

# Relevance of Oxidative Pathways in the Pathophysiology of Chronic Kidney Disease

Jonathan Himmelfarb, MD

*Division of Nephrology and Transplantation, Maine Medical Center,  
22 Bramhall Street, Portland, ME 04102, USA*

The expected life span for patients with advanced chronic kidney disease (CKD) is substantially shorter than that expected for age-, gender-, and race-matched members of the general population without kidney disease. For patients with stage 5 CKD or end-stage renal disease (ESRD), the single largest cause of morbidity and mortality is cardiovascular disease, which accounts for 50% of premature deaths [1,2]. Furthermore, although the prevalence of traditional cardiovascular risk factors exemplified by the Framingham Study are high in this population, the extent and severity of ensuing cardiovascular events remains disproportionate to the underlying risk factor profile [3]. Consequently, there have been considerable recent efforts to focus on so-called “nontraditional” risk factors for cardiovascular events in this patient population. Among examined nontraditional risk factors, there is increasing interest in oxidative stress, which is postulated to contribute to the excessive uremic cardiovascular risk. Other important and emerging considerations for evaluating cardiovascular risk in uremia include inflammation, malnutrition, and endothelial dysfunction, all of which phenomena are at least partially related to the dysmetabolism that produces increased oxidative stress [4]. This article outlines the scope of the evidence linking an increased burden of uremic oxidative stress to kidney disease and describes the emerging relationship of oxidative stress with acute-phase inflammation, endothelial dysfunction, and malnutrition. It reviews data suggesting that oxidative stress biomarkers are powerful predictors of

mortality risk in kidney disease. Finally, it reviews the growing evidence concerning the potential efficacy of antioxidants in patients with kidney disease.

## What is oxidative stress?

Oxidative stress is commonly viewed as a disturbance in the balance between oxidant production and antioxidant defense. A pro-oxidant imbalance can lead to the oxidation of macromolecules resulting in tissue injury. Oxygen is ubiquitous in the environment, and the pro-oxidant status of an organism is at least partly dependent on the state of oxygenation of the organism or cell. In eukaryotes, oxidative processes occur predominantly within the mitochondria, and the mitochondrial cytochrome oxidase enzyme complex accounts for most of the oxygen humans metabolize. The cytochrome oxidase enzyme complex transfers four electrons to oxygen in a coordinated reaction that produces two molecules of water as a byproduct. The enzyme complex contains four redox centers, each of which stores a single electron. The simultaneous reduction of the four redox centers results in no detectable reactive oxygen intermediates and thereby limits the production of reactive oxygen species. Nevertheless, mitochondrial oxygen can sometimes leak through the electron transport chain, resulting in the formation of reactive oxygen intermediates and free radicals, which can then diffuse out of the mitochondria and be a source of oxidant stress [5,6]. Recently, attention has been focused on uncoupling of the electron transport chain to ATP production in the mitochondria in disease states; this uncoupling may

---

*E-mail address:* [himmej@mmc.org](mailto:himmej@mmc.org)

alter redox balance, cell signaling, and induce apoptosis.

An additional important *in vivo* source of excess oxidants occurs through the action of another enzyme complex, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. NADPH oxidase is especially active within the endothelium and in leukocytes for the generation of reactive oxygen intermediates [7,8]. In particular, leukocytes use high levels of oxygen and the generation of reactive oxygen intermediates in host defense against pathogens in a process known as the respiratory burst. Leukocytes contain other enzymes, including superoxide dismutase, nitric oxide synthase, and myeloperoxidase (MPO), that contribute to the production of hydrogen peroxide, nitric oxide, peroxynitrite, and hypochlorous acid, respectively [9,10]. Recently a novel additional oxidative pathway within leukocytes has been described, whereby nitrite is converted to nitryl chloride and nitrogen dioxide through the MPO enzyme or through hypochlorous acid [11]. Ozone derived from singlet oxygen in leukocytes may also be a by-product of oxidative stress that contributes to atherosclerosis [12].

*In vivo*, critical oxygen intermediates are produced in minute quantities and have short biologic half-lives. The low concentrations and extreme reactivity make the *in vivo* detection of reactive oxygen species technically difficult. In response to these technical difficulties, a powerful strategy has emerged for understanding the underlying *in vivo* mechanisms of oxidative injury by detecting stable end products of oxidative chemistry produced by different reaction pathways as biomarkers. These biomarkers measure the oxidation of important macromolecules, including lipids, carbohydrates, proteins, amino acids, and DNA.

Although the concepts and pathophysiologic processes leading to an increase in oxidative stress may seem clear cut, unfortunately the use of the terms oxidative stress and oxidant stress are relatively nonspecific. Whereas oxidants are continuously being produced in living organisms, a multitude of antioxidant defense mechanisms are also constantly at work, and most biologic systems are not in redox equilibrium. In most biologic systems, reducing equivalents are constantly being generated, converted, and interconverted, indicating that a major component of antioxidant balance has to do with how extensively fed the cell or organism is at the time of

measurement. This limitation on the definition of oxidative stress requires that mechanisms thought to contribute to oxidative stress be investigated *in vivo* with a series of detailed biochemical assays rather than relying on a single biomarker.

### **Oxidative stress in the pathogenesis of atherosclerosis**

Conditions predisposing to atherosclerosis, including hypercholesterolemia, diabetes mellitus, tobacco use, and chronic kidney disease, are also commonly associated with increased oxidative stress, reduced vascular availability of nitric oxide, and inflammation. Steinberg and colleagues [13] first advanced the hypothesis that atherogenicity of low-density lipoproteins (LDL) is greatly increased upon oxidative modification. Oxidatively modified LDL is taken up by a family of scavenger receptors on monocytes leading to conversion into foam cells, an early step in the development of atherosclerosis (Fig. 1). Reactive oxygen species can directly promote LDL oxidation, stimulate vascular smooth muscle cell proliferation and migration, and potentiate the production of proinflammatory cytokines [14]. Reactive oxygen species can also activate several matrix metalloproteinases, which contribute to atherosclerotic plaque instability and rupture, thereby precipitating acute coronary syndromes [15].

Oxidative stress probably contributes to atherosclerosis risk by increasing the production of proinflammatory cytokines such as interleukin 6 (IL-6) and acute-phase proteins such as C-reactive protein (CRP) through activation of the transcription nuclear factor  $\kappa$ -B (NF- $\kappa$ B). NF- $\kappa$ B is a ubiquitous rapid-response transcription factor involved in inflammatory reactions through its action on expression of cytokines, chemokines, and cell-adhesion molecules [16,17] and has been identified *in situ* in human atherosclerotic plaques [18]. NF- $\kappa$ B activation leads to synthesis of IL-1 $\beta$ , tumor necrosis factor alpha, IL-6, and CRP. In addition to its role as a powerful risk marker, recent evidence suggests that CRP itself may directly contribute to the atherosclerotic process by leukocyte activation, by induction of endothelial dysfunction, and by attenuating endothelial progenitor cell survival, differentiation, and function [19–21]. CRP itself can potently increase the expression of NF- $\kappa$ B, thereby constituting a positive feedback loop that contributes to ongoing

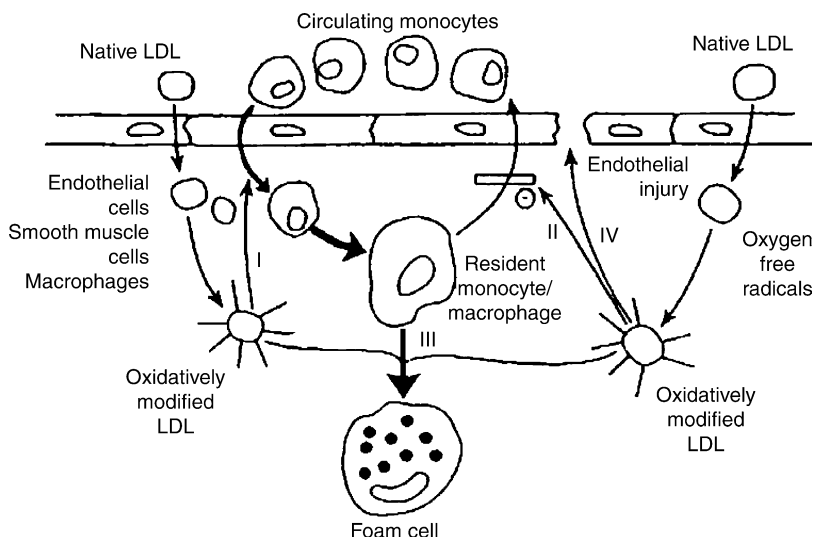


Fig. 1. Potential mechanisms for the role of oxidatively modified low-density lipoprotein (LDL) in atherosclerosis. (From Steinberg D, Parthasarathy S, Carew TE, et al. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989;320:919; with permission.)

proatherosclerotic risk [22]. NF- $\kappa$ B activation is controlled by the redox status of the cell, and intracellular reactive oxygen species may be common to all of the signaling pathways that lead to activation of NF- $\kappa$ B [23].

The vascular endothelium plays an important role in the regulation of arterial tone, local platelet aggregation, and vessel inflammation, in part through the release of nitric oxide [24]. Nitric oxide derived from endothelium has potent antiatherogenic properties manifested through inhibition of platelet aggregation, prevention of smooth muscle cell proliferation, and reduced expression of endothelial adhesion molecules. The reactive oxygen intermediate superoxide anion produced by NADPH oxidase reacts extremely rapidly with nitric oxide, resulting in the loss of nitric oxide bioactivity. The close relationship between increased oxidative stress biomarkers, inactivation of nitric oxide, and the subsequent cardiovascular event rate has been prospectively validated [25,26]. These observations collectively suggest that oxidative stress, inflammation, and endothelial dysfunction are all causally and synergistically linked to the pathogenesis of atherosclerosis.

Recent data suggest increased oxidative stress may also contribute to the pathogenesis of congestive heart failure [27]. Studies have shown that chronic volume overload directly increases oxidative stress [28,29]. Numerous biomarkers of

oxidative stress, including urinary isoprostane concentrations and plasma lipid peroxide levels, are elevated in patients with dilated cardiomyopathy and congestive heart failure in the absence of coronary atherosclerosis [30–32]. Excess myocardial NADPH oxidase activity has been detected in the myocardium of failing hearts when compared with the myocardium of explanted nonfailing hearts at the time of cardiac transplantation [33]. Many agents effective in the treatment of congestive heart failure, including carvedilol, blockers of the renin-angiotensin system, amiodarone, and probucol, are known to have antioxidative properties [34–36].

#### Prevalence of oxidative stress in kidney disease

Biomarkers of oxidative stress in kidney disease have been best studied in ESRD patients. In 1994, Maggi et al [37] reported that LDL isolated from uremic patients has greater susceptibility to copper-induced lipid peroxidation in vitro than LDL isolated from healthy subjects. This report was the first to suggest that oxidative stress may contribute to atherosclerosis in uremic patients. Since then, numerous studies using more direct in vivo biomarkers have shown that biomarkers of oxidative stress are altered in uremic patients (Box 1). The importance of uremic oxidative stress is emphasized by the role that lipid peroxidation

**Box 1. Assays for in vivo detection of oxidative stress in uremia**

*Lipids*

Malondialdehyde and other aldehydes  
Lipid peroxidation  
Oxidized LDL  
Exhaled ethane  
Advanced lipoxidation end products

*Arachidonic acid derivatives*

F2-isoprostanes  
Isolevuglandins

*Carbohydrates*

Reactive aldehydes  
Advanced glycosylation end products

*Amino acids*

Cysteine/cysteine  
Homocysteine/homocysteine  
Isoaspartate

*Proteins*

Thiol oxidation  
Carbonyl formation  
Advanced oxidation protein products

*DNA*

8 hydroxy 2' deoxyguanine

*Other*

Spin traps (electron paramagnetic resonance)  
Exhaled hydrogen peroxide

products, reactive aldehydes, and oxidized thiols play in the atherosclerotic process.

Reactive aldehydes are formed as the end products of numerous oxidative reactions, including oxidation of alcohol and amino groups and through the addition of oxygen to unsaturated double bonds. In particular, the formation of  $\alpha,\beta$ -unsaturated aldehydes as oxidation products is important in that the formation of two sites of reactivity frequently leads to the formation of cyclic adducts or crosslinks with other macromolecules [38].  $\alpha,\beta$ -Unsaturated aldehydes, or dicarbonyls, are also capable of reacting with protein nucleophiles to form advanced glycation end products [39]. Several groups have demonstrated that many such reactive aldehyde compounds, including

glyoxal, methylglyoxal, malondialdehyde, acrolein, and hydroxynonenal, are detectable at concentrations up to 10-fold higher in uremic plasma than in the plasma of healthy individuals [39,40]. In uremia, reactive aldehydes accumulate because of diminished renal catabolism and increased production, largely through MPO-catalyzed leukocyte activation. The importance of increased reactive aldehyde products is evidenced by their prominent role in the pathogenesis of atherosclerosis, including the oxidative modification of LDL. A variety of aldehyde adducts can be detected in macrophages and foam cells by immunohistochemical analysis from human atherosclerotic aortic lesions [38].

Thiol groups have an important antioxidant function as redox buffers [41]. Intracellular thiols, including glutathione and thioredoxin, which are found in millimolar concentrations, are crucial for maintaining the highly reduced environment within the cell [42]. Extracellular thiols also constitute an important component of antioxidant defense [43]. The plasma protein-reduced thiols (located primarily on the albumin molecule) are depleted in hemodialysis patients and are thus not able to participate in antioxidant defense [40]. In addition, there are diminished plasma glutathione levels and a profound decrease in glutathione peroxidase function in hemodialysis patients [44]. Furthermore, although protective reduced thiols are depleted in uremia, oxidized thiols that include homocysteine and cysteine accumulate in uremia and may have proatherogenic effects [45]. Homocysteine and cysteine can promote atherogenesis by changing endothelial function and increasing vascular smooth muscle cell activation and contribute to thrombosis by increasing tissue factor expression.

In uremic patients, lipid peroxidation can also contribute to atherosclerosis through the oxidation of LDL. The nonenzymatic free radical-induced peroxidation of arachidonic acid results in the formation of F2-isoprostanes [46]. The detection of free and esterified plasma F2-isoprostanes has been used to demonstrate an increase in lipid peroxidation in uremic individuals [47,48]. In addition, another family of highly reactive electrophiles can be generated by the free radical-induced lipid oxidation of arachidonic acid with the production of isolevuglandins. Isolevuglandin adducts to plasma proteins are elevated in ESRD patients compared with healthy individuals [49]. Levels of breath ethane, which results from the scission of lipid hydroperoxides, are also higher in hemodialysis patients than in healthy individuals

[50]. An important consequence of increased lipid peroxidation in uremic patients is the increased production of minimally oxidatively modified LDL [51]. This form of LDL is extremely atherogenic and is higher in the plasma of hemodialysis patients than in that of healthy individuals. These (and other) studies conclusively demonstrate that there is increased lipid peroxidation and oxidative stress in uremic patients.

Although the ESRD population is better studied, data increasingly suggest that patients with less severe varying degrees of kidney insufficiency are also predisposed to accelerated atherosclerosis and cardiovascular events, even in the absence of traditional cardiovascular risk factors [1,2]. Thus, more patients with stage 3 to 5 CKD will die of cardiovascular complications than will progress to develop ESRD that requires renal replacement therapy. Analysis from randomized clinical trials as well as community-based studies have identified the level of kidney dysfunction as an independent risk factor for adverse cardiovascular outcomes and all-cause mortality [52]. In the Chronic Renal Impairment in Birmingham Study, chronic kidney disease was clearly associated with low-grade inflammation, endothelial dysfunction, and platelet activation, even among patients with moderate renal impairment [53]. In a similar cross-sectional cohort analysis, multiple biomarkers of inflammation and oxidative stress were elevated in patients with stage 3 to 5 CKD compared with healthy subjects (Table 1) [54]. Levels of CRP and IL-6 have been found to be significantly higher in patients with renal insufficiency than in patients with normal kidney function in the Cardiovascular Health Study [55]. Other investigators using different biomarkers have demonstrated that CKD patients have evidence of increased oxidative stress including increases in lipid peroxidation, advanced oxidation protein products, and changes in glutathione content [56–59]. Thus, it is becoming increasingly clear that a surprisingly high prevalence of inflammation and oxidative stress, as well as a high rate of cardiovascular complications, is associated with the development of mild renal insufficiency.

#### **Are the levels of oxidative stress and inflammation linked in kidney disease?**

In numerous studies, elevations in the plasma levels of proinflammatory cytokines (especially

IL-6) and the acute-phase reactant CRP are the most powerful independent predictors of subsequent cardiovascular morbidity and mortality in uremic patients [60–70]. Total white blood cell count has also recently been reported as an independent biomarker of inflammation and mortality in dialysis patients [71]. Levels of plasmalogen, a phospholipid surrogate marker of oxidative stress, has also recently been associated with increased cardiovascular mortality in the dialysis population [72]. Levels of serum malondialdehyde, a lipid peroxidation biomarker of oxidative stress, have also been associated with cardiovascular morbidity in the dialysis population [73]. Recent studies have also used malondialdehyde-modified protein content and oxidized LDL content to predict mortality in uremic patients.

Although these studies show independent association between biomarkers of inflammation, oxidative stress, and subsequent cardiovascular morbidity and mortality, other investigations have also demonstrated an association between the levels of inflammatory and oxidative stress biomarkers. For instance, a positive correlation between elevated serum CRP levels and plasma thiobarbituric acid reaction substance (a bioassay measuring lipid peroxidation) have been observed in a hemodialysis patient cohort [74]. Plasma CRP levels are also correlated with plasma F<sub>2</sub>-isoprostane content in hemodialysis patients [47,48]. Similarly, a linkage between increased oxidative stress, inflammation, and endothelial dysfunction in hemodialysis patients has also recently been described [56]. A recent study identified a higher rate of oxidative stress in hemodialysis patients with increased inflammation and hypoalbuminemia than in normoalbuminemic hemodialysis patients [70]. These and other data suggest a bidirectional and synergistic linkage of inflammation and oxidative stress contributing to cardiovascular risk in uremic patients (Fig. 2).

#### **Oxidative stress and inflammation are linked in uremic patients through myeloperoxidase**

Stimulated leukocytes generate the reactive oxygen intermediate superoxide and its dismutation product hydrogen peroxide and secrete the heme enzyme MPO [75]. MPO is one of the most abundant proteins in leukocytes, constituting approximately 5% of neutrophil protein and 1% of monocyte protein. MPO has the unique

Table 1

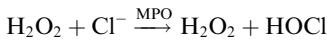
Comparison of inflammatory and oxidative stress biomarkers in patients with chronic kidney disease and healthy subjects

Biomarker	Healthy subjects median (range)	CKD patients median (range)	P
CRP (mg/L)	1.8 (0–28.6)	3.9 (0.6–28.4)	0.02
IL-6 (pg/mL)	2.1 (1.5–12.5)	6.4 (1.5–95.4)	0.001
Thiols (uM)	415 (262–497)	303 (193–435)	<0.001
Carbonyls (mmol/mg) protein	0.029 (0–0.154)	0.061 (0.020–0.134)	<0.001
F <sub>2</sub> -isoprostanes (ng/mL)	0.036 (0.019–0.179)	0.046 (0.025–0.156)	<0.001

Abbreviations: CKD, chronic kidney disease; CRP, C-reactive protein; IL-6, interleukin-6.

Modified from Oberg BP, McMenamin E, Lucas FL, et al. Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. *Kidney Int* 2004;65(3):1009–16; with permission.

property of converting chloride in the presence of hydrogen peroxide to hypochlorous acid:



MPO can also directly modulate vascular inflammatory responses by functioning as a vascular nitric oxide oxidase, thereby regulating nitric oxide availability [76]. Because MPO is released during acute inflammation, MPO-catalyzed oxidative injury and endothelial nitric oxide regulation provide a direct mechanistic linkage between inflammation, oxidative stress, and endothelial dysfunction.

A substantial body of evidence has accumulated to suggest that MPO is involved in inflammation and oxidative stress in patients with kidney disease. Catalytically active MPO can be released during the hemodialysis procedure, and 3-chlorotyrosine, an oxidative stress biomarker highly specific for MPO-catalyzed oxidation through hypochlorous acid, has been demonstrated in the plasma

proteins of dialysis patients but not in that of healthy subjects [77]. Uremic plasma containing advance oxidation protein products can induce MPO-dependent oxidation activity ex vivo in leukocytes [78]. Patterns of plasma protein oxidation observed in uremic patients (excess plasma protein carbonyl formation and oxidation of plasma protein thiol groups) can be replicated in normal plasma by the addition of hypochlorous acid, but not hydrogen peroxide and other oxidants [79]. These data strongly suggest that MPO may be a critical link between acute-phase inflammation and oxidative stress in the uremic patient population.

**Use of antioxidants in uremia**

Given the robust clinical and experimental data supporting the concept that an increase in oxidative stress contributes to cardiovascular disease in uremic patients, it is logical to hypothesize that antioxidant therapy may be beneficial in

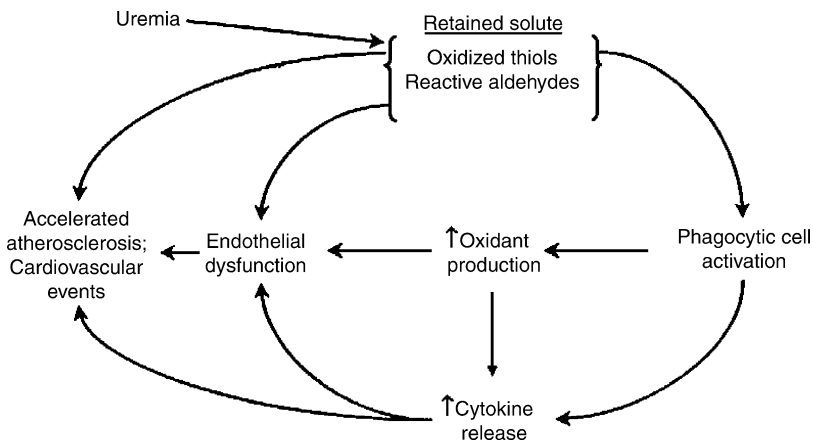


Fig. 2. Model of oxidative stress and cardiovascular complications in uremia. (From Himmelfarb J, Hakim RM. Oxidative stress in uremia. *Curr Opin Nephrol Hypertens* 2003;12:596; with permission.)

reducing these complications [80,81]. Enthusiasm for this concept, however, must be tempered by the knowledge that large, randomized clinical trials providing antioxidant therapy for either primary or secondary cardiovascular prevention in the general population have usually supported the null hypothesis. Notably, the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico study, the Heart Outcomes Prevention Evaluation study, the Study to Evaluate Carotid Ultrasound Changes in Patients Treated with Ramapril and Vitamin E, and the Heart Protection Study were all negative trials [82–85]. A recent meta-analysis confirms a lack of demonstrated efficacy with the use of antioxidant therapy in the general population. Given the weight of these large clinical trials, a high standard of evidence for clinical efficacy should be demonstrated before antioxidants are routinely recommended as therapy for uremic patients [81].

#### Antioxidant studies in dialysis patients

To date, several well-conducted, well-controlled published studies have suggested that the provision of antioxidant therapy to hemodialysis patients may have clinical efficacy in reducing the cardiovascular event rate. The Secondary Prevention with Antioxidants for Cardiovascular Disease in End-stage Renal Disease (SPACE) study examined the effects of administering 800 IU of alpha tocopherol daily in a randomized, clinical trial in hemodialysis patients [86]. A

clinically and statistically significant reduction in myocardial infarction and other cardiovascular events was noted in the alpha tocopherol-treated group compared with the placebo-treated group (Fig. 3). Despite the observed reduction in cardiovascular morbidity, however, overall survival between the two groups was not different. In another randomized, clinical trial, treatment of hemodialysis patients with the thiol-containing antioxidant *N*-acetylcysteine also significantly decreased cardiovascular events in the treated group compared with the placebo group [87]. Similar to the results of the SPACE study, this study also demonstrated no significant effect of treatment on overall mortality. These investigators have also demonstrated in a separate study that the use of *N*-acetylcysteine decreases plasma homocysteine concentration and improves endothelial function in dialysis patients, suggesting a direct mechanism for benefits of antioxidant therapy in this patient population [88]. Thus, there are now well-conducted published studies that suggest that providing antioxidants to dialysis patients may have some efficacy in decreasing the rate of cardiovascular events.

In addition to the above-mentioned studies with clinical end points, a number of published clinical studies of tocopherols used laboratory end points in dialysis patients. These studies have generally demonstrated either a decrease in the acute-phase inflammatory response or improved erythropoiesis. Most of these studies, however, involve small numbers of patients, are not powered to detect significant differences in clinical

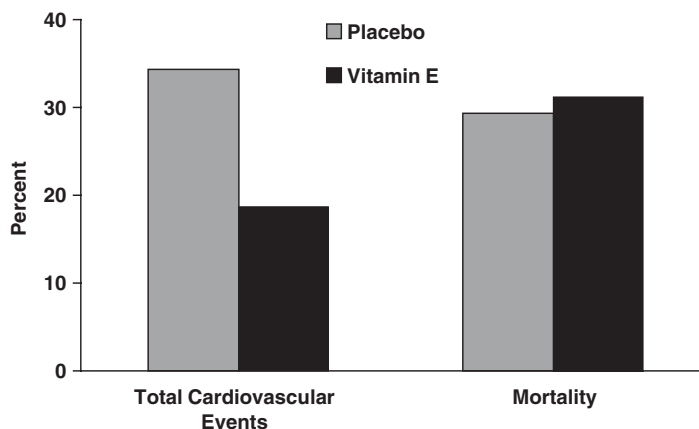


Fig. 3. Major end points of the SPACE study. (Adapted from Boaz M, Smetana S, Weinstein T, et al. Secondary prevention with antioxidants of cardiovascular disease in end-stage renal disease (SPACE): randomised placebo-controlled trial. *Lancet* 2000;356:1213–8; with permission.)

event rate, and employ antioxidant therapy for short periods. Thus, none of these studies reaches a level of evidence that would support the routine clinical use of antioxidants in dialysis patients. These studies do suggest that future randomized clinical trials should measure biomarkers of inflammation, erythropoiesis, and endothelial function in hopes of establishing a potential mechanism of beneficial effect of antioxidants and correlate with any demonstrated improved clinical outcomes.

### **Vitamin E–bonded hemodialyzers**

Although much of the excess oxidative stress observable in hemodialysis patients seems to be caused by uremia [40,89,90], it has also been suggested that the hemodialysis procedure itself may generate excess oxidant protection through inflammatory pathways. It is well known that the contact of blood with biomaterials used during the hemodialysis can elicit an organized inflammatory response that involves the activation of the body's defense against non-self, and the term hemodialysis membrane bioincompatibility has been used to describe these interactions [91,92]. Hemodialysis membranes can elicit different degrees of leukocyte and complement activation with consequent neutropenia and leukocyte degranulation, cytokine production, and reactive oxygen species production.

Because the dialysis procedure itself may elicit excess oxidative stress, an interesting approach to alleviating excess oxidative stress during hemodialysis involves incorporating antioxidant therapy into the dialysis membrane. Alpha tocopherol has been incorporated into the coating of commercial cellulose-based hemodialysis membrane (Excebrane; Terumo Corp., Tokyo, Japan). This dialyzer has numerous modifications, all made in an attempt to improve the biocompatibility of the membrane. The cellulose material is covered with a synthetic copolymer layer to cover free hydroxyl groups. The membrane further incorporates fluoro-resin, which theoretically could inhibit complement activation, and oleyl alcohol, in an effort to prevent platelet aggregation. Finally, vitamin E is bonded to the oleyl alcohol component of the membrane [93]. It is unclear if a dialysis membrane is an effective mode of vitamin E delivery, or if the Excebrane modifications will truly improve biocompatibility.

A number of surrogate rather than clinical end points have been investigated in small studies with

the use of vitamin E-coated hemodialysis membranes. These studies generally suggest that improvements are obtainable in serum vitamin E levels, red blood cell viscosity, endothelial dysfunction, and biomarkers of oxidative stress as compared with dialysis using untreated cellulose membranes [81]. A recent randomized, prospective, controlled study with 17 patients suggested there may be differences in the rate of progression of carotid intimal media thickness as a measure of atherosclerosis, with regression of carotid intimal thickness in the vitamin E–bonded dialyzer group [94]. Further studies using the rate of clinical cardiovascular events as primary end points will be required to determine if vitamin E–bonded hemodialyzers can decrease cardiovascular morbidity in the hemodialysis population.

### **Electrolyzed-reduced water treatment for hemodialysis**

An alternative and intriguing approach to improving oxidative stress in hemodialysis patients is the use of electrolyte-reduced water obtained by electrolysis for dialysate. Electrolyte-reduced water can scavenge reactive oxygen species through the production of active atomic hydrogen with high reducing ability. In a preliminary study, water for dialysate production was subjected to electrolysis through a solenoid valve resulting in electrolyte-reduced water. After 1 month of therapy, the use of electrolyte-reduced water partially restored total antioxidant status and decreased plasma lipid hydroperoxide levels [95]. Further studies evaluating this novel approach to reducing oxidative stress in dialysis patients are warranted.

### **Summary**

Patients with uremia (whether requiring renal replacement therapy or not) have a greatly increased cardiovascular risk that cannot be explained entirely by traditional cardiovascular risk factors. An increase in oxidative stress has been proposed as a nontraditional cardiovascular risk factor in this patient population. Using a wide variety of different biomarkers of increased oxidative stress status, numerous laboratories around the world have now unequivocally demonstrated that uremia is a state of increased oxidative stress. Recent data also suggest linkages between oxidative stress inflammation, endothelial dysfunction,

and malnutrition in the uremic population. These factors are probably synergistic in their effects on atherogenicity and risk of a cardiovascular event. The pathophysiology of increased oxidative stress in uremia is multifactorial, but the retention of oxidized solute by the loss of kidney function is probably a major contributor. Uremic oxidative stress can be characterized biologically by an increase in lipid peroxidation products and reactive aldehyde groups as well as by increased retention of oxidized thiols. Two recently published studies have suggested that antioxidative therapy may be particularly promising in reducing cardiovascular events in this patient population. Further definitive studies of antioxidant use are greatly needed.

### Acknowledgment

The author gratefully acknowledges the administrative assistance of Karen A. Kinne in the preparation of this article.

### References

- [1] Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis* 1998;32(5 Suppl 3):S112–9.
- [2] Baigent C, Burbury K, Wheeler D. Premature cardiovascular disease in chronic renal failure. *Lancet* 2000;356:147–52.
- [3] Cheung AK, Sarnak MJ, Yan G, et al. Atherosclerotic cardiovascular disease risks in chronic hemodialysis patients. *Kidney Int* 2000;58:353–62.
- [4] Stenvinkel P, Pecoits-Filho R, Lindholm B. Coronary artery disease in end-stage renal disease: no longer a simple plumbing problem. *J Am Soc Nephrol* 2003;14(7):1927–39.
- [5] Marnett LJ, Riggins JN, West JD. Endogenous generation of reactive oxidants and electrophiles and their reactions with DNA and protein. *J Clin Invest* 2003;111(5):583–93.
- [6] Papa S, Skulachev VP. Reactive oxygen species, mitochondria, apoptosis, and aging. *Mol Cell Biochem* 1997;174(1–2):305–19.
- [7] Babior BM. The NADPH oxidase of endothelial cells. *IUBMB Life* 2000;50(4–5):267–9.
- [8] Babior BM. The respiratory burst oxidase. *Adv Enzymol Relat Areas Mol Biol* 1992;65:49–95.
- [9] Winterbourn CC, Vissers MC, Kettle AJ. Myeloperoxidase. *Curr Opin Hematol* 2000;7(1):53–8.
- [10] Koppenol WH, Moreno JJ, Pryor WA, et al. Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. *Chem Res Toxicol* 1992;5(6):834–42.
- [11] Eiserich JP, Hristova M, Cross CE, et al. Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. *Nature* 1998;391:393–7.
- [12] Wentworth P Jr, Nieva J, Takeuchi C, et al. Evidence for ozone formation in human atherosclerotic arteries. *Science* 2003;302(5647):1053–6.
- [13] Steinberg D, Parthasarathy S, Carew TE, et al. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989;320(14):915–24.
- [14] Landmesser U, Harrison DG. Oxidant stress as a marker for cardiovascular events: OX marks the spot. *Circulation* 2001;104:2638–40.
- [15] Rajagopalan S, Meng XP, Ramasamy S, et al. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. *J Clin Invest* 1996;98(11):2572–9.
- [16] Barnes PJ, Karin M. Nuclear factor  $\kappa$ B—a pivotal transcription factor in chronic inflammatory disease. *N Engl J Med* 1997;336:1066–71.
- [17] Collins T, Cybulsky MI. NF- $\kappa$ B: pivotal mediator or innocent bystander in atherogenesis? *J Clin Invest* 2001;107(3):255–64.
- [18] Brand K, Page S, Rogler G, et al. Activated transcription factor nuclear factor- $\kappa$ B is present in the atherosclerotic lesion. *J Clin Invest* 1996;97(7):1715–22.
- [19] Verma S, Wang CH, Li SH, et al. A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. *Circulation* 2002;106(8):913–9.
- [20] Devaraj S, Xu DY, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. *Circulation* 2003;107(3):398–404.
- [21] Verma S, Kuliszewski MA, Li SH, et al. C-reactive protein attenuates endothelial progenitor cell survival, differentiation, and function: further evidence of a mechanistic link between C-reactive protein and cardiovascular disease. *Circulation* 2004;109(17):2058–67.
- [22] Verma S, Badiwala MV, Weisel RD, et al. C-reactive protein activates the nuclear factor- $\kappa$ B signal transduction pathway in saphenous vein endothelial cells: implications for atherosclerosis and restenosis. *J Thorac Cardiovasc Surg* 2003;126(6):1886–91.
- [23] Janssen-Heininger YMW, Poynter ME, Baeuerle PA. Recent advances towards understanding redox mechanisms in the activation of nuclear factor  $\kappa$ B. *Free Radic Biol Med* 2000;28:1317–27.
- [24] Furchgott R. Role of endothelium in response to vascular smooth muscle. *Circ Res* 1983;53:557–73.
- [25] Heitzer T, Schlinzig T, Krohn K, et al. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 2001;104:2673–8.

- [26] Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* 2000;87:840–4.
- [27] Mak S, Newton GE. The oxidative stress hypothesis of congestive heart failure: radical thoughts. *Chest* 2001;120(6):2035–46.
- [28] Belch JJ, Chopra M, Hutchison S, et al. Free radical pathology in chronic arterial disease. *Free Radic Biol Med* 1989;6(4):375–8.
- [29] Prasad K, Gupta JB, Kalra J, et al. Oxidative stress as a mechanism of cardiac failure in chronic volume overload in canine model. *J Mol Cell Cardiol* 1996;28(2):375–85.
- [30] Khaper N, Kaur K, Li T, et al. Antioxidant enzyme gene expression in congestive heart failure following myocardial infarction. *Mol Cell Biochem* 2003;251(1–2):9–15.
- [31] Yucl D, Aydogdu S, Senes M, et al. Evidence of increased oxidative stress by simple measurements in patients with dilated cardiomyopathy. *Scand J Clin Lab Invest* 2002;62(6):463–8.
- [32] Nonaka-Sarukawa M, Yamamoto K, Aoki H, et al. Increased urinary 15-F2t-isoprostane concentrations in patients with non-ischaemic congestive heart failure: a marker of oxidative stress. *Heart* 2003;89(8):871–4.
- [33] Heymes C, Bendall JK, Ratajczak P, et al. Increased myocardial NADPH oxidase activity in human heart failure. *J Am Coll Cardiol* 2003;41(12):2164–71.
- [34] Chin BS, Langford NJ, Nuttall SL, et al. Anti-oxidative properties of beta-blockers and angiotensin-converting enzyme inhibitors in congestive heart failure. *Eur J Heart Fail* 2003;5(2):171–4.
- [35] Book WM. Carvedilol: a nonselective beta blocking agent with antioxidant properties. *Congest Heart Fail* 2002;8(3):173–7, 190.
- [36] Sia YT, Lapointe N, Parker TG, et al. Beneficial effects of long-term use of the antioxidant probucol in heart failure in the rat. *Circulation* 2002;105(21):2549–55.
- [37] Maggi E, Bellazzi R, Falaschi F, et al. Enhanced LDL oxidation in uremic patients: an additional mechanism for accelerated atherosclerosis? *Kidney Int* 1994;45:876–83.
- [38] Uchida K. Role of reactive aldehyde in cardiovascular diseases. *Free Radic Biol Med* 2000;28(12):1685–96.
- [39] Miyata T, van Ypersele de Strihou C, Kurokawa K, et al. Alterations in nonenzymatic biochemistry in uremia: origin and significance of “carbonyl stress” in long-term uremic complications. *Kidney Int* 1999;55:389–99.
- [40] Himmelfarb J, McMonagle E, McMenamin E. Plasma protein thiol oxidation and carbonyl formation in chronic renal failure. *Kidney Int* 2000;58:2571–8.
- [41] Deneke SM. Thiol-based antioxidants. *Curr Top Cell Regul* 2000;36:151–80.
- [42] Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic Biol Med* 2001;30(11):1191–212.
- [43] Ueland PM, Mansoor MA, Guttormsen AB, et al. Reduced, oxidized and protein-bound forms of homocysteine and other aminothiols in plasma comprise the redox thiol status—a possible element of the extracellular antioxidant defense system. *J Nutr* 1996;126(4 Suppl):1281S–4S.
- [44] Ceballos-Picot I, Witko-Sarsat V, Merad-Boudia M, et al. Glutathione antioxidant system as a marker of oxidative stress in chronic renal failure. *Free Radic Biol Med* 1996;21(6):845–53.
- [45] Bostom AG, Lathrop L. Hyperhomocysteinemia in end-stage renal disease: prevalence, etiology, and potential relationship to arteriosclerotic outcomes. *Kidney Int* 1997;52:10–20.
- [46] Morrow JD, Hill KE, Burk RF, et al. A series of prostaglandin F<sub>2</sub>-like compounds are produced *in vivo* by humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc Natl Acad Sci U S A* 1990;87(23):9383–7.
- [47] Handelman GJ, Walter MF, Adhikarla R, et al. Elevated plasma F<sub>2</sub>-isoprostanes in patients on long-term hemodialysis. *Kidney Int* 2001;59:1960–6.
- [48] Ikingier TA, Morrow JD, Roberts LJ, et al. Plasma F<sub>2</sub>-isoprostane levels are elevated in chronic hemodialysis patients. *Clin Nephrol* 2002;58(3):190–7.
- [49] Salomon RG, Batoryeva E, Kaur K, et al. Isolevulinic acid-protein adducts in humans: products of free radical-induced lipid oxidation through the isoprostane pathway. *Biochim Biophys Acta* 2000;1485:225–35.
- [50] Handelman GJ, Rosales LM, Barbato D, et al. Breath ethane in dialysis patients and control subjects. *Free Radic Biol Med* 2003;35(1):17–23.
- [51] Ziouzenkova O, Asatryan L, Akmal M, et al. Oxidative cross-linking of ApoB100 and hemoglobin results in low density lipoprotein modification in blood. *J Biol Chem* 1999;274(27):18916–24.
- [52] Weiner DE, Tighiouart H, Amin MG, et al. Chronic kidney disease as a risk factor for cardiovascular disease and all-cause mortality: a pooled analysis of community-based studies. *J Am Soc Nephrol* 2004;15(5):1307–15.
- [53] Landray MJ, Wheeler DC, Lip GY, et al. Inflammation, endothelial dysfunction, and platelet activation in patients with chronic kidney disease: the chronic renal impairment in Birmingham (CRIB) study. *Am J Kidney Dis* 2004;43(2):244–53.
- [54] Oberg BP, McMenamin E, Lucas FL, et al. Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. *Kidney Int* 2004;65(3):1009–16.
- [55] Shlipak MG, Fried LF, Crump C, et al. Elevations of inflammatory and procoagulant biomarkers in

- elderly persons with renal insufficiency. *Circulation* 2003;107:87–92.
- [56] Annuk M, Zilmer M, Lind L, et al. Oxidative stress and endothelial function in chronic renal failure. *J Am Soc Nephrol* 2001;12:2747–52.
- [57] Bolton CH, Downs LG, Victory JGG, et al. Endothelial dysfunction in chronic renal failure: roles of lipoprotein oxidation and pro-inflammatory cytokines. *Nephrol Dial Transplant* 2001;16:1189–97.
- [58] Mezzano D, Pais EO, Aranda E, et al. Inflammation, not hyperhomocysteinemia, is related to oxidative stress and hemostatic and endothelial dysfunction in uremia. *Kidney Int* 2001;60:1844–50.
- [59] Witko-Sarsat V, Friedlander M, Nguyen-Khoa T, et al. Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. *J Immunol* 1998;161:2524–32.
- [60] Stenvinkel P. Inflammatory and atherosclerotic interactions in the depleted uremic patient. *Blood Purif* 2001;19:1053–61.
- [61] Arici M, Walls J. End-stage renal disease, atherosclerosis, and cardiovascular mortality: Is C-reactive protein the missing link? *Kidney Int* 2001;59:407–14.
- [62] Zimmermann J, Herrlinger S, Pruy A, et al. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. *Kidney Int* 1999;55(2):648–58.
- [63] Yeun Jy, Levine RA, Mantadilok V, et al. C-reactive protein predicts all-cause and cardiovascular mortality in hemodialysis patients. *Am J Kidney Dis* 2000;35:469–76.
- [64] Ikizler TA, Wingard RL, Harvell J, et al. Association of morbidity with markers of nutrition and inflammation in chronic hemodialysis patients: a prospective study. *Kidney Int* 1999;55(5):1945–51.
- [65] Stenvinkel P, Heimbürger O, Jøgestrand T. Elevated interleukin-6 predicts progressive carotid artery atherosclerosis in dialysis patients: association with *Chlamydia pneumoniae* seropositivity. *Am J Kidney Dis* 2002;39:274–82.
- [66] Pecoits-Filho R, Bárány P, Lindholm B, et al. Interleukin-6 is an independent predictor of mortality in patients starting dialysis treatment. *Nephrol Dial Transplant* 2002;17:1684–8.
- [67] Kimmel PL, Phillips TM, Simmens SJ, et al. Immunologic function and survival in hemodialysis patients. *Kidney Int* 1998;54(1):236–44.
- [68] Bologa RM, Levine DM, Parker TS, et al. Interleukin-6 predicts hypoalbuminemia, hypocholesterolemia, and mortality in hemodialysis patients. *Am J Kidney Dis* 1998;32(1):107–14.
- [69] Kimmel PL, Chawla LS, Amarasinghe A, et al. Anthropometric measures, cytokines and survival in haemodialysis patients. *Nephrol Dial Transplant* 2003;18(2):326–32.
- [70] Danielski M, Ikizler TA, McMonagle E, et al. Linkage of hypoalbuminemia, inflammation, and oxidative stress in patients receiving maintenance hemodialysis therapy. *Am J Kidney Dis* 2003;42(2):286–94.
- [71] Reddan DN, Klassen PS, Szczech LA, et al. White blood cells as a novel mortality predictor in haemodialysis patients. *Nephrol Dial Transplant* 2003;18(6):1167–73.
- [72] Stenvinkel P, Diczfalusy U, Lindholm B, et al. Phospholipid plasmalogen, a surrogate marker of oxidative stress, is associated with increased cardiovascular mortality in patients on renal replacement therapy. *Nephrol Dial Transplant* 2004;19(4):972–6.
- [73] Boaz M, Mataz Z, Biro A, et al. Serum malondialdehyde and prevalent cardiovascular disease in hemodialysis. *Kidney Int* 1999;56:1078–83.
- [74] Nguyen-Khoa T, Massy ZA, Pascal De Bandt J, et al. Oxidative stress and haemodialysis: role of inflammation and duration of dialysis treatment. *Nephrol Dial Transplant* 2001;16:335–40.
- [75] Podrez EA, bu-Soud HM, Hazen SL. Myeloperoxidase-generated oxidants and atherosclerosis. *Free Radic Biol Med* 2000;28(12):1717–25.
- [76] Eiserich JP, Baldus S, Brennan ML, et al. Myeloperoxidase, a leukocyte-derived vascular NO oxidase. *Science* 2002;296(5577):2391–4.
- [77] Himmelfarb J, McMenamin E, Loseto G, et al. Myeloperoxidase-catalyzed 3-chlorotyrosine formation in dialysis patients. *Free Radic Biol Med* 2001;31(10):1163–9.
- [78] Witko-Sarsat V, Gausson V, Nguyen AT, et al. AOPP-induced activation of human neutrophil and monocyte oxidative metabolism: a potential target for N-acetylcysteine treatment in dialysis patients. *Kidney Int* 2003;64(1):82–91.
- [79] Himmelfarb J, McMonagle E. Manifestations of oxidant stress in uremia. *Blood Purif* 2001;19:200–5.
- [80] Galle J, Seibold S. Has the time come to use antioxidant therapy in uremic patients? *Nephrol Dial Transplant* 2003;18(8):1452–5.
- [81] Gordon CA, Himmelfarb J. Antioxidant therapy in uremia—evidence-based medicine? *Semin Dial* 2004;17:327–32.
- [82] Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* 1999;354(9177):447–55.
- [83] Lonn EM, Yusuf S, Dzavik V, et al. Effects of ramipril and vitamin E on atherosclerosis: a study to evaluate carotid ultrasound changes in patients treated with ramipril and vitamin E (SECURE). *Circulation* 2001;103:919–25.
- [84] Yusuf S, Dagenais G, Pogue J, et al. Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med* 2000;342(3):154–60.
- [85] MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk

- individuals: a randomised placebo-controlled trial. *Lancet* 2002;360(9326):23–33.
- [86] Boaz M, Smetana S, Weinstein T, et al. Secondary prevention with antioxidants of cardiovascular disease in end-stage renal disease (SPACE): randomised placebo-controlled trial. *Lancet* 2000;356:1213–8.
- [87] Tepel M, van der Giet M, Statz M, et al. The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure: a controlled trial. *Circulation* 2003;107:992–5.
- [88] Scholze A, Rinder C, Beige J, et al. Acetylcysteine reduces plasma homocysteine concentration and improves pulse pressure and endothelial function in patients with end-stage renal failure. *Circulation* 2004;109(3):369–74.
- [89] Himmelfarb J, McMenamin E, McMonagle E. Plasma aminothiols oxidation in chronic renal failure. *Kidney Int* 2002;61(2):705–16.
- [90] Roselaar SE, Nazhat JB, Winyard PG, et al. Detection of oxidants in uremic plasma by electron spin resonance spectroscopy. *Kidney Int* 1995;48:199–206.
- [91] Cheung AK. Biocompatibility of dialysis membranes. *J Am Soc Nephrol* 1990;1:150–61.
- [92] Klinkmann H, Wolf H, Schmitt E. Definition of biocompatibility. *Contrib Nephrol* 1984;37:70–7.
- [93] Sasaki M, Hosoya N, Saruhashi M. Development of vitamin E-modified membrane. *Contrib Nephrol* 1999;127:49–70.
- [94] Kobayashi S, Moriya H, Aso K, et al. Vitamin E-bonded hemodialyzer improves atherosclerosis associated with a rheological improvement of circulating red blood cells. *Kidney Int* 2003;63(5):1881–7.
- [95] Huang KC, Yang CC, Lee KT, et al. Reduced hemodialysis-induced oxidative stress in end-stage renal disease patients by electrolyzed reduced water. *Kidney Int* 2003;64(2):704–14.