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Antioxidant defenses and lipid peroxidation in human blood plasma

(oxidants/polymorphonuclear leukocytes/ascorbate/plasma peroxidase)

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ABSTRACT **The temporal disappearance in human blood plasma of endogenous antioxidants in relation to the appearance of various classes of lipid hydroperoxides measured by HPLC postcolumn chemiluminescence detection has been investigated under two types of oxidizing conditions. Exposure of plasma to aqueous peroxyl radicals generated at a constant rate leads immediately to oxidation of endogenous ascorbate and sulfhydryl groups, followed by sequential depletion of bilirubin, urate, and α-tocopherol. Stimulating polymorphonuclear leukocytes in plasma initiates very rapid oxidation of ascorbate, followed by partial depletion of urate. Once ascorbate is consumed completely, micromolar concentrations of hydroperoxides of plasma phospholipids, triglycerides, and cholesterol esters appear simultaneously, even though sulfhydryl groups, bilirubin, urate, and α-tocopherol are still present at high concentrations. Nonesterified fatty acids, the only lipid class in plasma not transported in lipoproteins but bound to albumin, are preserved from peroxidative damage even after complete oxidation of ascorbate, most likely due to site-specific antioxidant protection by albumin-bound bilirubin and possibly by albumin itself. Thus, in plasma ascorbate and, in a site-specific manner, bilirubin appear to be much more effective in protecting lipids from peroxidative damage by aqueous oxidants than all the other endogenous antioxidants. Hydroperoxides of linoleic acid, phosphatidylcholine, and cholesterol added to plasma in the absence of added reducing substrates are degraded, in contrast to hydroperoxides of trilinolein and cholesterol linoleate. These findings indicate the presence of a selective peroxidase activity operative under physiological conditions. Our data suggest that in states of leukocyte activation and other types of acute or chronic oxidative stress such a simple regimen as controlled ascorbate supplementation could prove helpful in preventing formation of lipid hydroperoxides, some of which cannot be detoxified by endogenous plasma activities and thus might cause damage to critical targets.**

Free radical-mediated lipid peroxidation has been proposed to be critically involved in several disease states including cancer, rheumatoid arthritis, drug-associated toxicity, and postischemic reoxygenation injury, as well as in the degenerative processes associated with aging (1–3). Recently, evidence has accumulated suggesting that circulating lipid hydroperoxides play a pivotal role in atherogenesis (3–6) and thus coronary heart disease, the single most frequent cause of death in the United States and the western world. Lipid peroxidation initiates a series of events *in vitro* that eventually lead to enhanced uptake of low-density lipoproteins by macrophages and formation of lipid-laden foam cells, one of the earliest atherosclerotic lesions in the arterial intima. A detailed knowledge of the mechanisms of formation and breakdown of lipid hydroperoxides in human blood plasma, therefore, could prove helpful in the understanding and preventive treatment of atherosclerosis. Also, eliminating lipid hydroperoxides in plasma before they are taken up into peripheral tissues might have a great impact on the various other diseases associated with oxidative stress.

Human plasma is endowed with an array of antioxidant defense mechanisms. Important plasma antioxidants appear to be ascorbate (7), urate (8), α-tocopherol (9), albumin-bound bilirubin (10), and albumin itself (11). Protein sulfhydryl groups have also been suggested to contribute significantly to the antioxidant capacity of plasma (12), although their oxidation could also be considered oxidative damage, depending on the protein affected. Furthermore, transferrin (13) and ceruloplasmin (14) are considered preventive plasma antioxidants because they sequester transition metals, thereby preventing them from participating in free radical reactions. Finally, extracellular superoxide dismutase (15) and a selenium-dependent glutathione peroxidase (16–18) have been proposed to be involved in antioxidant defenses in human plasma.

In view of the central role of plasma in the transport and fate of lipids, and thus potentially lipid hydroperoxides, little is known about possible formation of lipid hydroperoxides in plasma and the relative contributions of each of the various endogenous antioxidants in preventing peroxidative damage to lipids. Furthermore, the fate of lipid hydroperoxides once they have been formed in plasma or taken up into it and the possible involvement of the various antioxidants therein are mainly unexplored. This prompted us to study the formation and degradation of lipid hydroperoxides in human plasma in relation to the consumption of endogenous antioxidants. The results give detailed insight into the effectiveness of the various antioxidants in preventing lipid peroxidation and lead to an alternative viewpoint of how plasma copes with oxidative stress.

MATERIALS AND METHODS

Materials. The chemicals used were the same as described (19). In addition, bilirubin, glutathione (reduced form) (GSH), 5,5′-dithiobis(2-nitrobenzoic acid), phorbol 12-myristate 13-acetate (PMA), cytochalasin B, and sodium borohydride were purchased from Sigma. 2,2′-Azobis(2-amidinopropane) hydrochloride (AAPH) was obtained from Polysciences (Warrington, PA), and sodium borate was from Fisher. Percoll and dextran T-500 were purchased from Pharmacia. Lipid hydroperoxide standards were prepared as described (19). The equipment used was the same as in ref. 19 except that a 10-μl flow cell was used in the fluorometer instead of a 5-μl flow cell.

Extraction of Plasma and Analysis of Lipid Hydroperoxides and Antioxidants. Plasma prepared from fresh heparinized blood (19) of a healthy 29-year-old male was incubated in the

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Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane) hydrochloride; GSH, reduced glutathione; PMA, phorbol 12-myristate 13-acetate; PMNs, polymorphonuclear leukocytes.

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presence of AAPH, activated polymorphonuclear leukocytes (PMNs), or lipid hydroperoxides as described below in a shaking waterbath at 37°C under air. At various time points, aliquots of between 200 μl and 500 μl were withdrawn and extracted with 4 vol of methanol and 20 vol of hexane. After centrifugation, aliquots of the two phases were analyzed for ascorbate, urate, α-tocopherol, and lipid hydroperoxides using HPLC with UV and chemiluminescence detection as reported (19–21). With the chemiluminescence assay, the detection limit for lipid hydroperoxides is ≈0.03 μM in plasma (21). For accurate ascorbate determination a fast extraction procedure was used by adding 50 μl of plasma to 200 μl of ice-cold methanol in an Eppendorf tube, spinning for 3 min at 13,600 × *g*, and immediately analyzing 50 μl of the supernatant. Bilirubin was determined in separate 50-μl plasma samples as described (10), and plasma sulfhydryl groups were measured in 100-μl samples with a method also described earlier (12).

Incubation of Plasma with AAPH. Seven milliliters of fresh plasma was kept for 5 min at 37°C, and then 500 μl of 750 mM AAPH (final concentration, 50 mM) was added. AAPH is a water-soluble azo compound that thermally decomposes and thereby produces peroxyl radicals at a known and constant rate (22, 23). Immediately after the addition of AAPH (time zero) and at the time points indicated, a 50-μl and a 500-μl aliquot were withdrawn and extracted with ice-cold methanol, and methanol followed by hexane, respectively. The methanol extract of the former sample and the hexane extract of the latter one were analyzed using HPLC with UV and chemiluminescence detection. Bilurubin was determined as described above. Plasma sulfhydryl groups (together with bilurubin as a relative standard) were measured in a separate experiment.

Incubation of Plasma with Stimulated PMNs. PMNs from 37 ml of fresh heparinized blood were isolated as described (24), washed twice with phosphate-buffered saline, pH 7.4 (PBS) and resuspended in 0.75 ml of PBS containing 5 mM glucose to give a final PMN concentration of ≈6.0×10^7 cells per ml. This step was followed by the addition of 3.45 ml of fresh plasma and 42 μg of cytochalasin B in 9 μl of dimethyl sulfoxide. After 5 min at 37°C and withdrawal of a first set of aliquots (see below) to determine the zero min values, the plasma/PMN mixture was divided into a 0.85-ml control sample, to which 1.2 μl of dimethyl sulfoxide was added and a 3.0-ml sample, to which 22 μg of PMA in 4.4 μl of dimethyl sulfoxide was added. Immediately after the addition of PMA and at the time points indicated, a 50-μl and a 200-μl aliquot were withdrawn, extracted with ice-cold methanol, and methanol followed by hexane, respectively, and analyzed as described above.

Stability of Lipid Hydroperoxides in Plasma. Lipid hydroperoxides in methanol or methanol/*tert*-butanol (19) were concentrated to ≈2 mM in a rotary evaporator under vacuum. The exact concentrations of the lipid hydroperoxides were determined by HPLC with chemiluminescence detection using linoleic acid hydroperoxide as a standard. Three milliliters of fresh plasma was incubated at 37°C for 3 min, and then ≈15 μl of the lipid hydroperoxide solution was added to give a concentration of 10.0 μM. Thirty seconds later and at the time points indicated, 250-μl aliquots were withdrawn, extracted with methanol and hexane, and the two phases were analyzed. The zero-min value was obtained by adding ≈12.5 μl of the lipid hydroperoxide solution diluted 1:10 to a mixture consisting of 0.25 ml of plasma and 1.0 ml of methanol. This step was followed by the addition of 5 ml of hexane and the normal analysis procedure.

RESULTS

To investigate the free radical-induced damage to plasma lipids and the relative effectiveness of the various plasma antioxidants in preventing this damage, we incubated fresh human blood plasma at 37°C in the presence of 50 mM of the water-soluble radical initiator AAPH. Under these conditions AAPH, through thermal decomposition produces peroxyl radicals at a constant rate of 3.0 μM/min (22, 23; see also ref. 10). As shown in Fig. 1, this initiated consumption of the plasma antioxidants in the temporal order: ascorbate, sulfhydryl groups > bilirubin > urate > α-tocopherol. During the first 50 min of the experiment the antioxidant defenses in plasma effectively protected all lipids from peroxidation. [Note that no detectable amounts of lipid hydroperoxides are present in fresh human plasma (21), although in two earlier reports (19, 20) two compounds producing chemiluminescence in the lipid peroxidation assay had been erroneously assigned to nonesterified fatty acid hydroperoxide and cholesterol ester hydroperoxide.] The initial phase of successful prevention of detectable lipid peroxidation coincided with the complete consumption of ascorbate (Fig. 1), suggesting that it was ascorbate that effectively protected plasma lipids from peroxidative damage. Once ascorbate was oxidized completely, micromolar concentrations of hydroperoxides of plasma phospholipids, triglycerides, and cholesterol esters appeared simultaneously, while the antioxidants bilurubin, urate, and α-tocopherol were consumed sequentially (Fig. 1). Nonesterified fatty acids appeared to be better protected against peroxidative damage than esterified fatty acids in the above lipid classes. Nonesterified fatty acids are present in plasma at a concentration of ≈0.5 mM, as compared with 12–14 mM esterified fatty acids (25). As can be seen in Fig. 1, 273 μM esterified fatty acid hydroperoxides were formed in plasma incubated for 5 hr with AAPH. Assuming a similar oxidation rate for nonesterified and esterified fatty acids, one would expect ≈10 μM nonesterified fatty acid hydroperoxides after 5 hr of incubation. However, in two of five experiments, no nonesterified fatty acid hydroperoxides could be detected (i.e., <0.03 μM in plasma), and in the three other experiments only very small amounts of nonesterified fatty acid hydroperoxides were formed (0.71 ± 0.54 μM) after 5 hr of incubation, their formation starting between 90 and 120 min of incubation with AAPH. No detectable amounts of cholesterol hydroperoxides were formed by incubation of plasma with AAPH.

One major source of oxidants in biological systems are activated PMNs. Upon specific membrane perturbation, PMNs exhibit a burst of oxygen consumption and start to generate a variety of oxidants such as superoxide radicals,

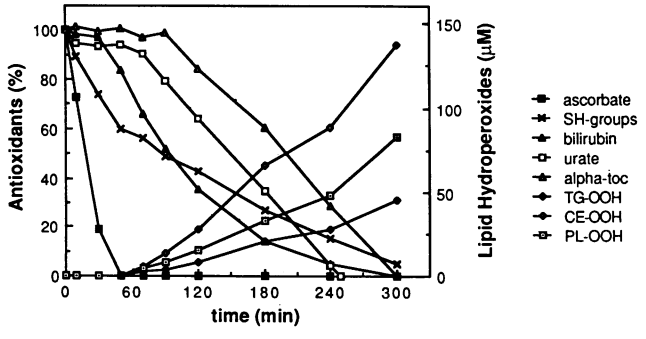

FIG. 1. Antioxidant defenses and lipid peroxidation in human plasma exposed to the water-soluble radical initiator AAPH. Plasma was incubated at 37°C in the presence of 50 mM AAPH. The levels of the antioxidants ascorbate (initial concentration, 72 μM), sulfhydryl groups (SH-groups, 425 μM), bilirubin (18 μM), urate (225 μM), and α-tocopherol (alpha-toc, 32 μM) are given as % of the initial concentrations. The levels of the lipid hydroperoxides triglyceride hydroperoxide (TG-OOH), cholesterol ester hydroperoxide (CE-OOH), and phospholipid hydroperoxide (PL-OOH) are given in μM concentrations (right ordinate). One experiment typical of four is shown.

hydrogen peroxide, and hypochlorous acid (26). Therefore, we added PMNs to plasma and stimulated them with PMA in the presence of cytochalasin B (27). As shown in Fig. 2, ascorbate was depleted very rapidly upon stimulation of PMNs with PMA. No lipid hydroperoxides could be detected during ascorbate depletion. However, once ascorbate was oxidized completely, detectable amounts of hydroperoxides of cholesterol esters, triglycerides, and phospholipids were formed simultaneously (Fig. 2). Their identity as hydroperoxides was confirmed by elimination of their chemiluminescence response in the lipid peroxidation assay after treatment of the samples with sodium borohydride (21) (data not shown). Neither nonesterified fatty acid hydroperoxides nor cholesterol hydroperoxides could be detected in plasma incubated with activated PMNs. During the oxidation of cholesterol esters, triglycerides, and phospholipids, urate was depleted partially, whereas the levels of bilirubin and α-tocopherol remained virtually unchanged (Fig. 2). The concentration of urate decreased to 68% of the initial value between 10 and 30 min after the addition of PMA and then remained constant. The cessation of urate oxidation 30 min after the addition of PMA most probably reflects the cessation of oxidant production by the PMNs. Interestingly, lipid peroxidation did not cease after this time but continued for at least another 90 min (Fig. 2). In the control experiment, where the PMNs were left unstimulated, no lipid hydroperoxides could be detected. Ascorbate was oxidized very slowly and continuously, the concentration dropping from 58 to 32 μM during the 2-hr incubation (data not shown); this is most probably due to autoxidation of ascorbate in plasma incubated at 37°C (ref. 28, see also Fig. 3*B*). The levels of bilirubin, urate, and α-tocopherol remained unchanged in the control experiment without PMA (the concentrations after 2 hr of incubation being 99%, 102%, and 102%, respectively, of the concentrations at the beginning of the experiment) (data not shown).

To determine the fate of lipid hydroperoxides in plasma once they have been formed or taken up into it, various lipid hydroperoxides were added to fresh plasma and incubated at 37°C. Significant differences in the stability of the various lipid hydroperoxides were seen (Fig. 3*A*). Whereas cholesterol linoleate hydroperoxide and trilinolein hydroperoxide were stable, hydroperoxides of phosphatidylcholine, linoleic

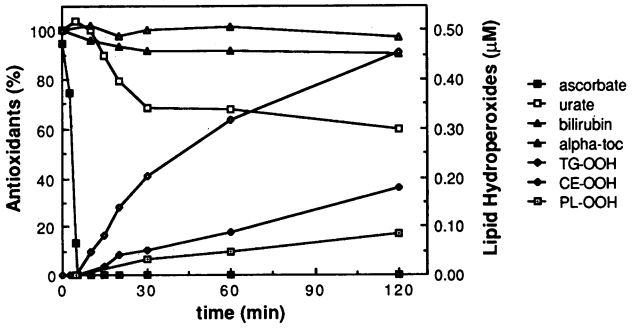

FIG. 2. Antioxidant defenses and lipid peroxidation in human plasma exposed to activated PMNs. Plasma was incubated at 37°C with freshly prepared PMNs at $\approx 1.3 \times 10^7$ cells per ml of plasma in the presence of cytochalasin B at 10 μg/ml. The PMNs were stimulated by the addition of PMA at 6.8 μg per $\approx 10^7$ cells at time zero. The levels of the antioxidants ascorbate (initial concentration, 58 μM), bilirubin (12 μM), urate (152 μM), and α-tocopherol (alpha-toc, 23 μM) are given as % of the initial concentrations. The ascorbate concentration before the addition of PMNs was 43 μM. The levels of the lipid hydroperoxides triglyceride hydroperoxide (TG-OOH), cholesterol ester hydroperoxide (CE-OOH), and phospholipid hydroperoxide (PL-OOH) are given in μM concentrations (right ordinate). Note the difference in the scales for lipid hydroperoxides between this figure and Fig. 1. One experiment typical of three is shown.

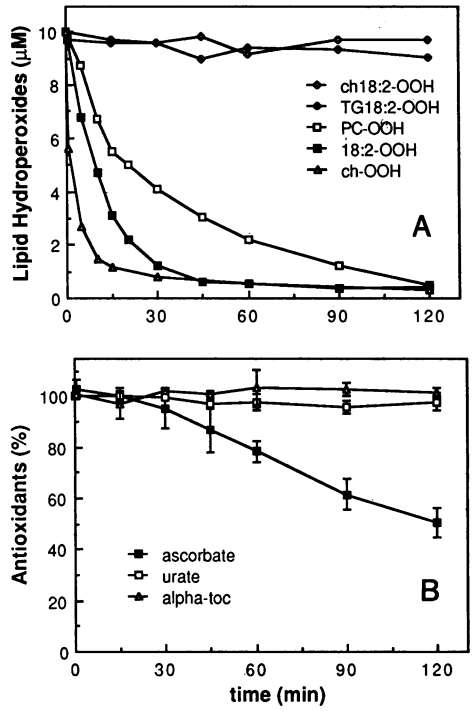

FIG. 3. Stability of various lipid hydroperoxides and antioxidants in human plasma. Ten μM of each lipid hydroperoxide was incubated separately in fresh plasma at 37°C. (*A*) The levels of the added lipid hydroperoxides cholesterol linoleate hydroperoxide (ch18:2-OOH), trilinolein hydroperoxide (TG18:2-OOH), phosphatidylcholine hydroperoxide (PC-OOH), linoleic acid hydroperoxide (18:2-OOH), and cholesterol-7-hydroperoxide (ch-OOH) are given in μM concentrations. (*B*) Mean values ± SD of the levels of the antioxidants ascorbate (initial mean concentration, 42 μM), urate (208 μM), and α-tocopherol (alpha-toc, 25 μM) for the five experiments shown in *A* are given as % of the initial concentrations.

acid, and cholesterol were degraded. Some of the added phosphatidylcholine hydroperoxide, before being degraded, reacted with endogenous cholesterol to produce cholesterol ester hydroperoxides (0.07 μM after 30 min of incubation), reflecting lecithin-cholesterol acyltransferase activity in plasma. The degradation of linoleic acid hydroperoxide occurred at an initial rate of 1.08 ± 0.22 μM/min (n = 4) as measured over the first 3 min of the experiment. The ability of plasma to degrade 10 μM linoleic acid hydroperoxide was drastically diminished by preincubation of plasma with 50 mM AAPH for 30 min at 37°C (rate of degradation, 0.06 μM/min as measured over the first 15 min of the experiment). In all incubations with the various lipid hydroperoxides, the concentrations of α-tocopherol and urate remained unchanged (Fig. 3*B*). The level of ascorbate decreased slowly with time (Fig. 3*B*), but no significant differences were observed between the different incubations. Linoleic acid hydroperoxide and phosphatidylcholine hydroperoxide when added at a concentration of 10 μM were very stable in PBS, incubated at 37°C (9.3 μM and 8.4 μM, respectively, left after 2-hr incubation), indicating that their degradation in plasma is not due to chemical instability. Cholesterol-7-hydroperoxide was less stable in PBS at 37°C (3.3 μM and 1.0 μM left from an initial 10 μM after 30-min and 2-hr incubation, respectively), and, thus, the very rapid decrease in the level of cholesterol-7-hydroperoxide added to plasma might be partly due to its chemical instability under the incubation conditions.

DISCUSSION

Human blood plasma is considered well equipped with both chain-breaking and preventive antioxidants to cope with

oxidative stress and prevent peroxidative damage to circulating lipids. Determining the total peroxyl radical-trapping antioxidant parameter of plasma by use of the water-soluble radical initiator AAPH, Ingold and colleagues (12) reported that plasma "resists" lipid peroxidation until all chain-breaking antioxidants have been completely consumed. In contrast, in this study we show that in plasma lipid peroxidation is prevented only as long as ascorbate is present and that sulfhydryl groups, urate, and α-tocopherol cannot prevent formation of *micromolar*—i.e., pathologically relevant (3)—concentrations of lipid hydroperoxides. Thus, the effectiveness of the various plasma antioxidants in protecting lipids against aqueous oxidants varies widely. In the experiment with AAPH described in Fig. 1, only 64% of the peroxyl radicals produced after completion of ascorbate oxidation are trapped by the remaining plasma antioxidants (see also below), and as much as 36% escape the antioxidant defenses, causing peroxidative damage to lipids. Ingold and associates, however, based their calculations of the total peroxyl radical-trapping antioxidant parameter on the premise that 100% of the peroxyl radicals generated by AAPH are trapped by the plasma antioxidants (12). The fundamental differences between our results and those of Ingold and colleagues most probably arise from the use of different incubation conditions and different techniques for measuring lipid peroxidation. Ingold and colleagues determined the rate of oxygen uptake, an indirect index of lipid peroxidation, using either highly diluted plasma in the presence of added linoleic acid or slightly diluted plasma in a pressure transducer chamber (12). We measure the lipid hydroperoxides themselves (21) in almost undiluted plasma.

Nonesterified fatty acid hydroperoxides are not formed in significant amounts in plasma under oxidative stress. This most probably is not due to rapid degradation of nonesterified fatty acid hydroperoxides after they have been formed, because the plasma peroxidase activity is virtually completely inactivated by preincubation of plasma with AAPH for >30 min—i.e., when nonesterified fatty acid hydroperoxides would be expected to accumulate. During the initial phase of oxidant challenge, peroxidation of nonesterified fatty acids and all the other lipid classes appears to be very effectively prevented by ascorbate. After that, peroxidative damage to lipids transported in lipoproteins becomes detectable, whereas nonesterified fatty acids, bound in plasma to albumin, are further protected. In the experiment with AAPH this site-specific antioxidant protection is likely to be provided by albumin-bound bilirubin, the consumption of which starts after ascorbate depletion (Fig. 1 and ref. 10), and possibly by albumin itself.

Such very effective, site-specific antioxidant protection against aqueous oxidants apparently is not provided for the esterified fatty acids of phospholipids, triglycerides, and cholesterol esters transported in lipoproteins. As mentioned above, under our experimental conditions formation of micromolar concentrations of hydroperoxides of these lipid classes is prevented neither by water-soluble sulfhydryl groups and urate nor by lipoprotein-associated α-tocopherol. These antioxidants merely reduce the rate of detectable lipid peroxidation by intercepting the chain reaction of the peroxidative process. This conclusion can be inferred from the experimental findings described in Fig. 1 that 273 μM lipid hydroperoxides (cholesterol ester hydroperoxides, triglyceride hydroperoxides, and phospholipid hydroperoxides) are formed between 50 and 300 min of incubation, as opposed to 750 μM peroxyl radicals generated during this period at a rate of 3.0 μM/min by decomposition of AAPH. Thus, the major portion of the peroxyl radicals generated are trapped by plasma sulfhydryl groups, urate, and α-tocopherol, causing consumption of these antioxidants; yet, concomitantly micromolar concentrations of lipid hydroperoxides are formed. It is particularly noteworthy that in the presence of activated PMNs the plasma α-tocopherol level remains unaffected despite complete consumption of ascorbate and subsequent lipid peroxidation (see Fig. 2). These observations exclude the possibility that the lack of measurable α-tocopherol oxidation is due to sparing of α-tocopherol by ascorbate. Our results with both AAPH and activated PMNs indicate that in plasma α-tocopherol is considerably less effective in preventing peroxidative damage to lipids induced by aqueous oxidants than in isolated low-density lipoproteins, where α-tocopherol has been reported to very effectively prevent lipid peroxidation (29). It is, therefore, conceivable that in plasma the main target of lipid peroxidation initiated by aqueous oxidants are not the lipids in the lipoproteins but those transported by transfer proteins between the lipoproteins. The lipids bound to transfer proteins can be expected to be more directly exposed to oxidants generated outside the lipoproteins and also to be less protected by α-tocopherol.

Taken together, our results strongly suggest that ascorbate plays a pivotal role in protecting plasma lipids from peroxidative damage initiated either by aqueous peroxyl radicals or by activated PMNs. Indeed, when the experiment described in Fig. 2 was performed with plasma from an individual whose plasma ascorbate concentration was very high (140 μM) due to dietary vitamin C supplementation, ascorbate depletion upon PMN stimulation was incomplete and no lipid hydroperoxides could be detected during the 2-hr incubation. Antioxidant protection of lipids by ascorbate *in vivo* should be even more extensive due to recycling of dehydroascorbate, the oxidation product of ascorbate. Dehydroascorbate appears to be taken up by erythrocytes, reduced to ascorbate by intracellular NADH and GSH, and then released again (30). Because ascorbate is the plasma antioxidant to become oxidized immediately upon leukocyte stimulation, an increased ratio of dehydroascorbate to ascorbate might be of diagnostic use as an early indicator of oxidative stress *in vivo* (31). Furthermore, a decrease in the ratio of urate to its oxidation product allantoin (32) would be indicative of sustained oxidative stress and would suggest that lipid hydroperoxides have been formed (see Fig. 2). Our findings also imply that ascorbate could prove helpful in the prevention and treatment of diseases and degenerative processes that may be associated with oxidative stress, such as cancer, atherosclerosis, rheumatoid arthritis, and aging, as well as in many clinical conditions characterized by generalized PMN activation, such as shock, sepsis, and trauma. Thus, our data provide evidence for an important role of ascorbate in human health and longevity beyond its well established functions in metabolism.

Exposure of plasma to either AAPH or stimulated PMNs results in different patterns of oxidation of the various antioxidants (compare Figs. 1 and 2). Besides the difference in α-tocopherol oxidation mentioned above, activation of PMNs does not lead to considerable oxidation of bilirubin, whereas bilirubin can trap the peroxyl radicals produced by AAPH (see also ref. 10). Quantitative differences were also observed: plasma ascorbate becomes much more rapidly oxidized when challenged by the oxidants released from activated PMNs than by AAPH, despite a drastically lower rate of subsequent lipid peroxidation. These differences suggest that the effects of activated PMNs on plasma antioxidants and lipids are not caused ultimately by peroxyl radicals. A more likely candidate is the myeloperoxidase-derived oxidant hypochlorous acid (26), which is known to be scavenged by ascorbate (33) and urate (34), but not by albumin-bound bilirubin (35). Hypochlorous acid may also be able to initiate lipid peroxidation (36).

Our observations that hydroperoxides of free fatty acids, phospholipids, and cholesterol, but not cholesterol esters and triglycerides, are degraded in plasma suggest the presence of

a selective peroxidase activity. It is conceivable that the degradation of phospholipid hydroperoxides is due to the consecutive action of the phospholipase A_2 activity associated with low-density lipoproteins (4) and the peroxidase activity that subsequently reduces the liberated fatty acid hydroperoxides. A plasma glutathione peroxidase different from the erythrocyte glutathione peroxidase has been purified and characterized recently (17, 18). The plasma glutathione peroxidase was found to have an apparent K_m value for GSH of between 4.3 mM (18) and 5.3 mM (17), which is more than 10^4 times the steady-state level of GSH in human plasma (37). Accordingly, no peroxidase activity was observed in plasma unless it was supplemented *in vitro* with exogenous GSH (16). In contrast, we find that selected lipid hydroperoxides are degraded in plasma in the absence of added reducing substrates. However, when we measured the degradation of 10 μM linoleic acid hydroperoxide using our chemiluminescence assay but the experimental conditions described in ref. 16—i.e., 20% plasma incubated for 3 min at 37°C, we, too, failed to observe statistically significant degradation of the peroxidic substrate. Compatible with our observation, Terao *et al.* (38) recently reported that arachidonic acid hydroperoxide is reduced to the corresponding alcohol in plasma in the absence of added reducing substrates. Because the GSH level of isolated plasma is only $\approx$0.34 μM (37), and we observe virtually complete degradation of 10 μM selected lipid hydroperoxides, it is very unlikely that this hydroperoxide degrading activity is the plasma glutathione peroxidase, unless its K_m value for GSH in plasma is much lower than reported for the isolated enzyme *and* oxidized glutathione is recycled by (an)other reducing substrate(s) present in plasma. Such possibilities require further investigation.

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