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High density lipoprotein is the major carrier of lipid hydroperoxides in human blood plasma from fasting donors

(atherosclerosis/low density lipoprotein/oxidation/ubiquinol/metabolism)

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ABSTRACT **Analysis of untreated fresh blood plasma from healthy, fasting donors revealed that high density lipoprotein (HDL) particles carry most (≈85%) of the detectable oxidized core lipoprotein lipids. Low density lipoprotein (LDL) lipids are relatively peroxide-free. *In vitro* the mild oxidation of gel-filtered plasma from fasting donors with a low, steady flux of aqueous peroxyl radicals initially caused preferential oxidation of HDL rather than LDL lipids until most ubiquinol-10 present in LDL was consumed. Thereafter, LDL core lipids were oxidized more rapidly. Isolated lipoproteins behaved similarly. Preferential accumulation of lipid hydroperoxides in HDL reflects the lack of antioxidants in most HDL particles compared to LDL, which contained 8–12 α-tocopherol and 0.5–1.0 ubiquinol-10 molecules per particle. Cholesteryl ester hydroperoxides (CEOOHs) in HDL and LDL were stable when added to fresh plasma at 37°C for up to 20 hr. Transfer of CEOOHs from HDL to LDL was too slow to have influenced the *in vitro* plasma oxidation data. Incubation of mildly oxidized LDL and HDL with cultured hepatocytes afforded a linear removal of CEOOHs from LDL (40% loss over 1 hr), whereas a fast-then-slow biphasic removal was observed for HDL. Our data show that HDL is the principal vehicle for circulating plasma lipid hydroperoxides and suggest that HDL lipids may be more rapidly oxidized than those in LDL *in vivo*. The rapid hepatic clearance of CEOOHs in HDL could imply a possible beneficial role of HDL by attenuating the build-up of *oxidized* lipids in LDL.**

Oxidative modification of low density lipoprotein (LDL) has been implicated in the formation of lipid-laden foam cells, which is an early and important step in the development of atherosclerotic lesions (1). Oxidized high density lipoprotein (HDL), in contrast, is not avidly taken up by macrophages (2) and does not lead to foam cell formation. Furthermore HDL has been reported to inhibit endothelial cell-mediated LDL modification (2, 3) and to substantially reduce the cellular uptake and degradation of native and oxidatively modified LDL (2, 4).

Radical oxidants, including those produced by cells present in the artery wall, have been shown to oxidize the lipid component of LDL before detectable alteration of the apoprotein B (1, 5). By using ultrasensitive HPLC assays, various classes of lipid hydroperoxides (LOOHs) have been detected in human plasma from nonfasting donors (6). We have also detected LOOHs, both in plasma from fasting and nonfasting subjects, but presumably in a component other than LDL since this lipoprotein contained less LOOHs than the plasma from which it was obtained (7). Here we identify the major LOOH-containing particle as HDL and examine possible mechanisms by which HDL might preferentially accumulate LOOHs.

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MATERIALS AND METHODS

Apart from the following, the chemicals, solvents, and buffers used were the same as described previously (7). Uricase (EC 1.7.3.3) and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma, and superfine Sephadex G-25 was purchased from Pharmacia. LOOH standards were prepared as described (8). Ubiquinol-10 ($CoQH_2$) and ubiquinol-9 were prepared as described (9) and used within 24 hr.

Plasma, HDL, and LDL. Plasma was prepared from freshly obtained heparinized blood of fasting (≥12 hr), healthy male (n = 8) and female (n = 6) donors (25–36 years old). The use of "fasted plasma" simplified the experimental system by eliminating chylomicrons as potential LOOH carriers and labile (nonequilibrated) lipid sources/sinks. LDL and HDL fractions were isolated by two methods (specified in the figure legends). Method I used procedure 7 in ref. 10 and effectively separates LDL from the higher density plasma proteins that include HDL as the major neutral lipid-carrying particle. Method II, which affords purified LDL and HDL fractions, was based on procedure 15 (single centrifugation), also given in ref. 10, and was adapted for the Beckman TL-100 centrifuge in the same way the authors had modified the two-density LDL preparation (compare procedures 7 and 8). Thus in method II, 0.6 ml of phosphate-buffered saline (PBS) (d = 1.006 g/ml) was successively underlayered with 0.6 ml of PBS plus KBr (1.20 g/ml) and 0.7 ml of plasma plus KBr (1.33 g/ml) and centrifuged at 436,000 × g (TLA 100.2 rotor) for 40 min. Syringe extraction of the visible LDL (≈1.06 g/ml) and HDL (≈1.15–1.25 g/ml) density bands afforded lipoproteins with no detectable cross-contamination (judged by polyacrylamide gel electrophoresis) and only slight contamination with albumin (<3% in LDL and ≈10% in HDL) (7, 10).

For some experiments, plasma was incubated for 10 min at 25°C in the presence of ascorbate oxidase and/or uricase (each at 1 unit/ml) prior to isolation of the lipoproteins. Preliminary experiments showed complete depletion of plasma ascorbate and/or urate within 5 min. In other experiments ascorbate, urate, and other small aqueous solutes were removed by centrifuge-assisted percolation of plasma or lipoproteins through superfine Sephadex G-25 (4°C, 600 × g), a procedure that caused oxidation of ≈15% of the $CoQH_2$ but barely detectable oxidation of core lipids [i.e., <10 nM before vs. 30–50 nM cholesteryl ester hydroperoxides (CEOOHs) after treatment of the lipoprotein].

Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane) hydrochloride; CEOOH, cholesteryl ester hydroperoxide; CE, cholesteryl ester; ChOH, cholesterol; CoQ, ubiquinone-10; $CoQH_2$, ubiquinol-10; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); HDL, high density lipoprotein; LDL, low density lipoprotein; LOOH, lipid hydroperoxide; PLOOH, phospholipid hydroperoxide; α-TocH, α-tocopherol; LDL_{ox}, oxidized LDL; HDL_{ox}, oxidized HDL; Ch18:2, cholesteryl linoleate; Ch18:2-OOH, cholesteryl linoleate hydroperoxide.

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Oxidation of HDL, LDL, and Plasma. The isolated lipoproteins were diluted to their estimated concentration in the parent plasma, and controlled steady oxidation was achieved by incubation with 2,2'-azobis(2-amidinopropane) hydrochloride (AAPH) as described for LDL (7). For plasma oxidation, the potential actions of lecithin–cholesterol acyltransferase were investigated by preincubation of some samples with the thiol agent DTNB (1.0 mM) followed by careful gel filtration to remove excess reagent and the released thiolate. Postsampling oxidation (i.e., during lipoprotein isolation) was arrested by chilling the 0.6-ml plasma aliquots on ice and adding ascorbate (3 mM) (7).

Analysis. Fresh or oxidized plasma or isolated lipoprotein fractions were processed and analyzed for LOOHs, unoxidized lipids, and antioxidants as described (7). Additional experiments have verified that the methanol/hexane extraction procedure employed in this work affords efficient separation of phospholipids from neutral lipids, and Folch extraction of the lower aqueous methanol portion of a plasma extract, including the precipitated protein, yielded neither detectable α-tocopherol (α-TocH), cholesterol (ChOH), nor cholesteryl esters (CEs). Typical detection limits determined by standard addition of CEOOHs and phospholipid hydroperoxides (PLOOHs; detected as phosphatidylcholine hydroperoxide) were about 3 nM and 10 nM, respectively. Ubiquinol-9 added to isolated LDL or HDL was <10% oxidized during the extraction/analysis procedures, which indicates that substantial formation of LOOHs during the work-up was unlikely to occur since the ubiquinol (independent of incorporation into lipoproteins) would be more readily oxidized than plasma lipids (7). Analysis of the aqueous methanol portion of the extract revealed no detectable ChOH, α-TocH, or neutral lipids, thus allowing internal standardization of LOOHs and antioxidants against ChOH. CEOOHs present in plasma or formed during oxidation were mostly those derived from cholesteryl linoleate (Ch18:2) and cholesteryl arachidonate, with a typical ratio of cholesteryl linoleate hydroperoxide (Ch18:2-OOH) to cholesteryl arachidonate hydroperoxide of ≈5. Identities of the CEOOHs were verified by coinjection of fresh standards and by elimination of the chemiluminescence-positive signals by $NaBH_4$ treatment (11). In general, core lipid oxidation has been assessed from the ratio of the concentration of Ch18:2-OOH to the concentration of Ch18:2, which is conveniently expressed in parts per million (ppm).

CEOOH Transfer. Oxidized HDL (HDL_{ox}) and LDL (LDL_{ox}) were prepared by incubating ascorbate- and urate-free plasma with 1.5 mM AAPH at 37°C for 45 min, removing excess AAPH by gel filtration, and isolating the plasma lipoproteins (method II). The resulting LDL_{ox} and HDL_{ox} contained typically 800–1200 ppm and 500–800 ppm of CEOOHs, respectively. For LDL and HDL, one core LOOH per particle corresponds to ≈1600 and 30,000 ppm, respectively. LDL_{ox} and HDL_{ox} were then added to the untreated parent plasma such that LDL_{ox}/total LDL ≈ 0.2 and HDL_{ox}/total HDL ≈ 0.4. The supplemented plasmas were divided into 800-μl aliquots in separate argon-flushed small plastic vials and incubated in the dark at 37°C. An aliquot (200 μl) of each sample was analyzed directly for total LOOHs and lipids before LDL and HDL fractions were prepared from the remaining 600 μl by using method I.

Hepatoma Cells. HepG2 cells were grown at 37°C to ≈70% confluence (5×10^5 cells per dish) in Dulbecco's modified Eagle's medium containing fetal calf serum (10% vol/vol), glutamine (1 mM), penicillin (100 units/ml), and streptomycin (0.1 mg/ml) in 5% CO_2 in a humidified incubator. The medium was changed, and the cells were incubated for two additional 24-hr periods: first in the same medium with lipoprotein-depleted serum and then in Chelex-treated PBS supplemented with human serum albumin (1%). Cells were then washed and incubated at 37°C in 5.5 ml of Chelex-treated PBS plus albumin before either LDL_{ox} or HDL_{ox} was added. Aliquots of the medium were removed at different times and analyzed for unoxidized lipids and CEOOHs.

RESULTS

HPLC chemiluminescence analysis (Fig. 1) of fresh plasma from healthy fasting donors revealed small amounts of LOOHs in most samples (Table 1). From 14 different donors, CEOOHs were detectable in plasma from 10 subjects, in HDL from 12 subjects, but in LDL from only 2 subjects. HDL carried 85% of total plasma CEOOHs and all of the detectable PLOOHs. While HDL and LDL carried approximately equal numbers of molecules of CEOOH *per particle*, the CEs in HDL on a *per lipid* basis were over 20-fold "more oxidized" than those in LDL [i.e., 11 vs. 0.4 ppm (1 ppm = 1×10^{-6} Ch18:2-OOH/Ch18:2), respectively]. Blood analyses were reproducible, and the results of three separate extractions of a single sample varied by 12.4% and 8.6% for CEOOHs in plasma and HDL, respectively, after normalization of the CEOOH values for the ChOH content of the extracts. The presence of detectable LOOHs in plasma and HDL was not due to a pro-oxidant activity of ascorbate and urate exerted during the extraction, as removal of these antioxidants by enzymic treatment of a separate sample prior to extraction actually increased (by 50%) the amounts of LOOHs detected. Day-to-day variations for a single donor were within ≈15%. In each case the LOOH content in plasma could be accounted for by the sum of LOOHs in HDL and LDL, the major lipid-bearing particles in fasted plasma. The mean plasma CEOOH concentration in our survey is com-

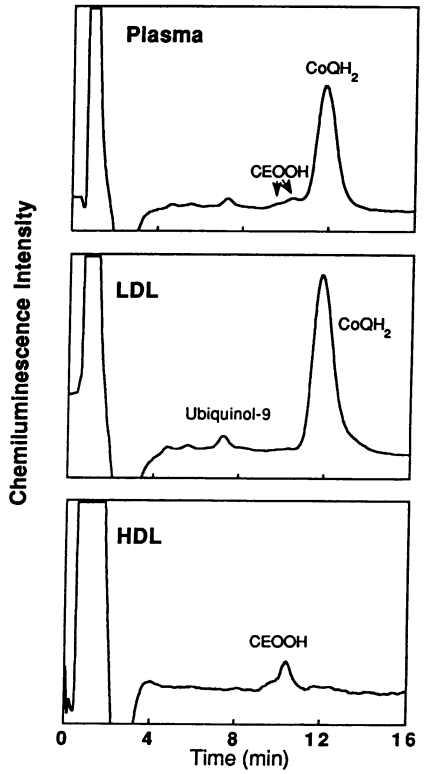

FIG. 1. HPLC chemiluminescence detection of LOOHs in fresh fasted human plasma and its lipoproteins. Hexane extracts of plasma, LDL, and HDL (corresponding to an equimolar amount of ChOH) were prepared (method I) and analyzed as described (7). The negative peak eluting near 3 min is caused by α-TocH's chemiluminescence quenching; ubiquinol-9 (≈7 min) and $CoQH_2$ (12 min) give positive peaks due to their rapid autoxidation in the presence of the alkaline "isoluminol" solution (6, 7).

Table 1. Distribution of LOOHs and antioxidants in fresh fasted plasma

LOOH or antioxidant	Plasma	LDL		HDL*	
		Plasma†	Molecules per particle,‡ mol/mol	Plasma†	Molecules per particle,‡ mol/mol
CEOOH,§ nM	4.2 ± 2.7	0.6 ± 1.0	0.0004	3.3 ± 1.8	0.0004
PLOOH,¶ nM	3.2 ± 3.8	ND‖	ND‖	4.2 ± 4.0‖	0.0005
α-TocH, μM	16.8 ± 5.4	10.7 ± 3.9	8.5	3.9 ± 1.6	0.31
β-Carotene, μM	0.8 ± 0.5	0.5 ± 0.3	0.4	0.2 ± 0.1	0.01
$CoQH_2$ + CoQ, μM	1.4 ± 0.5	1.1 ± 0.5	0.7	0.2 ± 0.2	0.015
$CoQH_2$/CoQ, mol/mol	1.6 ± 1.3	4.3 ± 2.6		0.5 ± 0.4	

Errors represent standard deviations but include only interdonor variation in ChOH normalized data. ND, below detection limits.
*HDL refers to the $d > 1.1$ g/ml fraction from the LDL preparation method (7). Comparison of this HDL-enriched fraction with purified HDL (compare with Fig. 2) showed no differences in ChOH-corrected LOOH or antioxidant contents.
†Data have been normalized to a plasma ChOH concentration = 1.0 mM, and the lipoprotein data are based on an LDL ChOH concentration = 0.80 mM and an HDL ChOH concentration = 0.20 mM in plasma.
‡Based on 550 and 35 molecules of ChOH per LDL and HDL, respectively.
§As mentioned in the text, CEOOHs were detectable in plasma from 10 subjects, in HDL from 12 subjects, but in LDL from only 2 subjects. LOOHs were more easily detected in HDL than in plasma because, on a *per lipid* basis, the LOOH concentration was much higher in this lipoprotein and because the relative $CoQH_2$ concentration was lower, so it interfered less strongly with the detection of CEOOH.
¶PLOOHs were detected as hydroperoxides of phosphatidylcholine, the major class of phospholipids in lipoproteins.
‖$n = 5$.

parable with that reported previously (6) (i.e., 4.2 nM vs. ≈3 nM, respectively). Linked with high plasma CEOOH concentrations was the presence of PLOOHs in HDL ($r = 0.92$, $n = 8$).

Table 1 also shows the concentrations of the major lipoprotein-associated antioxidants. As shown previously (5, 7), α-TocH is quantitatively the major antioxidant in LDL. Although the concentrations of α-TocH and carotenoids in HDL are similar to those in LDL when expressed per ChOH, the small size of HDL particles means that most *particles* are actually devoid of all known lipid antioxidants. The plasma level of $CoQH_2$ correlated positively ($r = 0.84$, $n = 10$) with α-TocH. High concentrations of the latter have been linked to low incidences of ischemic heart disease (12). Both α-TocH and $CoQH_2$ showed some negative correlation with the plasma LOOH concentration ($r = -0.32$ and -0.33 for $CoQH_2$ and α-TocH, respectively, $n = 10$), but the best index for the plasma lipoprotein lipid oxidation state was the $CoQH_2$-to-ubiquinone-10 (CoQ) ratio, which showed a correlation of $r = -0.82$ ($n = 10$) with plasma CEOOHs and $r = -0.79$ with CEOOHs in HDL ($n = 12$). This correlation is reflected further by a higher $CoQH_2$-to-CoQ ratio in LDL compared with that in HDL.

The observed uneven distribution of plasma LOOHs may be attributed to a number of differences between LDL and HDL including (*i*) the *relative ease* with which HDL and LDL lipids *become oxidized*, (*ii*) the *transfer* of oxidized core lipids from LDL to HDL and vice versa, or (*iii*) the *rate* of CEOOH *clearance* (e.g., by endothelial cells or the liver) from LDL and HDL. We have attempted to address each of these possibilities.

Relative Oxidizability of LDL and HDL Lipids. Exposure of *isolated* LDL to a low and constant flux of aqueous peroxyl radicals in the absence of aqueous antioxidants caused immediate oxidation of lipoprotein lipids but with a period during which lipid oxidation was strongly inhibited (Fig. 2*A*), corresponding to the consumption of $CoQH_2$ (7). In contrast, *isolated* HDL obtained from the same donor and oxidized under the same conditions gave a constant rate of CEOOH formation throughout the entire incubation (Fig. 2*B*; cf. ref. 13). The ratio of accumulating PLOOH to CEOOH was higher in HDL (1.1) than in LDL (0.3) (data not shown), and this may be explained in part by the higher relative phospholipid concentration in HDL (42% of lipid mass vs. 26% for LDL). After the period of strong inhibition, the rate of LDL core lipid oxidation was 2-fold greater than that of HDL when expressed in ppm. Since, however, LDL contained ≈4 times

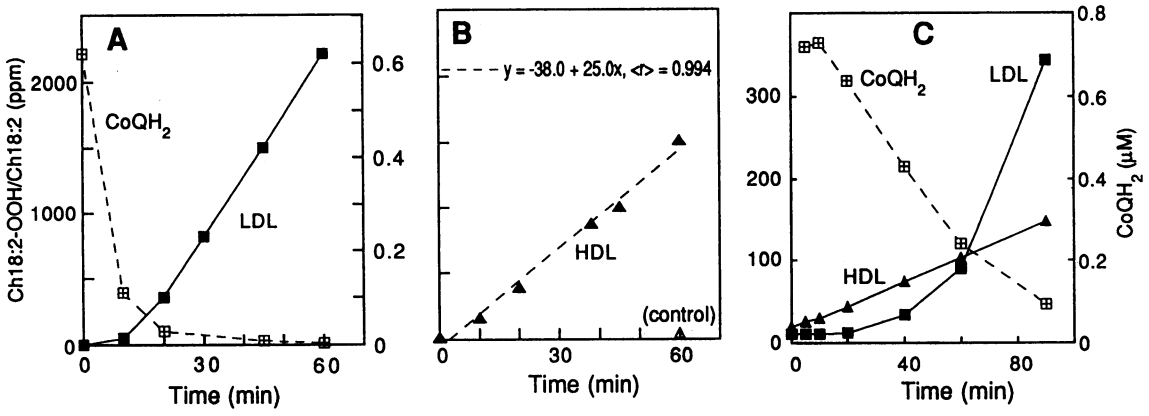

FIG. 2. Peroxyl radical-mediated oxidation of lipoprotein lipids in either isolated LDL (*A*), HDL (*B*), or fresh human plasma (*C*). Controlled oxidation of isolated ascorbate- and urate-free LDL (1.8 μM), HDL (11.7 μM), or plasma (≈20% diluted) was effected at 37°C by a mild, steady flux of aqueous peroxyl radicals generated from AAPH (1.0 mM in *A* and *B*, 0.5 mM in *C*). HDL was prepared by using method II. At various time points, samples were withdrawn, processed, and analyzed as described in *Materials and Methods*. In the absence of AAPH (open symbols), no significant oxidation of lipids in isolated HDL or LDL occurred. Three independent experiments using higher AAPH concentrations (i.e., 1.0, 1.5, or 2.0 mM) gave consistent results with correspondingly faster LOOH formation.

more CEs than HDL (i.e., 0.8 vs. 0.2 mM Ch18:2, respectively), the total amounts of CEOOHs formed in LDL in the later, less-inhibited stage of oxidation was ≈8-fold higher than that in HDL. This implies more extensive radical propagation in the larger lipid core of LDL (see *Discussion*).

The relative susceptibilities of LDL vs. HDL lipids to oxidation were also measured in plasma. To do this, ascorbate- and urate-depleted plasma from a fasting donor was exposed to aqueous radicals and subsequently separated into LDL and HDL fractions. To inhibit possible exchange of oxidized surface and core lipids within the lipoproteins, half the plasma was pretreated with DTNB, a sulfhydryl reagent that inhibits lecithin–ChOH acyltransferase (14). The temporal accumulation of CEOOHs in each fraction is shown in Fig. 2*C*. Data from the nontreated plasma were virtually identical (data not shown), suggesting that ChOH esterification has little influence on this time scale. As indicated by the peroxide scales of Fig. 2, CEOOHs accumulated more rapidly in the preisolated lipoproteins than in plasma (even allowing for a 2-fold higher AAPH concentration in the former). Whether this arises from the presence of aqueous peroxyl radical scavengers remaining in gel-filtered plasma (e.g., protein sulfhydryls and bilirubin) or from a peroxidase activity in the plasma (cf. ref. 15 and below) is unclear at this stage. However, in accord with the *isolated* lipoprotein oxidation data (Fig. 2 *A* and *B*), the whole plasma oxidation data do show that, *initially*, HDL core lipids are oxidized more rapidly than those in LDL. The point at which LDL core lipid oxidation becomes faster than that of HDL corresponds closely to the disappearance of $CoQH_2$ in the LDL fraction. HDL lipid oxidation proceeded at a constant rate throughout the experiment, suggesting that $CoQH_2$ in LDL did not influence the extent of HDL oxidation. Other experiments using higher radical fluxes showed that after the initial lag period CEOOHs accumulated at 2- to 4-fold higher rates in LDL than in HDL, depending on the plasma donor. Owing to their instability in plasma (15), PLOOHs were not analyzed.

Transfer of CEOOHs Between HDL and LDL. LDL_{ox} or HDL_{ox} was incubated in fresh (fasted) plasma under argon, and LDL and HDL fractions were subsequently reisolated from the incubates. At 4°C, no transfer was discerned in either direction even after 48 hr. Incubation of fresh plasma at 37°C with either LDL_{ox} or HDL_{ox} for 24 hr resulted in transfer of only ≈0.5%/hr and 2%/hr of CEOOHs from LDL to HDL and HDL to LDL, respectively (data not shown). The rate of CEOOH transfer varied somewhat between donors and was inhibited completely in the presence of 1.5 mM DTNB (data not shown). These rates of CEOOH transfer are too slow to influence our plasma oxidation experiments (Fig. 2*C*) with *or* without DTNB. Interestingly, CEOOHs within LDL_{ox} or HDL_{ox} were stable at 37°C in freshly isolated plasma for at least 20 hr, unlike free fatty acid hydroperoxides and PLOOHs (15). Additional experiments indicated that CEOOHs in LDL and HDL are also stable for at least 2 hr in fresh whole blood (data not shown).

Clearance of CEOOHs in LDL and HDL by Hepatocytes. The stability of CEOOHs in plasma and whole blood suggests that they must be cleared from the circulation by interaction with non-blood components (e.g., endothelial cells or the liver, the major lipoprotein catabolizing tissue). As human HepG2 hepatoma cells have been used as a model for the hepatic clearance of lipoprotein CEs (16, 17), we chose to test whether these cells can also metabolize CEOOHs from LDL_{ox} and HDL_{ox}. In the presence of HepG2 cells, a rapid loss of CEOOHs relative to CEs was observed compared to controls in which no cells were present (Fig. 3). The rate was linear for LDL_{ox}, giving rise to ≈7% loss per 10 min. For HDL the curve was biphasic, with an initial rapid phase of clearance (≈50% in the first 10 min) before basal rates were achieved. In neither case did CEOOHs accumulate within the cells. We expressed the loss of CEOOHs relative to CEs because the sterile culture dishes were responsible for a nonspecific loss of both unoxidized and oxidized CEs, especially from HDL_{ox}.

DISCUSSION

Our survey of plasma from fasting healthy donors (Table 1) shows an uneven distribution of both LOOHs and $CoQH_2$ between LDL and HDL. Thus, while HDL is the principal vehicle for plasma core LOOHs, LDL lipids are virtually peroxide-free, and the redox status of coenzyme Q_{10} correlates negatively with the plasma LOOH concentration among different donors. These observations are in agreement with our quantitative *in vitro* evaluation of LDL oxidation (7) in which we found that endogenous $CoQH_2$ strongly inhibited the earliest stages of LDL oxidation initiated by peroxyl radicals generated in the aqueous or lipid phase or by oxidants released from activated neutrophils. In particular, when LDL was exposed to a *steady* flux of aqueous peroxyl radicals (ROO·), LOOHs were formed very slowly until about 80% of the $CoQH_2$ was oxidized to CoQ. Thereafter LOOHs formed about 20 times faster in spite of the continued presence of α-TocH and other lipid antioxidants, which were

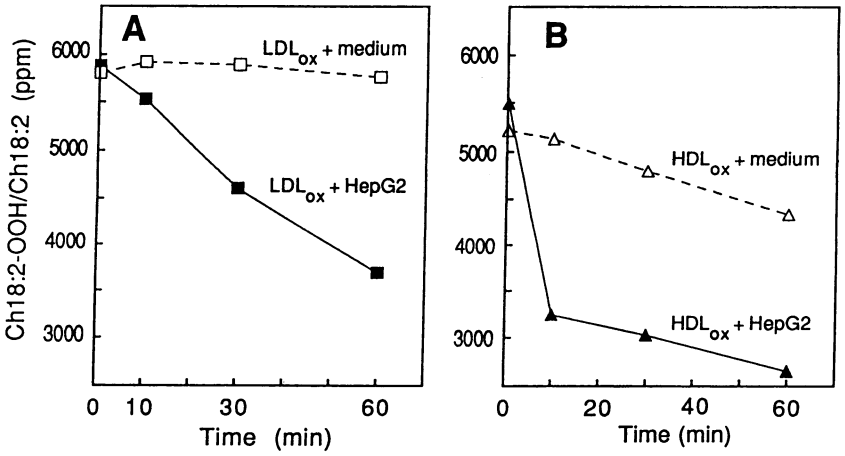

FIG. 3. Removal of CEOOHs from LDL_{ox} (*A*) and HDL_{ox} (*B*) by HepG2 cells. Cells grown to near confluence as described in *Materials and Methods* were incubated with either LDL_{ox} (0.25 mg of total mass per ml) or HDL_{ox} (0.16 mg of total mass per ml) containing 4–6 or 1–2 molecules of CEOOH per particle, respectively. The results shown are representative of four independent experiments showing the time-dependent removal of CEOOHs from the medium.

only slightly depleted by the low radical flux (cf. Fig. 2*A*).§ Together, these findings support the notion that $CoQH_2$ has an important *in vivo* antioxidant protective function for lipids in lipoproteins, particularly LDL, and that the redox status of coenzyme Q_{10} may be a useful *early* marker for the assessment of oxidative LDL modification.

The *linear* oxidation of HDL (Fig. 2*B*) (i.e., the lack of an antioxidant dependent lag period) is hardly surprising as the majority (50–70%) of HDL particles are devoid of lipid antioxidant molecules. In LDL, once endogenous $CoQH_2$ is depleted, the same radical initiation rates afford faster oxidation of core lipids than that in HDL in spite of the continued presence of more than six molecules of α-TocH per LDL particle.¶ This is most likely due to the shorter apparent radical chain length, $\chi = [LOOH]/[ROO\cdot]$, for the "uninhibited" oxidation of HDL (Fig. 2*A*) compared to that calculated for the second, linear region of the "α-TocH-inhibited" LDL oxidation (Fig. 2*B*, time > 40 min)—namely, $\chi_{LDL} = 6$ vs. $\chi_{HDL} = 1.1$. This may merely reflect a more limited supply of polyunsaturated fatty acids in the tiny HDL particles (e.g., 35 Ch18:2 per HDL vs. 600 per LDL), although a higher antioxidant contribution from the HDL apoprotein (51% by weight in HDL vs. 23% in LDL) cannot be discounted.

Relative oxidation rates of HDL and LDL in *plasma* are determined by at least two factors: the extent of radical propagation within lipoprotein particles (see above) *and* the relative ease with which radicals initiate lipid oxidation chains across the lipid/water interface. By comparing relative Ch18:2-OOH formation rates for the *isolated* lipoproteins with those for the same lipoproteins in *plasma*, we estimate that radical initiation across the lipid/water interface in LDL is 2- to 3-fold faster than that in HDL. Since HDL and LDL present similar surface areas to the aqueous environment,‖ it would appear that the surface of LDL is more readily breached by ROO· than that of HDL (see *Note Added in Proof*).

The transfer of CEOOHs (i.e., Ch18:2-OOH) between lipoproteins is considerably slower than published rates of *in vitro* transfer of unoxidized CEs from isolated HDL to LDL (19). A relatively slow CEOOH transfer could result from selectivity of lipid transfer protein toward the nonoxidized lipid. Regardless of the mechanism, however, it is clear that the transfer rates of CEOOHs between HDL and LDL are too slow to substantially influence plasma CEOOH distribution either in our *in vitro* plasma oxidation (Fig. 2*C*) or, indeed, *in vivo* since CEs of circulating HDL are turned over several times per day and our HepG2 data indicate that HDL CEOOHs are cleared even more rapidly than the unoxidized CEs.

Our experiments with HepG2 cells indicate that core LOOHs even when present in very low levels within different lipoproteins, particularly HDL, may be detoxified rapidly. The mechanism(s) of this detoxification is not well understood at present, but we note that in no instance could CEOOHs be detected in the cells. Recent results suggest that the rapid removal of CEOOHs from HDL_{ox} is due to a more rapid "selective uptake" of oxidized over unoxidized CEs by HepG2 cells (W. Sattler and R.S., unpublished data). Removal of CEOOHs from HDL_{ox} and LDL_{ox} by these cells suggests that liver is capable of efficiently detoxifying low levels of circulating core LOOHs.

In summary, the preferential presence of LOOHs in the HDL of fresh *ex vivo* fasted plasma is consistent with our *in vitro* oxidation and CEOOH transfer data—i.e., LOOHs may accumulate more rapidly in HDL than in LDL *in vivo* because of the antioxidant action of $CoQH_2$ in the LDL and because the transfer of the core LOOH is (in fasted plasma at least) too slow to redistribute these LOOHs to other plasma lipoproteins. The finding that HepG2 cells, as a model for liver, appear to be capable of efficiently detoxifying circulating core LOOH in HDL suggests a beneficial function of HDL in the hepatic clearance of circulating oxidized lipids. Other possible influences upon the distribution of plasma LOOHs between different lipoproteins are uptake of oxidized lipids from cells and/or more labile plasma components (i.e., chylomicrons and very low density lipoprotein).

Note Added in Proof. Recent findings suggest that the higher peroxidation rate of LDL vs. HDL is at least partly due to a pro-oxidant activity of α-TocH (ref. 20; V.W.B., K. U. Ingold, and R.S., unpublished data), which is present at a higher concentration in LDL than HDL.

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§The mechanism(s) of the remarkable antioxidant effectiveness of $CoQH_2$ in LDL is under investigation. Here we merely wish to point out that apparent radical chain lengths, χ, calculated in the earliest stages of LDL oxidation initiated by AAPH are considerably less than unity (i.e., $\chi = 0.3–0.8$) and that as little as 0.2 molecule of $CoQH_2$ per LDL (i.e., ≈40% of the $CoQH_2$ present in freshly isolated LDL) substantially inhibits oxidation. These observations suggest that $CoQH_2$ may be intercepting ROO· in the aqueous phase as well as breaking lipid radical chains. Judged by known kinetic parameters (18), it seems likely that there is also some degree of α-TocH "sparing" by $CoQH_2$ during LDL oxidation. Such sparing *alone*, however, cannot explain the sharp increase in LOOH formation after $CoQH_2$ is consumed when LDL is exposed to a *steady* flux of peroxyl radicals.

¶Radical oxidation chains in LDL may be terminated by α-TocH, and experiments using much higher AAPH concentrations show that depletion of α-TocH influences the LOOH formation rate (C. Suarna, R. T. Dean, and R.S., unpublished data).

‖The calculated average surface areas of LDL and HDL in plasma are 1.3 and 1.8 m^2/ml, respectively, based on molarities and particle diameters. However, since the protein-to-phospholipid ratio for HDL is higher than that for LDL [i.e., 2.0 and 1.1 (wt/wt), respectively], the actual *lipid*/water interface area of LDL might well be about the same or even slightly higher than that of HDL.