Peroxy nitrite-induced oxidation of plasma lipids is enhanced in stable hemodialysis patients

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Peroxy nitrite-induced oxidation of plasma lipids is enhanced in stable hemodialysis patients.

Background. The relationship between end-stage renal disease (ESRD), hemodialysis, and oxidative stress is controversial. To determine whether ESRD causes oxidative stress, we measured basal levels of plasma F2-isoprostanes as a marker of lipid peroxidation in vivo, and peroxynitrite-stimulated formation of F2-isoprostanes, as a marker of the oxidizibility of plasma lipids in vitro, before and after routine hemodialysis.

Methods. Total plasma F2-isoprostanes were measured by gas chromatography-mass spectrometry (GC-MS) before and after the oxidation of plasma lipids with the peroxynitrite-generating compound, 3-morpholino-sydnonimine (SIN-1), in 23 patients with ESRD patients undergoing regular hemodialysis, and 14 controls. Plasma vitamin E concentrations were measured by high-performance liquid chromatography (HPLC).

Results. There was no difference in basal plasma concentrations of F2-isoprostanes in the ESRD group prior to hemodialysis, 246 ± 20 pg/mL, compared to controls, 252 ± 28 pg/mL, or immediately on completion of hemodialysis, 236 ± 14 pg/mL. Incubation of control plasma with SIN-1 caused the formation of F2-isoprostanes with plasma concentrations increasing to 987 ± 54 pg/mL at 6 hours. The formation of F2-isoprostanes stimulated by SIN-1 was markedly enhanced in the plasma obtained from patients undergoing hemodialysis at 1861 ± 174 pg/mL, P < 0.001, and SIN-1–induced formation of F2-isoprostanes was further increased in plasma obtained immediately after hemodialysis at 2437 ± 168 pg/mL, P < 0.001. Incubation of plasma with SIN-1 resulted in the net consumption of vitamin E.

Conclusion. Although basal plasma F2-isoprostanes were similar in patients with ESRD compared with controls, the presence of oxidative stress in patients with ESRD was unmasked when the plasma was stressed by peroxynitrite generated from SIN-1, and this was enhanced further by hemodialysis.

Key words: F2-isoprostanes, chronic renal failure, SIN-1, lipid peroxidation, dialysis.

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Over the past decade, several studies have shown that reactive oxygen species (ROS) play a key role in the pathology in a wide variety of clinical and experimental diseases [1–4]. It is generally agreed that measurement of plasma or urinary F2-isoprostanes is the “gold standard” for the assessment of oxidative stress in vivo [5–7]. F2-isoprostanes are isomeric forms of prostaglandin F2α formed by nonenzymatic oxidation of arachidonyl lipids. Parameters such as plasma malondialdehyde or 4-hydroxynonenal are less reliable markers of oxidative injury in vivo [8]. The major fraction of plasma F2-isoprostanes are esterified to plasma lipids and are presumably cleaved in vivo by a phospholipase to yield free F2-isoprostanes. Thus, plasma levels of free F2-isoprostanes represent the balance between the formation of esterified F2-isoprostanes, their subsequent hydrolysis from tissue or plasma lipids, and metabolism, which may be influenced by co-morbid disease [9–11]. Cardiovascular disease is the most important cause of morbidity and mortality in patients with end-stage renal disease (ESRD) [12], and the susceptibility of plasma lipids to undergo oxidative modification may be an important contributory factor. For example, there is widespread consensus that oxidative modifications of low-density lipoprotein (LDL) within the arterial wall represent a key early event in the development of the atherosclerotic plaque. This hypothesis is supported by the observation that there is increased susceptibility of LDL to metal ion–dependent oxidation in vitro in several groups of patients known to be at increased risk of developing atherosclerosis [13]. This method requires the isolation of the LDL fraction by differential centrifugation in each subject, followed by measuring the formation of conjugated dienes after the addition of copper to LDL suspensions. Typically, LDL oxidation is characterized by a lag phase, followed by
propagation of the oxidation reaction [14]. The lag phase, which varies in duration with the antioxidant capacity of LDL, is used as an index of oxidizibility of LDL. The lag phase is shortened in patients with chronic renal failure who require regular hemodialysis (ESRD) suggesting increased oxidizibility of LDL [15]. This method is, however, time consuming and does not take into account the effect of antioxidants in whole plasma. Furthermore, the initiation of oxidation of LDL by metal ions is unlikely to be an important pathway in vivo. Furthermore, the assessment of oxidative stress using other indices has yielded conflicting data [8, 16–18]. In 1995, we reported that the peroxynitrite donor, 3-morpholinosydnonimine (SIN-1), could cause the oxidation of lipids in whole plasma to form esterified \( F_2 \)-isoprostanes. This was in contrast to the inability of copper or myoglobin to induce oxidation of lipids in whole plasma. It seemed to us that since SIN-1 could cause oxidation of plasma lipids that one could assess the oxidizibility of plasma lipids by measuring the capacity of plasma components to limit SIN-1–induced formation of \( F_2 \)-isoprostanes in whole plasma. This technique has the advantage that normal plasma components, which are removed during the isolation of LDL, are still present. This technique might also allow the efficacy of therapeutic interventions such as antioxidant supplementation protocols or the use of vitamin-E–coated dialysis membranes to be assessed [19].

**METHODS**

**Chemicals**

All chemicals were purchased from Sigma (Poole, Dorset, UK) unless otherwise stated.

**Subjects**

Twenty-three stable ESRD patients (age, 62 ± 18 years old; male = 11, female = 12) on regular three times a week hemodialysis for more than 6 months were recruited from the Nephrology and Dialysis Unit of the R. Silvestrini Hospital in Perugia, Italy. Patients were well dialyzed (Kt/V >1.3) using fistula access, with standard bicarbonate dialysate (AMM quality) and unfraccionated porcine heparin, using either biocompatible or bioincompatible dialysis membranes. The synthetic membrane comprised polysulphone (Polysulfone® Fresenius A.G., Bad Homburg, Germany) \( N = 5 \), polyamide (Polyflux Gambro AB, Stockholm, Sweden) \( N = 2 \), polymethylmethacrylate (Excebrane® Serie Terumo Co., Japan) \( N = 1 \), and Serie B1 (Toray Co., Japan) \( N = 1 \), or polycrylonitrile (AN69, Hospal) \( N = 1 \) membranes. The cellulose and/or modified cellulose membranes comprised cuprammonium rayon dialyzers (Cilrans® Terumo Co., Japan) \( N = 8 \), and cellulose acetate \( N = 4 \), or cuprammonium rayon-polyethylenglycol membranes \( N = 1 \). Primary renal diseases were chronic glomerulonephritis \( N = 6 \), glomerulosclerosis \( N = 4 \), chronic interstitial nephropathy \( N = 1 \), membranoproliferative glomerulonephritis \( N = 2 \), diabetic nephropathy \( N = 1 \), gouty nephropathy \( N = 1 \), chronic pyelonephritis \( N = 3 \), polycystic kidney disease \( N = 2 \), or no definitive diagnosis possible \( N = 3 \). Of these 23 patients, two were diabetic, eight patients had evidence of cardiovascular, cerebrovascular, or peripheral vascular disease. Exclusion criteria were chronic infections, including hepatitis C virus, any preceding acute inflammatory disease, and malignancy. No patients were prescribed antioxidants such as vitamins C or E, or fibrates. Fourteen healthy controls age 50 ± 15 years old with no clinical history or signs of renal disease were recruited from the same local population. No control subjects were taking any antioxidant supplements. All the healthy volunteers and patients gave informed consent according to the guidelines of the Ethical Committee of the R. Silvestrini Hospital, Perugia, Italy.

**Sample collection**

Blood samples were collected into ethylenediamine-tetraacetic acid (EDTA) tubes. Plasma was separated by centrifugation at 1300g for 20 minutes at 4°C. Aliquots of the plasma were transferred to Eppendorf tubes and stored at −80°C until analysis.

**Oxidation of plasma by SIN-1**

On the day of study, 1 to 2 mL of plasma was thawed. SIN-1 (Alexis Ltd., UK) in phosphate-buffered saline (PBS) (pH 7.4) was added at 10% of the plasma volume to give a final concentration of 1 mmol/L SIN-1. Plasma and SIN-1 were incubated at 37°C for 0 to 6 hours. Aliquots of plasma were removed (800 μL at t = 0, and 200 μL at subsequent time points) during SIN-1 incubation. Further oxidation of plasma lipids was quenched in the removed aliquots by the addition of butylated hydroxytoluene (BHT) 1 mmol/L final concentration and Trolox dissolved in methanol 100 μmol/L final concentration.

**Extraction and measurement of \( F_2 \)-isoprostanes**

Total \( F_2 \)-isoprostanes (esterified plus free \( F_2 \)-isoprostanes) were quantified by gas chromatography-mass spectrometry (GC-MS) after base hydrolysis of lipids in whole plasma, purification, and derivatization. In brief, 1 ng of \([\text{H}]_8\)-iso-prostaglandin \( F_{3a} \) (PGF\(_{3a}\)) (Cayman Co., Ann Arbor, MI, USA) were added as internal standards to 200 to 800 μL of plasma containing BHT. A half volume of 25% potassium hydroxide was added to plasma to give a final concentration of 8.3% potassium hydroxide and incubated at 37°C for 45 minutes. To extract \( F_2 \)-isoprostanes, the pH was adjusted to 3, and the samples were extracted on a TC18 solid-phase extraction cartridge (Waters, UK), converted to the pentafluoro-
Table 1. Clinical biochemistry and hematology (mean \pm SEM)

<table>
<thead>
<tr>
<th></th>
<th>End-stage renal failure (N = 21)</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol mg/dL</td>
<td>200 \pm 11</td>
<td>120–210</td>
</tr>
<tr>
<td>Triglycerides mg/dL</td>
<td>197 \pm 25</td>
<td>50–170</td>
</tr>
<tr>
<td>Creatinine mg/dL</td>
<td>9 \pm 0.43</td>
<td>0.5–1.2</td>
</tr>
<tr>
<td>Urea mg/dL</td>
<td>165 \pm 6.5</td>
<td>11–50</td>
</tr>
<tr>
<td>Hemoglobin g/dL</td>
<td>11.0 \pm 0.3</td>
<td>14–16.1 (M)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.5–16.5 (F)</td>
</tr>
<tr>
<td>Hematocrit %</td>
<td>33 \pm 0.8</td>
<td>36–54</td>
</tr>
</tbody>
</table>

Table 1. Clinical biochemistry and hematology (mean \pm SEM)

benzyl ester, purified by thin-layer chromatography and analyzed as the trimethylsilyl ether as previously described [20]. Detection was performed by selected ion monitoring gas chromatography negative ion chemical ionization/mass spectrometry. Samples were analyzed as the pentafluorobenzyl ester trimethylsilyl ether derivatives monitoring the M-181 ions, m/z 569 for endogenous \(^2\)-isoprostanes, and m/z 573 for [\(^2\)H\(_4\)-8-iso-PGF\(_2\alpha\)]. The \(^2\)-isoprostanes elute as a series of chromatographic peaks over ~20 seconds, and quantification is based on the primary peak that coelutes with the deuterated 8-iso-PGF\(_2\alpha\) internal standard. All results were calculated by reference to the deuterated 8-iso-PGF\(_2\alpha\) internal standard. The inter assay coefficient of variation of this procedure is ~7%.

Measurement of plasma uric acid and \(\alpha\)- and \(\gamma\)-tocopherol

Uric acid levels (\(\mu\)mol/L) were determined by reverse-phase high-performance liquid chromatography (HPLC) coupled with electrochemical detection [21]. Plasma vitamin E (\(\alpha\)- and \(\gamma\)-tocopherol) concentrations were quantified by HPLC assay [22]. Absolute vitamin E levels were corrected for plasma triglyceride concentrations (\(\mu\)mol/ mmol triglyceride).

Statistical analyses

All results are expressed as mean \pm SEM. Data was analyzed using the Wilcoxon signed-rank test for paired nonparametric data. Multiple comparisons were compared by analysis of variance (ANOVA). Differences were considered statistically significant if the \(P\) value was <0.05.

RESULTS

Oxidizibility of plasma lipids in controls and patients with ESRD

The baseline biochemistry and hematology data in the patients with ESRD are shown in Table 1. The concentration of total \(^2\)-isoprostanes in plasma taken from the 23 patients with ESRD immediately before a hemodialysis session (246 \pm 20 pg/mL) was not significantly different from normal controls (252 \pm 28 pg/mL, \(P = NS\)).

The kinetics of oxidation of plasma lipids obtained before hemodialysis by SIN-1 was studied in 17 out of the 23 patients with ESRD and 13 healthy control subjects. Addition of SIN-1 to plasma caused a time-dependent increase in total \(^2\)-isoprostanes over 6 hours (Fig. 1). Pilot studies, in which the formation of total \(^2\)-isoprostanes was followed up for up to 24 hours, did not show any further increment beyond that observed at 6 hours (data not shown). Despite the absence of any differences in the basal concentration of total \(^2\)-isoprostanes between controls and patients with ESRD, the SIN-1–induced formation of \(^2\)-isoprostanes was approximately doubled in ESRD patients (1861 \pm 174 pg/mL vs. 987 \pm 54 pg/mL; \(P < 0.0001\)) (Fig. 1). This demonstrates that plasma lipids from patients with ESRD are more susceptible to oxidation by SIN-1. Having established the time course for these reactions in vitro, subsequent studies simply compared the concentration of total \(^2\)-isoprostanes (at \(t = 0\) hour) to those generated by SIN-1 at 6 hours.

Effect of hemodialysis on oxidizibility of plasma lipids

To assess the effect of dialysis on the oxidizibility of plasma lipids, blood was collected before and within 5 minutes of the end of a hemodialysis session, and the SIN-1–induced formation of \(^2\)-isoprostanes from plasma lipids determined at 6 hours in 23 patients with ESRD. Following dialysis, the basal concentration of total \(^2\)-isoprostanes was 236 \pm 14 pg/mL, and this was not significantly different from predialysis concentrations (246 \pm 20 pg/mL). However, the hemodialysis procedure markedly enhanced the SIN-1–induced formation of \(^2\)-isoprostanes, with peak levels increasing from 1841 \pm 166...
pg/mL to 2437 ± 168 pg/mL; \( P < 0.001 \) (Fig. 2) in the 23 subjects studied.

**Effect of dialysis membrane on the oxidizibility of plasma lipids**

The oxidizibility of plasma lipids was compared in the two groups of patients undergoing dialysis with either synthetic (\( N = 10 \)) or cellulose membranes (\( N = 13 \)). As shown in Figure 3, there was no significant difference between the two types of membranes used (\( P = NS \)).

**Effect of SIN-1 on plasma urate and tocopherol concentrations**

The effect of SIN-1 oxidation on plasma concentrations of uric acid, as well as \( \alpha- \) and \( \gamma- \)tocopherol was determined in a subgroup of four patients before and after hemodialysis and in four healthy controls. Basal uric acid levels were higher in patients with ESRD (361 ± 17 \( \mu \)mol/L) compared with healthy controls (210 ± 29 \( \mu \)mol/L; \( P < 0.01 \)), consistent with a decreased renal elimination of uric acid in the ESRD group. Plasma uric acid decreased following hemodialysis (361 ± 17 \( \mu \)mol/L to 123 ± 21 \( \mu \)mol/L, \( P < 0.01 \)). Addition of SIN-1 to plasma resulted in the net consumption of uric acid, although there were no differences between the amount of uric acid consumed when ESRD and controls or before and after dialysis specimens were compared (Fig. 4A).

The interpretation of vitamin E (as \( \alpha- \)tocopherol) concentration in plasma is complicated by differences in plasma lipid levels and by the acute hemococoncentration that occurs with plasma solutes that are not filtered during hemodialysis. Plasma vitamin E levels were signifi-
cantly decreased in ESRD patients after correction for triglyceride concentration compared to normal controls, but there was no significant change in vitamin E levels before and after dialysis. The vitamin E concentrations were ESRD, before hemodialysis, 12 ± 2 μmol/mmol triglyceride; after hemodialysis, 15 ± 2 μmol/mmol tryglyceride; and controls, 34 ± 6 μmol/mmol triglyceride.

Following the addition of SIN-1 to plasma there was a time-dependent consumption of vitamin E. This was minimal in the plasma of normal controls over the 6-hour incubation with SIN-1 (Fig. 4B). However, a marked consumption of vitamin E in plasma was observed after 2 hours of incubation with SIN-1. The consumption of vitamin E was enhanced further in the plasma of patients immediately following hemodialysis and was consistent with increased oxidation of plasma lipids as evidenced in Figure 2.

The basal plasma concentration of γ-tocopherol was 1.4 ± 0.3 μmol/L in normal controls and 1.7 ± 0.3 μmol/L and 2.3 ± 0.5 μmol/L in patients before and after hemodialysis, respectively. There were no significant changes in plasma concentrations of γ-tocopherol during the 6-hour incubation with SIN-1, which remained at over 90% of its basal levels in both patients and controls (data not shown).

DISCUSSION

The aim of this study was to measure basal oxidative stress in patients with ESRD undergoing hemodialysis and to assess whether hemodialysis causes further oxidative injury. At present it is not clear whether the measurement of free or esterified F2-isoprostanes represents the best marker for systemic oxidative stress in vivo. Most studies have used measurement of free F2-isoprostanes [23, 24]. However, decreased elimination of free F2-isoprostanes due to chronic renal failure, or enhanced removal or generation during hemodialysis, makes the assessment of lipid peroxidation in ESRD more difficult.

In a pilot study, we measured the plasma concentrations of free F2-isoprostanes in 26 patients with chronic renal failure patients who did not require hemodialysis. There was a modest but significant increase in plasma free F2-isoprostanes in patients with chronic renal failure compared to controls (chronic renal failure 35 ± 5 pg/mL vs. control 20 ± 2 pg/mL, P < 0.01). The advantage of measuring esterified F2-isoprostanes is that the problem of renal elimination is excluded. However, dynamic changes, such as those that may be generated during hemodialysis, may occur too slowly to detect immediately at the end of the dialysis session, since lipid peroxidation commences with initiation of oxidation followed by propagation, and this process takes a few hours in vitro. Our initial observation that only modest differences of plasma-free F2-isoprostanes were observed between patients with chronic renal failure and controls led us to develop a new method to assess the oxidizibility of plasma lipids with SIN-1 in ESRD patients. This method measures oxidative stress under basal conditions in vivo as determined by basal plasma total F2-isoprostanes, and then quantitates the generation of F2-isoprostanes in vitro after stressing plasma lipids by the addition of the peroxynitrite generator SIN-1.

There was no difference between basal plasma F2-isoprostanes in patients with ESRD or controls, and plasma levels did not increase after hemodialysis. This was a surprising observation since it was recently reported in a definitive study by Handelman et al [25] that basal esterified F2-isoprostanes were increased by ~sixfold in patients undergoing regular intermittent hemodialysis, but that a single episode of hemodialysis did not significantly enhance the formation of esterified F2-isoprostanes. We did, however, observe a significant increase in the oxidizibility of plasma lipids by peroxynitrite in patients with ESRD, and this effect was enhanced by hemodialysis. Thus, our data suggest that there is increased consumption of plasma antioxidants, and are consistent with the presence of oxidative stress in patients with ESRD, which is enhanced by hemodialysis. One alternative possibility is that an altered lipid profile in the patients with chronic renal failure could increase the levels of the substrate for SIN-1–induced oxidation, namely arachidonic acid. However, several studies have consistently shown that patients with chronic renal failure, including those on hemodialysis, exhibit a significantly lower plasma concentration of arachidonic acid [26–28] suggesting that this is not the mechanism for increased F2-isoprostane generation by SIN-1. Moreover, the observation that the oxidation of plasma lipids was enhanced further following hemodialysis is consistent with consumption or depletion of endogenous antioxidants. The explanation for the discrepancy between our data, and that published by Handelman et al [25] may lie in fundamental differences of clinical practice between the United States and Europe, in terms of vascular access and the reuse of dialyzers. Many patients in the study by Handelman et al [25] were dialyzed through central venous access catheters and had an elevated C-reactive protein (CRP), suggesting chronic inflammation. It is not stated whether the dialysis membranes were reused, but this is standard practice in many dialysis units in the United States. In the current study, all patients were dialyzed through native arteriovenous fistulas, and with single use dialysis membranes, and European Pharmacopoeia Standard dialysate water. There are some data to suggest that reusing dialysis membranes may be associated with an increased risk of cardiac mortality, and other studies have shown a strong correlation between increased CRP levels and cardiac deaths [29]. Indeed, Handelman et al [25] showed a positive correla-
tion between CRP levels and basal F\textsubscript{2}-isoprostane concentrations. This suggests that chronic inflammation leads to oxidative stress in ESRD. In the present study, we did not measure the CRP levels at the time of blood collection. However, on reviewing the routine monthly CRP data available from 13 of the patients in this study, we observed a median concentration of CRP of 6.5 mg/L (normal range, <10 mg/L). Both interleukin-6 (IL-6) and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) were also measured (ELISA) in these patients at around the time when these patients were studied. The median concentrations of IL-6 and TNF-\(\alpha\) were 3.3 pg/mL (normal range, < 3.1 pg/mL) and 15.9 pg/mL (normal range, <15.6 pg/mL), respectively. Therefore, the absence of chronic inflammation in our patient population may be the major reason why there was no overt basal oxidative stress in our patient group compared to our controls.

The key observation in our present study is that even though basal concentrations of total F\textsubscript{2}-isoprostanes in patients with ESRD were not significantly different from healthy controls, subtle differences in endogenous defenses against oxidative stress could be revealed by stressing the plasma lipids with peroxynitrite, using the peroxynitrite generator SIN-1 [30]. One of the key advantages of this method is that it evaluates the oxidizibility of plasma lipids without the need to separate and isolate LDL, for in vitro studies. In a recent study, Thomas, Davies, and Stocker [31] exposed human plasma from normal subjects to 5 mmol/L SIN-1 and also observed a minimal consumption of \(\alpha\)-tocopherol and urate over the first 6 hours of incubation. In the present study, there was an increased consumption of vitamin E, while uric acid consumption was minimal. Thomas, Davies, and Stocker also observed a rapid consumption of ascorbate, bilirubin, and ubiquinol-10. Although plasma ascorbate concentrations were not measured in the current study, previous investigations have shown that plasma ascorbate concentrations in patients with ESRD before hemodialysis are decreased by about 50% compared to control values, and this decreases by a further 50% after hemodialysis [12]. Other studies have suggested that plasma concentrations of other antioxidants (such as thiols, selenium, glutathione peroxidase, and coenzyme Q\textsubscript{10}) are also decreased in patients with chronic renal failure because of increased consumption during oxidative stress. Decreased plasma concentrations of vitamin E have been observed in other studies [32] and this is confirmed in the present study.

This study demonstrates unequivocally that the plasma of patients undergoing hemodialysis is more susceptible to oxidation by SIN-1 and this is further enhanced immediately after dialysis. This may be related to removal of low-molecular-weight water-soluble antioxidants by hemodialysis [33], or consumption of antioxidants by free radicals generated during extracorporeal circulation in bioincompatible materials. Free radicals may be generated by leukocyte activation, due to membrane and/or dialysate interactions, contaminated dialysate, mechanical stress induced by peristaltic pumps, chemical stimuli induced by the uremic environment, and abrupt changes in pH and solute concentrations associated with hemodialysis [34]. Moreover, during hemodialysis, the release of proinflammatory cytokines and chemotactic factors may activate leukocytes and the endothelium. Thus, chronic hemodialysis patients may be at increased risk of free radical–mediated injury. This may be important in the development and subsequent progression of cardiovascular complications. In particular, LDL lipids might be at risk of oxidation when exposed to a high flux of reactive oxygen or nitrogen species as observed in the subendothelium during the genesis of the atherosclerotic plaque. Several groups have been interested in the relative oxidative stress imposed by different membranes used in chronic hemodialysis [35]. Although it was not a primary aim of this study to compare the effect of different types of dialyzer membranes on SIN-1–induced oxidation of plasma lipids, we were not able to demonstrate any statistically significant difference between synthetic and cellulosic-based membranes. However, F\textsubscript{2}-isoprostane generation was slightly greater, in particular following dialysis, in those patients who used the cellulosic membranes.

This study has highlighted a new approach to assess the oxidizibility of plasma lipids. In our method we used the water-soluble SIN-1 because it produces species (namely, \(\text{O}_2^-\) and \(\text{NO}^-\) further reacting to form \(\text{ONOO}^-\)) that are likely to be important factors in LDL oxidation in the subendothelium [36]. Moreover, we chose to assess lipid oxidizibility in whole plasma instead of its subfractions since it characterizes the oxidizibility of all plasma lipoproteins in their biologic milieu rather than just one lipoprotein class in a buffer system. The study of the oxidizibility of plasma represents a valid approach to ex vivo evaluation of the risk of lipid peroxidation in health and disease. This method has wide applicability, is readily reproducible, and will help to assess therapeutic strategies aimed to prevent lipid peroxidation during hemodialysis and the development of cardiovascular disease.

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