Sevelamer prevents uremia-enhanced atherosclerosis progression in apolipoprotein E deficient (apoE-/-) mice
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Sevelamer prevents uremia-enhanced atherosclerosis progression in apolipoprotein E deficient (apoE⁻/⁻) mice

Short title: Sevelamer prevents atherosclerosis in uremic mice

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Abstract

**Background**—The novel phosphate binder sevelamer has been shown to prevent the progression of aortic and coronary calcification in uremic patients. Whether it also decreases the progression of atheromatous plaques is unknown. The aim of our study was to examine the effect of sevelamer administration on the development of atherosclerosis and aortic calcification in the uremic apoE⁻/⁻ mouse as an established model of accelerated atherosclerosis.

**Methods and Results**—Female mice were randomly assigned to 4 groups: 2 groups of non-uremic mice (sevelamer vs. control) and 2 groups of uremic mice (sevelamer vs. control). Sevelamer was given at 3% with chow. The increase in serum phosphorus concentration and calcium-phosphorus product observed in uremic control mice were prevented by sevelamer. Serum total cholesterol was increased in the 2 uremic mouse groups and remained unchanged in response to sevelamer. After 8 weeks of sevelamer treatment, uremic mice exhibited a significantly lower degree of atherosclerosis (p < 0.001) and vascular calcification, compared with uremic control mice. Of interest, sevelamer exerted an effect on both intima and media calcification (p = 0.005) in uremic mice. Among possible mechanisms involved, we found no evidence for the modulation by sevelamer of inflammation or selected uremic toxins. In contrast, nitrotyrosine staining as a measure of oxidative damage was significantly decreased in response to sevelamer treatment in control and uremic mice (p < 0.005).

**Conclusion**—Sevelamer delays not only vascular calcification, but also atherosclerotic lesion progression in uremic apoE⁻/⁻ mice. It opens the possibility of a
cholesterol-independent sevelamer action on atheroma formation, via effects on mineral metabolism, oxidative stress, or both.

**Key Words:** atherosclerosis, sevelamer hydrochloride, uremia, calcification, oxidative stress
Introduction

Chronic renal failure (CRF) is associated with numerous metabolic and endocrine disturbances, including abnormalities of calcium and phosphate metabolism and an inflammatory syndrome.\textsuperscript{1,2} The latter occur early in the course of renal failure and contribute to the development and progression of arteriosclerosis, atherosclerosis, and vascular calcification.\textsuperscript{3,4} After stratification for age, gender, race, and the presence or absence of diabetes cardiovascular mortality in dialysis patients is 10 to 20 times higher than in the general population.\textsuperscript{5}

Until recently, it seemed impossible to slow or even halt the progression of uremic arteriopathy and arterial calcification. The result of the prospective, randomized “Treat-To-Goal” study changed this view. It showed that it was possible to retard the progression of vascular calcification, based on electron-beam computerized tomography (EBCT) technique allowing a quantitative and reproductive assessment of calcium deposition in the vessel wall.\textsuperscript{6} In this study, the administration of the calcium-free and aluminum-free phosphate binder sevelamer hydrochloride to chronic hemodialysis patients over a time period of 12 months, effectively led to a significantly slower progression of aortic and carotid calcification than the administration of calcium-containing phosphate binders. However, the EBCT technique can not distinguish whether calcium deposits are localized in plaques (intima) or in the vascular media, respectively. Even more importantly, it also does not enable a concomitant assessment of the progression of atherosclerotic vessel wall lesions.

Therefore, we decided to address these questions more directly in a uremic mouse model of accelerated atherosclerosis \textsuperscript{7-9} and vascular calcification.\textsuperscript{9} We used one of
the most common models for the study of atherogenic mechanisms, namely the apoE<sup>−/−</sup> mouse. This genetically engineered animal generates atherosclerotic lesions within weeks after birth that exhibit features similar to those found in humans. We created CRF in these mice according to a recently established method and reported increased atheroma progression compared to non-CRF controls. In addition, uremia was associated with more extensive aortic calcium deposits, both in the media and the intima.

The goal of our study was to examine the effect of sevelamer administration on the development of atherosclerosis and aortic calcification in the above experimental mouse model of CRF.

**Methods**

**Animals**

Female apoE<sup>−/−</sup> mice were primarily obtained from Charles Rivers Breeding Laboratories (Wilmington, MA). All procedures were in accordance with NIH guidelines for the care and use of experimental animals (NIH publication No. 85-23). The mice were housed in polycarbonate cages in pathogen free, temperature controlled (25°C) facility, with a strict 12-hour light-dark cycle. The mice had free access to chow diet and water. The diet (Harlan Teklad Global Diet 2018, Harlan, United Kingdom) contained 18.9% protein, 6% fat, 1.01% calcium, 0.65% phosphorus and 1.54 IU/g vitamin D3.

**Experimental procedure and diet**
At the age of 8 weeks, mice were randomly assigned to one of the following 4 groups, with 12 animals in each group: 2 groups of non-CRF animals (sevelamer group vs. placebo group), and 2 groups of CRF animals (sevelamer CRF group vs. placebo CRF group). As previously described we used a two-step procedure to create uremia.\(^9\) Briefly, we applied cortical electrocautery to the right kidney through a 2-cm flank incision and performed left total nephrectomy through a similar incision 2 weeks later; control animals received sham operation that included decapsulation of both kidneys. Special care was taken to avoid damage to the adrenals. Blood samples were taken two weeks after nephrectomy, and animals of the CRF group with an urea level > 20 mM (normal mouse serum urea, \(\leq 12\) mM) were subsequently randomized to two CRF subgroups: one CRF subgroup was fed for 8 weeks on sevelamer-containing diet whereas the other CRF subgroup received placebo diet for the same period of time. Likewise, control non-CRF mice were fed on sevelamer or placebo-containing diets. Sevelamer was administered together with the diet as a 3% mix with animal chow. At the end of the study, each mouse was anesthetized with ketamin/xylasine anesthesia (100 mg/kg, 20 mg/kg) and whole blood was collected via cardiac puncture. The heart and aorta were dissected free down to the renal arteries and removed. For immunohistochemistry and aortic calcification, the heart with the aortic root was separated from more distal aorta as reported previously.\(^9\) The rest of the aorta was used for quantification of atherosclerotic lesions (see below).

**Serum biochemistry**

Serum urea, total cholesterol, phosphorus and calcium were measured using a Hitachi 917 autoanalyser (Roche, Meylan, France) and intact parathyroid hormone
(iPTH) was by enzyme immunoassay (Immunotopics, San Clemente, CA, USA) measured as reported previously.\textsuperscript{9}

For the analysis of circulating fetuin-A at day of sacrifice, mouse sera were fractionated on 10\% polyacrylamide gels, blotted to nitrocellulose and probed with polyclonal rabbit anti-mouse fetuin-A antibody at a dilution of 1:5000 in hybridization buffer (PBS with 5\% skim milk and 0.1\% Tween 20) for 1 hr at 37 °C. After washing the blots with PBS including 0.1\% Tween20, a secondary antibody conjugated with horseradish peroxidase (Vector Laboratories, Biovalley, Marne la Vallée, France) was added at a dilution of 1:5000 in hybridization buffer for 1 h at 37°C. Bound antibody was detected by chemiluminescence using luminol and X-ray film. Fluorographs were analysed using flatbed scanner and Multianalysis\textsuperscript{®} software package (Biorad, Munich, Germany). We performed dilutions of the serum in the range of 100-fold or 200-fold. All samples were measured on one day, using same blot with same antibodies and identical luminal to circumvent any inter-assay variability.

Analysis of uremic toxins was performed in placebo and sevelamer CRF mice and in placebo control non-CRF mice\textsuperscript{10}: uric acid, indoxyl sulfate, hippuric acid, indole acetic acid (IAA) and 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (cmpf). After deproteinization of the serum samples by heat denaturation, analyses were performed with reversed-phase high performance liquid chromatography (HPLC). Indoxyl sulfate and IAA were determined by fluorescence detection (excitation 280 nm, emission 340 nm). Uric acid, hippuric acid and cmpf were analyzed by UV detection at 254 nm. Calibration curves of the 5 compounds were used to calculate the concentrations in each sample.\textsuperscript{10}
We used enzyme immunoassay for the determination of mouse serum amyloid A (SAA) in mouse serum (ELISA kit MG45182, IBL Hamburg, Germany) to evaluate inflammation in CRF apoE⁻/⁻ mice.¹¹

**Quantification of atherosclerotic lesions**

Evaluation of the atherosclerotic plaque area was made by the “en face” method.¹² Briefly, the aortas were carefully freed of connective and adipose tissue under a dissection microscope, opened longitudinally and stained with Oil red O. The quantification was made with a Histolab software (Microvision Instruments, Evry, France) as reported previously.⁹ The extent of atherosclerosis was expressed as the percent of surface area of the aorta covered by lesions.¹²

**Quantitative and qualitative evaluation of aorta calcification**

We performed von Kossa staining in cryo-sections of aortic tissue to evaluate calcium deposits inside and outside atheromatous plaques, respectively. These locations of calcification are supposed to reflect intima and media calcification. The precision and the accuracy of this method have been reported elsewhere with a semi-automated measurement software.⁹,¹³ Data were expressed as the relative proportion of calcified area to total surface area of either atherosclerotic lesions or vessel area outside atheromatous plaques as reported previously.⁹

**Quantification of nitrotyrosine, monocyte-macrophage (MOMA) infiltration and collagen in aortic root lesions**

Lesion nitrotyrosine expression, MOMA infiltration and collagen content were assessed as described previously.¹⁴ Briefly, for nitrotyrosine analysis the sections
were preincubated in peroxidase blocking solution (Dako Cytomation, Trappes, France) before incubation with biotinylated nitrotyrosine monoclonal mouse antibody (Cayman Chemical, SpiBio, Massy, France). The sections were treated with peroxidase-labeled streptavidin (Dako) for 15 min followed by reaction with diaminobenzidine/hydrogen peroxidase. For MOMA infiltration, aortic sections were incubated with 10% normal goat serum at room temperature, and incubated with a primary rat monoclonal antibody against mouse macrophages (clone MOMA-2; BioSource International, Camarillo, CA). The secondary antibody was a biotin-horseradish peroxidase-conjugated goat anti-rat IgG (Vector Laboratories, Biovalley, Marne la Vallée, France). Immunostainings were visualized after incubation with a peroxidase detection system (Vectastain ABC kit, Vector Laboratories) using 3-amino 9-ethyl carbazole (Sigma Aldrich) as substrate. The lesion collagen content was determined by staining with Sirius red.

**Statistical analysis**

Data were analyzed by analysis of variance (ANOVA) taking in account one or two factors (treatment and uremic state) as appropriate, and chi square. Results were expressed as means ± SEM. Differences between groups were considered significant when p < 0.05.

**Results**

**Serum biochemistry**

At sacrifice (10 weeks of uremia), serum urea concentration was significantly increased in CRF mice compared with non-CRF mice (Table 1). Serum urea concentration was slightly higher in CRF mice treated with sevelamer than in CRF
mice receiving placebo diet (Table 1). The body weight of CRF mice was 10% lower than that of non-uremic mice; no difference in body weight was observed between the 2 uremic groups (Table 1).

Total serum cholesterol was 24% higher in CRF mice than in non-CRF mice (Table 1). However, no difference was observed between the sevelamer-fed and the placebo-fed CRF groups. Sevelamer was effective in decreasing serum phosphorus, the calcium-phosphorus (Ca x P) product and iPTH concentration in uremic mice (Table 1). Western-blot analysis of serum fetuin-A did not show a significant change in response to uremia or sevelamer treatment (data not shown). SAA and serum uremic toxin concentrations were significantly increased in CRF mice (except for uric acid, IAA, and cmpf). Sevelamer treatment did not lead to a change of SAA or uremic toxin concentrations in CRF sevelamer mice (Figure 1).

Quantification of atherosclerotic lesions
At sacrifice, the thoracic aorta lesion area (%) of apoE⁻/⁻ mice on placebo diet increased 2-fold compared with non-uremic control littermates on placebo diet. The administration of sevelamer led to a decrease in aortic lesion area in uremic mice down to the level of non-uremic controls fed a placebo diet (Figure 2, 3). Figure 3 shows a typical feature of thoracic aorta plaques in CRF mouse with or without sevelamer treatment. Sevelamer did not modify aortic lesion area in non-uremic mice.

Quantification of aorta calcification
Uremic apoE⁻/⁻ mice on placebo diet exhibited a marked increase in aortic intima (Figure 4), media (Figure 5) and both combined (Figure 6), compared to non-uremic
control mice, and the dramatic decrease in the progression of type of calcification in response to sevelamer, down to the level of non-uremic mice fed on placebo diet. Figure 7 shows calcium deposits in characteristic feature of CRF mouse aorta with or without sevelamer treatment. Interestingly, the prevention of the progression of intima calcification was more effective than that of media calcification. No effect of sevelamer on aorta calcification was observed in non-uremic mice.

**Quantification of nitrotyrosine expression, monocyte-macrophage (MOMA) infiltration and collagen in aortic lesions**

Sevelamer treatment led to a highly significant reduction in nitrotyrosine expression in atheromatous plaques in uremic as well as in non-uremic mice (Table 2) (by Fisher's Exact Test, $p < 0.005$). The percentage of cross-sectional lesion area occupied by macrophages, as revealed by MOMA-2 staining, was comparable for the four groups (by ANOVA, effect of uremia on MOMA staining, $p = \text{NS}$; effect of sevelamer on MOMA staining, $p = 0.051$; interaction, $p = \text{NS}$). Aortic collagen content was markedly increased in uremic mice on placebo diet, as compared to non-uremic control mice. However, sevelamer treatment did not lead to a change of collagen content in either non-uremic or uremic mice (by Anova, effect of uremia on collagen deposition, $p = 0.001$; effect of sevelamer on collagen deposition, $p = \text{NS}$; interaction, $p = \text{NS}$).

**DISCUSSION**
The present study shows for the first time that the phosphate binder sevelamer is capable of preventing the uremia-enhanced atherosclerosis progression in apoE−/− mice.

This effect was observed in the absence of a change in serum total cholesterol levels. In addition, our study confirms sevelamer’s inhibitory effect on the progression of vascular calcification, as reported in end-stage renal disease patients, and shows for the first time an effect on both intima and media calcification. The fact that the observed beneficial effect of sevelamer was apparently cholesterol-independent was unexpected, since this drug not only binds phosphate, but also cholesterol in the intestinal lumen and thereby exerts cholesterol-lowering effects, at least in humans. The fact that serum total cholesterol remained unchanged in uremic apoE−/− mice in response to sevelamer treatment does however not exclude possible changes of LDL and/or HDL cholesterol concentrations, which are abnormal in uremic animals.

In CRF mice serum VLDL, IDL, and LDL cholesterol concentrations were increased compared with non-CRF mice, whereas HDL cholesterol remained the same in the two groups. Unfortunately, we were unable to perform serum lipoprotein determinations in the mice of this study due to insufficient availability of blood at the time of sacrifice. Alternatively, one could also envisage mechanisms other than a reduction of lipoprotein cholesterol subfractions in the observed anti-atherosclerotic effect of sevelamer. The observation that its administration did not lead to changes in atheromatous lesion progression in non-uremic apoE−/− mice might point to a beneficial action on the process of the uremia-linked acceleration of atherosclerosis. An interference with the enhanced oxidative stress and/or the inflammatory state of CRF represents possible alternative mechanisms.
Oxidative stress is being increasingly suggested to play a central role in the pathogenesis of cardiovascular disease in uremia.\textsuperscript{1,6} Others as well as our group have shown a high expression of nitrotyrosine as a marker of oxidative stress in atheromatous lesions of uremic apoE\textsuperscript{\textminus} mice.\textsuperscript{7,8,14} Recently, we observed that the administration of the antioxidant N-acetylcysteine led to a reduction of atheromatous lesion progression in uremic apoE\textsuperscript{\textminus} mice.\textsuperscript{14} This reduction was associated with a decrease of nitrotyrosine expression in the aortic lesions. In the present study, a significant reduction of atherosclerotic plaque nitrotyrosine staining was also observed in response to sevelamer treatment in both CRF and control mice, compared with placebo treatment. The mechanisms by which sevelamer might modify oxidative stress or whether it indirectly influences local iNOS activity in the vessel wall remain to be defined.\textsuperscript{17}

As to the possible implication of inflammation, we wondered whether fetuin-A deficiency might be involved. Fetuin-A is associated with inflammation and links vascular calcification to mortality in patients on dialysis. Activated acute-phase response and fetuin-A also may account for accelerated atherosclerosis in uremia.\textsuperscript{18,19} However, in the present mouse model western-blot analysis of serum fetuin-A did not show significant changes in response to uremia and/or sevelamer. Fetuin-A may not play the same role in mice as in humans. It is also of note that sevelamer treatment did not decrease serum SAA, which is a major acute–phase protein in vertebrates and has been associated with atherosclerosis in mice\textsuperscript{20}, and that it did not modify vascular infiltration by inflammatory monocytes/macrophages. Considering these negative findings, the beneficial effect of sevelamer on atherosclerosis progression and vascular calcification in apoE\textsuperscript{\textminus} mouse model seemed to be independent from an anti-inflammatory effect. Since post-hoc analyses
of the “Treat-To-Goal” study showed that sevelamer-treated dialysis patients experienced a relative reduction in highly sensitive C-reactive protein\textsuperscript{21}, it remains unclear to which extent sevelamer’s beneficial effects on atherosclerosis and calcium deposition in mice involves arterial wall inflammation or not. Hyperphosphatemia and an increased Ca x P product are recognized factors of cardiovascular morbidity and mortality in advanced CRF.\textsuperscript{22} To what extent high serum phosphorus, calcium, or Ca x P product contribute to uremic arteriopathy, and in particular to the accelerated atherosclerosis of uremic apoE\textsuperscript{−/−} mice, in addition to the induction of marked vascular calcification remains to be evaluated. In uremic apoE\textsuperscript{−/−} mice treated by sevelamer, the serum phosphorus concentration was well controlled as compared to placebo-treated uremic mice, as was the Ca x P product and iPTH. Since the rapid progression of vascular calcification was inhibited by sevelamer in CRF apoE\textsuperscript{−/−} mice in association with the reduced progression of atherosclerosis one could think of a possible link between these two processes. We observed a more marked effect of sevelamer on intima vascular calcification compared with media calcification. Together with the disturbances in lipid metabolism and inflammation, calcification is now generally recognized as an integral part of the atherosclerotic process. The majority of atheromatous lesions in CRF patients are calcified, much more frequently than in general population\textsuperscript{23,24}, and this may be an aggravating factor in lesion progression. Whether the slowed progression of arterial calcification in dialysis patients treated by sevelamer\textsuperscript{6} was mainly due to reduced intima calcification, media calcification, or both cannot be determined based on EBCT imaging technique. In the uremic apoE\textsuperscript{−/−} mice of the present study, calcium deposition at both vascular sites was decreased by sevelamer and especially inside atheromatous lesions. Since serum calcium, which was elevated in uremic placebo-
treated mice, remained unchanged in sevelamer-treated mice the beneficial effect of sevelamer on vascular calcification could be explained at least partially by the better control of phosphorus whose serum concentration decreased. Of note, high phosphorus concentrations have been shown to induce the expression of osteoblast-specific proteins in vascular smooth muscle cells in vitro and to promote the deposition of apatite into extracellular matrix.\textsuperscript{25-27} The clinical relevance of these in vitro data could recently be confirmed in arterial media calcifications of dialysis patients.\textsuperscript{28} Whether these or similar effects play a role in atheroma calcification and perhaps even progression as well is unclear.

Other mechanisms explored in the present study include sevelamer effects on uremic toxins. Interestingly, it was shown that sevelamer could adsorb partially lipophilic uremic compounds such as indoxyl sulfate in the intestinal lumen.\textsuperscript{29} Recently, indoxyl sulfate-induced endothelial toxicity has been reported.\textsuperscript{30} In the present study, we failed to observe a reduction of the serum concentration of four different uremic toxins in response to sevelamer in CRF apoE\textsuperscript{-/-} mice. Of note, serum uric acid was not elevated in CRF mice. Moreover, sevelamer did not decrease it, in contrast to its effect in uremic patients.\textsuperscript{31} This discrepancy is probably due to the fact that rodents, but not man, are equipped with uricase activity. Since there is a substantial number of defined and as yet undefined uremic toxin molecules\textsuperscript{32}, it remains possible that sevelamer exerts beneficial effects on other uremic toxins.

In conclusion, sevelamer prevents not only vascular calcification, but also atherosclerotic lesion progression in uremic apoE\textsuperscript{-/-} mice. The observed reduction of atherosclerosis progression in the absence of changes of serum total cholesterol in uremic mice may be of both theoretical and practical importance. It opens the
possibility of a cholesterol-independent sevelamer action on atheroma formation, possibly via effects on mineral metabolism or oxidative stress. It may thus be of major importance for patients with CKD if the findings obtained in mice can be extrapolated to the condition in human beings.

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REFERENCES


Figure legends

Figure 1
Serum uremic toxins and serum A amyloid (SAA) concentrations in apoE−/− mice without chronic renal failure (non-CRF), with CRF, and with CRF plus sevelamer treatment (CRF-Sev), respectively (n = 7-9 animals per group for uremic toxins analysis, n = 5 animals in the CRF-Sev group for SAA analysis). Indoxyl sulfate, hippuric acid, and SAA levels were increased in CRF apoE−/− mice, compared with control apoE−/− mice (p < 0.05 by ANOVA). In contrast, uric acid, indole acetic acid, and 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (cmpf) levels were similar in CRF and control apoE−/− mice. Sevelamer treatment did not modify any of these parameters (p = NS).

Figure 2
Atherosclerotic lesion cross-section area in thoracic aorta in apoE−/− mice without chronic renal failure (non-CRF), with CRF. By ANOVA, effect of uremia on atherosclerosis progression, p = 0.02; effect of sevelamer on atherosclerosis prevention, p < 0.001; interaction, p = 0.03 (n = 7-11 per group).

Figure 3
Representative images of regression of atherosclerotic lesions in thoracic aorta in control apoE−/− mice with chronic renal failure (CRF) and CRF sevelamer treated apoE−/− mice (CRF sevelamer ). Magnification (x2.5) of the object after 2% eosin staining (n = 7-11 per group).
**Figure 4**
Proportion of intima calcified area to total surface area of atherosclerotic lesions in apoE\(^{-/-}\) mice without chronic renal failure (non-CRF) and CRF apoE\(^{-/-}\) mice. By ANOVA, effect of uremia on calcification progression, \(p = 0.03\); effect of sevelamer on calcification prevention, \(p = 0.002\); interaction, \(p = 0.003\) (\(n = 7-11\) per group).

**Figure 5**
Proportion of media calcified area to total surface area outside of atherosclerotic lesions in apoE\(^{-/-}\) mice without chronic renal failure (non-CRF) and CRF apoE\(^{-/-}\) mice. By ANOVA, effect of uremia on calcification progression, \(p = \text{NS}\); effect of sevelamer on calcification prevention, \(p = \text{NS}\); interaction, \(p < 0.05\) (\(n = 7-11\) per group).

**Figure 6**
Proportion of intima and media calcified area together to total surface area in apoE\(^{-/-}\) mice without chronic renal failure (non-CRF) and CRF apoE\(^{-/-}\) mice. By ANOVA, effect of uremia on calcification progression, \(p < 0.05\); effect of sevelamer on calcification prevention, \(p = 0.005\); interaction, \(p = 0.004\) (\(n = 7-11\) per group).

**Figure 7**
Representative images of regression of vascular calcification in control apoE\(^{-/-}\) mice with chronic renal failure (CRF control) and in apoE\(^{-/-}\) mice with chronic renal failure treated with sevelamer (CRF sevelamer). Magnification (x2.5) of the object after 2% eosin and von Kossa silver nitrate staining (calcification in black). M, media; P, plaque; L, lumen.
Table 1. Effect of CRF and sevelamer on body weight and serum biochemistry

<table>
<thead>
<tr>
<th></th>
<th>Non-CRF control</th>
<th>Non-CRF sevelamer</th>
<th>CRF control</th>
<th>CRF sevelamer</th>
<th>Effect of CRF / sevelamer / interaction</th>
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</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>24.9 ± 0.84</td>
<td>25.3 ± 0.86</td>
<td>22.9 ± 0.30</td>
<td>22.4 ± 0.45</td>
<td>p &lt; 0.005 / NS / NS</td>
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<td>Urea (mM)</td>
<td>8.24 ± 0.39</td>
<td>8.70 ± 0.35</td>
<td>27.2 ± 1.30</td>
<td>30.1 ± 1.18</td>
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<td>Calcium (mM)</td>
<td>2.34 ± 0.03</td>
<td>2.37 ± 0.03</td>
<td>2.57 ± 0.04</td>
<td>2.57 ± 0.02</td>
<td>p &lt; 0.0001 / NS / NS</td>
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<tr>
<td>Phosphorus (mM)</td>
<td>1.95 ± 0.09</td>
<td>1.80 ± 0.11</td>
<td>2.23 ± 0.12</td>
<td>1.63 ± 0.08</td>
<td>p = NS / &lt; 0.001 / &lt; 0.05</td>
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<tr>
<td>Ca X P (mM²)</td>
<td>4.51 ± 0.22</td>
<td>4.27 ± 0.28</td>
<td>5.66 ± 0.36</td>
<td>4.12 ± 0.22</td>
<td>p = NS / &lt; 0.05 / &lt; 0.05</td>
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<tr>
<td>iPTH (µg/mL)</td>
<td>48.1 ± 12.2</td>
<td>44.1 ± 13.8</td>
<td>124 ± 29.3</td>
<td>37.2 ± 4.26</td>
<td>p = 0.05 / &lt; 0.005 / &lt; 0.01</td>
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<td>Total cholesterol (mM)</td>
<td>11.1 ± 0.50</td>
<td>9.02 ± 0.49</td>
<td>14.5 ± 0.52</td>
<td>14.40 ± 0.82</td>
<td>p &lt; 0.0001 / NS / NS</td>
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Values at sacrifice (n = 6-12 per group). CRF, chronic renal failure; iPTH, intact parathyroid hormone; Ca X P, calcium phosphate X product. Data were analysed by analysis of variance (ANOVA) taking in account two factors (CRF state and sevelamer treatment).
Table 2. Anti-oxidative sevelamer-dependent decrease in nitrotyrosine expression in plaques of non-chronic renal failure (CRF) apolipoprotein E<sup>-/-</sup> mice

<table>
<thead>
<tr>
<th></th>
<th>Non-CRF control</th>
<th>Non-CRF sevelamer</th>
<th>CRF control</th>
<th>CRF sevelamer</th>
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<tr>
<td>Percentage of</td>
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<tr>
<td>animals with</td>
<td>64 %&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0 %</td>
<td>94 %&lt;sup&gt;†&lt;/sup&gt;</td>
<td>37 %</td>
<td>&lt;0.005</td>
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<td>positive nitro-</td>
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<td>tyrosine staining</td>
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(n = 6-12 per group). Data were analyzed by Fisher's Exact Test for the global evaluation.

* p = 0.0013 vs non CRF sevelamer, † p = 0.0007 vs CRF sevelamer
Figure 1
Figure 2

Aortic lesion area (%) vs CRF and Non-CRF conditions with placebo and sevelamer treatment.

- **Placebo**
  - Non-CRF: 0.8%
  - CRF: 1.8%

- **Sevelamer**
  - Non-CRF: 0.6%
  - CRF: 1.4%

Legend:
- □: placebo
- ■: sevelamer
Regression of atherosclerotic lesions in thoracic aorta

CRF control

CRF sevelamer

Figure 3
Figure 4

Intima calcification lesion area (%)
Figure 5
Intima and media calcification lesion area (%)

Figure 6
Regression of calcification

CRF control

CRF sevelamer

Figure 7