The effect of structured personal care on RNA oxidation: A 19-year follow-up of the randomized trial Diabetes Care in General Practice (DCGP)

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ABSTRACT

Aims: The urinary marker of RNA oxidation, 8-oxo-7,8-dihydroguanosine (8-oxoGuo), but not the corresponding marker of DNA oxidation, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), is a prognostic biomarker in patients with type 2 diabetes (T2D). The aim of the present study was to investigate the effect of structured personal care (individualized multifactorial treatment) versus standard care on RNA oxidation level in patients with T2D and to assess if the effect of structured personal care on all-cause and diabetes-related mortality was modified by RNA oxidation level.
Methods: Urine samples were analyzed for 8-oxoGuo/8-oxodG from 1381 newly diagnosed T2D patients from the cluster randomized trial Diabetes Care in General Practice cohort, and 970 patients were reexamined after six years of intervention.
Results: The yearly variation in RNA oxidation levels were not significantly different between the structured personal care group and standard care group. The effect of treatment on all-cause and diabetes-related mortality was not modified by the level of RNA oxidation.
No changes in DNA oxidation were seen.
Conclusions: Structured personal care does not influence RNA oxidation level nor is it better for patients with high RNA oxidation level. Thus, structured personal care may not impact the disease-related aspects identified by RNA oxidation level in T2D patients.

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1. Introduction

Diabetes is an increasing global burden affecting 425 million people in 2017, and of which 90% are patients with type 2 diabetes.[1] Despite improved survival of diabetes patients in recent years,[2] the increasing number of patients calls for continuous optimization of diabetes care to prevent related complications.

Evidence shows that oxidative stress is involved in the pathogenesis of type 2 diabetes complications and can damage nucleic acids.[3,4] Nucleic acid oxidation markers are believed to reflect intracellular mechanisms not achieved by traditional extracellular markers.[5] In two large, independent prospective cohort studies we have recently documented that the urinary nucleic acid oxidation marker for RNA, 8-oxo-7,8-dihydroguanosine (8-oxoGuo), but not for DNA, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), is prognostic for all-cause and cardiovascular mortality in patients with type 2 diabetes irrespective of disease duration.[6–9] However, to our knowledge, recommended treatment with the potential of lowering nucleic acid oxidation markers has not been studied in large prospective cohorts.

In the randomized controlled trial Diabetes Care in General Practice (DCGP), patients with type 2 diabetes were allocated to six years of either intervention to support structured personal care (individualized multifactorial treatment) for type 2 diabetes or standard care.[10,11] After 19 years of follow-up the intervention group experienced almost 20% reduction in incidence of the aggregate outcome "any diabetes-related outcome" and myocardial infarction.[11] This effect was to a major degree confined to women.[12]

The aim of this study was to assess the effect of an intervention of structured personal care in patients with type 2 diabetes compared with standard care on urinary nucleic acid oxidation markers. The second aim was to investigate if the effect of the intervention on hard clinical outcomes could be explained by high or low nucleic acid oxidation level at baseline. For both aims, sex differences were explored.

2. Methods

2.1. Study population

In this study, 1381 patients with newly diagnosed type 2 diabetes aged ≥40 years from the DCGP study were included. Of these, 97.5% were considered to have type 2 diabetes.[10] Patients were enrolled between 1 March 1989 and 28 February 1992. During the last year of inclusion, only the 71 general practitioners (GPs) in the structured care group recruited patients for the intervention.[11] The eligibility criteria were raised blood glucose and/or symptoms of hyperglycemia. Subsequently, diagnosis was confirmed in a major laboratory by fasting whole blood or plasma glucose concentration ≥ 7.0/8.0 mmol/l. Exclusion criteria were severe mental illness, life-threatening somatic disease or unwillingness to participate. The intervention was terminated on 26 September 1995, and 970 patients completed 6-year follow-up. Due to emigration in 1992, the vital status of one patient could not be assessed. Detailed information about study design has previously been published.[10]

2.2. Intervention

In the structured personal care group, the GP was asked to work together with the patient to define the optimal goals for controlling important risk factors with focus on glycemic control.[10] Both three-monthly follow-up and annual screening for diabetes complications were supported by sending a questionnaire to the GP one month prior the next expected consultation.

The GPs were offered six annual half-day seminars, through which they were introduced to possible solutions to therapeutic problems, and they received annual descriptive feedback reports on individual patients.[10] Folders and leaflets on diet and pharmacological treatment were made for both doctors and patients. In general, the importance of diet was emphasized, and doctors were advised to postpone, if possible, the beginning of glucose-lowering drugs until at least 3 months after diabetes diagnosis to see the effect of a potential weight loss.[10] Moreover, the GPs were advised to advocate for simple dietary rules and increased physical exercise. The GPs were encouraged to get an agreement with the overweight patients on a small, realistic weight reduction. The GPs were allowed to deviate from the recommendations in order to individualize treatment.[10]

In the standard care group, GPs were allowed to choose any treatment and to adjust it over time.[10] The standard care practices were not contacted by the study coordinating center during the trial period after inclusion had ended.

2.3. Urinary biomarkers of DNA and RNA oxidation analyses

The urinary nucleic acid oxidation markers were measured using freshly voided morning spot urine samples collected near the time of enrollment and again at the end of intervention (median follow-up: 5.7 years). Analyses were conducted at the Laboratory of Clinical Pharmacology, Rigshospitalet and Glostrup Hospital, between 2009 and 2010, with ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) using the Acquity UPLC system (Waters Corp., Milford, USA) and API 3000 triple quadrupole mass spectrometer (Sciex, Toronto, Canada). UPLC-MS/MS is a validated method for measuring urinary nucleic acid oxidation products and the urine sample analyses of 8-oxodG (DNA) and 8-oxoGuo (RNA) were corrected for urinary creatinine levels.[13,14] The urinary creatinine levels were analyzed by the Jaffe method. Storage of urine samples were kept at −80 °C until analysis.

2.4. Outcomes

Primary outcomes were the yearly changes in the two nucleic acid oxidation markers calculated as the difference between the marker at the end of the intervention and the marker at baseline, divided by the time between these two measurements. Secondary outcomes were time from baseline until death, either all-cause or diabetes-related mortality.

2.4.1. Assessment of vital status

Vital status was certified on 1 January 2009 by the Danish Civil Registration System.[15] In accordance with previous publications,[11] the definition of diabetes-related mortality was based on the Danish National Register of Causes of Death[16] including ≥1 of following entries: sudden death or death from myocardial infarction, stroke, peripheral vascular disease, renal disease, hyperglycemia, or hypoglycemia.[11]

2.5. Statistics

Descriptive statistics of baseline variables were reported as medians (interquartile range, IQR) or numbers (%).

For the primary outcomes a robust method to account for non-normal distribution and outliers was chosen. The primary outcomes were compared between the structured personal care group and the standard care group by the difference of their medians; 95% confidence intervals and p-values were obtained by non-parametric bootstrap using 10,000 replicates. To account for possible differential dropout we used the medians of the non-missing outcomes weighted by the inverse probability of not having dropped out among those still alive at the end of the intervention[17]; these probabilities were estimated from a logistic regression model including treatment group, baseline level of the nucleic acid oxidation biomarker, inclusion in the original trial (1989–1991 or 1991–1992), age and sex as covariates. This analysis was repeated stratified on sex. The unadjusted medians with IQR were also reported which include all patients observed at baseline and follow-up, thus not accounting for patient dropout or death.

The secondary outcomes were compared between the structured personal care group and the standard care group in Cox proportional hazard regression models (Main treatment effect model). The treatment effects were then contrasted between sex (Treatment contrasted by sex model) and patients with high and low oxidation (defined as above or below the median; for 8-oxodG: 2.1 nmol/mmol creatinine and for 8-oxoGuo: 3.6 nmol/mmol creatinine) (Treatment contrasted by oxidation model) separately and combined (Treatment contrasted by sex and oxidation). All analyses were adjusted for nucleic acid oxidation marker level at baseline (continuous or dichotomous, respectively), age, sex, smoking status, cohabitation status, physical activity, education, body mass index (BMI), hypertension, microalbuminuria, glycated hemoglobin, total cholesterol, triglycerides, serum creatinine, retinopathy, peripheral neuropathy and history of myocardial infarction or stroke. To justify the proportional hazard assumption, the baseline hazard was estimated separately for the age categories: 40–50, 50–60, 60–70, 70–80, ≥80 years and the serum creatinine categories: ≤80, 80–90, 90–100, ≥100 μmol/l. To account for the clustering of patients in practices, the models were fitted using a robust variance estimator.

R 3.5.0 was used for the statistical analyses.[18] A significance level of 5% was used.

2.6. Ethics

The study protocol was approved by the ethics committee of Copenhagen and Frederiksberg. All patients gave their informed consent and the study was in accordance with the Helsinki declaration. The original study with follow-up was registered at Clinicaltrials.gov, number NCT01074762.

Table 1
Baseline characteristics.

Baseline characteristics	No. of patients (standard/structured personal care)	Standard care	Structured personal care
Sociodemographic			
Age [years; median (IQR)]	620/761	65.2 (56.2–73.4)	65.5 (55.6–73.7)
Sex [Men; n, (%)]	620/761	329 (53.1)	404 (53.1)
Cohabitation status [living alone; n, (%)]	606/743	198 (32.7)	236 (31.8)
Education [basic level; n, (%)]	588/723	459 (78.1)	574 (79.4)
Urinary biomarker outcomes			
8-oxodG [nmol/mmol u-creatinine; median (IQR)]	609/744	2.10 (1.60–2.76)	2.11 (1.60–2.77)
8-oxoGuo [nmol/mmol u-creatinine; median (IQR)]	608/744	3.55 (2.82–4.67)	3.69 (2.94–4.80)
U-creatinine [mmol (IQR)]	611/747	8.60 (6.10–12.10)	8.90 (5.90–12.00)
Clinical			
Body mass index [kg/m^2; median (IQR)]	619/745	28.7 (26.0–32.2)	29.4 (26.2–33.0)
Hypertension [n, (%)]	620/761	458 (73.9)	568 (74.6)
History of MI [n, (%)]	618/759	55 (8.9)	63 (8.3)
History of stroke [n, (%)]	619/760	28 (4.5)	33 (4.3)
Retinopathy [n, (%)]	559/668	25 (4.5)	30 (4.5)
Peripheral neuropathy [n, (%)]	609/751	123 (20.2)	140 (18.6)
Biochemical			
Glycosylated hemoglobin (%, DCCT)	512/624	10.2 (8.7–11.9)	10.2 (8.6–11.7)
Glycosylated hemoglobin (mmol/mol; IFCC)		88 (72–107)	88 (70–104)
Total cholesterol [mmol/l; median (IQR)]	610/740	6.2 (5.5–7.2)	6.2 (5.3–7.1)
Fasting triglycerides [mmol/l; median(IQR)]	610/736	1.99 (1.39–2.96)	1.97 (1.42–2.85)
Serum creatinine [μmol/l; median (IQR)]	611/740	88 (79–100)	90 (80–101)
Urinary albumin [mg/l; median (IQR)]	595/723	11.8 (5.7–27.2)	11.5 (6.0–29.4)
Behavioral			
Current smoking [n, (%)]	604/742	208 (34.4)	264 (35.6)
Low physical activity [n, (%)]	604/741	162 (26.8)	210 (28.3)

Sociodemographic, clinical and biochemical characteristics for standard care group and structured personal care group are presented as medians with interquartile range (IQR) or as numbers with percentages (%).

3. Results

3.1. Patient characteristics

Baseline characteristics are presented in Table 1. All sociodemographic, clinical, biochemical and behavioral characteristics were similar across treatment groups (Table 1).

3.2. Primary outcomes

The yearly change in nucleic acid oxidation markers from baseline to the end of intervention did not differ between the structured personal care and the standard care group (Table 2). 50% of those in the structured personal care group that was still alive when the intervention ended had a yearly change in RNA oxidation ±0.031 95% CI (−0.005 to 0.074) and for standard care group 0.001 95% CI (−0.026 to 0.056), p-value = 0.45. 50% of those, in the structured personal care group, that was still alive when the intervention ended had a yearly change in DNA oxidation ±0.006 95% CI (−0.023 to 0.032) and for standard care group −0.001 95% CI (−0.028 to 0.025), p-value = 0.77.

Stratified results for sex showed no significantly differences between treatment groups on yearly change in nucleic acid oxidation markers (Table 3).

Table 2
Overall treatment effect.

Variable	Standard care Unadjusted median (IQR)	Standard care Median (bootstrapped 95% confidence interval)	Structured personal care Unadjusted median (IQR)	Structured personal care Median (bootstrapped 95% confidence interval)	P-value
Yearly change in 8-oxodG (nmol/mmol creatinine/year)	−0.001 (−0.092 to 0.094)	−0.001 (−0.028 to 0.025)	0.006 (−0.093 to 0.101)	0.006 (−0.023 to 0.032)	0.77
Yearly change in 8-oxoGuo (nmol/mmol creatinine/year)	0.001 (−0.129 to 0.167)	0.001 (−0.026 to 0.056)	0.031 (−0.118 to 0.205)	0.031 (−0.005 to 0.074)	0.45

Table shows the yearly change in nucleic acid oxidation markers in the two treatment groups and respective p-values from comparing the medians.

Table 3
Overall treatment effect by sex.

Sex	Variable	Standard care Unadjusted median (IQR)	Standard care Median (bootstrapped 95% confidence interval)	Structured personal care Unadjusted median (IQR)	Structured personal care Median (bootstrapped 95% confidence interval)	P-value
Women	Yearly change in 8-oxodG (nmol/mmol creatinine)	0.000 (−0.106 to 0.113)	−0.001 (−0.047 to 0.052)	0.013 (−0.098 to 0.109)	0.011 (−0.036 to 0.052)	0.78
Women	Yearly change in 8-oxoGuo (nmol/mmol creatinine)	0.036 (−0.190 to 0.209)	0.033 (−0.046 to 0.109)	0.032 (−0.135 to 0.187)	0.029 (−0.021 to 0.087)	0.92
Men	Yearly change in 8-oxodG (nmol/mmol creatinine)	−0.005 (−0.088 to 0.076)	−0.001 (−0.033 to 0.027)	0.002 (−0.090 to 0.099)	0.003 (−0.037 to 0.037)	0.89
Men	Yearly change in 8-oxoGuo (nmol/mmol creatinine)	−0.008 (−0.105 to 0.112)	−0.008 (−0.041 to 0.051)	0.031 (−0.092 to 0.234)	0.031 (−0.023 to 0.103)	0.30

Table shows the yearly change in nucleic acid oxidation markers in the two treatment groups for each sex and respective p-values from comparing the medians.

3.3. Secondary outcomes

There was no significant effect of treatment on all-cause survival when adjusted for the urinary nucleic acid oxidation markers or modified by a high or low level of urinary nucleic acid oxidation markers after multiple adjustments (Fig. 1). No significant treatment differences were seen within each sex.

There was no significant effect of treatment on diabetes-related mortality, except differences in the oxidation markers were seen for men in the low RNA oxidation group who had a 50% increase in mortality rate (HR 1.50, 95% CI 1.02–2.20) and women who had a 41% decrease (HR 0.59, 95% CI 0.35–0.99) (Fig. 1, gray lines with a rhombus).

4. Discussion

This study did not show any differences in yearly change in urinary nucleic acid oxidation markers between structured personal care versus standard care during six years of intervention. Moreover, our study did not find any difference in the treatment effect on survival among those with high versus low urinary nucleic acid oxidation. There were no sex
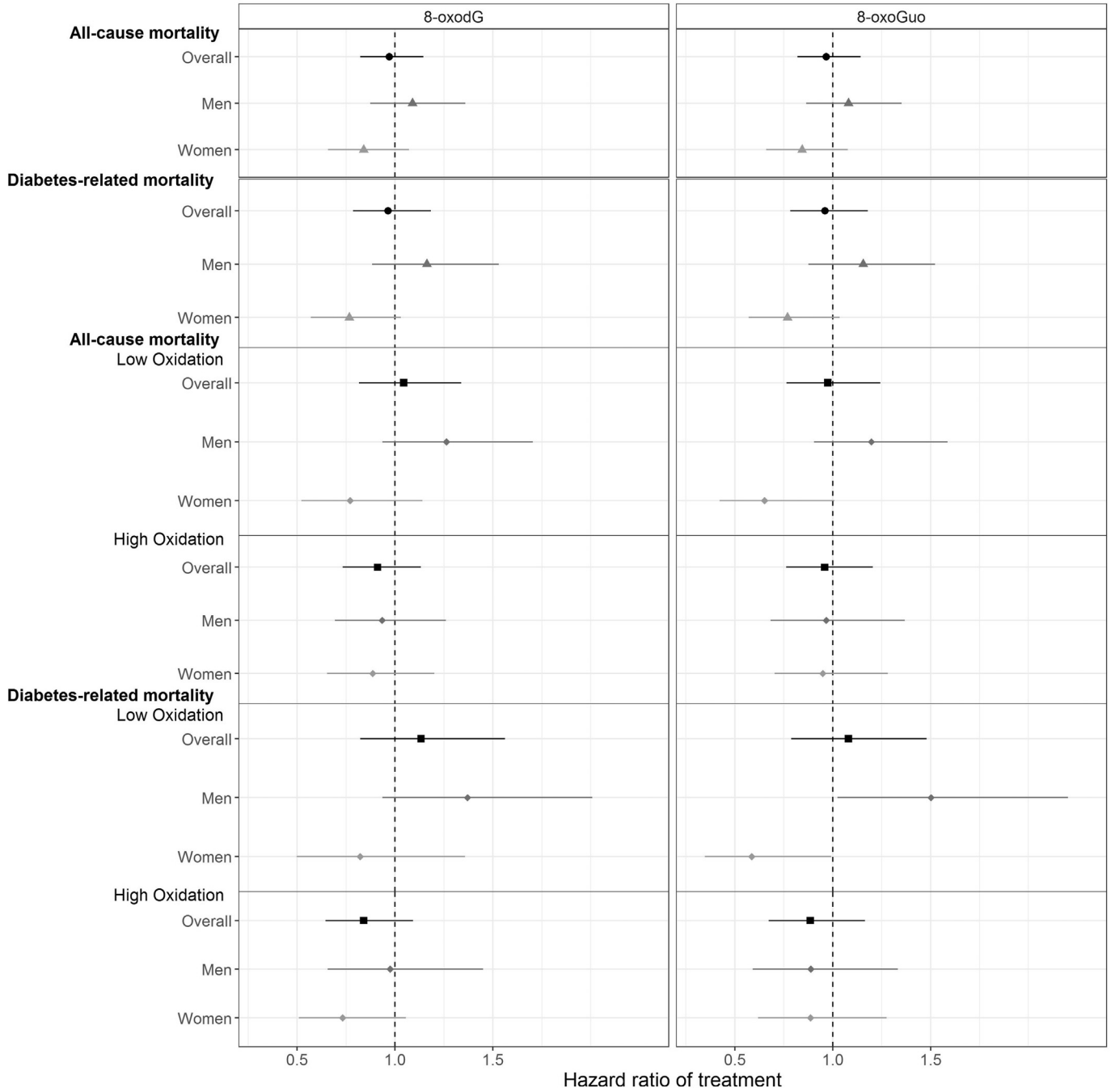

Fig. 1. Effect of treatment on all-cause and diabetes-related mortality and modified by the level of nucleic acid oxidation markers and sex. Main treatment effect model (black lines with circles): Cox model adjusted for treatment, nucleic acid oxidation marker level at baseline, age, sex, smoking status, cohabitation status, physical activity, education, body mass index (BMI), presence or absence hypertension and of microalbuminuria, glycated hemoglobin, total cholesterol, triglycerides, serum creatinine, presence or absence of retinopathy and of peripheral neuropathy, history of MI, and stroke. Treatment contrasted by sex model (gray lines with triangles): As 'Main treatment effect model' but included an interaction term between treatment and sex. Treatment contrasted by oxidation model (black lines with squares): As 'Main treatment effect model' except for nucleic acid oxidation markers were grouped into high and low oxidation (according to median). Treatment contrasted by sex and oxidation model (gray lines with rhombus): As 'Treatment contrasted by oxidation model' but included interactions between treatment and grouped nucleic acid oxidation markers, sex and treatment, and sex and grouped nucleic acid oxidation markers.

differences found between treatment groups, except for the low RNA oxidation group on diabetes-related mortality.

The original DCGP study did not show an effect of structured personal care versus standard care on all-cause mortality during 19 years of follow-up.[11] However, the original DCGP study found that classic clinical risk factors for diabetic complications were reduced by structured personal care.[10] We found that RNA oxidation was prognostic for death in our original study,[7] and changes in RNA oxidation reflected changes in mortality,[8] but it does not seem to be related to the differences in treatment regimen.

More evidence on nucleic acid oxidation markers as prognostic markers has been produced in this field of research. A new case-cohort study of 3766 patients with type 2 diabetes from the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial shows that plasma 8-oxodG is prognostic for all-cause and cardiovascular mortality.[19] As a cautionary remark, the method used is ELISA, and the manufacture's homepage states that approximately 23% of the measured nucleic acid oxidation markers originates from 8-oxoGuo (8-OHG),[20] thus it may be more accurate to say that this methodology produces a marker of nucleic acid oxidation than of 8-oxodG alone. To our knowledge, the impact of treatment was not investigated in the study. However, we have recently shown that RNA oxidation can identify high risk groups in patients with type 2 diabetes and microalbuminuria after an intensified multifactorial treatment.[21]

Although studies on the prognostic aspects of nucleic acid oxidation markers in patients with type 2 diabetes have been validated in large prospective studies,[6–8] it is still uncertain whether the markers are related to treatment and/or can be used to monitor treatment. Meanwhile, it is recognized that reactive oxygen species are involved in the pathogenesis of diabetic complications,[22,23] and reactive oxygen species can damage macromolecules such as DNA and RNA.[24–26] In our previous study, we found that change in urinary levels of RNA oxidation from high to low or vice versa was prognostic for mortality in patients with type 2 diabetes.[8] Therefore, if pathways involved in the formation of oxidatively generated damage to RNA could be manipulated a potential causative treatment would be available.[22]

Examples of studies showing induced changes in urinary nucleic acid oxidation markers are limited but involve both lifestyle and pharmacological interventions. In healthy humans, olive oil was shown to decrease the biomarker of DNA oxidation by 13% in a randomized controlled trial.[27] Moreover, in healthy smokers quitting cigarettes, a 16% reduction was seen in DNA oxidation four weeks after cessation compared with persistent smokers.[28] Exercise and caloric restriction in healthy humans have also been shown to reduce DNA and RNA oxidation in white blood cells but not in urine.[29] In terms of pharmacological treatment, clarithromycin was shown to increase both DNA and RNA oxidation but trimethoprim lowered DNA oxidation in healthy humans.[30] Whether the abovementioned examples are applicable and beneficial in patients with type 2 diabetes is uncertain.

The current evidence highlights the need to better understand of the protective and pathogenic processes in diabetic complications[23] and the development of further clinical biomarkers could hopefully contribute to further insights. For example, in the Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG) trial,[31] other mechanism than merely HbA_{1c} reduction seem to be involved,[31] and animal studies found implications of oxidative stress involvement.[32] Future studies are needed to elaborate the effect of SGLT-2 inhibitors on nucleic acid oxidation markers in a clinical setting, which our group is currently investigating.[33] If empagliflozin has an effect on nucleic acid oxidation markers the markers could be used to monitor treatment, and a randomized controlled trial with long-term follow-up could clarify if nucleic acid oxidation markers could be used as predictive biomarkers.

Whereas DNA oxidation has been implicated in mutagenic processes, some would argue that oxidatively generated damage to RNA is an epiphenomenon and not directly involved in disease pathogenesis (primarily studied in neurodegenerative diseases).[34] However, evidence on RNA oxidation in various diseases are increasingly being appreciated.[25,26] Our group has previously shown that RNA and DNA oxidation is primarily determined by environmental, non-genetic factors[35] which leaves ample room for manipulation of RNA and DNA oxidation by intervention.

The aim of this post-hoc study was to gain novel insights in the clinical utility of the urinary nucleic acid oxidation markers, and thus further research is needed to evaluate urinary nucleic acid oxidation markers as predictive biomarkers in patients with type 2 diabetes.

We investigated the impact of sex on RNA and DNA oxidation according to intervention. No differences were seen between sexes. A previous study has shown that structured personal care decreased mortality rate in women but not in men.[12] According to our study, this sex difference in mortality does not seem to be related to nucleic acid oxidation levels. To the best of our knowledge, this has not been done previously in a large cohort of patients with type 2 diabetes.

4.1. Strengths and limitations

Despite only one available urine sample per patient at enrollment and again at the end of intervention we used a validated analytical method of urinary nucleic acid oxidation markers with UPLC-MS/MS.[13]

The strengths of this study rest on the large number of patients, the prospective randomized trial design, the long-term follow-up, and the low lost-to-follow-up rate due to the Danish registries. We used robust statistical methods and, as this is a post hoc-analysis of a randomized controlled trial, corrected for a comprehensive selection of confounders to minimize bias.

The intervention was multifaceted, i.e. included both non-pharmacological and pharmacological treatment. Thus, we did not investigate specific treatment subelements between the trial arms on RNA and DNA oxidation. Moreover, antioxidant supplements were not registered in the study.

5. Conclusions

We conclude that six years of structured personal care did not affect the urinary markers of nucleic acid oxidation. Furthermore, the effects of structured personal care on all-cause and diabetes-related mortality were not expressed differently for different levels of the markers of nucleic acid oxidation; in fact, no such treatment effects were found.

Future randomized controlled trials with up-to-date anti-diabetic treatment and long-term follow-up should be performed to assess the potential of using urinary markers of nucleic acid oxidation as biomarkers for monitoring treatment in patients with type 2 diabetes.

Contribution statement

HEP and NdFO are guarantors. NdFO is responsible for the original DCGP study. LKK researched data, contributed to interpretation, wrote the draft of the manuscript and reviewed and edited the manuscript. MKG and VS did the statistical analyses. All authors made substantial contribution to the research question, design, analyses and interpretation of data. All authors have approved the final version of the manuscript for submission.

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