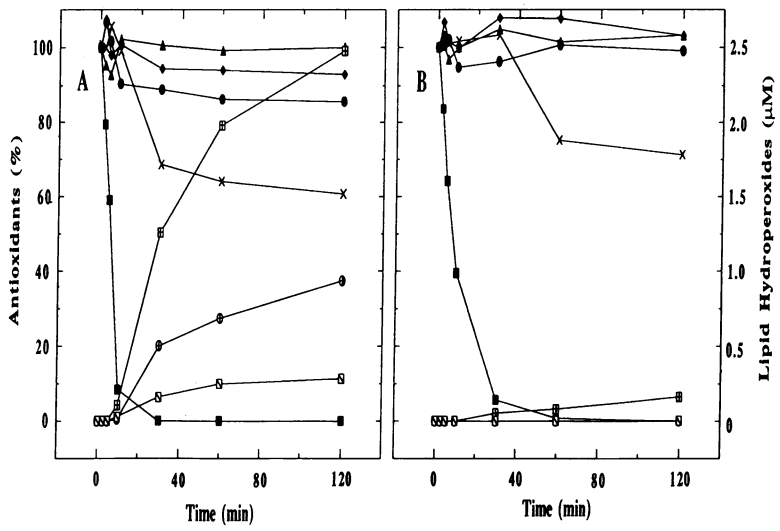

FIG. 4. Antioxidant defenses and lipid peroxidation in human LDL exposed to activated PMN in the absence (*A*) and presence (*B*) of supplemented ascorbate (50 μM). LDL was incubated at 37°C with freshly isolated PMN (5×10^6 cells per ml), and the cells were stimulated with PMA (1 μg/ml) at time zero. The levels of the antioxidants ubiquinol-10 (initial concentration, 0.81 μM), lycopene (0.58 μM), β-carotene (0.34 μM), α-tocopherol (27.7 μM), and urate (19.8 μM) are given as the percentage of the initial concentrations (left ordinate). The levels of the lipid hydroperoxides are given in μM (right ordinate). Note the difference in scales for lipid hydroperoxides between this figure and Figs. 1–3. Symbols are the same as in Fig. 1. One experiment typical of three is shown for the hexane and aqueous-methanol phases.

confirming that under these experimental conditions radicals were formed within LDL (29).

Fig. 4*A* shows that activation of human PMN with PMA caused rapid and almost complete consumption of ubiquinol-10 in LDL within the first 10 min of incubation, during which period lipid peroxidation was inhibited strongly. Following this, peroxidation of LDL lipids proceeded at relatively high rates between 10 and 30 min and then continued at low rates for the remaining duration of the experiment. α-Tocopherol, lycopene, and β-carotene were consumed steadily but only partially (5–15%), while ≈40% of urate was consumed between 10 and 30 min. In contrast to the cell-free control (see above), we consistently observed complete consumption of ubiquinol-10 but not other antioxidants, together with formation of small concentrations of lipid hydroperoxides in LDL coincubated with PMN for 2 hr in the absence of PMA. Typically, the amounts of hydroperoxides formed in this control incubation corresponded to 10% of those produced in the appropriate PMA-containing sample. When LDL was supplemented with 50 μM ascorbate prior to exposure to PMA-activated PMN, hydroperoxides of phospholipids and triglycerides were not detected, and substantially reduced amounts of cholesterol ester hydroperoxides appeared after 30 min (Fig. 4*B*). Ubiquinol-10 consumption proceeded slowly from the beginning of the reaction, indicating that ubiquinol-10 successfully competed with ascorbate for oxidants.

DISCUSSION

Recent reports (11, 13, 14) have suggested that one or more presently unidentified antioxidants apart from α-tocopherol and carotenoids may be responsible for the resistance of LDL to oxidation. Here we demonstrate that ubiquinol-10 is an endogenous antioxidant of LDL with outstanding reactivity towards oxidants. Regardless of the source and nature of the oxidant used (Figs. 1, 3, and 4) or whether ascorbate was present or not (Fig. 2), ubiquinol-10 was always the first lipid-soluble antioxidant to be consumed. More importantly, rates of LDL peroxidation were always low while ubiquinol-10 was present but increased markedly after its consumption, even though α-tocopherol, lycopene, and β-carotene were still present at high concentrations. We conclude that ubiquinol-10 is a more efficient antioxidant than either tocopherols or carotenoids in inhibiting LDL oxidation.

The experimental approach chosen to investigate LDL-associated antioxidant defenses differed from that of previous studies (3, 11–14, 30) in several important aspects: (*i*) in view of the lability of reactive antioxidants towards autoxidation, we used LDL isolated in the shortest feasible time (30 min); (*ii*) we placed special emphasis on rapid, selective, and sensitive analysis of antioxidants and, in particular, lipid hydroperoxides; and (*iii*) we employed mild oxidizing conditions to increase the likelihood of detecting low concentrations of reactive antioxidant(s) over a practical time scale. The method for the rapid isolation of LDL (21) used throughout this study yielded preparations that were free of detectable amounts of lipid hydroperoxides. However, LDL preparations were contaminated with small amounts of the water-soluble antioxidants urate and ascorbate. Urate is inefficient in inhibiting oxidation of lipids in plasma (7) and LDL (29), whereas ascorbate is highly efficient in these activities (7, 8, 29). In line with this, a lag period in AAPH-induced appearance of detectable LDL lipid hydroperoxides occurred only when the lipoprotein preparation contained small amounts of ascorbate (compare Figs. 1 and 2), and addition of ascorbate to the LDL preparation significantly inhibited cell-mediated lipid peroxidation (Fig. 4). These observations illustrate that LDL-associated antioxidants lower the rate of lipid peroxidation (by scavenging chain-propagating lipid peroxyl radicals), while ascorbate prevents initiation of lipid peroxidation (by trapping aqueous radicals before they can attack the LDL lipids).

Although previously implied (18) and suggested (17), we are unaware of studies directly demonstrating antioxidant activity of LDL-associated ubiquinol-10. More importantly, whereas ubiquinol-10 protected lipids from peroxidation about as efficiently as α-tocopherol in studies using either artificial or mitochondrial membranes (15, 17), our data indicate that in LDL ubiquinol-10 is a more efficient antioxidant than vitamin E. This may be best illustrated by the ≈35-fold increase in chain length of lipid peroxidation in LDL following consumption of ubiquinol-10.‡ Taken together with the absence of markedly increased α-tocopherol consumption following complete oxidation of ubiquinol-10, an interaction between the two antioxidants in LDL, i.e., a sparing effect of ubiquinol-10 on α-tocopherol, seems unlikely. Therefore, our results suggest that ubiquinol-10 may represent the primary lipid-soluble antioxidant in LDL. However, to verify unambiguously such a function of ubiquinol-10, an analysis and comparison of the sum of the contributing antioxidant parameters of all individual antioxidants (i.e., ubiquinol-10, tocopherols, and ca-

‡From the data in Fig. 2 we calculate that lipid peroxidation proceeded initially (0–15 min) with a chain length of ≈0.2 molecule of lipid hydroperoxides formed per aqueous peroxyl radical generated, whereas immediately after complete ubiquinol-10 consumption (2–3 hr), the chain length was ≈7 and remained constant for at least 2 hr (not shown). We assume that this second phase of inhibited lipid peroxidation represents the phase referred to as the "lag period" in previous work on LDL oxidation (2, 3, 11–14).

rotenoids) to the total radical trapping antioxidant parameter (9, 31) of LDL remains to be completed.

The apparent higher antioxidant activity of LDL-associated ubiquinol-10 in comparison to that in bilayers (15–17), or to LDL-associated α-tocopherol (this study), raises a number of interesting issues, including relative concentrations, intra- versus inter-particle mobility and physical location of the lipid-soluble antioxidants. Regarding the latter, the radical scavenging activity of ubiquinols in homogeneous solution is independent of the length of the isoprenoid chain, while in microsomes or mitochondria it decreases strongly with increasing isoprenoid chain length (32). The location of ubiquinone in membranes or lipoproteins is unknown. However, there is some evidence that in biological membranes a substantial proportion of ubiquinol-10 is present in a separate phase rather than intercalated between adjacent lipid chains or concentrated at the center of the hydrocarbon core (33). Thus, while the availability of the antioxidant active benzoquinol moiety of ubiquinol-10 to react with lipid-centered radicals could be limited in membranes, this may not be the case in LDL. [Note that the length of the extended 10 isoprene groups of ubiquinol-10 is longer than the thickness of a biological membrane, whereas LDL is a particle with a diameter about twice as thick as a membrane.]

Although we did not investigate the mechanisms of lipid peroxidation induced by stimulated PMN, we noted that LDL lipids were peroxidized by these cells even in the absence of a stimulus, suggesting that LDL itself activated oxidant production by PMN (see also ref. 34) and/or that lipid peroxidation was partly due to oxidants other than those produced following activation of the NADPH-oxidase. Cell activation appeared to be independent of small amounts of peroxides, as the LDL initially added to the PMN was free of detectable amounts of lipid hydroperoxides.

For recognition by the scavenger receptors, substantial oxidative modification of LDL is required. This is achieved only after massive lipid peroxidation and complete consumption of endogenous α-tocopherol (3, 13, 30). As we used oxidative conditions that usually resulted in consumption of not more than 20% of the endogenous α-tocopherol, our finding that ubiquinol-10 is more potent than vitamin E in protecting LDL lipids from peroxidation cannot directly be interpreted as an important mechanism for inhibitory generation of high-uptake forms of LDL. However, LDL oxidized to a lesser extent than that required for altered uptake by macrophages may possess bioactivities of potential relevance to atherogenesis (3, 35, 36). Furthermore, our results suggest that oxidant-mediated consumption of α-tocopherol is always preceded by that of ubiquinol-10. Considering the "oxidative theory" of atherosclerosis (2, 3), our results suggest that LDL-associated ubiquinol-10 is an important anti-risk factor for the development of this disease. Tissues of humans suffering from heart disease are deficient in coenzyme Q_{10} (37). Since endogenous ubiquinol-10 is derived from both dietary sources and biosynthesis via the mevalonate pathway, our findings may have nutritional as well as clinical implications, the latter regarding the use of cholesterol-lowering drugs through inhibition of 3-hydroxy-3-methylglutaryl CoA reductase (38).

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