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Review

Eicosapentaenoic Acid as a Potential Therapeutic Approach to Reduce Cardiovascular Risk in Patients with End-Stage Renal Disease on Hemodialysis: A Review

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Abstract
Background: Patients with end-stage renal disease on hemodialysis have excess cardiovascular disease (CVD) burden with substantially increased CV event rates compared with the general population. ***Summary:*** Traditional interventions that, according to standard clinical guidelines, reduce CV risk such as antihypertensive therapy, diet, exercise, and statins are not similarly effective in the hemodialysis population. This raises the question of whether additional risk factors, such as enhanced inflammation and oxidative stress, may drive the increased CVD burden in hemodialysis patients. Eicosapentaenoic acid (EPA), an omega-3 polyunsaturated fatty acid, is incorporated into the atherosclerotic plaque as well as membrane phospholipid bilayers and produces beneficial effects on inflammatory and oxidative mechanisms involved in atherosclerotic plaque formation and progression. EPA levels and the ratio of EPA to the omega-6 polyunsaturated fatty acid arachidonic acid (AA) are reduced in hemodialysis patients. Serum EPA levels have been inversely correlated with proinflammatory cytokines, and the EPA/AA ratio has been inversely associated with CV events in hemodialysis cohorts. Three recent studies involving over 800 hemodialysis patients and follow-up of 2–3 years suggest that EPA therapy may improve clinical outcomes in this patient population as evidenced by significant reductions in cardiovascular mortality, all-cause mortality, and/or CV events. ***Key Messages:*** Further studies with high-purity EPA are warranted in patients on hemodialysis, especially given the fact that other interventions including antihypertensives, diet, exercise, and statins have not provided meaningful benefit.

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Introduction

According to the United States Renal Data System, there were more than 661,000 patients with end-stage renal disease (ESRD) in the US in 2013, of whom 63.7% were receiving hemodialysis [1]. The prevalence of ESRD in the US continues to increase by about 21,000 cases per year, whereas ESRD incidence has largely stabilized over the last decade except in patients aged 45–74 years, in whom it continues to increase. This latter observation has important significance because older individuals are the most susceptible to acute coronary events.

Patients on hemodialysis have increased cardiovascular disease (CVD) burden with substantially increased rates of adverse CV events. CV risk is higher for adults with ESRD on hemodialysis compared with age-matched healthy individuals; for adults over 75 years of age, CV risk remains up to 10 times higher for patients on hemodialysis [2]. Mortality rates for patients starting hemodialysis are 16 times higher compared with the general population, with CVD responsible for nearly 40% of these deaths [3]. The elevated CV morbidity and mortality combined with the increasing prevalence of ESRD underscore the need to identify and establish safe and effective therapeutic interventions to reduce adverse CV outcomes in the vulnerable population of patients on hemodialysis.

Unmet Needs with Traditional Approaches to Mitigation of Cardiovascular Risk in Hemodialysis Patients

Evidence-based approaches for reducing CV risk in patients on hemodialysis are limited because such patients are usually excluded from large clinical outcomes trials [4]. The following is a brief overview of data in the hemodialysis population relating to standard therapeutic interventions that are used to decrease CV risk in the general population.

Antihypertensive Therapy

Epidemiologic studies suggest that higher blood pressure is paradoxically associated with decreased mortality in patients on hemodialysis [5–7]. Several studies have evaluated the effects of different antihypertensive drug classes (beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, or calcium channel blockers) on outcomes in hemodialysis patients, but they have generally involved a limited number of patients with estimates of effect size that are often insignificant [8]. In general, interventions that improve elevated blood pressure have not been shown to consistently reduce CVD events in patients with ESRD on hemodialysis. A possible exception is a meta-analysis of 5 studies suggesting that antihypertensive medications may reduce CV events in hemodialysis patients compared with control subjects (hazard ratio [HR]: 0.69, 95% confidence interval [CI]: 0.56–0.84 in inverse-weighted fixed-effects model, and HR: 0.62, 95% CI: 0.45–0.86 in random-effects model) [8]. However, the broad-based applicability of this meta-analysis is questionable due to substantial heterogeneity across the 5 studies with respect to clinical outcomes, reflecting different study designs and study populations.

Weight Loss

Although weight loss in overweight and obese subjects is associated with improved outcomes in the general population, it has the opposite effect in the hemodialysis population. Obesity has been associated with longer survival and reduced CV mortality in patients on hemodialysis [6, 9]. Moreover, weight loss has been shown to be an independent risk factor for adverse outcomes in patients on maintenance hemodialysis [10]. Several hypotheses have

been offered to explain these counterintuitive relationships, including greater malnutrition and less stable hemodynamics among hemodialysis patients with a lower body mass index [6].

Statin Therapy

Statin therapy for decreasing low-density lipoprotein cholesterol (LDL-C) is the backbone of atherosclerotic CVD risk reduction management strategies in both the primary and secondary prevention settings. However, multiple studies have shown that a statin-based approach for reducing CV risk may not be suitable in patients on hemodialysis [11]. Two large international trials addressed this issue. The 4D study evaluated atorvastatin in 1,255 type 2 diabetes patients on hemodialysis, and the AURORA study evaluated rosuvastatin in 2,776 patients undergoing maintenance hemodialysis [12, 13]. Neither statin improved the primary endpoint of CV mortality, nonfatal myocardial infarction (MI), or nonfatal stroke compared with control after median follow-up periods of 4.0 and 3.8 years, respectively (relative risk [RR]: 0.92, 95% CI: 0.77–1.10, $p = 0.37$ in the 4D study, and HR: 0.96, 95% CI: 0.84–1.11, $p = 0.59$ in the AURORA study).

The SHARP study, which evaluated simvastatin plus ezetimibe in chronic kidney disease patients with no known history of MI or coronary revascularization, included a subgroup of 3,023 patients on dialysis (2,527 hemodialysis/496 peritoneal dialysis) [14]. In the hemodialysis subgroup, simvastatin plus ezetimibe did not reduce risk of a first major atherosclerotic event defined as coronary death, nonfatal MI, nonhemorrhagic stroke, or any arterial revascularization procedure (RR: 0.95; 95% CI: 0.78–1.15).

A meta-analysis based largely on the 4D, AURORA, and SHARP studies concluded that statins, when used in patients on hemodialysis, have little or no effect on all-cause mortality (HR: 0.96; 95% CI: 0.88–1.04), CV mortality (HR: 0.94; 95% CI: 0.82–1.07), or major CV events (HR: 0.95; 95% CI: 0.87–1.03) [15]. A subsequent meta-analysis suggested that beneficial effects of statin therapy in patients with renal dysfunction are significantly related to the level of residual kidney function [16]. Of particular interest was the observation that there was no clear relationship between LDL-C reduction and CV benefit in patients on hemodialysis [16, 17].

Proposed Pathophysiologic Factors Associated with Increased Cardiovascular Risk in Hemodialysis Patients

The type and severity of atherosclerosis in patients on hemodialysis differs from that in the general population and likely contributes to the differing pathophysiology of CVD between the 2 groups. Whereas obstructive atherosclerotic coronary artery disease is the principal manifestation in the general population, the pathophysiology of CVD in ESRD includes other disorders such as arteriosclerosis, cardiomyopathy, and increased sudden cardiac death due to arrhythmias [11]. It has been suggested that statins may not impact the excess CV events caused by these additional disorders [16]. ESRD patients on hemodialysis have a high incidence of comorbid conditions, notably type 2 diabetes mellitus and hypertension, but these traditional risk factors cannot account for the increased CVD burden. Other risk factors, including oxidative stress and inflammation may be important in terms of explaining the excess CV events in hemodialysis patients (Fig. 1). Intradialytic hypotension and chronic anemia may also be contributing factors but are beyond the scope of this review [4, 16].

Oxidative Stress and Inflammation

Patients undergoing hemodialysis have higher levels of oxidative stress and inflammation than age-matched individuals not on hemodialysis. This may be attributed to a variety

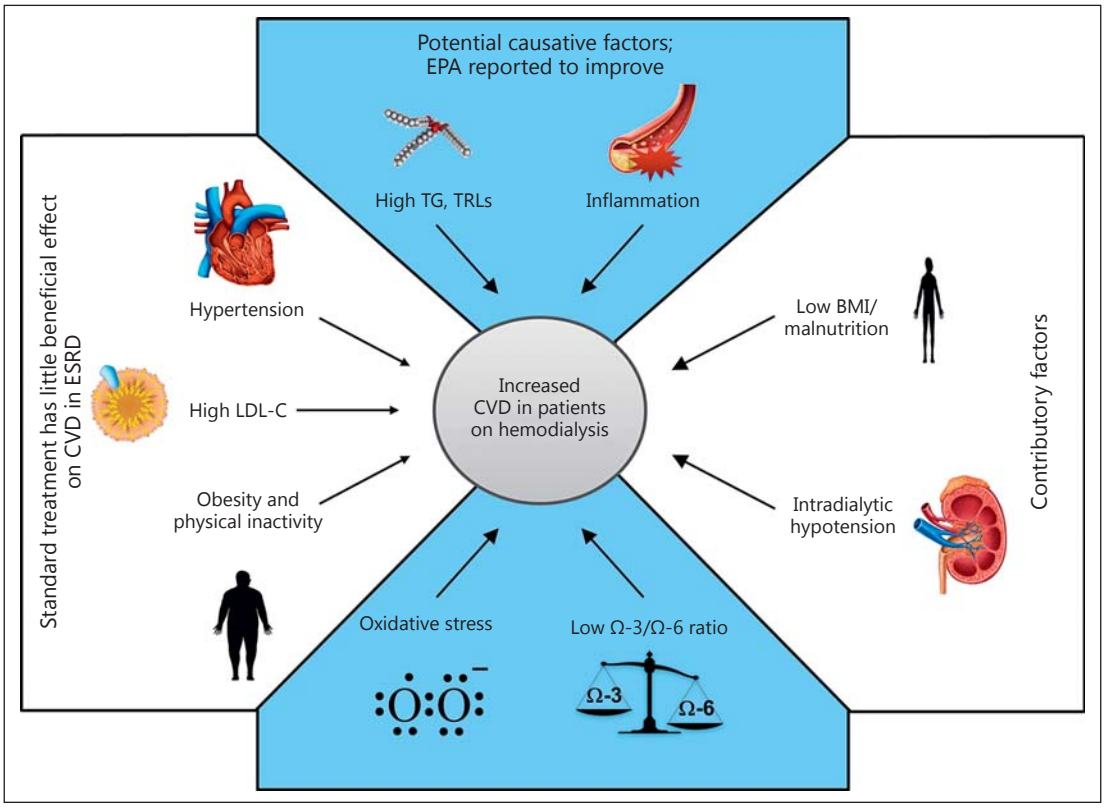

Fig. 1. Potential mechanisms involved in the increased CVD burden in patients with ESRD on hemodialysis with features that may be impacted by EPA. Interventions that improve factors shown in the left panel (hypertension, high LDL-C, and obesity/physical inactivity) are typically associated with CVD risk reduction in the general population but have not been found to reduce CVD risk in patients with ESRD on hemodialysis. Factors shown in the right panel may contribute to increased CVD risk in patients with ESRD on hemodialysis; interventions are supportive in nature for these factors. Factors shown in the shaded panels may contribute to CVD risk in both the general and ESRD populations; EPA has been reported to have beneficial effects on these potentially important CVD causative factors (see text for citations). BMI, body mass index; CVD, cardiovascular disease; EPA, eicosapentaenoic acid; ESRD, end-stage renal disease; LDL-C, low-density lipoprotein cholesterol; Ω-3, omega-3 fatty acid; Ω-6, omega-6 fatty acid; TG, triglycerides; TRLs, triglyceride-rich lipoproteins.

of factors including interactions between the blood and dialyzer membrane, subclinical infection, indwelling catheters, activation of the renin-angiotensin-aldosterone system, impaired intestinal barrier function, impaired nitric oxide metabolism, and decreased production of numerous antioxidant and cytoprotective molecules [11, 18]. During the development of atherosclerosis, oxidative stress is known to cause endothelial dysfunction, which promotes monocyte activation, adhesion to endothelial surfaces, and infiltration into the arterial wall [17, 19, 20]. Oxidative stress also stimulates oxidation of LDL and increases levels of triglyceride (TG)-rich lipoprotein (TRL) remnants including very-low-density lipoprotein and chylomicrons. These products increase expression of scavenger receptors, thereby allowing their uptake by macrophages and other resident phagocytic cells in the arterial wall. Together, these mechanisms promote transformation of macrophages into foam cells, a key step in the atherosclerosis process [17, 19].

High-density lipoprotein (HDL) may help to protect against foam cell formation by preventing lipoprotein oxidation, preventing cholesterol influx into macrophages, and stimulating nitric oxide production and blocking chemokine production by endothelial cells. However, in ESRD, the structure and function of HDL is altered, which impairs its capacity for antioxidant, anti-inflammatory, and reverse cholesterol transport activities [21]. This effect may be accompanied by impaired cholesterol metabolism in the remaining functional kidney and vasculature, even though serum cholesterol and LDL-C are usually normal or reduced in patients maintained on hemodialysis [11]. The metabolism of TRLs may also be impaired in ESRD. This is thought to be related to deficiencies in lipoprotein lipase, hepatic lipase, and LDL receptor-related protein [11]. The net result is an accumulation of TRL remnants, which are prone to oxidation and give rise to lipoprotein particles with enhanced atherogenicity. Together, these mechanisms may help to explain why traditional interventions, such as statins, show little or no benefit in CVD prevention in outcomes studies in hemodialysis patients.

Eicosapentaenoic Acid as a Potential Therapeutic Approach to Decrease Cardiovascular Risk in Hemodialysis Patients

The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) recommends dietary intake of food sources rich in the omega-3 polyunsaturated fatty acids (PUFAs) at least twice weekly as part of well-balanced nutrition in hemodialysis patients [4]. Omega-3 PUFAs include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). While both EPA and DHA have potential beneficial CV effects [22], DHA has been reported to have the unwanted off-target effect of raising LDL-C [23]. Recently, a review article described the beneficial effects of EPA on multiple steps in the atherosclerotic process, from protecting against endothelial dysfunction to reducing plaque formation, progression, and vulnerability [17]. Interventional studies of omega-3 PUFAs have investigated the potential effectiveness of PUFA treatment in reducing CV risk in patients on hemodialysis. These include studies of treatment with fish oil dietary supplements containing EPA, DHA, and other ingredients as well as studies of purified products that contain both EPA and DHA or only EPA; results were varied [24–29]. Here, we examine the interventional outcomes studies of EPA-only therapy in hemodialysis patients as well as the effects of EPA on factors such as lipids, inflammation, and oxidative stress, which are likely to play a role in the increased CVD risk in this patient population.

EPA Outcomes Studies

Perhaps the most direct evidence for EPA as a therapeutic approach to decreasing CV risk in hemodialysis patients comes from 3 CV outcomes studies that included over 800 subjects undergoing hemodialysis. The effects of this intervention on CV outcomes in these studies are summarized in Table 1 [26–28].

In a prospective, open-label, controlled clinical study, 179 hemodialysis patients were randomized to receive purified EPA 1.8 g/day (n = 89) or control (n = 90) [26]. After a 2-year follow-up, patients randomized to EPA had significant decreases in CV death (HR: 0.20; 95% CI: 0.04–0.91; p = 0.037), CV events including acute MI, stroke, and aortic-disease-related events (HR: 0.50; 95% CI: 0.26–0.96; p = 0.039), and combined outcome of all-cause death and CV events (HR: 0.49; 95% CI: 0.26–0.90; p = 0.021) compared with the control group [26]. Notably, these effects of EPA were independent of TG and C-reactive protein levels [26]. In a 3-year longitudinal observational cohort study, 176 maintenance hemodialysis patients received either a purified formulation of EPA 1.8 g/day (n = 51) or no EPA (n = 125). The EPA

Table 1. Effects of EPA on cardiovascular outcomes in hemodialysis patients

First author [Ref.], year	Study design	EPA dose, g/day	Follow-up, years	Clinical outcome (EPA vs. control)
Nasu [26][a], 2013	179 subjects (EPA [*n* = 89] vs. control [*n* = 90]); prospective, randomized, open-label trial	1.8	2	EPA decreased CV death by 80% ($p = 0.037$), CV events by 50% ($p = 0.039$), and CV death or events by 51% ($p = 0.021$)
Inoue [27], 2015	176 subjects (EPA [*n* = 51] vs. no EPA [*n* = 125]); longitudinal, observational cohort study	1.8	3	EPA decreased all-cause mortality by 58% ($p = 0.034$)
Umemoto [28][a], 2015	459 subjects (EPA [*n* = 106] vs. no EPA [*n* = 353]); both groups received standard therapy; longitudinal, observational study	0.9	3	EPA decreased all-cause mortality by 47% ($p = 0.023$) and CV mortality by 59% ($p = 0.029$)

CV, cardiovascular; EPA, eicosapentaenoic acid. [a] Preliminary evidence; data from European Society of Cardiology abstract.

patients experienced a statistically significant 58% reduction in all-cause mortality (HR: 0.42; 95% CI: 0.16–0.94; $p = 0.034$) [27]. In another study, 459 patients on maintenance hemodialysis received standard therapy with either the addition of purified EPA 0.9 g/day ($n = 106$) or no EPA added (control; $n = 353$) and were followed for 3 years [28]. Propensity score analysis using a multivariate logistic model was used to compare outcomes in the EPA group versus the control group. EPA significantly decreased all-cause mortality by 47% (HR: 0.53; 95% CI: 0.31–0.92; $p = 0.023$) and CV mortality by 59% (HR: 0.41; 95% CI: 0.18–0.91; $p = 0.029$) compared with the control cohort [28].

Limitations of these studies include open-label treatment, longitudinal observational design, and restriction to Japanese patient populations, where background omega-3 fatty acid consumption and long-term survival for dialysis populations are high relative to Western populations. In addition, 2 of the studies should be considered preliminary as they have not yet been published in full [26, 28]. The results should therefore be appropriately interpreted, especially when generalizing to Western populations. However, it should be noted that outcomes data for CV death, all-cause mortality, and CV events appear to be internally consistent between the 3 studies. It may also be worth noting that in these 3 studies, all patients were receiving hemodialysis and thus were exposed to the same disease processes. The studies also included extensive follow-up through 2–3 years and, most importantly, the endpoints included were stringent (i.e., "hard endpoints": CV death, CV mortality, and all-cause mortality). Taken together, the substantial reductions observed in these 3 independent studies suggest a potential for benefit with EPA and that further investigation of the effects of EPA on CV outcomes in hemodialysis patients may be warranted to gain further insight into the possible role of EPA-only therapy in this patient population.

Effects of EPA on Lipids, Inflammation, and Oxidative Stress

For the hemodialysis population, the effects of EPA on lipids, inflammation, and oxidative stress are likely to play a role in the increased CVD burden observed in this patient population.

Effects of Plasma EPA on Lipids and Lipoproteins

Several studies have reported that hemodialysis patients have low plasma levels of EPA and, as a result, lower ratios of EPA to the proinflammatory omega-6 fatty acid arachidonic acid (AA) compared with control populations [30–33]. In a study of 461 hemodialysis patients, low ratios of EPA/AA and omega-3/omega-6 fatty acids were closely associated with carotid atherosclerosis as measured by ultrasound [34]. In a single-center observational study, serum EPA and AA levels were measured in a cohort of 517 Japanese patients on maintenance hemodialysis and compared with 122 age- and gender-matched controls [35]. Serum EPA/AA ratios were lower in hemodialysis patients compared with controls and, after adjusting for age and other confounding variables, were inversely associated with CVD events.

In EPA interventional studies, treatment with EPA has been shown to significantly improve the EPA/AA ratio in hemodialysis patients [28, 36]. In the outcomes study described earlier where EPA was found to reduce all-cause mortality in hemodialysis patients, significant improvements were observed in the EPA/AA ratio as well [28]. These results were consistent with data from a non-hemodialysis patient population of hypercholesterolemic patients in whom the ratio of EPA to AA was found to be predictive of cardiovascular risk, with higher EPA to AA values being more protective against cardiovascular events [37]. In a crossover study, 59 hemodialysis patients with a low EPA/AA ratio were given EPA 1.8 g/day or control treatment for 3 months, with a 1-month washout between study periods [36]. EPA significantly improved the EPA/AA ratio ($p < 0.0001$), which was accompanied by reductions in advanced glycation end products [36].

Regarding other atherogenic lipid parameters, in a cohort of 22 hemodialysis patients with relatively high baseline levels of remnant-like particle cholesterol (RLP-C; >7.5 mg/dL) and oxidized LDL (oxLDL; >150 ng/mg), EPA 1.8 g/day for 3 months significantly reduced levels of RLP-C by 52%, oxLDL by 38%, and TGs by 42% compared with baseline (all $p < 0.01$) [38]. By 3 months after discontinuing EPA, each parameter increased and approached baseline values [38]. In the crossover study of 59 hemodialysis patients mentioned above, improvement was also observed in the lipid profile [36]. These data are in line with an early pilot study of 12 hemodialysis patients which showed that daily fish oil rich in EPA significantly reduced TG and total cholesterol levels after 4 and 13 weeks [39].

In addition to beneficial effects on lipid/lipoprotein parameters including TRLs and the ratios of EPA/AA and omega-3/omega-6 fatty acids in hemodialysis patients described above, EPA also improved the lipid profile in other patient populations. Icosapent ethyl, a pure ethyl ester of EPA, significantly reduced levels of TGs, non-HDL cholesterol, key atherogenic lipid/lipoprotein parameters including RLP-C, apolipoprotein C-III, and oxLDL, and other markers of inflammation in statin-treated patients with high TG levels (200 to <500 mg/dL) in the ANCHOR study and in patients with very high TG levels (500 to ≤2,000 mg/dL) in the MARINE study [40–44].

Effects of EPA on Inflammation

EPA is known to exert beneficial effects on a wide array of inflammatory processes in experimental systems and in multiple patient populations. Both omega-6 (e.g., AA) and omega-3 (e.g., EPA) PUFAs are incorporated into cell membrane phospholipid bilayers, serve as structural components that ensure the fluidity and stability of the membrane, and act as gatekeepers in the cell [45]. In addition, AA and EPA are converted into a number of important bioactive lipids that have the potential to affect multiple classes of pro- and anti-inflammatory mediators including eicosanoids and cytokines [17, 46]. The eicosanoids are lipid mediators derived from AA and EPA found in membrane phospholipids; cyclooxygenase enzymes convert AA and EPA into prostanoids, and lipoxygenase enzymes convert them into leukotrienes and other lipid products [46]. AA is converted into prostanoids containing 2 double

bonds and leukotrienes containing 4 double bonds, which typically have proinflammatory and/or prothrombotic effects [46, 47]. Treatment with EPA leads to increased incorporation into membrane phospholipid bilayers in experimental models, and also leads to an increase in EPA/AA ratios and multiple patient populations. Importantly, EPA is converted into prostanoids containing 3 double bonds and leukotrienes with 5 double bonds, mediators that may exhibit anti-inflammatory and/or antithrombotic effects [46, 47]. Thus, the ratio of EPA/AA is an indicator of inflammation and, as noted earlier, is an indicator of CVD in hemodialysis patients; treatment with EPA has been shown to improve this ratio in hemodialysis patients [36].

Regarding other mediators of inflammation, in a study of 42 hemodialysis patients, pentraxin-3 and interleukin (IL)-1 levels were found to be significantly lower in the cohort with high plasma EPA levels than those with low plasma EPA levels; the pentraxin-3 level was also negatively correlated with the EPA/AA ratio [48]. Pentraxin-3 is of particular interest because it is an early sensitive marker of hemodialysis-induced inflammation [49] and has been associated with CVD [50] and higher comorbidity [51] in hemodialysis patients. It may be of interest to note that it has been reported that the combination of EPA with a statin reduces pentraxin-3 levels more than a statin alone, although these studies were not in hemodialysis patients [52, 53].

EPA levels in a study of 42 hemodialysis patients were negatively correlated with concentrations of the proinflammatory cytokines tumor necrosis factor-α ($r = -0.497$; $p < 0.05$) and IL-6 ($r = -0.468$; $p = 0.03$) [54]. In populations other than hemodialysis patients, EPA has been shown to increase the levels of anti-inflammatory cytokines and reduce the levels of the proinflammatory cytokines [44, 55, 56].

In experimental models, EPA has been shown to have beneficial effects on inflammation-driven atherosclerotic processes such as monocyte adhesion to endothelial cells [57, 58]. In addition, oxLDL and RLP-C may stimulate foam cell formation; EPA has been shown to lower the levels of both of these atherogenic parameters in patients with hypertriglyceridemia and in hemodialysis patients [38, 44, 59].

The resolution of inflammation is also an important mechanism in the prevention of chronic inflammation, and specialized pro-resolving mediators such as resolvins act to counter-regulate proinflammatory responses and help lead to resolution [60, 61]. Resolvins can be derived from omega-3 fatty acids, and studies have suggested that omega-3 fatty acid supplementation can increase specialized pro-resolving mediators, including in patients with chronic renal impairment [60–65]. The e-series resolvins can be derived from EPA, and a recent study using a murine model of atherosclerosis demonstrated that treatment with the EPA-derived resolvin E1 reduced atherosclerotic lesions by 35% ($p < 0.05$) in the absence of atorvastatin or by 51% ($p < 0.001$) in the presence of atorvastatin [66].

Effects of EPA on Oxidative Stress

Several preclinical studies suggest that EPA has antioxidant effects that reduce oxidative stress and improve endothelial function. EPA inhibited lipid peroxidation in membrane vesicles with normal or elevated cholesterol levels as well as glucose-induced pathologic changes in the structural organization of membrane lipids, including development of cholesterol crystalline domains [67, 68]. EPA also exhibited antioxidant effects in human umbilical vein and glomerular endothelial cells, where it improved the balance between nitric oxide and peroxynitrite [69, 70]. These antioxidant effects are thought to be due to intercalation of EPA into the membrane lipid bilayer [68].

In hemodialysis patients, EPA has been shown to reduce oxLDL levels in patients with relatively high baseline oxLDL and RLP-C, as previously noted [38]. These antioxidant effects are supported by other clinical and ex vivo studies, although not in hemodialysis

populations. In a Japanese study, administration of EPA 1.8 g/day for 3 months restored endothelium-dependent vasodilation measured by peak forearm blood flow during reactive hyperemia in hyperlipidemic patients [71]. Improvements in endothelial function were also realized when EPA was added to statin therapy in patients with type 2 diabetes or coronary artery disease [72, 73]. Higher serum EPA also improved vascular endothelial dysfunction via its anti-inflammatory effect in Japanese hemodialysis patients [48]. EPA has also been shown to reduce oxidation of human LDL in a concentration-dependent manner in an ex vivo study [68]. Consistent with this observation, icosapent ethyl at a dose of 4 g/day for 12 weeks reduced oxLDL levels compared with placebo by 13.3% ($p < 0.0001$) in statin-treated patients with high TG levels (200 to <500 mg/dL) in the ANCHOR study and by 6.6% ($p = 0.055$) in patients with very high TG levels (500 to ≤2,000 mg/dL) in the MARINE study [44].

Discussion

The increased CV risk seen in patients on hemodialysis does not appear to be driven by dyslipidemia and other traditional risk factors involved in the general population. Indeed, interventions that target these traditional risk factors, such as statins, antihypertensive drugs, and weight loss do not lower CV risk in this population even though they are effective at lowering CV risk in other populations. Instead, oxidative stress and chronic inflammation appear to be important drivers of CVD in the hemodialysis population. Chronic inflammation is known to increase CV risk in various chronic immunoinflammatory conditions such as rheumatoid arthritis [74–76], HIV [77], and Crohn's disease [78]. The increased CVD risk in these disorders is also not driven solely by dyslipidemia or other traditional risk factors.

EPA reduces levels of TGs, TRLs, and other atherogenic lipid parameters, and also affects multiple steps in the development and progression of atherosclerosis including inflammation and oxidative stress [17]. These pleiotropic effects of EPA are not all shared by DHA [79], and therefore EPA may be unique among omega-3 PUFAs regarding potential benefit to patients on hemodialysis. In a notable randomized, multicenter, double-blind, controlled study ($n = 206$) by Svensson et al. [25], treatment of chronic hemodialysis patients with purified EPA+DHA did not lead to a significant reduction in CV events and death, but did result in a significant reduction in the number of secondary MIs. The authors suggest that the reduction in MIs might be due to the omega-3 PUFA's anti-inflammatory, antithrombotic, or antioxidant effects [25]. It may be of interest that the dose of EPA was less than 1 g/day and thus relatively low compared with the EPA-only studies discussed earlier [26–28].

In the authors' opinion, the potential benefit/risk profile of EPA in hemodialysis patients appears favorable based on evidence available to date. Administration of EPA would be expected to help correct the relative EPA deficiency reported in hemodialysis patients [30–32] and increase the EPA/AA ratio that has been inversely associated with adverse CV events and markers of inflammation in this population [35, 54]. The potential benefit in reducing CV risk would be expected to be associated with low treatment risk, given the well-characterized safety profile reported in the literature [40, 41]; however, this profile needs to be confirmed in hemodialysis patients. Of note, EPA is cleared via beta-oxidation, and its accumulation is not likely to be problematic because it competes with AA and other PUFAs for storage in membrane phospholipid bilayers [80]. Interestingly, due to its lipophilic nature, EPA is stored in the plasma membrane where it can be expected to provide a consistent reservoir of EPA, even in the hemodialysis patient population where plasma drug levels may be variable.

Conclusions

Consistent data from 3 separate, although somewhat limited, studies suggest that EPA therapy may potentially improve CV outcomes in the high-risk population of patients with ESRD on chronic hemodialysis, a population with significant unmet clinical need. The increased CVD burden seen in these patients appears to be driven, in part, by chronic inflammation and oxidative stress independent of traditional CVD risk factors and therapies. Efforts to reduce CV risk in ESRD patients on hemodialysis may benefit from focusing on these drivers. Subject to further clinical investigation and confirmation, particularly in Western populations, EPA may be an effective therapeutic option because of its potential beneficial pleiotropic effects, which may counter pathologic processes that lead to the development and progression of inflammation and oxidative stress at multiple downstream sites of action in vascular disease states. Further clinical evaluation of EPA in patients on hemodialysis is warranted.

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References

1 United States Renal Data System: Incidence, Prevalence, Patient Characteristics, and Treatment Modalities. 2015 USRDS Annual Data Report Volume 2: Epidemiology of Kidney Disease in the United States. Bethesda, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2015.
2 Foley RN, Parfrey PS, Sarnak MJ: Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis 1998;32(suppl 3):S112–S119.
3 de Jager DJ, Grootendorst DC, Jager KJ, van Dijk PC, Tomas LM, Ansell D, et al: Cardiovascular and noncardiovascular mortality among patients starting dialysis. JAMA 2009;302:1782–1789.
4 K/DOQI Clinical Practice Guidelines for Cardiovascular Disease in Dialysis Patients. Am J Kidney Dis 2005; 45(suppl 3):S1–S153.
5 Zager PG, Nikolic J, Brown RH, Campbell MA, Hunt WC, Peterson D, et al: "U" curve association of blood pressure and mortality in hemodialysis patients. Medical Directors of Dialysis Clinic, Inc. Kidney Int 1998;54: 561–569.
6 Kalantar-Zadeh K, Kopple JD, Kilpatrick RD, McAllister CJ, Shinaberger CS, Gjertson DW, et al: Association of morbid obesity and weight change over time with cardiovascular survival in hemodialysis population. Am J Kidney Dis 2005;46:489–500.
7 Li Z, Lacson E Jr, Lowrie EG, Ofsthun NJ, Kuhlmann MK, Lazarus JM, et al: The epidemiology of systolic blood pressure and death risk in hemodialysis patients. Am J Kidney Dis 2006;48:606–615.
8 Agarwal R, Sinha AD: Cardiovascular protection with antihypertensive drugs in dialysis patients: systematic review and meta-analysis. Hypertension 2009;53:860–866.
9 Abbott KC, Glanton CW, Trespalacios FC, Oliver DK, Ortiz MI, Agodoa LY, et al: Body mass index, dialysis modality, and survival: analysis of the United States Renal Data System Dialysis Morbidity and Mortality Wave II Study. Kidney Int 2004;65:597–605.

10 Wiesholzer M, Harm F, Schuster K, Putz D, Neuhauser C, Fiedler F, et al: Initial body mass indexes have contrary effects on change in body weight and mortality of patients on maintenance hemodialysis treatment. J Ren Nutr 2003;13:174–185.
11 Epstein M, Vaziri ND: Statins in the management of dyslipidemia associated with chronic kidney disease. Nat Rev Nephrol 2012;8:214–223.
12 Wanner C, Krane V, Marz W, Olschewski M, Mann JF, Ruf G, et al: Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. N Engl J Med 2005;353:238–248.
13 Fellstrom BC, Jardine AG, Schmieder RE, Holdaas H, Bannister K, Beutler J, et al: Rosuvastatin and cardiovascular events in patients undergoing hemodialysis. N Engl J Med 2009;360:1395–1407.
14 Baigent C, Landray MJ, Reith C, Emberson J, Wheeler DC, Tomson C, et al: The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. Lancet 2011;377:2181–2192.
15 Palmer SC, Craig JC, Navaneethan SD, Tonelli M, Pellegrini F, Strippoli GF: Benefits and harms of statin therapy for persons with chronic kidney disease: a systematic review and meta-analysis. Ann Intern Med 2012;157:263–275.
16 Hou W, Lv J, Perkovic V, Yang L, Zhao N, Jardine MJ, et al: Effect of statin therapy on cardiovascular and renal outcomes in patients with chronic kidney disease: a systematic review and meta-analysis. Eur Heart J 2013;34:1807–1817.
17 Borow KM, Nelson JR, Mason RP: Biologic plausibility, cellular effects, and molecular mechanisms of eicosapentaenoic acid (EPA) in atherosclerosis. Atherosclerosis 2015;242:357–366.
18 Vaziri ND: Oxidative stress in uremia: nature, mechanisms, and potential consequences. Semin Nephrol 2004;24:469–473.
19 Glass CK, Witztum JL: Atherosclerosis. The road ahead. Cell 2001;104:503–516.
20 Montezano AC, Touyz RM: Reactive oxygen species and endothelial function – role of nitric oxide synthase uncoupling and Nox family nicotinamide adenine dinucleotide phosphate oxidases. Basic Clin Pharmacol Toxicol 2012;110:87–94.
21 Vaziri ND, Navab K, Gollapudi P, Moradi H, Pahl MV, Barton CH, et al: Salutary effects of hemodialysis on low-density lipoprotein proinflammatory and high-density lipoprotein anti-inflammatory properties in patient with end-stage renal disease. J Natl Med Assoc 2011;103:524–533.
22 Mozaffarian D, Wu JH: Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. J Am Coll Cardiol 2011;58:2047–2067.
23 Wei MY, Jacobson TA: Effects of eicosapentaenoic acid versus docosahexaenoic acid on serum lipids: a systematic review and meta-analysis. Curr Atheroscler Rep 2011;13:474–483.
24 Lok CE, Moist L, Hemmelgarn BR, Tonelli M, Vazquez MA, Dorval M, et al: Effect of fish oil supplementation on graft patency and cardiovascular events among patients with new synthetic arteriovenous hemodialysis grafts: a randomized controlled trial. JAMA 2012;307:1809–1816.
25 Svensson M, Schmidt EB, Jorgensen KA, Christensen JH: N-3 fatty acids as secondary prevention against cardiovascular events in patients who undergo chronic hemodialysis: a randomized, placebo-controlled intervention trial. Clin J Am Soc Nephrol 2006;1:780–786.
26 Nasu M, Seino K, Tamura Y, Suzuki M, Iwabuchi Y, Maeda N, et al: Eicosapentaenoic acid restrains the development of the cardiovascular events independent of triglyceride and C-reactive protein reduction in Japanese hemodialysis patients (abstract P1428). Eur Heart J 2013;34(suppl 1):271.
27 Inoue T, Okano K, Tsuruta Y, Tsuruta Y, Tsuchiya K, Akiba T, et al: Eicosapentaenoic acid (EPA) decreases the all-cause mortality in hemodialysis patients. Intern Med 2015;54:3133–3137.
28 Umemoto N, Ishii H, Sakamoto T, Kamoi D, Aoyama T, Takahashi H, et al: Administration of eicosapentaenoic acid reduces cardiovascular and all-cause mortality in chronic hemodialysis patients (abstract P3753). Eur Heart J 2015;36(suppl 1):662.
29 Marrs JC, Saseen JJ: Effects of lipid-lowering therapy on reduction of cardiovascular events in patients with end-stage renal disease requiring hemodialysis. Pharmacotherapy 2010;30:823–829.
30 Friedman AN, Moe SM, Perkins SM, Li Y, Watkins BA: Fish consumption and omega-3 fatty acid status and determinants in long-term hemodialysis. Am J Kidney Dis 2006;47:1064–1071.
31 Madsen T, Christensen JH, Svensson M, Witt PM, Toft E, Schmidt EB: Marine n-3 polyunsaturated fatty acids in patients with end-stage renal failure and in subjects without kidney disease: a comparative study. J Ren Nutr 2011;21:169–175.
32 Nakamura N, Fujita T, Kumasaka R, Murakami R, Shimada M, Shimaya Y, et al: Serum lipid profile and plasma fatty acid composition in hemodialysis patients – comparison with chronic kidney disease patients. In Vivo 2008;22:609–611.
33 Nishimura M, Tokoro T, Nishida M, Hashimoto T, Kobayashi H, Yamazaki S, et al: Lower levels of serum eicosapentaenoic acid in hemodialysis patients under 60 years of age (abstract Sa599). NDT Plus 2010;3(suppl 3):iii244.
34 Umemoto N, Ishii H, Kamoi D, Aoyama T, Sakakibara T, Takahashi H, et al: Reverse association of omega-3/omega-6 polyunsaturated fatty acids ratios with carotid atherosclerosis in patients on hemodialysis. Atherosclerosis 2016;249:65–69.

35 Shoji T, Kakiya R, Hayashi T, Tsujimoto Y, Sonoda M, Shima H, et al: Serum n-3 and n-6 polyunsaturated fatty acid profile as an independent predictor of cardiovascular events in hemodialysis patients. Am J Kidney Dis 2013;62:568–576.
36 Inagaki H, Yokota N, Sato Y, Chiyotanda S, Fukami K, Fujimoto S: EPA ethyl ester increases EPA/AA ratio and decreases carboxylmethyl lysine in dialysis patients (abstract SP504). Nephrol Dial Transplant 2014;29(suppl 3):iii240.
37 Itakura H, Yokoyama M, Matsuzaki M, Saito Y, Origasa H, Ishikawa Y, et al: Relationships between plasma fatty acid composition and coronary artery disease. J Atheroscler Thromb 2011;18:99–107.
38 Ando M, Sanaka T, Nihei H: Eicosapentanoic acid reduces plasma levels of remnant lipoproteins and prevents in vivo peroxidation of LDL in dialysis patients. J Am Soc Nephrol 1999;10:2177–2184.
39 Hamazaki T, Nakazawa R, Tateno S, Shishido H, Isoda K, Hattori Y, et al: Effects of fish oil rich in eicosapentaenoic acid on serum lipid in hyperlipidemic hemodialysis patients. Kidney Int 1984;26:81–84.
40 Bays HE, Ballantyne CM, Kastelein JJ, Isaacsohn JL, Braeckman RA, Soni PN: Eicosapentaenoic acid ethyl ester (AMR101) therapy in patients with very high triglyceride levels (from the Multi-center, plAcebo-controlled, Randomized, double-blINd, 12-week study with an open-label Extension [MARINE] trial). Am J Cardiol 2011; 108:682–690.
41 Ballantyne CM, Bays HE, Kastelein JJ, Stein E, Isaacsohn JL, Braeckman RA, et al: Efficacy and safety of eicosapentaenoic acid ethyl ester (AMR101) therapy in statin-treated patients with persistent high triglycerides (from the ANCHOR study). Am J Cardiol 2012;110:984–992.
42 Ballantyne CM, Bays HE, Braeckman RA, Philip S, Stirtan WG, Doyle RT Jr, et al: Icosapent ethyl (eicosapentaenoic acid ethyl ester): effects on plasma apolipoprotein C-III levels in patients from the MARINE and ANCHOR studies. J Clin Lipidol 2016;10:635–645.
43 Ballantyne CM, Bays HE, Philip S, Doyle RTJ, Braeckman RA, Stirtan WG, et al: Icosapent ethyl (eicosapentaenoic acid ethyl ester): effects on remnant-like particle cholesterol from the MARINE and ANCHOR studies. Atherosclerosis 2016;253:81–87.
44 Bays HE, Ballantyne CM, Braeckman RA, Stirtan WG, Soni PN: Icosapent ethyl, a pure ethyl ester of eicosapentaenoic acid: effects on circulating markers of inflammation from the MARINE and ANCHOR studies. Am J Cardiovasc Drugs 2013;13:37–46.
45 Endo J, Arita M: Cardioprotective mechanism of omega-3 polyunsaturated fatty acids. J Cardiol 2016;67: 22–27.
46 Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A: Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am J Clin Nutr 2004;79:935–945.
47 Adkins Y, Kelley DS: Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. J Nutr Biochem 2010;21:781–792.
48 Kamada Y, Kamekawa D, Kamiya K, Shimizu R, Matsunaga A, Akiyama A, et al: Effects of serum eicosapentaenoic acid on vascular inflammation and endothelial dysfunction in Japanese hemodialysis patients (abstract 10140). Circulation 2011;124:A10140.
49 Sjoberg B, Qureshi AR, Anderstam B, Alvestrand A, Barany P: Pentraxin 3, a sensitive early marker of hemodialysis-induced inflammation. Blood Purif 2012;34:290–297.
50 Xu Y, Ding X, Zou J, Liu Z, Jiang S, Xu S, et al: Plasma pentraxin 3 is associated with cardiovascular disease in hemodialysis patients. Ren Fail 2011;33:998–1004.
51 El Sebai AA, El Hadidi ES, Abdel Al H, El Sayed EY: Pentraxin 3 in hemodialysis patients: relationship to co-morbidities. Basic Res J Med Clin Sci 2015;4:204–211.
52 Nishio R, Shinke T, Otake H, Nakagawa M, Nagoshi R, Inoue T, et al: Stabilizing effect of combined eicosapentaenoic acid and statin therapy on coronary thin-cap fibroatheroma. Atherosclerosis 2014;234:114–119.
53 Niki T, Wakatsuki T, Yamaguchi K, Taketani Y, Oeduka H, Kusunose K, et al: Effects of the addition of eicosapentaenoic acid to strong statin therapy on inflammatory cytokines and coronary plaque components assessed by integrated backscatter intravascular ultrasound. Circ J 2016;80:450–460.
54 Perunicic-Pekovic GB, Rasic ZR, Pljesa SI, Sobajic SS, Djuricic I, Maletic R, et al: Effect of n-3 fatty acids on nutritional status and inflammatory markers in haemodialysis patients. Nephrology (Carlton) 2007;12:331–336.
55 Satoh-Asahara N, Shimatsu A, Sasaki Y, Nakaoka H, Himeno A, Tochiya M, et al: Highly purified eicosapentaenoic acid increases interleukin-10 levels of peripheral blood monocytes in obese patients with dyslipidemia. Diabetes Care 2012;35:2631–2639.
56 Cawood AL, Ding R, Napper FL, Young RH, Williams JA, Ward MJ, et al: Eicosapentaenoic acid (EPA) from highly concentrated n-3 fatty acid ethyl esters is incorporated into advanced atherosclerotic plaques and higher plaque EPA is associated with decreased plaque inflammation and increased stability. Atherosclerosis 2010; 212:252–259.
57 Grenon SM, Aguado-Zuniga J, Hatton JP, Owens CD, Conte MS, Hughes-Fulford M: Effects of fatty acids on endothelial cells: inflammation and monocyte adhesion. J Surg Res 2012;177:e35–e43.
58 Yamada H, Yoshida M, Nakano Y, Suganami T, Satoh N, Mita T, et al: In vivo and in vitro inhibition of monocyte adhesion to endothelial cells and endothelial adhesion molecules by eicosapentaenoic acid. Arterioscler Thromb Vasc Biol 2008;28:2173–2179.
59 Ballantyne CM, Bays HE, Philip S, et al: Icosapent ethyl (eicosapentaenoic acid ethyl ester): effects on remnant-like particle cholesterol from the MARINE and ANCHOR studies. Atherosclerosis 2016;253:81–87.

60 Barden AE, Mas E, Mori TA: n-3 Fatty acid supplementation and proresolving mediators of inflammation. Curr Opin Lipidol 2016;27:26–32.
61 Serhan CN: Pro-resolving lipid mediators are leads for resolution physiology. Nature 2014;510:92–101.
62 Calder PC: Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance. Biochim Biophys Acta 2015;1851:469–484.
63 Gdula-Argasinska J, Czepiel J, Wozniakiewicz A, Wojton K, Grzywacz A, Wozniakiewicz M, et al: n-3 Fatty acids as resolvents of inflammation in the A549 cells. Pharmacol Rep 2015;67:610–615.
64 Barden A, Mas E, Croft KD, Phillips M, Mori TA: Short-term n-3 fatty acid supplementation but not aspirin increases plasma proresolving mediators of inflammation. J Lipid Res 2014;55:2401–2407.
65 Mas E, Barden A, Burke V, Beilin LJ, Watts GF, Huang RC, et al: A randomized controlled trial of the effects of n-3 fatty acids on resolvins in chronic kidney disease. Clin Nutr 2016;35:331–336.
66 Salic K, Morrison MC, Verschuren L, Wielinga PY, Wu L, Kleemann R, et al: Resolvin E1 attenuates atherosclerosis in absence of cholesterol-lowering effects and on top of atorvastatin. Atherosclerosis 2016;250:158–165.
67 Mason RP, Jacob R, Beauregard G, Rowe J: Comparative lipid antioxidant effects of omega-3 fatty acids in combination with HMG-CoA reductase inhibitors (abstract). J Clin Lipidol 2011;5:201.
68 Mason RP, Jacob RF: Eicosapentaenoic acid inhibits glucose-induced membrane cholesterol crystalline domain formation through a potent antioxidant mechanism. Biochim Biophys Acta 2015;1848:502–509.
69 Mason RP, Jacob RF: Eicosapentaenoic acid inhibited glucose-induced membrane lipid peroxidation and cholesterol crystalline domain formation (abstract 161). J Clin Lipidol 2014;8:343.
70 Mason RP, Jacob RF, Dawoud H, Wagner MR, Mahmud FJ, Sherratt SCR, et al: Eicosapentaenoic acid and atorvastatin active metabolite, alone or in combination, reversed glucose- and oxidized LDL-induced endothelial dysfunction measured ex vivo in rats (abstract). J Am Coll Cardiol 2016;67(suppl):2320.
71 Yamakawa K, Shimabukuro M, Higa N, Asahi T, Ohba K, Arasaki O, et al: Eicosapentaenoic acid supplementation changes fatty acid composition and corrects endothelial dysfunction in hyperlipidemic patients. Cardiol Res Pract 2012;2012:754181.
72 Sasaki J, Miwa T, Odawara M: Administration of highly purified eicosapentaenoic acid to statin-treated diabetic patients further improves vascular function. Endocr J 2012;59:297–304.
73 Toyama K, Nishioka T, Isshiki A, Ando T, Inoue Y, Kirimura M, et al: Eicosapentaenoic acid combined with optimal statin therapy improves endothelial dysfunction in patients with coronary artery disease. Cardiovasc Drugs Ther 2014;28:53–59.
74 Choy E, Ganeshalingam K, Semb AG, Szekanecz Z, Nurmohamed M: Cardiovascular risk in rheumatoid arthritis: recent advances in the understanding of the pivotal role of inflammation, risk predictors and the impact of treatment. Rheumatology (Oxford) 2014;53:2143–2154.
75 Levy L, Fautrel B, Barnetche T, Schaeverbeke T: Incidence and risk of fatal myocardial infarction and stroke events in rheumatoid arthritis patients. A systematic review of the literature. Clin Exp Rheumatol 2008;26:673–679.
76 Meune C, Touze E, Trinquart L, Allanore Y: High risk of clinical cardiovascular events in rheumatoid arthritis: levels of associations of myocardial infarction and stroke through a systematic review and meta-analysis. Arch Cardiovasc Dis 2010;103:253–261.
77 Zanni MV, Abbara S, Lo J, Wai B, Hark D, Marmarelis E, et al: Increased coronary atherosclerotic plaque vulnerability by coronary computed tomography angiography in HIV-infected men. AIDS 2013;27:1263–1272.
78 Andersen NN, Jess T: Risk of cardiovascular disease in inflammatory bowel disease. World J Gastrointest Pathophysiol 2014;5:359–365.
79 Mason RP, Sherratt SCR, Jacob RF: Eicosapentaenoic acid inhibits oxidation of ApoB-containing lipoprotein particles of different size in vitro when administered alone or in combination with atorvastatin active metabolite compared with other triglyceride-lowering agents. J Cardiovasc Pharmacol 2016;68:33–40.
80 Arterburn LM, Hall EB, Oken H: Distribution, interconversion, and dose response of n-3 fatty acids in humans. Am J Clin Nutr 2006;83(6 suppl):1467S–1476S.