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## Urinary nucleic acid oxidation product levels show differential associations with pharmacological treatment in patients with type 2 diabetes

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### ABSTRACT

The relationship between RNA and DNA oxidation and pharmacological treatment has not been systematically investigated in patients with type 2 diabetes (T2D). We aimed to investigate the association between pharmacological treatments and levels of urinary markers of nucleic acid oxidation in T2D patients. Vejle Diabetes Biobank cohort data was nested into nationwide registry data. Multiple logistic regression was used to associate drug usage with risk of high (above median) RNA and DNA oxidation. Data from 2664 T2D patients (64% male, age range: 25–75) were included. Questionnaire-validated lipid lowering drug use was associated with low RNA oxidation (Odds ratio, OR 0.71, 95% CI: [0.59–0.87]). Insulin and non-specific antidiabetic drugs were associated with low DNA oxidation (insulin: OR 0.60, 95% CI [0.49–0.73]). Oral antidiabetics were associated with high DNA oxidation and RNA oxidation (OR 1.30, 95% CI [1.10–1.53] and OR 1.26, 95% CI [1.07–1.29]). Our findings indicate that diabetes-related drugs are associated with RNA and DNA oxidation and further studies are required to determine causality in T2D patients.

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8-Oxo-7,8-dihydro-2-deoxyguanosine; 8-oxo-7,8-dihydroguanosine; clinical pharmacology; oxidative stress; type 2 diabetes

### Introduction

Oxidative stress is implicated in the pathogenesis of diabetic vascular complications [1]. Notably, only a few trials were specifically designed to test antioxidants in patients with diabetes [2], but overall clinical trials with antioxidants have been disappointing [3].

Multifactorial treatment is needed to reduce mortality in patients with type 2 diabetes [4]; however, mortality is not normalised [5]. Even in treated patients macrovascular complications are the primary cause of death [5], and biomarkers like glycated haemoglobin (HbA<sub>1c</sub>) have limitations as prognostic and predictive biomarkers for mortality and treatment effect, respectively [6,7]. A prognostic biomarker identifies the likelihood of an outcome such as death or disease development in patients with that particular disease

(risk stratification), and are usually studied in observational study designs [7]. A predictive biomarker identifies the likelihood of an effect from an exposure, e.g. a new pharmacological treatment vs. control, in patients with and without the biomarker and are usually studied in a randomised placebo-controlled trial [7]. Some biomarkers may have both properties and they can be difficult to distinguish [7].

In two independent cohorts of patients with type 2 diabetes [8–10] we have established that the urinary marker of oxidative stress, RNA oxidation product 8-oxoGuo, is a prognostic biomarker for death; however, it is unknown whether 8-oxoGuo levels are influenced by pharmacological treatment. It is also unknown if DNA oxidation product 8-oxodG levels are affected by pharmacological treatment. We hypothesise that

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urinary nucleic acid oxidation marker levels are associated with pharmacological treatment.

In this explanatory study our aim was to investigate whether pharmacological treatment (antidiabetics and diabetes-related drugs) was associated with the urinary levels of RNA oxidation (8-oxoGuo) and DNA oxidation (8-oxodG) products, respectively, in patients with type 2 diabetes. To obtain more accurate information on drug use, we used both the prescriptions from the Danish National Prescription Registry <90 days prior to the day of examination and the pharmacological treatment declared in the patient questionnaires.

## Materials and methods

### Study cohort

Patients with type 2 diabetes aged between 25 and 75 years from 31 December 2006 onwards were recruited to the Vejle Diabetes Biobank [11]. Patients identification was achieved using the unique 10-digit Danish Personal Identification Number and the Danish Civil Registration System [12], and inclusion was based on the presence of at least 1 of the following conditions: high HbA<sub>1c</sub> value [one value of HbA<sub>1c</sub> ≥ 6.6% (48.6 mmol/mol) in the laboratory database from 1996–2006], minimum three HbA<sub>1c</sub> measurements in the laboratory database from 2002–2006, antidiabetic medication prescriptions in the Danish National Prescription Registry, and/or diabetes diagnosis registered in the Danish National Patient Registry [11]. Additionally, based on the results of the questionnaire administered on the day of the health examination, 57 patients with self-reported type 2 diabetes (2%) were included. Participants diagnosed with type 1 diabetes and those not acknowledging having diabetes were excluded.

### Study design

In this study we used the questionnaire data (exercise, specific antidiabetic medications, and other medications), the health examination (systolic blood pressure in sitting position after 5 min rest measured with Omron M5 Professional [Osaka, Japan], height and weight to calculate body mass index [BMI]), biochemical measurements (urinary albumin, HbA<sub>1c</sub> and low-density lipoprotein [LDL]) and measurements of urinary 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) were included. All measurements were analysed as previously described in the Vejle Diabetes Biobank [11]. Further information

on the cohort and the full study protocol can be found online [11].

The drug prescription history of the patients was also derived from the Danish National Prescription Registry [13]. Exposure to treatment at the time of examination and urine sampling was defined as having at least one redeemed medical prescriptions within 3 months prior the day of examination. Medical prescriptions were grouped according to the Anatomical Therapeutic Chemical (ATC) classification system and included insulin and analogues (A10A), blood glucose lowering drugs excluding insulin (A10B), antithrombotic agents (B01), cardiac therapy agents (C01), antihypertensives (C02), diuretics (C03), β-blocking agents (C07), calcium blocking agents (C08), agents acting on renin-angiotensin system (C09), and lipid modifying agents (C10) [14]. Each drug was identified at the fifth level of the ATC classification system. Two thousand six hundred sixty-four patients were available for the primary outcomes in the multiple logistic regression analyses because of missing values.

### Ethics

The study was conducted within the principles of the Helsinki Declaration, and included informed consent from each patient. Approval of the study was granted by the local ethics committee of the Southern Region of Denmark on April 3 2013 (S-20080097, amendment protocol 37831) and reported to the Danish Data Protection Agency.

### Measurements of urinary nucleic acids oxidation products

A validated method based on ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was used to detect 8-oxoGuo levels and 8-oxodG levels in urinary creatinine corrected spot urine samples [15,16].

Our recently published data showed a lower limit of quantification of 1.0 nM for both 8-oxoGuo and 8-oxodG, and the accuracy was 98.7% for 8-oxoGuo and 95.7% for 8-oxodG [15]. The average between-day precision was 1.5% for 8-oxoGuo and 3.4% for 8-oxodG, while the average within-day precision was 2.9% for 8-oxoGuo and 3.7% for 8-oxodG [15]. Specificity was accomplished by measuring the quantifier and qualifier ions (two characteristic fragmentation ions) and using the relevant acceptance criteria for the signal ratio between the quantifier and qualifier ions [15].

Spot urine measurements of 8-oxoGuo and 8-oxodG were available for 2727 patients. Collection of urine samples was achieved between March 2007 and May 2010. The urine samples were kept for up to 5 years poststudy, stored at  $-80^{\circ}\text{C}$ . Sample analyses were carried out between August 2012 and December 2013, stored at  $-20^{\circ}\text{C}$  during. Storage of urine at  $-20^{\circ}\text{C}$  maintains stable content of the nucleic acid oxidation products throughout 15 years [17].

## Outcomes

The primary outcome was RNA oxidation product, urinary 8-oxoGuo excretion. For comparison DNA oxidation product, urinary 8-oxodG excretion, was included.

## Statistics

For both the registry and questionnaire data, differences in pharmacological treatment and urinary nucleic acid oxidation products were performed with multiple logistic regressions for low (below median) vs. high (above median) 8-oxoGuo and 8-oxodG, respectively. Based on previous literature [18,19], the analyses were adjusted for sex, systolic blood pressure (continuous), BMI (groups:  $<25\text{ kg/m}^2$ ,  $25\text{--}30\text{ kg/m}^2$ ,  $>30\text{ kg/m}^2$ ), HbA<sub>1c</sub> levels (continuous), LDL (continuous), urinary albumin (continuous), and age groups ( $<50$  years,  $50\text{--}59$  years,  $60\text{--}69$  years, and  $>70$  years). Complete case analyses were performed. Significance level was set at 0.05 and reported.

Furthermore, correction for multiple testing was performed using the Bonferroni method separately for RNA oxidation and DNA oxidation in the registry data and patient questionnaires, respectively, and Bonferroni corrected *p*-values and cut-offs were reported [20].

For the baseline variables medians and interquartile range for continuous variables and numbers with percentages for categorical variables were reported.

R version 3.4.4 was used for the statistical analyses [21].

## Results

### Study population

Table 1 shows age, sex, common biochemical measurements, and drug treatment. High RNA oxidation group was defined as above the median of 2.74 nmol/mmol creatinine and the low RNA oxidation group as below median of 2.74 nmol/mmol creatinine in 2727 patients

with type 2 diabetes. High DNA oxidation group was defined as above the median of 1.70 nmol/mmol creatinine and low DNA oxidation group as below median of 1.70 nmol/mmol creatinine.

Since there were very few acarbose prescriptions ( $<3$ ), these were excluded for the primary analyses. Due to missing values (total missing values = 63, Table 1), 2664 patients were available for the primary analyses.

**Table 1.** Baseline characteristics and drug prescriptions in patients.

Variable	Level	Total (n = 2727)
8-oxoGuo	nmol/mmol creatinine	2.74 (2.26–2.96)
8-oxodG	nmol/mmol creatinine	1.68 (1.28–2.21)
Age	Years	64.0 (58–69)
Sex	Women	1062 (38.9)
	Men	1665 (61.1)
HbA <sub>1c</sub>	% DCCT	6.8 (6.3–7.5)
	Missings (n)	11
Systolic blood pressure	mmHg	148 (135–162)
	Missings (n)	31
Low-density lipoprotein	mmol/L	2.2 (1.8–2.8)
	Missings (n)	9
Urinary albumin	mg/L	6.6 (4.2–11.9)
	Missings (n)	0
Body mass index	$<25\text{ kg/m}^2$	325 (12.0)
	$25\text{--}30\text{ kg/m}^2$	1036 (38.1)
	$>30\text{ kg/m}^2$	1358 (49.9)
	Missings (n)	8
Exercise	Yes	1992 (73.2)
	Missings (n)	7
Insulin	Users	649 (23.8)
Insulin excl. oral combinations	Users	348 (12.8)
Metformin	Users	1373 (50.3)
Metformin combinations	Users	65 (2.4)
Sulfonylureas	Users	709 (26.0)
Thiazolidinediones	Users	13 (0.5)
Acarbose	Users	$<3$
DPP-4 inhibitors	Users	55 (2.0)
GLP-1 analogues	Users	23 (0.8)
Meglitinides	Users	14 (0.5)
All oral antidiabetics	Users	1722 (63.1)
All oral antidiabetics (insulin included)	Users	2070 (75.9)
All oral antidiabetics (insulin excluded)	Users	1421 (52.1)
Statins	Users	1583 (58.0)
Other lipid lowering agents	Users	68 (2.5)
ACE inhibitors	Users	1003 (36.8)
AT2 antagonists	Users	604 (22.1)
$\beta$ -Blockers	Users	636 (23.3)
Calcium blockers	Users	635 (23.3)
Other hypertensive agents	Users	19 (0.7)
Renin inhibitors	Users	9 (0.3)
Thiazides	Users	487 (17.9)
Indapamide	Users	9 (0.3)
Furosemides	Users	343 (12.6)
Aldosterone	Users	56 (2.1)
All diuretics	Users	827 (30.3)
Cardiac therapy	Users	179 (6.6)
Antithrombotic agents	Users	1114 (40.9)

Medians with upper and lower quartile are provided for continuous variables and numbers with percentages for categorical variables if not otherwise specified. Medical treatment was according to ATC codes found in the Danish National Prescription Registry defined as  $\geq 1$  prescription  $<90$  days prior the day of the examination.

## Primary outcomes

### RNA oxidation

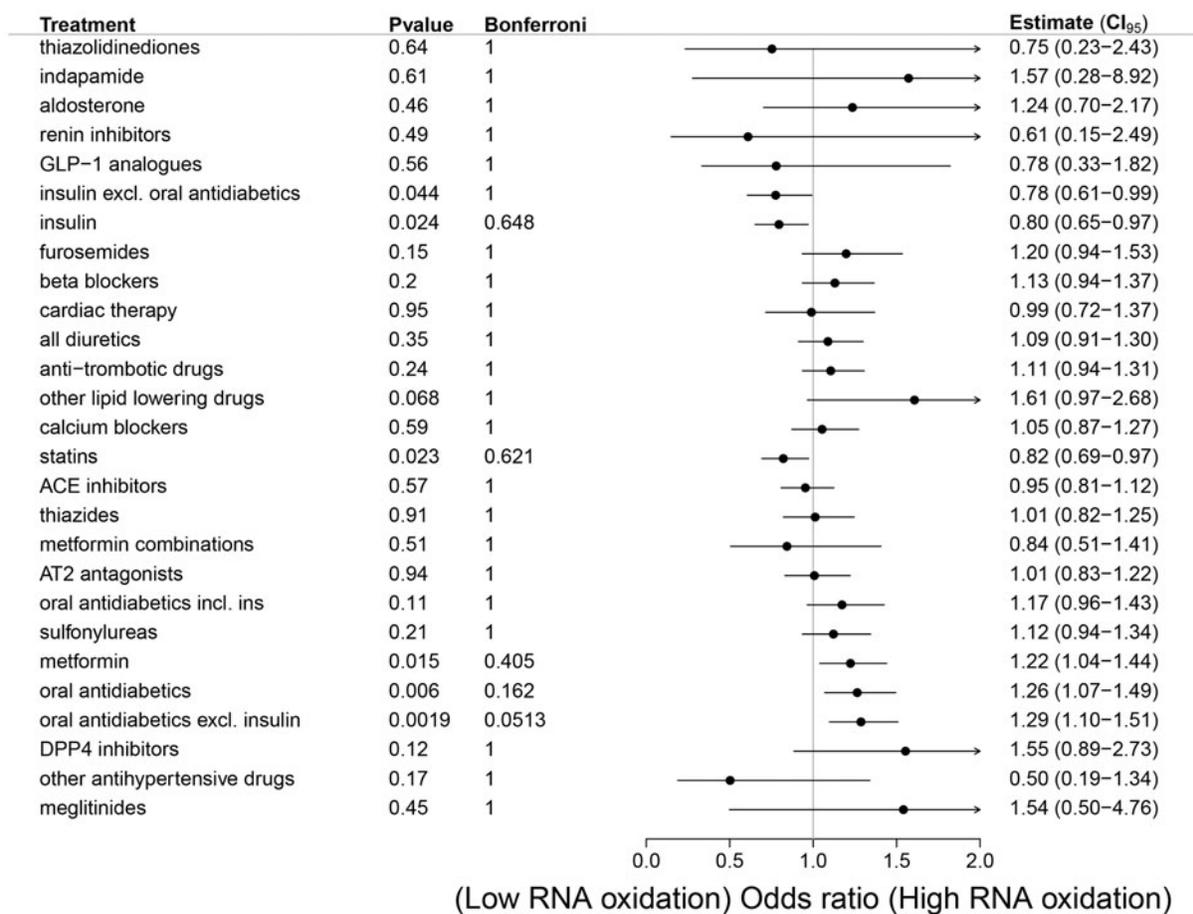
Figures 1 and 2 show the odds ratio (OR) for urinary levels of high and low RNA oxidation products according to treatment based on the registry and questionnaire data. In both the registry and the questionnaire data, association with low RNA oxidation was seen for statins/lipid lowering drugs (OR 0.82, 95% CI [0.69–0.97];  $p$ -value = 0.02 and OR 0.71, 95% CI [0.59–0.87];  $p$ -value < 0.001). In the registry data, insulin use was associated with low RNA oxidation (OR 0.80, 95% CI [0.65–0.97];  $p$ -value = 0.02); the same was found in the questionnaire data, albeit not significant (OR 0.84, 95% CI [0.69–1.03];  $p$ -value = 0.10). Conversely, in both the registry and questionnaire data, metformin use was associated with high RNA oxidation (OR 1.22, 95% CI [1.04–1.44];  $p$ -value = 0.02 and OR 1.20, 95% CI [1.02–1.42];  $p$ -value = 0.03).

There were no significant associations between statins or metformin and RNA oxidation after adjustment for multiple testing in the registry data (Bonferroni corrected  $p$ -value cut-off:  $0.05/27 = 0.002$ ). For the questionnaire data, association between low RNA oxidation and lipid lowering drugs remained significant after adjustment for multiple testing (Bonferroni corrected  $p$ -value cut-off:  $0.05/16 = 0.003$ ).

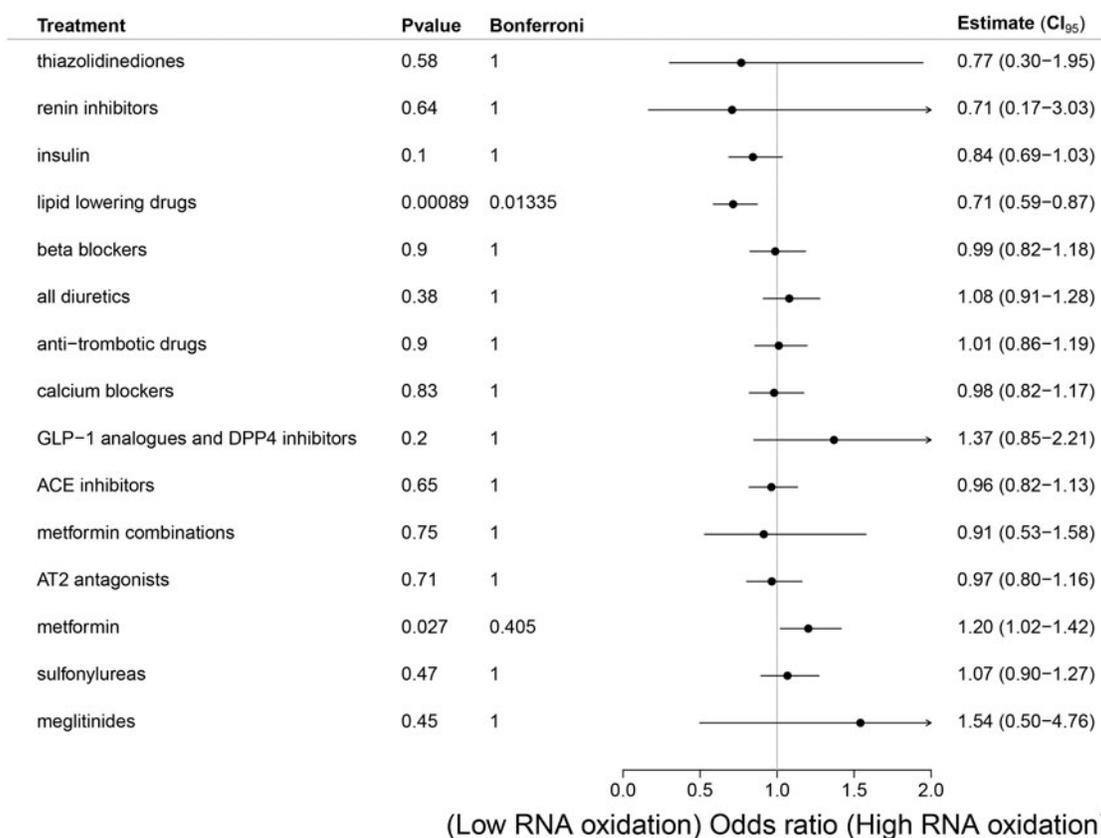
In addition, for nonpharmacological treatment in questionnaire data, there was a marginally significant risk of high RNA oxidation in nonexercising patients (OR 1.20, 95% CI [1.00–1.43];  $p$ -value = 0.05, data not shown).

### DNA oxidation

Figures 3 and 4 show OR for urinary levels of high and low DNA oxidation products according to treatment based on registry and questionnaire data. In both the registry and questionnaire data, association with low



**Figure 1.** Forest plot showing results of multiple logistic regression analyses with high vs. low RNA oxidation as outcome and according to pharmacological treatment derived from the Danish National Prescriptions Registry. Odds ratio (OR) with 95% confidence limits and  $p$ -values based on complete cases ( $n = 2664$ ) and Bonferroni corrected  $p$ -values are shown. Multiple logistic regression models were adjusted for sex, systolic blood pressure, body mass index (BMI), glycosylated haemoglobin (HbA<sub>1c</sub>) levels, low-density lipoprotein (LDL) cholesterol, urinary albumin, and age.



**Figure 2.** Forest plot showing results of multiple logistic regression analyses with high vs. low RNA oxidation as outcome and according to pharmacological treatment derived from the patient questionnaires. Odds ratio (OR) with 95% confidence limits and *p*-values based on complete cases ( $n = 2664$ ) and Bonferroni corrected *p*-values are shown. Multiple logistic regression models were adjusted for sex, systolic blood pressure, body mass index (BMI), glycosylated haemoglobin (HbA<sub>1c</sub>) levels, low-density lipoprotein (LDL) cholesterol, urinary albumin, and age.

DNA oxidation was seen for insulin use (OR 0.60, 95% CI [0.49–0.73]; *p*-value <0.001 and OR 0.58, 95% CI [0.47–0.71]; *p*-value <0.001), also when patients on oral antidiabetics were excluded. In the registry data, use of any oral antidiabetic (insulin excluded) was associated with high DNA oxidation (OR 1.41, 95% CI [1.20–1.64]; *p*-value <0.001). In both the registry and questionnaire data, use of statins/lipid lowering drugs (OR 0.83, 95% CI [0.71–0.98]; *p*-value = 0.03 and OR 0.65, 95% CI [0.54–0.79], *p*-value <0.001),  $\beta$ -blockers (OR 0.66, 95% CI [0.54–0.79]; *p*-value <0.001 and OR 0.69, 95% CI [0.58–0.83]; *p*-value <0.001), any diuretic (OR 0.70, 95% CI [0.59–0.83]; *p*-value <0.001 and OR 0.63, 95% CI [0.54–0.75]; *p*-value <0.001), and antithrombotic agents (both OR 0.72, 95% CI [0.61–0.85]; *p*-value <0.001) was associated with low DNA oxidation. In the registry data, use of cardiac therapies including cardiac glycosides, antiarrhythmics, cardiac stimulants and related vasodilators (OR 0.66, 95% CI [0.48–0.92]; *p*-value = 0.01) and furosemides (OR 0.65, 95% CI [0.51–0.83]; *p*-value <0.001) was associated with low DNA oxidation. In the

registry data, results for calcium blockers and aldosterone were not significantly different after adjustment for multiple testing (Bonferroni corrected *p*-value cut-off:  $0.05/27 = 0.002$ ). For the questionnaire data, significance remained except for calcium blockers after adjustment for multiple testing (Bonferroni corrected *p*-value cut-off:  $0.05/16 = 0.003$ ).

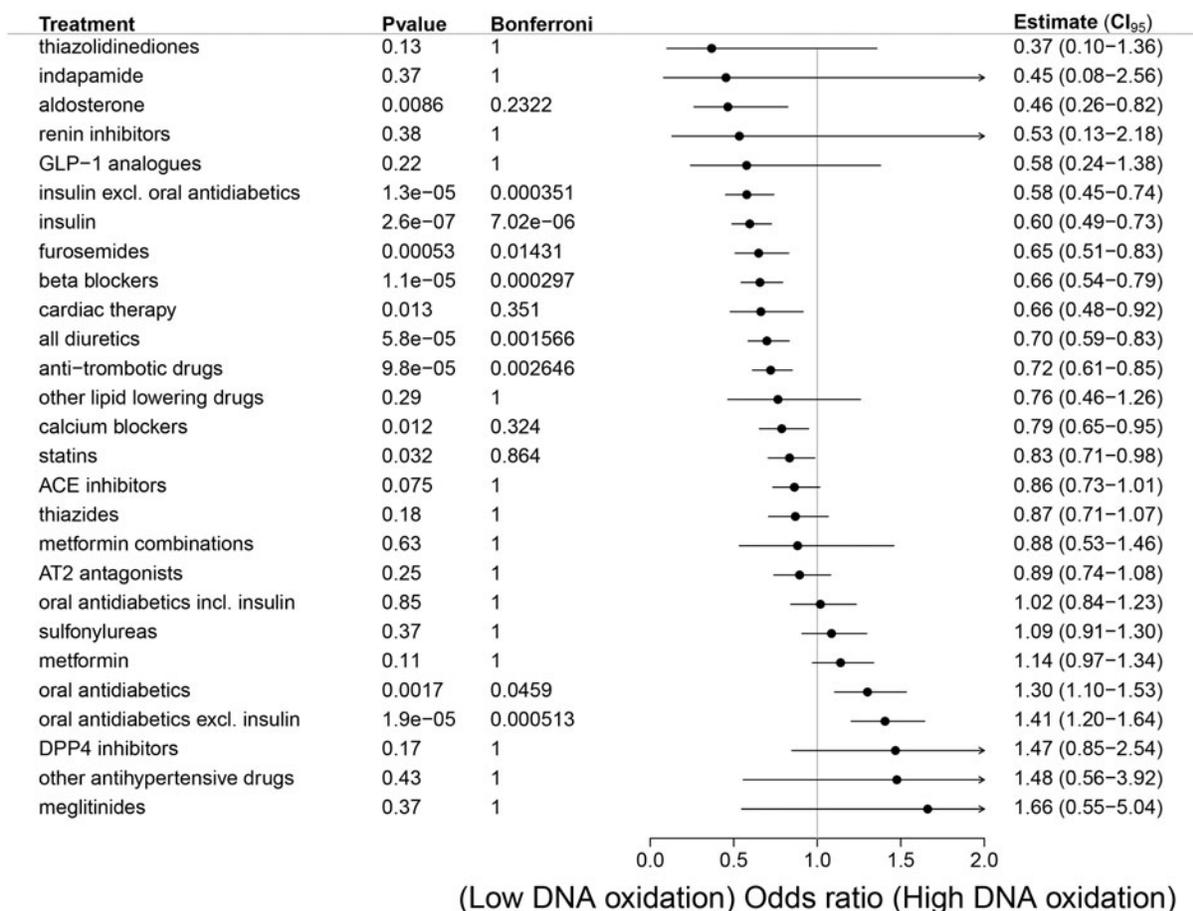
In addition, for nonpharmacological treatment in questionnaire data, there was no significant risk of high DNA oxidation in nonexercising patients (OR 0.92, 95% CI [0.77–1.10]; *p*-value = 0.37, data not shown).

## Discussion

### Key findings

To the extent of our knowledge, this is the first study to systematically investigate the influence of use of registered drugs on oxidative stress measured as RNA and DNA oxidation.

In questionnaire data, lipid lowering drug use was associated with lower RNA oxidation after corrections



**Figure 3.** Forest plot showing results of multiple logistic regression analyses with high vs. low DNA oxidation as outcome and according to pharmacological treatment derived from the Danish National Prescriptions Registry. Odds ratio (OR) with 95% confidence limits and *p*-values based on complete cases ( $n = 2664$ ) and Bonferroni corrected *p*-values are shown. Multiple logistic regression models were adjusted for sex, systolic blood pressure, body mass index (BMI), glycosylated haemoglobin (HbA<sub>1c</sub>) levels, low-density lipodystrophy (LDL) cholesterol, urinary albumin, and age.

for multiple testing but not for any antidiabetic drug. On the contrary, in both the registry and questionnaire data, lower DNA oxidation was observed in insulin users while high DNA oxidation was associated with any combination of oral antidiabetic drugs not including insulin. Further, lower DNA oxidation was associated with use of many treatments not targeting glycaemia (statins, certain antihypertensive drugs, diuretics, cardiac therapy, and antithrombotic drugs).

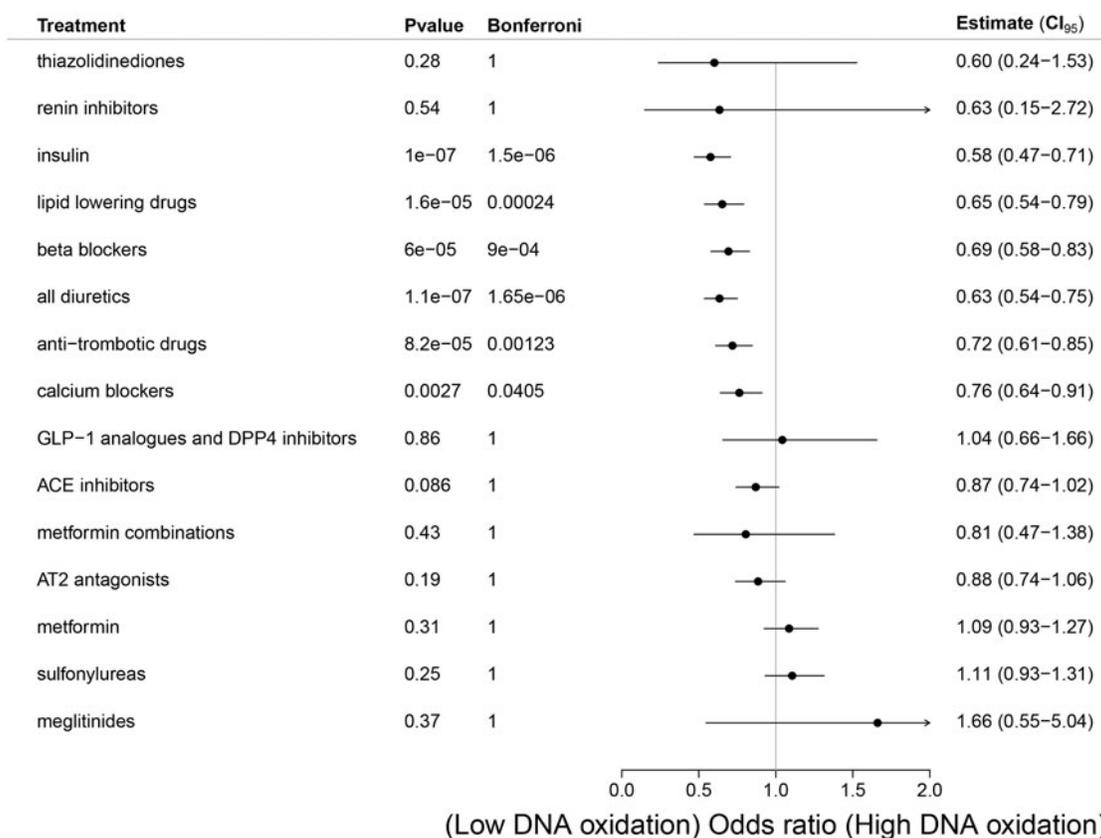
### Findings in context

This study shows clear dissociation of RNA oxidation and DNA oxidation is in agreement with previous studies [8,9,18]. Previously, we have found that diseases like type 2 diabetes show a dissociation of DNA and RNA oxidation, we now find that also drug treatment has different associations with DNA and RNA oxidation,

supporting our view of compartmentalisation of oxidative stress [22].

We have previously found that RNA but not DNA oxidation was prognostic for all-cause and cardiovascular mortality [8–10]. Another recent study with the ELISA method found DNA oxidation in plasma to be prognostic for these outcomes [23].

The key findings in our study suggest that the RNA oxidation product 8-oxoGuo as a prognostic biomarker should be further adjusted in our prediction models by pharmacological treatment, i.e. lipid lowering drugs. This cross-sectional study cannot deal with causality. However, in our first study, the Diabetes Care in General Practice cohort, the patients with type 2 diabetes were treatment-naïve and still elevated RNA oxidation was a prognostic biomarker [8]. Moreover, RNA oxidation was still a prognostic biomarker in the same patients 6 years after diagnosis who now were treatment-engaged [9]. These survival analyses were



**Figure 4.** Forest plot showing results of multiple logistic regression analyses with high vs. low DNA oxidation as outcome and according to pharmacological treatment derived from the patient questionnaires. Odds ratio (OR) with 95% confidence limits and *p*-values based on complete cases ( $n = 2664$ ) and Bonferroni corrected *p*-values are shown. Multiple logistic regression models were adjusted for sex, systolic blood pressure, body mass index (BMI), glycosylated haemoglobin (HbA<sub>1c</sub>) levels, low-density lipoprotein (LDL) cholesterol, urinary albumin, and age.

adjusted for treatment and the same analyses were conducted for DNA oxidation [9].

Oxidative stress can cause oxidative modification to RNA and DNA [24]. Although clinical antioxidant trials have been disappointing, despite robust evidence for a pathophysiological role of oxidative stress, antioxidant effects of already available diabetes-related treatment have been suggested [25,26].

In this study we used a hypothesis-generating approach to study if pharmacological treatment was associated with RNA oxidation and DNA oxidation levels in patients with type 2 diabetes.

In a previous cross-over study with healthy men we found that trimethoprim lowered DNA oxidation while clarithromycin increased both RNA and DNA oxidation [27]. These examples demonstrated that both RNA and DNA oxidation can be pharmacologically altered. For clarithromycin the increase is intriguing in light of the controversy about clarithromycin and cardiovascular mortality [28]. Moreover, in a general population only DNA but not RNA oxidation was associated with statin

use [29] in contrast to our population of type 2 diabetes.

To the best of our knowledge, no registered treatments lowering RNA or DNA oxidation exist, although such a treatment is much warranted. We have previously shown that urinary nucleic acid oxidation markers are minimally determined by genetics which makes the possibility of manipulating oxidative stress both practically and theoretically possible [30].

However, clinical trials with antioxidants failed to reproduce the findings in mechanistic studies and animal models [2,31]. The majority of clinical trials have investigated  $\beta$ -carotene [32], vitamin C, and vitamin E, which are scavengers of already formed free radicals, and thus, a nonetiological treatment [2,31]. Moreover, the scavenging antioxidants clear oxidants stoichiometrically, but oxidants are formed continuously and may require intervention in the generation of these free radicals [25]. Thus, recent research points to another approach with the “new antioxidants” which includes increasing the intracellular antioxidant defence and the

control of the production of free radicals [31]. We assume the urinary markers are reflecting the intracellular oxidative processes and therefore could be a valuable tool in future investigations.

In our study none of the antidiabetic drugs was associated with high RNA oxidation after adjustment for multiple testing. Assuming that RNA oxidation is involved in the pathogenesis of diabetic complications [22], this is in agreement with the notion that diabetic macrovascular complications is primarily prevented by lipid and blood pressure control [33]. Moreover, statin treatment is known to exert antioxidant effects (one of its many pleiotropic effects) [34]. Thus, the lower RNA oxidation seen in patients using lipid lowering drug should be further investigated for the predictive potential as a biomarker for monitoring effects of statins since we have found that RNA oxidation is prognostic for cardiovascular death in patients with type 2 diabetes. We have previously shown in healthy young men enrolled into a randomised controlled trial that statin treatment did not alter the predefined outcome of 20% change in urinary RNA and DNA oxidation [15]. If such or a larger effect of statins exists for patients with type 2 diabetes, characterised by high oxidative stress, it is undetermined.

As far as we know, the inverse relationship between pharmacological treatment and DNA oxidation is new. The associations between low DNA oxidation and both diabetes-specific and non-specific treatments might be due to a compartmentalised effect of the drugs on DNA oxidation, e.g. epigenetic, since markers of oxidative stress can be compartmentalised into intranuclear- (DNA oxidation), cytosolic- (RNA oxidation), and plasma lipid damage (lipid peroxidation) [27]. However, mechanistic studies are needed to investigate the potential compartmentalisation on the molecular/cellular level.

Unexpectedly, we showed recently that the absolute risk of death tended to be higher in those with low DNA oxidation [10]. This inverse relationship between treatment and DNA oxidation could be due to confounding by indication as more patients had microalbuminuria, higher blood pressure, and HbA<sub>1c</sub>, although we took these confounders into account in our models. Mechanistic studies and randomised controlled trials are needed to show a cause-effect relationship.

In agreement with the diverse role of oxidative stress [35], others have very recently found that lower plasma levels of specific oxidation products were associated with higher incidence of cardiovascular events in patients with type 2 diabetes in a subcohort of the Veterans Affairs diabetes trial (VADT) and in a nested

case-control subgroup from the Action to Control Cardiovascular Risk in Diabetes (Accord) trial [36].

### **Strengths and limitations**

This study is cross-sectional and consequently cannot reveal causality. In agreement our aim was to find potential associations between pharmacological treatment and nucleic acid oxidation markers before investing in costly and time-consuming interventional studies.

The cut-off levels of high versus low RNA and DNA oxidation were based on our previous studies where RNA oxidation levels in urine above the median were associated with increased mortality risk rate [9,37].

The Danish National Prescription Registry is by far the preferred gold standard for obtaining valid patient information; however, depending on the outcome of interest, self-reported questionnaire findings may be favoured especially for behavioural aspects [38]. For this reason, even though the Danish National Prescription Registry is considered reliable we have no indicators of drug compliance [13,39]. Consequently we complemented our analyses with patient questionnaires.

However, dose-escalation was not addressed in this study. We only addressed the current use of drugs and did not investigate switching of drugs over time as the study design was cross-sectional. Moreover, the investigated drugs are frequently co-prescribed in diabetes care. We studied both individual compounds and regimens of antidiabetic drugs, but the issue of mutual confounding due to polypharmacy cannot be excluded.

The medical condition of each patient with type 2 diabetes was assessed in the original Vejle Diabetes Biobank and revealed that only 56% reached HbA<sub>1c</sub> level below 7.0, 28% achieved blood pressure target of 140/90 mmHg, 34% met the criteria for lipids target, and 44% of patients with indication for statin use were not treated [11]. This may have influenced our results.

To our surprise, use of first line treatment metformin was relatively low (50.3% plus 2.4% metformin combinations; Table 1). The reason could be that the cohort consisted of patients with type 2 diabetes with varying disease duration. Our findings between 2007–2010 are consistent with the original findings from the Vejle Diabetes Biobank in 2006 prior to inclusion start date, where 56% were on oral antidiabetics exclusively and 67% on oral antidiabetics and insulin [11]. We noted that that between 2007–2010, this has increased to 63.1 and 75.9% (Table 1).

Some drugs were seldom prescribed and power becomes an issue. Almost no patients in the study were

prescribed the recently marketed antidiabetic drugs that for the first time was shown to decrease cardiovascular mortality and related outcomes in patients with type 2 diabetes like EMPA-REG (empagliflozin) [40], LEADER (liraglutide) [41], and SUSTAIN-6 (semaglutide) [42]. Future studies should investigate interventions with follow-up on these pharmacological treatments and RNA oxidation and DNA oxidation as predictive biomarkers, and we are currently investigating the effect of empagliflozin [43].

Lastly, we did not take diet into account since data was not available. This is unfortunate since for example the Mediterranean diet is known to improve oxidative stress, cardiovascular risk factors and major cardiovascular events [31,44]. However, we found a trend for nonexercising patients were associated with high RNA oxidation in this study. In the Vejle Diabetes Biobank, 66% of the patients with type 2 diabetes were former or current smokers [11]. We have previously studied the effect of smoking on DNA and RNA oxidation in the Vejle Diabetes Biobank Cohort and found no significant effects in patients with type 2 diabetes [45].

## Conclusions

Taken together, this study provides insights of associations between urinary nucleic acid oxidation markers and drug use determined by uniquely detailed pharmacological treatment records of 2664 patients with type 2 diabetes derived from both the Danish National Prescription Registry and patient questionnaires. These findings are in agreement with clear differences in urinary nucleic acid oxidation products. More studies are needed to settle if oxidative modifications to DNA and RNA are indirect related to drug efficacy on disease activity or directly caused by the pharmacological nature of the drugs.

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