

Changes in oxidative nucleic acid modifications and inflammation following one-week treatment with the bile acid sequestrant sevelamer: Two randomised, placebo-controlled trials

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ABSTRACT

Aims: Sevelamer has been reported to have anti-oxidative and anti-inflammatory effects as well as effects on glycaemic control and plasma lipids. The aim of this study was to determine the effects of one-week treatment with sevelamer on oxidative nucleic acid modifications and inflammation markers.

Methods: Two double-blinded studies including 30 patients with type 2 diabetes (T2D) and 20 healthy individuals were conducted. Participants were randomised to one week of treatment with sevelamer (1600 mg three times daily) or placebo. RNA and DNA oxidation, measured by urinary excretion of 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and (8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), and markers of inflammation were determined before and after the intervention.

Results: In patients with T2D there was no significant placebo-corrected reduction in 8-oxoGuo or 8-oxodG. However, a reduction in 8-oxoGuo was observed within the group treated with sevelamer (Δ 8-oxoGuo/creatinine (median[IQR]): -0.04 [-0.24 ; 0.01] nmol/mmol, $p = 0.02$). A sevelamer-mediated reduction in interleukin-2 ($p = 0.04$) and a trend towards reduction in interleukin-6 ($p = 0.053$) were found in patients with T2D.

Conclusions: This study reveals a potential effect of sevelamer treatment on inflammation and possible oxidative RNA modifications. The potential protective effects of sevelamer in terms of cardiovascular disease in patients with T2D need further investigation.

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1. Introduction

Sevelamer is a non-absorbable, amine-based resin used in patients with chronic kidney disease for the treatment of hyperphosphatemia. Maximum recommended dose is 1600 mg three times a day. This amine-based polymer is highly protonated at physiological pH, which allows binding and increased faecal excretion of negatively charged phosphate.¹ In addition, sevelamer has been reported to reduce plasma cholesterol and to elicit improvement of glycaemic control in patients with type 2 diabetes (T2D).^{2,3} In vitro studies have demonstrated a marked bile acid-sequestering potential of sevelamer, which might

explain these additional effects of sevelamer on lipid and glucose metabolism.⁴ The current study involving patients with T2D has also investigated the effects of sevelamer on plasma lipids and glycemia. These results have previously been reported elsewhere.³ In brief, the seven-day treatment course with sevelamer in patients with T2D caused clinically significant placebo-corrected reductions in fasting plasma glucose as well as postprandial glucose excursions as measured by AUC_{240 min}, whereas no placebo-corrected changes in plasma glucose concentrations were observed in healthy subjects treated with sevelamer. In addition, sevelamer treatment elicited reductions in fasting plasma LDL compared to placebo in patients with T2D and healthy individuals. In line with previous studies reporting an altered and less diverse gut microbiota in subjects with metabolic syndrome and in patients with T2D,^{5,6} we observed significant differences in baseline species richness and overall composition when comparing baseline microbiota of patients with T2D with the microbiota of healthy subjects. However,

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sevelamer treatment elicited no significant changes in gut microbiota species richness in patients with T2D or healthy subjects, as measured by phylogenetic diversity and number of operational taxonomic units. All reported adverse events were mild and well described in relation to treatment with sevelamer carbonate. Exclusively gastro-intestinal adverse events were observed with the vast majority concerning changes in stool consistency. The data on adverse events from the study including patients with T2D have previously been reported.³ In these patients, no difference in incidence of events was evident between subjects treated with sevelamer (5/20) and those receiving placebo (3/10). More than half (7/12) of sevelamer treated healthy subjects reported adverse events, whereas no events were observed after treatment with placebo in this group.

In addition to the lipid and glucose lowering effects following sevelamer treatment, anti-oxidative and anti-inflammatory effects in combination with a reduction in plasma concentration of advanced glycation end products (AGEs) have been shown.² Studies in patients with chronic kidney disease showed reductions in lipid peroxidation (measured as 8-isoprostanes), inflammation (measured as tumor necrosis factor alpha (TNF α)), and AGEs (measured as *N*-carboxymethyl-lysine (CML)) following treatment with sevelamer compared to calcium carbonate treatment.^{2,7} The potential anti-inflammatory and anti-oxidative effects of sevelamer have been hypothesized to be mediated through a binding and prevented absorption of AGEs in the intestinal tract.² AGEs modify intracellular signalling and increase the production of reactive oxygen species (ROS) and inflammatory cytokines.⁸ Along these lines, reduction of dietary AGEs has been found to mimic the results of sevelamer treatment on oxidative stress and inflammation.⁹

Increased risk of cardiovascular disease remains a major challenge in the treatment and care of patients with T2D.¹⁰ We have previously suggested reduction of oxidative RNA modifications as a potential new treatment target in patients with T2D.^{11,12} Whole-body RNA oxidation can be estimated by measurement of urinary excretion of 8-oxo-7,8-dihydroguanosine (8-oxoGuo),¹³ which has been demonstrated as a prognostic factor of all-cause and cardiovascular mortality in patients with T2D.^{12,14,15} Furthermore, changes in oxidative RNA modifications are associated with changes in all-cause mortality hazard ratio in this patient group.¹⁵ Historically, oxidative DNA modifications has attracted the predominant focus, but no association between oxidative DNA modifications and mortality in patients with T2D has been established.^{12,14,15} However, a study has reported slightly higher levels of urinary excretion of the oxidative DNA modifications marker 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in patients with T2D and complications compared to patients without complications among certain age groups.¹⁶ A recent study confirmed an association between oxidative nucleic acid modifications, measured in plasma using the ELISA method, and mortality in patients with T2D.¹⁷

The effects of sevelamer on both glycemia and plasma lipids suggest a treatment potential in patients with T2D¹⁸ – additional potential effects on oxidative stress and inflammation might make this treatment option even more relevant. Thus, the aim of this study was to determine the effect of one-week treatment with sevelamer on urinary excretion of 8-oxoGuo and 8-oxodG in patients with T2D and healthy individuals. In addition, this study determined the effect of sevelamer treatment on inflammatory markers and examined the potential correlation between these markers and oxidative nucleic acid modifications.

2. Materials and methods

2.1. Study design

In these double-blinded, randomised, placebo-controlled trials with parallel-groups, we investigated the effect of sevelamer on oxidative stress and inflammation markers in 30 patients with T2D and 20 healthy individuals with normal glucose tolerance. Randomisation

was performed using [random.org](https://www.random.org). Thus, investigators and participants were blinded with respect to intervention groups throughout the study. Participants were randomised to seven days of treatment with either sevelamer 1600 mg three times daily (20/12 patients with T2D and healthy subjects, respectively) or placebo (10/8 patients with T2D and healthy subjects, respectively). Maximum recommended dose of sevelamer was applied in order to obtain the largest possible treatment effect in terms of bile acid sequestration. Two identical study days including standardized 4-h liquid meal tests were performed for each participant. The meal tests were carried out after administration of the first dose of study drug and again on the seventh day of treatment. A study diary was used for monitoring study drug compliance and patients with T2D were instructed to pause ongoing metformin treatment seven days prior to the first study day and throughout the treatment course.

As previously described,³ participants arrived at the study centre on both study days after an overnight absolute fast and were instructed to fully empty their bladder before initiation of the meal test. Baseline blood samples for measurements of inflammatory markers were drawn after a 10-min rest and heat-up of the hand. At time 0 min, sevelamer or placebo powder (1600 mg) and a liquid mixed meal (Danone, Paris, France) comprising 1260 kJ (36.8 g carbohydrate, 11.6 g protein and 12.0 g of lipid) were ingested. Following the 4-h study day, the participants were instructed to perform a urine sample, which was collected and stored at -20°C until analysis. Urine concentration of 8-oxodG has been found stable (within 10% of original measured concentration) for up to 15 years stored at -20°C .¹⁹ The primary objectives of the study including patients with T2D have been published elsewhere.³

2.2. Biochemistry analyses

Interferon gamma (IFN- γ), tumor necrosis factor alpha (TNF α), interleukin-2 (IL-2), IL-6, IL-8, and IL-10 were measured by the V-PLEX Proinflammatory Panel 1 (human) Kit (Meso Scale Diagnostics, Rockville, Maryland, USA). hsCRP was analysed using the Cobas® 6000 analyser (Roche, Basel, Switzerland). sCD163 was measured by in-house sandwich ELISA as described elsewhere.²⁰ Urinary excretion of 8-oxoGuo and 8-oxodG was determined at the Laboratory of Clinical Pharmacology, Bispebjerg-Frederiksberg Hospital – Rigshospitalet, using ultra-performance liquid chromatography tandem mass-spectrometry (UPLC-MS/MS). The UPLC-MS/MS method has been described elsewhere.²¹ Measurements of oxidized nucleosides were adjusted to urinary flow using urine creatinine.

The study was performed in a specialized research facility at Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Gentofte Hospital, after approval from the Ethics Committee of the Capital Region of Denmark (reg. no. H-2-2013-148) and registration at [ClinicalTrials.gov](https://www.clinicaltrials.gov) (ID: NCT02061124). Written informed consent was received from participants prior to inclusion in the study, which was conducted in accordance with the principles of the Declaration of Helsinki (7th Revision, 2013).

2.3. Statistical analysis

Results are reported as medians with interquartile range (IQR) unless otherwise stated. Comparisons between delta values for placebo and sevelamer-treated groups have been performed using unpaired *t*-test for normally distributed data or Mann-Whitney *U* test in cases of non-Gaussian distribution of data. In addition, assessment of changes during the one-week treatment course within the different groups of patients with T2D or healthy individuals were carried out using one sample *t*-test for normally distributed data or Wilcoxon signed rank test in cases of non-Gaussian distribution of data. A two-way ANOVA test for interaction between sex and treatment was conducted to investigate the effect of the numeric difference in sex distribution in patients

with type 2 diabetes. Correlation between oxidative stress and inflammation markers was evaluated using Pearson correlation. Statistical analyses and graphs have been performed in R version 3.4.1.²² Differences with a two-tailed P value <0.05 were considered statistically significant. No adjustments for multiple comparisons have been performed for analyses of the parameters outlined above.

The sample size was calculated with plasma glucagon-like peptide 1 area under curve as the main outcome, as reported elsewhere.³ However, in previous studies, small sample sizes (i.e. $n = 30$ (15 in each group)) has been shown sufficient to detect changes in urinary excretion of oxidative nucleic acid modifications.²³

3. Results

3.1. Study participants

The baseline characteristics demonstrated similar distributions of age, disease duration (for patients with T2D), fasting plasma glucose, glycated haemoglobin A1c (HbA1c), and body mass-index (BMI) in groups treated with sevelamer or placebo, which was the case in both the study including patients with T2D and the study including healthy individuals with normal glucose tolerance (Table 1). All participants completed the study and were included in the analyses. No changes in ongoing pharmacologic treatment were made for any of the participants during the study period. The baseline characteristics of the patients with T2D have been published previously.³ The most common background treatment in participants with diabetes included statins (simvastatin, atorvastatin), antihypertensive agents (ACE-inhibitors, AT2 receptor antagonist, calcium-channel blockers, diuretics and beta-blockers) and acetylsalicylic acid that are all commonly used drugs in patients with T2D. Overall, no relevant differences in background treatment were evident between the treatment groups. Subjects treated with glucose-lowering treatments other than metformin were excluded from the study, which should imply a relatively uncomplicated T2D study population. Along these lines, only a single participant had established cardiovascular disease. Relevant exclusion criteria included liver disease or history of hepatobiliary disorder, gastrointestinal disease, previous intestinal resection, cholecystectomy or any major intra-abdominal surgery, nephropathy, hypo- or hyperthyroidism, active or recent malignant disease, antibiotic treatment within previous six months prior to inclusion and tobacco smoking.³

3.2. Baseline levels of oxidative stress and inflammation

The patients with T2D presented a trend towards statistically significant higher baseline excretion rate of urine 8-oxoGuo/creatinine compared to healthy individuals (median [IQR]: 1.59 [1.35; 1.78] vs. 1.33 [1.20; 1.55] nmol/mmol, $p = 0.05$), but similar baseline concentration of urine 8-oxodG (median [IQR]: 1.03 [0.80; 1.37] vs. 0.91 [0.73; 1.23] nmol/mmol, $p = 0.44$) (Fig. 1A+1B). At baseline, patients with T2D compared to healthy individuals presented with higher concentrations

of TNF α (median [IQR]) 2.51 [1.96; 2.82] vs. 2.03 [1.59; 2.24] pg/ml, $p = 0.045$) and IL-8 (median [IQR]: 12.90 [10.09; 17.80] vs. 10.20 [8.38; 12.30] pg/ml, $p = 0.02$), a tendency towards higher concentrations of sCD163 (median [IQR]: 1.85 [1.47; 2.57] vs. 1.59 [1.40; 1.98] mg/l, $p = 0.06$), and lower concentrations of IL-6 (median [IQR]: 0.68 [0.49; 0.83] vs. 0.85 [0.68; 1.09] pg/ml, $p = 0.01$). The additional analysed inflammation markers (i.e. hsCRP, IFN- γ , IL-2, and IL-10) demonstrated no differences between patients with T2D and healthy individuals.

3.3. Sevelamer and changes in oxidative stress and inflammation

We found no significant reduction of 8-oxoGuo/creatinine in the patients with T2D treated with sevelamer compared to placebo (Δ 8-oxoGuo/creatinine (median [IQR]: -0.04 [-0.24 ; 0.01] vs. 0.02 [-0.07 ; 0.06] nmol/mmol, $p = 0.11$), and in addition, no difference in 8-oxodG/creatinine was evident (Table 2). However, a significant reduction in 8-oxoGuo/creatinine (Δ median[IQR]: -0.04 [-0.24 ; 0.01], $p = 0.02$) and a marginal tendency towards reduction in 8-oxodG/creatinine (Δ median[IQR]: -0.05 [-0.10 ; 0.05], $p = 0.09$) were observed within the group of patients with T2D treated with sevelamer (Fig. 2 and Table 2). No effects of treatment with sevelamer on 8-oxoGuo or 8-oxodG were observed between or within the groups of healthy individuals (Fig. 2 and Table 3).

Treatment with sevelamer caused a reduction in plasma concentrations of IL-2 compared to placebo in patients with T2D (Δ median [IQR]: -0.03 [-0.08 ; 0.00] vs. 0.03 [-0.04 ; 0.13] pg/ml, $p = 0.04$), whereas no change was observed in healthy individuals. We found a trend towards reduction of IL-6 in patients treated with sevelamer compared to placebo treated patients with T2D (Δ median [IQR]: -0.04 [-0.19 ; 0.03] vs. 0.03 [0.01 ; 0.14] pg/ml, $p = 0.053$). No effects of treatment with sevelamer were observed for the additional analysed inflammatory markers in patients with T2D (Table 2) or healthy individuals (Table 3).

Potential effects of difference in sex distribution between treatment groups on changes in inflammation and oxidative stress was evaluated using a two-way ANOVA test. We found a significant interaction between sex and treatment for Δ 8-oxodG/creatinine ($p = 0.04$), however post-hoc investigation of Δ 8-oxodG/creatinine least-squares means for separate groups using Tukey adjustment revealed no significant difference between the sex stratified treatment groups. No interaction was evident for additional investigated markers.

3.4. Correlation between markers of oxidative stress and inflammation

We conducted the following post-hoc analysis to explore the potential relationship between oxidative stress and inflammation. Using Pearson correlation, we found a significant correlation between urine 8-oxoGuo/creatinine and plasma TNF- α in patients with T2D ($r = 0.49$ (95% confidence interval (CI): 0.16 ; 0.72), $p < 0.01$) at baseline. This significant correlation between the markers was confirmed at the

Table 1
Subject characteristics.

	Patients with T2D			Healthy individuals		
	Placebo ($n = 10$)	Sevelamer ($n = 20$)	P value	Placebo ($n = 8$)	Sevelamer ($n = 12$)	P value
Sex (F/M)	5/5	4/16	0.21 ^a	3/5	5/7	1.00 ^a
Age (years)	65.5 [54.8; 67.0]	60.0 [48.8; 64.3]	0.21	55.5 [47.5; 65.8]	57.5 [50.3; 67.0]	0.67
Duration of T2D (years)	5.5 [2.8; 8.8]	4.0 [2.8; 6.3]	0.41	N/A	N/A	N/A
FPG (mmol/l)	7.7 [5.9; 9.3]	8.0 [7.4; 8.4]	0.71	5.9 [5.2; 6.2]	5.8 [5.2; 5.9]	0.56
HbA1c (mmol/mol)	48.0 [44.3; 51.0]	44.5 [43.0; 52.3]	0.57	32.5 [30.0; 36.3]	33.0 [31.0; 36.5]	0.67
BMI (kg/m ²)	26.9 [25.4; 28.9]	28.9 [27.5; 31.0]	0.14	28.3 [26.8; 28.8]	28.0 [26.8; 30.6]	0.85

Values are medians with interquartile range. P values compare placebo to sevelamer within the groups of patients with type 2 diabetes and healthy individuals, respectively. FPG, fasting plasma glucose; N/A, not applicable; T2D, type 2 diabetes mellitus.

^a Pearson chi-square test.

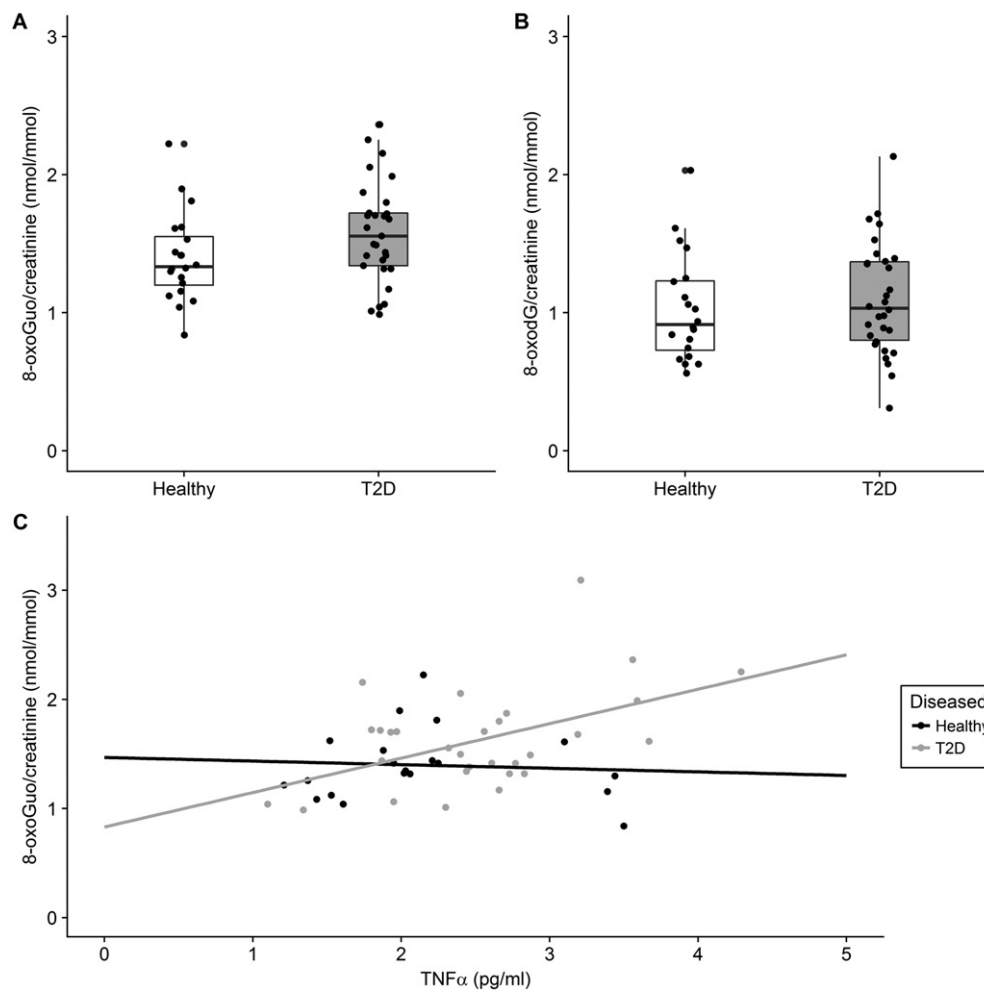


Fig. 1. Urinary excretion rates of 8-oxoGuo and 8-oxodG, and correlation between TNF α and 8-oxoGuo at baseline A) Baseline urinary excretion rates of 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and B) 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in healthy individuals with normal glucose tolerance and patients with type 2 diabetes (T2D). Boxplot and individual values (points) are presented. C) Correlation between plasma tumor necrosis factor alpha (TNF α) and urine 8-oxoGuo/creatinine at baseline in healthy individuals with normal glucose tolerance (black) ($r = -0.07$ [95% confidence interval (95%CI): $-0.50;0.38$, $p = 0.76$]) and patients with T2D (grey) ($r = 0.49$ [95%CI: $0.16;0.72$, $p < 0.01$]). The correlation coefficients were significantly different ($p = 0.049$).

last visit ($r = 0.44$ (CI: $0.09;0.69$), $p = 0.01$), but not found when delta values of TNF- α were correlated to delta values of 8-oxoGuo. No correlation was observed between urine 8-oxoGuo/creatinine and plasma TNF- α at baseline in healthy individuals ($r = -0.07$ (CI: $-0.50;0.38$),

$p = 0.76$) (Fig. 1C). A statistically significant difference between the calculated correlation coefficients were evident ($p = 0.049$). Only a borderline significant correlation was found between urine 8-oxodG/creatinine and plasma TNF- α in patients with T2D ($r = 0.36$

Table 2
Oxidative stress and inflammatory markers in patients with type 2 diabetes.

	Placebo (n = 10)		Sevelamer (n = 20)		P value
	Baseline	Δ Placebo	Baseline	Δ Sevelamer	
Oxidative stress markers					
8-oxoGuo/creatinine (nmol/mmol)	1.69 [1.43; 1.71]	0.02 [-0.07; 0.06]	1.53 [1.34; 1.82]	-0.04 [-0.24; 0.01]*	0.11
8-oxodG/creatinine (nmol/mmol)	1.25 [0.76; 1.50]	0.04 [-0.05; 0.07]	1.00 [0.82; 1.35]	-0.05 [-0.10; 0.05]	0.25
Inflammation markers					
hsCRP (mg/l)	0.89 [0.48; 1.63]	-0.04 [-0.11; 0.55]	1.34 [0.76; 2.04]	-0.04 [-0.22; 0.27]	0.44
TNF α (pg/ml)	2.59 [2.08; 2.86]	0.08 [-0.07; 0.20]	2.45 [1.95; 2.74]	0.10 [-0.11; 0.34]	0.48
IFN- γ (pg/ml)	6.30 [5.27; 8.32]	1.08 [-1.09; 3.13]	4.91 [3.68; 6.85]	0.09 [-0.52; 1.16]	0.77
sCD163 (mg/l)	1.92 [1.37; 2.52]	-0.03 [-0.20; 0.04]	1.85 [1.55; 2.64]	-0.05 [-0.19; 0.07]	0.88
IL-2 (pg/ml)	0.18 [0.13; 0.31]	0.03 [-0.04; 0.13]	0.23 [0.16; 0.30]	-0.03 [-0.08; 0.00]*	0.04
IL-6 (pg/ml)	0.60 [0.45; 0.69]	0.03 [0.01; 0.14]	0.73 [0.55; 0.83]	-0.04 [-0.19; 0.03]	0.05
IL-8 (pg/ml)	13.45 [11.32; 18.18]	-0.67 [-1.53; 2.35]	11.10 [9.90; 17.40]	0.40 [-0.58; 1.22]	0.38
IL-10 (pg/ml)	0.26 [0.21; 0.27]	0.01 [-0.02; 0.02]	0.34 [0.26; 0.39]	0.00 [-0.03; 0.05]	0.91

Values are medians with interquartile range. Delta values (Δ) describe the difference between baseline and end of treatment within the group with * illustrating $P < 0.05$. The specified P values compare Δ Placebo to Δ Sevelamer using unpaired t-test for normally distributed data or Mann-Whitney U test in cases of non-Gaussian distribution of data. 8-oxoGuo, 8-oxo-7,8-dihydro-guanosine; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; hsCRP, high-sensitivity C-reactive protein; IFN- γ , interferon gamma; IL, interleukin; sCD163, soluble CD163; soluble CD163; TNF α , tumor necrosis factor alpha.

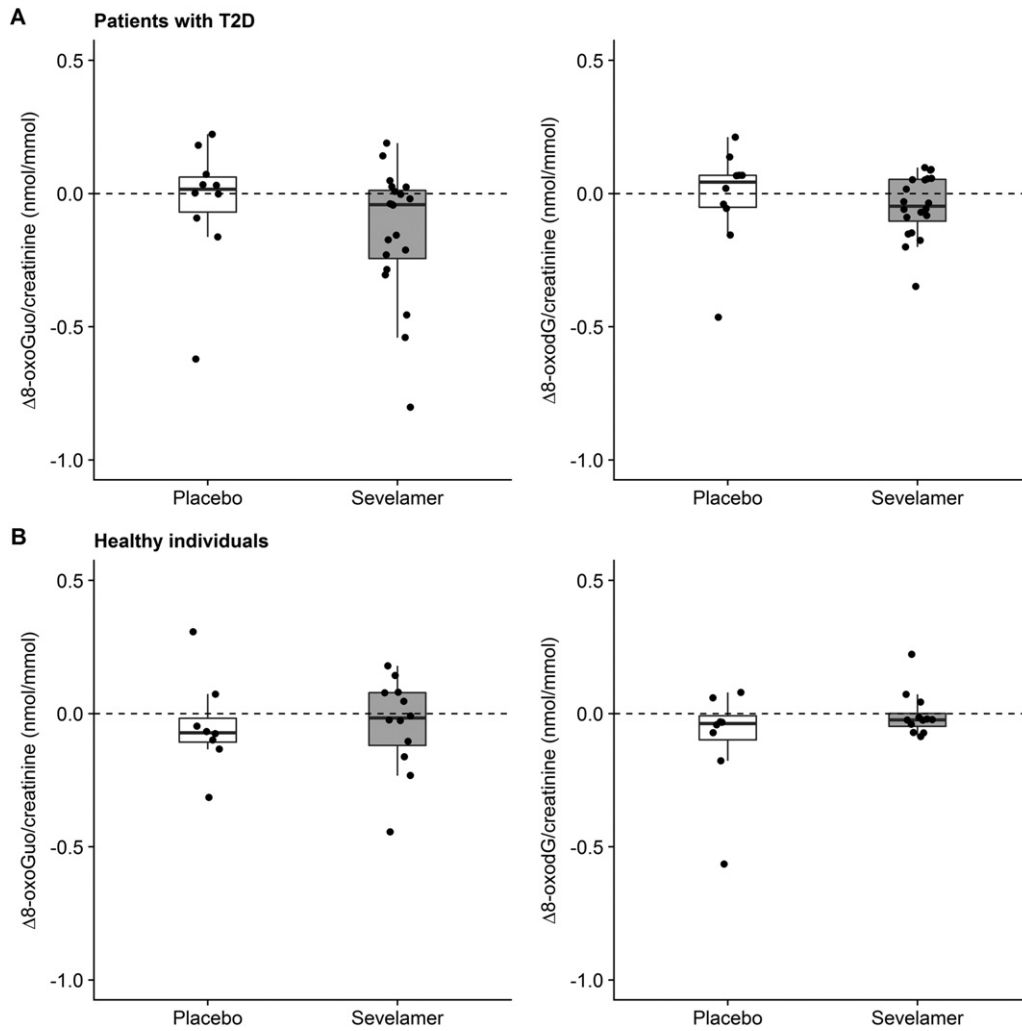


Fig. 2. Effects of treatment with sevelamer on urinary excretion of 8-oxoGuo and 8-oxodG Each symbol illustrates the difference (delta value (Δ)) between baseline and end of treatment for a single participant. Bars show medians with interquartile range. A) Patients with type 2 diabetes. B) Healthy individuals with normal glucose tolerance. 8-oxo-7,8-dihydroguanosine (8-oxoGuo); 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG).

(CI: $-0.01;0.63$), $p = 0.05$). Once again, no significant correlation between 8-oxodG/creatinine and TNF- α were seen in healthy individuals ($r = -0.25$ (CI: $-0.63;0.21$), $p = 0.28$) (Appendix A, Supplementary Fig. 1).

Furthermore, we found a statistically significant correlation between urine 8-oxodG/creatinine and IL-10 ($r = 0.37$ (CI: $0.02;0.65$), $p = 0.04$) and a borderline statistically significant correlation between urine 8-oxoGuo/creatinine and IL-10 ($r = 0.36$ (CI: $-0.00;0.64$), $p = 0.05$) in

Table 3
Oxidative stress and inflammatory markers in healthy individuals.

	Placebo (n = 8)		Sevelamer (n = 12)		P value
	Baseline	Δ Placebo	Baseline	Δ Sevelamer	
Oxidative stress markers					
8-oxoGuo/creatinine (nmol/mmol)	1.28 [1.17; 1.47]	-0.07 [-0.11; -0.02]	1.42 [1.28; 1.55]	-0.02 [-0.12; 0.08]	0.57
8-oxodG/creatinine (nmol/mmol)	1.00 [0.73; 1.30]	-0.04 [-0.10; -0.01]	0.91 [0.76; 1.10]	-0.02 [-0.05; 0.00]	0.31
Inflammation markers					
hsCRP (mg/l)	1.03 [0.40; 2.41]	-0.23 [-0.82; 0.00]	1.01 [0.84; 2.41]	-0.15 [-0.78; 0.04]	0.85
TNF α (pg/ml)	2.01 [1.55; 2.47]	0.07 [-0.02; 0.12]	2.05 [1.79; 2.24]	0.15 [0.00; 0.25]	0.26
IFN- γ (pg/ml)	4.40 [2.67; 5.42]	0.76 [-0.12; 1.08]	4.36 [3.61; 7.52]	0.21 [-0.56; 0.93]	0.57
sCD163 (mg/l)	1.40 [1.01; 1.55]	0.04 [-0.02; 0.18]	1.75 [1.51; 2.09]	0.05 [-0.03; 0.12]	0.70
IL-2 (pg/ml)	0.29 [0.20; 0.47]	-0.05 [-0.08; 0.02]	0.21 [0.15; 0.43]	-0.00 [-0.10; 0.04]	0.79
IL-6 (pg/ml)	0.69 [0.64; 0.81]	-0.05 [-0.10; 0.07]	1.08 [0.90; 1.54]	0.01 [-0.15; 0.07]	1.00
IL-8 (pg/ml)	8.45 [8.09; 9.47]	0.91 [-0.07; 1.28]	11.05 [9.78; 12.95]	-0.50 [-1.20; 0.99]	0.37
IL-10 (pg/ml)	0.29 [0.22; 0.33]	0.02 [-0.06; 0.04]	0.26 [0.21; 0.41]	0.00 [-0.06; 0.04]	0.68

Values are medians with interquartile range. Delta values (Δ) describe the difference between baseline and end of treatment within the group with † illustrating $P < 0.05$. The specified P values compare Δ Placebo to Δ Sevelamer using unpaired t-test for normally distributed data or Mann-Whitney U test in cases of non-Gaussian distribution of data. 8-oxoGuo, 8-oxo-7,8-dihydro-guanosine; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; hsCRP, high-sensitivity C-reactive protein; IFN- γ , interferon gamma; IL, interleukin; sCD163, soluble CD163; TNF α , tumor necrosis factor alpha.

patients with T2D, but there was no significant correlation between these inflammatory markers in healthy individuals. No significant correlations to urine 8-oxoGuo/creatinine or 8-oxodG/creatinine were observed with the remaining inflammatory markers (i.e. CRP, IFN γ , sCD163, IL-2, IL-6, and IL-8) (Appendix A, Supplementary Figs. 1 + 2).

4. Discussion

In this study, short-term treatment with sevelamer caused no significant reductions in oxidative RNA and DNA modifications when compared to placebo treatment. However, a significant reduction was observed within the treated group of patients with T2D, whereas a similar effect was not evident in healthy individuals. A trend towards higher baseline excretion rate of oxidative RNA modifications seemed to be evident in the patients with T2D compared to healthy individuals, which might explain the difference in treatment effects within these groups.

Reduction of oxidative RNA modifications could be a relevant new therapeutic target in patients with T2D due to the association with all-cause and cardiovascular mortality in this patient group.^{12,14,15} Sevelamer is approved for the treatment of hyperphosphatemia in patients with chronic kidney disease, but additional beneficial effects on glycemia^{2,3} and blood lipid status^{2,3,24–26} could implicate a possible treatment potential of this drug in patients with T2D.¹⁸ A previous study has demonstrated treatment with sevelamer for two months to elicit a reduction in 8-isoprostanes (a measure of lipid peroxidation²⁷) compared to calcium carbonate treatment.² We have previously demonstrated that short term (i.e. one-week) treatment with other drugs was sufficient to modify urinary excretion of oxidative nucleic acid modifications,²⁸ which prompted us to explore the potential short term effects of sevelamer. Our findings indicate that sevelamer may affect oxidative RNA modifications and studies with longer treatment periods are required to investigate if a more profound effect of sevelamer treatment exists.

We did not observe sevelamer treatment to reduce oxidative DNA modifications. However, a minor trend towards a reduction in 8-oxodG was observed within the group of patients with T2D treated with sevelamer. We have previously observed different effects of an intervention on RNA and DNA oxidation.²⁸ The intracellular localization as well as the structure of the nucleic acids seem pivotal for the sources that generate and prevent oxidative modifications.¹¹ A compartmentalization of oxidative stress has been suggested.²⁸

Low-grade inflammation promotes insulin resistance,²⁹ and thus, anti-inflammatory effects might be beneficial in patients with T2D. The short-term treatment with sevelamer was observed to affect only IL-2 and trend towards reduction of IL-6 in patients with T2D. None of the remaining inflammatory markers were affected. Previous studies have shown sevelamer treatment to induce changes in plasma concentrations of TNF- α , hsCRP, IL-6, and IL-10, but all of these studies included considerably longer treatment periods compared to the present study.^{2,24,25,30}

Oxidative stress and inflammation are two essential mechanisms for cellular signalling and protection. However, there is a fine line between these essential cellular mechanisms and the damaging potential of both.^{31,32} The association between oxidative stress and inflammation is intriguing and a very complex field that has been recognized for many years, but details remain unclarified. Two clear interactions are known: The first is oxidative burst, which is a mechanism including production of reactive oxygen species (ROS) from neutrophil granulocytes and macrophages by the enzyme NADPH oxidase. ROS are released from these cells to eliminate pathogens.³³ The second example is the ability of ROS to modulate nuclear factor-kappa B (NF κ B). NF κ B is a transcription factor that controls expression of numerous genes involved in the innate immune system.³³ Furthermore, oxidative stress and inflammation are known to modify each other in multiple ways.³³ To explore the association between oxidative stress and inflammation, we conducted post-hoc correlation tests between the measured markers at baseline. Interestingly, we found a quite strong correlation between TNF- α and

8-oxoGuo that was only evident in patients with T2D. A previous in vitro study showed that TNF- α induced reactive oxygen species generation in cardiac myocytes.³⁴ Other researchers have suggested a linkage between oxidative stress and inflammation through release of glutathionylated peroxiredoxin-2 that stimulates TNF- α production,³² thus, providing possible explanations to a correlation between the markers. An observational study conducted in patients with rheumatic arthritis showed reduced urinary excretion of 8-oxodG after 14 weeks treatments of infliximab.³⁵ This encourages us and hopefully others to explore the targeted effect of monoclonal antibody TNF- α therapy on oxidative RNA modifications. The difference between the correlation coefficients among patients with T2D and healthy individuals might suggest that patients with T2D are more prone to oxidative RNA modifications and/or inflammation.

As previously reported,³ plasma concentrations of unconjugated fractions of the two primary bile acids (cholic acid (CA) and chenodeoxycholic acid (CDCA)) as well as the main secondary bile acid (deoxycholic acid (DCA)) were measured in the present studies. Findings from in vitro studies and animal models indicate anti-inflammatory properties of the specific bile acid receptors TGR5 and FXR.^{36,37} Thus, TGR5 activation has been reported to elicit anti-inflammatory activities by reducing cytokine production in leukocytes, whereas FXR activation has been shown to regulate bacterial overgrowth and contribute to the mucosal resistance against microbes. In addition, TGR5 and FXR activation have been demonstrated to reduce hepatic inflammation via suppression of NF- κ B signalling.^{36,37} In the present studies, no effects of sevelamer on fasting plasma concentrations of CA, CDCA or DCA were evident in patients with T2D or healthy subjects. Placebo-corrected reductions in AUC_{240 min} for CDCA and DCA were observed following sevelamer treatment in patients with T2D, whereas no effect on CA was seen in this group of patients. A similar decrease in AUC_{240 min} for CDCA was also evident in the healthy subjects, whereas no changes in AUC_{240 min} for CA or DCA were observed compared to placebo. The observed changes in plasma bile acid concentrations are supported by findings from a study by Brufau et al.³⁸ However, the overall knowledge on bile acid-mediated impact on inflammation as well as the potential influence of changes in plasma bile profile remain limited and to be further clarified.³⁶

The randomised, double-blinded, and placebo-controlled designs constitute a strength of the two studies. Furthermore, the UPLC-MS/MS method used for analysis of urinary excretion of 8-oxoGuo and 8-oxodG is preferred method when compared to ELISA due to high specificity.³⁹

The collection of the baseline urine sample 4 h after administration of the first dose of study drug poses a limitation to the study. The half-life of injected 8-oxodG in plasma is approximately 2.5 h based on animal studies.⁴⁰ Thus, we do not expect an acute (hours) effect of sevelamer treatment on urinary excretion of oxidative modification, and if any it would have diminished the observed effect of sevelamer treatment in terms of 8-oxoGuo and 8-oxodG. We adjusted urinary oxidative nucleic acid concentrations to urinary flow using urine creatinine. The use of this method instead of a 24-h urine collection seems reasonable due to no evidence or indication of sevelamer-mediated treatment effects on urine creatinine. The unfortunate numeric, but not statistically significant difference in sex distribution between treatment groups was investigated using a two-way ANOVA. An interaction between sex and treatment group was only observed when evaluating changes in 8-oxodG/creatinine, but no difference in least squares means between sex stratified treatment groups was evident. Thus, gender distribution should not affect the interpretation of additional results. Finally, the relatively small sample size of both studies constitutes a limitation of the study.

5. Conclusion

The pleiotropic effects of sevelamer, including improved glycaemic control, reduction in LDL cholesterol, and potentially reduced oxidative

stress and inflammation point to a potential beneficial effect of sevelamer in terms of cardiovascular disease in patients with T2D. The potential relevance is supported by a previous study demonstrating reduced cardiovascular morbidity and mortality after treatment with the bile acid sequestrant cholestyramine in patients with dyslipidemia.⁴¹

In conclusion, this study shows promising results of sevelamer treatment and suggests a possible effect on oxidative RNA modifications and inflammation in patients with T2D, but not in healthy individuals. These findings should be further explored in studies with a longer intervention period.

Declaration of competing interest

AB has received a lecture fee from AstraZeneca. EL has received an unrestricted grant from Boehringer Ingelheim for an investigator-initiated study. TV has received lecture fees from, participated in advisory boards of, consulted for, and/or received research grants from Amgen, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, MSD/Merck, Novo Nordisk, and Sanofi. HP has received an unrestricted grant from Boehringer Ingelheim for an investigator-initiated study. FKK has received lecture fees from, participated in advisory boards of, consulted for, and/or received research grants from Amgen, AstraZeneca, Boehringer Ingelheim, Carmot Therapeutics, Eli Lilly, MSD/Merck, Norgine, Novo Nordisk, Sanofi, and Zealand Pharma. KK and TH declare no conflicts of interest.

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Contribution statement

AB and ELL are the guarantors of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. AB designed the study and performed the human experimental studies, contributed to analysis of study results, and the preparation of the manuscript. ELL contributed to the conduction of 8-oxoGuo and 8-oxodG measurements, the analysis, and interpretation of study results, and the preparation of the manuscript. KK contributed to the interpretation of study results, data analysis methods, and the preparation of the manuscript. TV contributed to the study design and interpretation of study results. HEP and TH contributed to the analysis of 8-oxoGuo 8-oxodG and to the interpretation of data. FKK initiated and designed the study and contributed to interpretation of study results. All authors had access to the study data and reviewed and approved the final manuscript.

Data sharing statement

Data that support the findings of the present study are available on request to the corresponding author, given it does not violate the laws of the Danish Data Protection Agency.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jdiacomp.2019.107446>.

References

- Ramsdell R. Renagel: a new and different phosphate binder. *ANNA J* 1999;26:346-7.
- Vlassara H, Uribarri J, Cai W, et al. Effects of sevelamer on HbA1c, inflammation, and advanced glycation end products in diabetic kidney disease. *Clin J Am Soc Nephrol* 2012;7:934-42. <https://doi.org/10.2215/CJN.12891211>.
- Brønden A, Mikkelsen K, Sonne DP, et al. Glucose-lowering effects and mechanisms of the bile acid-sequestering resin sevelamer. *Diabetes Obes Metab* 2018;20:1623-31. <https://doi.org/10.1111/dom.13272>.
- Braunlin W, Zhorov E, Guo A, et al. Bile acid binding to sevelamer HCl. *Kidney Int* 2002;62:611-9. <https://doi.org/10.1046/j.1523-1755.2002.00459.x>.
- Karlsson FH, Tremaroli V, Nookaew I, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 2013;498:99-103. <https://doi.org/10.1038/nature12198>.
- Le Chatelier E, Nielsen T, Qin J, et al. Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013;500:541-6. <https://doi.org/10.1038/nature12506>.
- Yubero-Serrano EM, Woodward M, Poretzky L, Vlassara H, Striker GE. Effects of sevelamer carbonate on advanced glycation end products and antioxidant/pro-oxidant status in patients with diabetic kidney disease. *Clin J Am Soc Nephrol* 2015;10:759-66. <https://doi.org/10.2215/CJN.07750814>.
- Uribarri J, del Castillo MD, de la Maza MP, et al. Dietary advanced glycation end products and their role in health and disease. *Adv Nutr* 2015;6:461-73. <https://doi.org/10.3945/an.115.008433>.
- Uribarri J, Cai W, Peppas M, et al. Circulating glycotoxins and dietary advanced glycation endproducts: two links to inflammatory response, oxidative stress, and aging. *J Gerontol* 2007;62A:427-33. <https://doi.org/10.1093/gerona/62.4.427>.
- Tancredi M, Rosengren A, Svensson A-M, et al. Excess mortality among persons with type 2 diabetes. *N Engl J Med* 2015;373:1720-32. <https://doi.org/10.1056/NEJMoa1504347>.
- Poulsen HE, Specht E, Broedbaek K, et al. RNA modifications by oxidation: a novel disease mechanism? *Free Radic Biol Med* 2012;52:1353-61. <https://doi.org/10.1016/j.freeradbiomed.2012.01.009>.
- Kjær LK, Cejvanovic V, Henriksen T, et al. Cardiovascular and all-cause mortality risk associated with urinary excretion of 8-oxoGuo, a biomarker for RNA oxidation, in patients with type 2 diabetes: a prospective cohort study. *Diabetes Care* 2017;40:1771-8. <https://doi.org/10.2337/dc17-1150>.
- Poulsen HE, Nadal LL, Broedbaek K, Nielsen PE, Weimann A. Detection and interpretation of 8-oxodG and 8-oxoGua in urine, plasma and cerebrospinal fluid. *Biochim Biophys Acta* 2014;1840:801-8. <https://doi.org/10.1016/j.bbagen.2013.06.009>.
- Broedbaek K, Siersma V, Henriksen T, et al. Urinary markers of nucleic acid oxidation and long-term mortality of newly diagnosed type 2 diabetic patients. *Diabetes Care* 2011;34:2594-6. <https://doi.org/10.2337/dc11-1620>.
- Broedbaek K, Siersma V, Henriksen T, et al. Association between urinary markers of nucleic acid oxidation and mortality in type 2 diabetes: a population-based cohort study. *Diabetes Care* 2013;36:669-76. <https://doi.org/10.2337/dc12-0998>.
- Liu X, Gan W, Zou Y, et al. Elevated levels of urinary markers of oxidative DNA and RNA damage in type 2 diabetes with complications. *Oxid Med Cell Longev* 2016;1-7. <https://doi.org/10.1155/2016/4323198>.
- Thomas MC, Woodward M, Li Q, et al. Relationship between plasma 8-OH-deoxyguanosine and cardiovascular disease and survival in type 2 diabetes mellitus: results from the ADVANCE trial. *J Am Heart Assoc* 2018;7:1-9. <https://doi.org/10.1161/JAHA.117.008226>.
- Brønden A, Hansen M, Sonne DP, Rohde U, Vilsbøll T, Knop FK. Sevelamer in a diabetologist's perspective: a phosphate-binding resin with glucose-lowering potential. *Diabetes Obes Metab* 2015;17:116-20. <https://doi.org/10.1111/dom.12355>.
- Loft S, Olsen A, Møller P, Poulsen HE, Tjønneland A. Association between 8-oxo-7,8-dihydro-2'-deoxyguanosine excretion and risk of postmenopausal breast cancer: nested case-control study. *Cancer Epidemiol Biomarkers Prev* 2013;22:1289-96. <https://doi.org/10.1158/1055-9965.EPI-13-0229>.
- Møller HJ, Hald K, Moestrup SK. Characterization of an enzyme-linked immunosorbent assay for soluble CD163. *Scand J Clin Lab Invest* 2002;62:293-9. <https://doi.org/10.1080/003655102760145852>.
- Rasmussen ST, Andersen JT, Nielsen TK, et al. Simvastatin and oxidative stress in humans: a randomized, double-blinded, placebo-controlled clinical trial. *Redox Biol* 2016;9:32-8. <https://doi.org/10.1016/j.redox.2016.05.007>.
- R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL, <https://www.R-project.org/>. 2016. <https://www.r-project.org/>.
- Morillas-Ruiz J, Zafrilla P, Almar M, et al. The effects of an antioxidant-supplemented beverage on exercise-induced oxidative stress: results from a placebo-controlled double-blind study in cyclists. *Eur J Appl Physiol* 2005;95:543-9. <https://doi.org/10.1007/s00421-005-0017-4>.
- Ferramosca E, Burke S, Chasan-Taber S, Ratti C, Chertow GM, Raggi P. Potential antiatherogenic and anti-inflammatory properties of sevelamer in maintenance hemodialysis patients. *Am Heart J* 2005;149:820-5. <https://doi.org/10.1016/j.ahj.2004.07.023>.
- Stinghen AEM, Gonçalves SM, Bucharles S, et al. Sevelamer decreases systemic inflammation in parallel to a reduction in endotoxemia. *Blood Purif* 2010;29:352-6. <https://doi.org/10.1159/000302723>.
- Shantouf R, Budoff MJ, Ahmadi N, Tian J, Flores F, Kalantar-Zadeh K. Effects of sevelamer and calcium-based phosphate binders on lipid and inflammatory markers in hemodialysis patients. *Am J Nephrol* 2008;28:275-9. <https://doi.org/10.1159/000111061>.

27. Lawson JA, Rokach J, FitzGerald GA. Isoprostanes: formation, analysis and use as indices of lipid peroxidation in vivo. *J Biol Chem* 1999;274:24441-4. <https://doi.org/10.1074/jbc.274.35.24441>.
28. Larsen EL, Cejvanovic V, Kjær LK, et al. Clarithromycin, trimethoprim, and penicillin and oxidative nucleic acid modifications in humans: randomised, controlled trials. *Br J Clin Pharmacol* 2017;83:1643-53. <https://doi.org/10.1111/bcp.13261>.
29. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* 2005;115:1111-9. <https://doi.org/10.1172/JCI200525102>.
30. Navarro-González JF, Mora-Fernández C, de Fuentes MM, Donate-Correa J, Cazaña-Pérez V, García-Pérez J. Effect of phosphate binders on serum inflammatory profile, soluble CD14, and endotoxin levels in hemodialysis patients. *Clin J Am Soc Nephrol* 2011;6:2272-9. <https://doi.org/10.2215/CJN.01650211>.
31. Sies H. Oxidative stress: a concept in redox biology and medicine. *Redox Biol* 2015;4:180-3. <https://doi.org/10.1016/j.redox.2015.01.002>.
32. Salzano S, Checconi P, Hanschmann E-M, et al. Linkage of inflammation and oxidative stress via release of glutathionylated peroxiredoxin-2, which acts as a danger signal. *Proc Natl Acad Sci* 2014;111:12157-62. <https://doi.org/10.1073/pnas.1401712111>.
33. Lugin J, Rosenblatt-Velin N, Parapanov R, Liaudet L. The role of oxidative stress during inflammatory processes. *Biol Chem* 2014;395:203-30. <https://doi.org/10.1515/hsz-2013-0241>.
34. Suematsu N, Tsutsui H, Wen J, et al. Oxidative stress mediates tumor necrosis factor- α -induced mitochondrial DNA damage and dysfunction in cardiac myocytes. *Circulation* 2003;107:1418-23. <https://doi.org/10.1161/01.CIR.0000055318.09997.1F>.
35. Kageyama Y, Takahashi M, Ichikawa T, Torikai E, Nagano A. Reduction of oxidative stress marker levels by anti-TNF- α antibody, infliximab, in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2008;26:73-80.
36. Chavez-Talavera O, Tailleux A, Lefebvre P, Staels B. Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease. *Gastroenterology* 2017;152:1679-1694.e3. <https://doi.org/10.1053/j.gastro.2017.01.055>.
37. Zhu C, Fuchs CD, Halilbasic E, Trauner M. Bile acids in regulation of inflammation and immunity: friend or foe? *Clin Exp Rheumatol* 2016;34:25-31 Suppl 98.
38. Brufau G, Stellaard F, Prado K, et al. Improved glycemic control with colesevelam treatment in patients with type 2 diabetes is not directly associated with changes in bile acid metabolism. *Hepatology* 2010;52:1455-64. <https://doi.org/10.1002/hep.23831>.
39. Weimann A, Broedbaek K, Henriksen T, Stovgaard ES, Poulsen HE. Assays for urinary biomarkers of oxidatively damaged nucleic acids. *Free Radic Res* 2012;46:531-40. <https://doi.org/10.3109/10715762.2011.647693>.
40. Loft S, Larsen PN, Rasmussen A, et al. Oxidative DNA damage after transplantation of the liver and small intestine in pigs. *Transplantation* 1995;59:16-20. <https://doi.org/10.1097/00007890-199501150-00004>.
41. The Lipid Research Clinics Coronary Primary Prevention Trial Results. I. Reduction in incidence of coronary heart disease. *JAMA* 1984;251:351-64. <https://doi.org/10.1001/jama.1984.03340270029025>.