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Oxidatively Damaged DNA/RNA and 8-Isoprostane Levels Are Associated With the Development of Type 2 Diabetes at Older Age: Results From a Large Cohort Study

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OBJECTIVE

Oxidative stress is believed to play an important role in the pathophysiology of type 2 diabetes, but the few cohort studies that have assessed the association of oxidative stress biomarkers with type 2 diabetes incidence were small and reported inconclusive results.

RESEARCH DESIGN AND METHODS

We examined the associations of urinary oxidized guanine/guanosine (OxGua) levels (a biomarker of DNA/RNA oxidation) and urinary 8-isoprostane levels (a biomarker of lipid peroxidation) with type 2 diabetes incidence in 7,828 individuals initially without diabetes from a population-based German cohort study with 14 years of follow-up. Hazard ratios (HRs) (95% CIs) per 1 SD were obtained using multivariable-adjusted Cox proportional hazards regression models.

RESULTS

In the total population, weak but statistically significant associations with type 2 diabetes incidence were observed for OxGua levels (HR [95% CI] per 1 SD 1.05 [1.01; 1.09]) and 8-isoprostane levels (1.04 [1.00; 1.09]). Stratified analyses showed that associations of both biomarkers with type 2 diabetes incidence were absent in the youngest age-group (50–59 years) and strongest in the oldest age-group (65–75 years) of the cohort, with HR of OxGua levels 1.14 (1.05; 1.23) per 1 SD and of 8-isoprostane levels 1.22 (1.02; 1.45) per 1 SD.

CONCLUSIONS

These results from a large cohort study support suggestions that an imbalanced redox system contributes to the development of type 2 diabetes but suggest that this association becomes clinically apparent at older ages only, possibly as a result of reduced cellular repair capacity.

Free radicals are natural products of mitochondrial energy synthesis. However, excessive, unbalanced free radical production can cause mitochondrial DNA damage and mitochondrial dysfunction, which subsequently can lead to cell senescence and/or cell apoptosis (1). Furthermore, free radicals can damage fatty acids, DNA, RNA, and proteins as well as other cellular components (2–4). Oxidative stress occurs

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when free radicals overpower the cellular antioxidant defense system through either an increase in free radicals or a decrease in cellular antioxidants. Oxidative stress has been suggested to play an important role for the development and progression of type 2 diabetes by inducing insulin resistance and β-cell dysfunction (5–7).

Oxidative stress can be measured by using different biomarkers. The 8-isoprostane concentration in urine samples is one of the most accurate ways to determine lipid peroxidation in vivo because of its long-term stability (8). The 8-isoprostane molecule and other F2-isoprostanes are peroxidation products of arachidonic acid (9). While the 8-isoprostane molecule concentration in urine samples is a biomarker reflecting lipid peroxidation, oxidized guanine/guanosine (OxGua) molecule concentrations are reliable urinary biomarkers for DNA/RNA oxidation (10). Base excision repair processes correct oxidatively damaged DNA and RNA strands, and the OxGua molecules are released into the urine (11).

Several cross-sectional studies have shown that 8-hydroxy-7,8-dihydro-2′-deoxyguanosine (8-OHdGuo) levels are increased in patients with type 2 diabetes compared with healthy control individuals (12–17). However, no study looking into incident type 2 diabetes is available for OxGua levels so far. With respect to F2-isoprostanes, three small, community-based cohort studies with 138–222 incident type 2 diabetes patients investigated the association with type 2 diabetes incidence but yielded inconclusive and conflicting results (18–20). Whereas statistically significantly positive associations that did not persist after full model adjustment were observed in the Framingham Heart Study (18) and the Coronary Artery Risk Development Study in Young Adults study (20), a statistically significant inverse association between F2-isoprostanes and incident type 2 diabetes was observed in the Insulin Resistance Atherosclerosis Study (19). Because of these conflicting results of relatively small studies, the primary aim of the current study was to investigate potential associations of urinary OxGua and 8-isoprostane levels with incident type 2 diabetes using data from a large, population-based cohort study of 1,328 individuals with recorded incident type 2 diabetes. The secondary aim was to assess potential heterogeneity between the sexes and among age-groups. The tertiary aim was to identify characteristics of the study participants that are associated with urinary OxGua and/or 8-isoprostane levels.

RESEARCH DESIGN AND METHODS

Study Population

The ESTHER study (Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung) is an ongoing population-based cohort study whose design has been reported elsewhere in detail (21,22). Briefly, the cohort was initiated during 2000 and 2002 in Saarland, a federal state in southwest Germany. At baseline, 9,940 participants aged 50–75 years were recruited by their general practitioners (GPs) during a general health checkup. The ethics committees of the Medical Faculty of the University of Heidelberg and the Medical Association of Saarland approved the study, and the study is conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all study participants.

At baseline, participants were excluded if they had not donated a urine sample ($n = 160$) or in whom urinary OxGua and 8-isoprostane levels could not be measured ($n = 257$), had a history of diabetes defined by physician-reported diagnosis or antidiabetic medication prescriptions ($n = 1{,}405$), had potentially undiagnosed diabetes defined by HbA_{1c} >6.5% (48 mmol/mol) ($n = 278$), or were lost to follow-up for diabetes incidence right after baseline ($n = 12$). Hence, the final sample size consisted of 7,828 study participants.

Oxidative Stress Serum Marker Measurement

The urine collection in the ESTHER study, the 8-isoprostane and OxGua level measurements, and their long-time stability at −80 °C have been previously described in detail (23–25). In brief, at baseline, spot urine samples were taken during the health checkup, and there was no rule for a time distance to the last urination. Almost all urine samples were collected in the morning (97.7%), and the time of day was not associated with the urinary oxidative stress biomarker measurements (data not shown). The 8-isoprostane and OxGua levels were measured with ELISA kits (8iso1 kit; Detroit R&D, Detroit, MI; and DNA/RNA Oxidative Damage Kit; Cayman, Ann Arbor, MI). The DNA/RNA Oxidative Damage ELISA kit detects three OxGua species: 8-hydroxyguanine, 8-OHdGuo, and 8-hydroxyguanosine. 8-Hydroxyguanine is formed from the attack of hydroxyl radicals on the guanine nucleobase of the DNA or RNA strand. Its nucleoside forms 8-OHdGuo and 8-hydroxyguanosine are products of the oxidation of the guanosine nucleobase of the DNA and RNA strands, respectively. To correct for variability in dilution of the biomarkers in the urine samples, the measurements were standardized by urinary creatinine levels and expressed in nanomoles per millimole creatinine (8-isoprostane levels) or in micrograms per gram creatinine (OxGua levels).

Covariate Assessment

Sociodemographic characteristics, lifestyle factors, family history of diabetes, history of stroke, and history of cancer were assessed by self-administered questionnaires. Self-reported cancer diagnoses were complemented by information from the Saarland Cancer Registry, which recorded cancer diagnoses since the early 1970s. All cancer diagnoses except nonmelanoma skin cancer qualified for the history of cancer definition. Heart failure and coronary heart disease (CHD) were taken from a standardized form, which was used by GPs to document the health checkup. Participants with a self-reported history of myocardial infarction were added to the CHD group. Height, weight, and systolic and diastolic blood pressure were measured during the health checkup. Total cholesterol and HDL cholesterol were assessed in serum samples by enzymatic chromatography (analytes: Chol2 2100, HDLC3 450; Roche) and C-reactive protein (CRP) by immunoturbidimetry on a cobas 8000 C701 analyzer (analyte: CRPL3 500; Roche). Serum creatinine concentrations were assessed with a kinetic Jaffé method on a cobas 8000 C701 analyzer (analyte: CREJ2 3000). The estimated glomerular filtration rate (eGFR) was calculated with the creatinine-based Chronic Kidney Disease Epidemiology Collaboration equation (26).

Outcome Ascertainment

Type 2 diabetes incidence in the ESTHER study was ascertained by four different sources as described previously (27). In brief, study participants were asked in mailed standardized questionnaires at 2-, 5-, 8-, 11-, and 14-year follow-up to document currently prescribed drugs (source 1: drugs of Anatomical Therapeutic Chemical Classification code A10) and answer the question about whether diabetes had been diagnosed after the baseline examination (source 2: self-reported diagnoses). At the 2- and 5-year follow-up, all self-reported diabetes diagnoses were validated by mailing standardized questionnaires to the study participants' GPs; cases of self-reported type 2 diabetes not confirmed by GPs were not used. At the 8-, 11-, and 14-year follow-up, all study participants' GPs were mailed standardized questionnaires that asked for, among other things, new diabetes diagnoses during the past 3 years (source 3: GP-reported diagnoses). Again, if the GP did not confirm a study participant's self-reported diabetes diagnosis, it was not used. Furthermore, to identify participants with potentially undiagnosed incident diabetes, HbA_{1c} was measured at the 8-, 11-, and 14-year follow-up (source 4: $HbA_{1c} \geq 6.5\%$ [48 mmol/mol] [28]). In summary, 9% of the ultimately used incident type 2 diabetes cases were based on HbA_{1c} measurements only, 4% were based on self-reported diagnoses only, and 87% were based on GP-reported diagnoses or prescribed antidiabetic medication.

Statistical Analyses

Associations of baseline characteristics with 8-isoprostane and OxGua levels were evaluated in linear regression models. In this analysis, the distribution of the biomarkers was transformed to a normal distribution by applying the natural logarithm ($\log_e$). The best-fitting model for each biomarker was found by backward selection, trimming out all variables that were not statistically significantly associated with the biomarker (stay criterion was $P < 0.05$).

Cox proportional hazards models were used to derive hazard ratios (HRs) and 95% CIs for the association of OxGua and 8-isoprostane levels with type 2 diabetes incidence. OxGua and 8-isoprostane levels were operationalized as continuous variables to assess risk increase by 1 SD and in quintiles of OxGua and 8-isoprostane levels, using the lowest quintile as the reference. Three statistical models were developed, with an increasing inclusion of variables. Model 3 includes all potential risk factors for either type 2 diabetes or oxidative stress biomarkers, which are available in the ESTHER study. All continuous variables were modeled as such because no deviations from linear associations with incident diabetes were observed. Model 2 includes only risk factors of type 2 diabetes that were found to be statistically significantly associated with type 2 diabetes incidence in the ESTHER study (determined in a Cox proportional hazards model with backward variable selection, using $P < 0.05$ as stay criterion). Model 1 uses the identified diabetes risk factors of model 2 that were also found to be statistically significantly associated with an oxidative stress biomarker [determined in separate linear regression models for $\log_e$(OxGua) and $\log_e$(8-isoprostane) levels with backward selection, using $P < 0.05$ as the entry criterion] (Supplementary Table 1). On the basis of subject matter knowledge, we also checked that model 1 did not contain variables that could be intermediates on the pathway from high oxidative stress to type 2 diabetes, and this was not the case. Thus, model 1 is the simplest possible model with sufficient adjustment for confounders and is not overadjusted, which could be the case for models 2 and 3. Therefore, model 1 is considered the main model, and all stratified and sensitivity analyses have been carried out only with model 1. Analyses were stratified by sex and age-group. Sensitivity analyses were carried out with competing-risks modeling (cause-specific Cox proportional hazards regression with death as the competing risk) and shorter follow-up time (using only events up to the 5- and 10-year follow-up). Furthermore, we tested for interactions between OxGua/8-isoprostane levels and diabetes risk factors (variables of model 2) by adding interaction terms to main model 1.

Multiple imputation was used to impute missing baseline covariate values. The proportion of missing values was <5% for all variables with the exception of HDL, triglycerides, and alcohol consumption, which had proportions of missing values of 38%, 14%, and 9%, respectively. To our knowledge, data were missing at random, which is the assumption of the multiple imputation. Separately by sex, 20 complete data sets were imputed with the SAS 9.3 procedure PROC MI (SAS Institute, Cary, NC) using the Markov chain Monte Carlo method. The variables of model 3 were used for the imputation model. All multivariable analyses were performed in the 20 imputed data sets, and results of the individual data sets were combined by the SAS 9.3 procedure PROC MIANALYZE. All analyses were performed with SAS 9.3, and all statistical tests were two-sided using an α-level of 0.05.

RESULTS

The analyzed study sample of 7,828 individuals without diabetes comprised more women (57.1%) than men, and the median age (interquartile range) was 62 years (57; 67). The median (interquartile range) levels of OxGua and 8-isoprostane concentrations were 146 μg/g (107; 202) creatinine and 0.20 nmol/mmol (0.16; 0.27) creatinine, respectively. There was a low positive correlation between the two oxidative stress biomarkers (Pearson correlation coefficient $r = 0.342$; $P < 0.001$). Cross-sectional associations of baseline characteristics and the two oxidative stress biomarkers are shown in Table 1. Female sex, moderate or high physical activity, and the eGFR were statistically significantly positively associated with the levels of both oxidative stress biomarkers. Furthermore, there was a consistent lack of association with both biomarkers for age, vegetable consumption, use of antihypertensive medication, CRP levels, systolic blood pressure, a family history of diabetes, CHD, and heart failure. OxGua levels were also associated with triglyceride levels (inversely) and a history of stroke. 8-Isoprostane levels were also positively associated with low education, BMI, smoking, high alcohol consumption, use of lipid-lowering medication (which is a proxy for dyslipidemia), total cholesterol, HDL cholesterol, HbA_{1c}, and a history of cancer. Daily fruit consumption and multivitamin supplementation were inversely associated with urinary 8-isoprostane levels.

During a median follow-up time of 10.6 years, 1,328 cases of incident type 2 diabetes were recorded. Tables 2 and

Table 1—Baseline characteristics of the diabetes-free study population of the ESTHER cohort and their associations with log_e(OxGua) and log_e(8-isoprostane) levels in linear regression models

Baseline characteristic	n[a]	%	Mean (SD)	Log_e(OxGua) β (P)[b]	Log_e(8-isoprostane) β (P)[b]
Age (years) per 1 SD	7,828	—	61.7 (6.6)	NS	NS
Female	4,468	57.1	—	**0.265 (<0.001)**	**0.115 (<0.001)**
Education[c]					
Low	5,647	73.7	—	NS	**0.046 (0.007)**
Medium	1,147	15.0	—	NS	−0.008 (0.691)
High	869	11.3	—	Ref	Ref
BMI (kg/m^2) per 1 SD	7,820	—	27.3 (4.2)	NS	**0.023 (<0.001)**
Smoking history					
Never	3,912	51.5	—	Ref	Ref
Former	2,414	31.8	—	NS	**0.033 (0.010)**
Current (0–15 g tobacco/day)	551	7.3	—	NS	**0.211 (<0.001)**
Current (>15 g tobacco/day)	718	9.5	—	NS	**0.342 (<0.001)**
Alcohol consumption[d]					
Abstainer	2,152	30.3	—	Ref	Ref
Moderate	4,427	62.4	—	NS	−0.002 (0.871)
High	518	7.3	—	NS	**0.088 (<0.001)**
Moderate or high amount of physical activity[e]	5,137	65.8	—	**0.038 (0.008)**	**0.042 (<0.001)**
Daily vegetable consumption	2,725	35.6	—	NS	NS
Daily fruit consumption	4,747	62.5	—	NS	**−0.038 (<0.001)**
Multivitamin supplementation	3,104	41.6	—	NS	**–0.050 (<0.001)**
Antihypertensive medication	3,114	39.9	—	NS	NS
Lipid-lowering medication	803	10.3	—	NS	**0.081 (<0.001)**
HbA_{1c} per 1 SD	684	—		NS	**0.014 (0.008)**
%			5.5 (0.4)		
mmol/mol			37.0 (2.1)		
Total cholesterol (mg/dL) per 1 SD	7,828	—	221.4 (50.9)	NS	**0.019 (<0.001)**
HDL cholesterol (mg/dL) per 1 SD	7,828	—	54.6 (15.5)	NS	**0.015 (0.027)**
Triglycerides (mg/dL) per 1 SD	7,828	—	127.7 (78.2)	**−0.016 (0.024)**	NS
CRP (mg/L) per 1 SD	7,828	—	4.0 (8.6)	NS	NS
Systolic blood pressure (mmHg) per 1 SD	7,828	—	139.0 (19.4)	NS	NS
eGFR (mL/min/1.73 m^2) per 1 SD	1,399	—	80.6 (20.6)	**0.017 (0.015)**	**0.053 (<0.001)**
Family history of diabetes	2,716	35.3	—	NS	NS
CHD	812	10.4	—	NS	NS
History of stroke	204	2.7	—	**0.084 (0.049)**	NS
Heart failure	701	9.0	—	NS	NS
History of cancer	586	7.5	—	NS	**0.062 (0.002)**

Boldface type indicates statistical significance at $P < 0.05$. NS, not significant; Ref, reference. [a]Numbers shown were drawn from the data set without imputed missing values. Therefore, numbers do not always add up to the total of $n = 7{,}828$ because of missing values. [b]Multivariable model consisting of the statistically significant ($P < 0.05$) variables shown with results in the table. [c]Definition of low, medium, and high education were ≤9, 10–11, and ≥12 years of school, respectively. [d]Definition of moderate alcohol consumption: women >0 to <20 g ethanol/day and men >0 to <40 g ethanol/day. Definition of high alcohol consumption: women ≥20 g ethanol/day and men ≥40 g ethanol/day. [e]Definition of moderate or high amount of physical activity: >1 h of vigorous and >1 h of light physical activity/week.

3, respectively, show the associations of OxGua and 8-isoprostane levels with type 2 diabetes incidence. Both continuous OxGua (HR [95% CI] per 1 SD 1.05 [1.01; 1.09]) and 8-isoprostane (1.04 [1.00; 1.09]) variables were statistically significantly associated with type 2 diabetes incidence in main model 1. Using categorized variables, only the top OxGua quintile was associated with an increased type 2 diabetes risk in main model 1 compared with the bottom quintile (HR [95% CI] 1.21 [1.02; 1.43]). Results for models 2 and 3 were very similar to the results of model 1 but not statistically significant for OxGua levels. In sensitivity analysis modeling the competing mortality risk, the results were almost identical (Supplementary Table 2). A further sensitivity analysis with shorter follow-up times showed that the associations of both biomarkers would have been almost the same if only incident cases were used that occurred in the first 10 years of follow-up and that both biomarkers were not associated with early events in the first 5 years of follow-up (Supplementary Table 3).

Table 4 shows the sex- and age-stratified results. Whereas the association of OxGua levels with type 2 diabetes incidence was stronger among men than women, the interaction by sex was not statistically significant, and there was no sex difference for the 8-isoprostane levels. Consistently for both oxidative stress biomarkers, no association was observed in the youngest age-group (50–59 years), and the strongest association was observed in the oldest age-group (65–75 years). However, the interaction terms of neither OxGua nor 8-isoprostane levels with age were

Table 2—Associations of OxGua levels with type 2 diabetes incidence

Modeling (μg/g creatinine)	n_{total}	n_{cases}	Model 1[a]	Model 2[b]	Model 3[c]
Per 1 SD[d]	7,828	1,328	**1.05 (1.01; 1.09)**	1.04 (1.00; 1.09)	1.04 (1.00; 1.09)
≤98.9	1,565	285	Ref	Ref	Ref
>98.9 to ≤130.7	1,566	246	0.96 (0.81; 1.15)	0.98 (0.82; 1.16)	0.98 (0.83; 1.17)
>130.7 to ≤165.2	1,566	263	1.06 (0.90; 1.26)	1.02 (0.86; 1.21)	1.02 (0.86; 1.21)
>165.2 to ≤221.8	1,565	255	1.06 (0.89; 1.26)	1.03 (0.87; 1.23)	1.03 (0.87; 1.23)
>221.8	1,566	279	**1.21 (1.02; 1.43)**	1.13 (0.96; 1.34)	1.14 (0.96; 1.35)

Data are HR (95% CI) unless otherwise indicated. Boldface type indicates statistical significance at $P < 0.05$. Ref, reference. [a]Model adjusted for alcohol consumption, triglycerides, and HDL cholesterol. [b]Model adjusted for BMI, smoking, alcohol consumption, use of antihypertensive medication, systolic blood pressure, HbA_{1c}, triglycerides, HDL cholesterol, eGFR, and family history of diabetes. [c]Model adjusted for all baseline characteristics shown in Table 1. [d]1 SD = 275 μg/g creatinine.

statistically significant (Table 4). In addition, no interactions with other covariates were observed (data not shown). These results were reproduced in the sensitivity analysis using the competing risk model (Supplementary Table 4).

CONCLUSIONS

In this large, population-based cohort study of older adults, increased OxGua and 8-isoprostane levels were statistically significantly associated with type 2 diabetes incidence, but associations were weak. In addition, both oxidative stress biomarkers were not associated with type 2 diabetes incidence in participants <60 years of age. The strongest and statistically significant associations were observed in the oldest age-group of the cohort (65–75 years).

To our knowledge, this study is the first to assess the association of OxGua levels and type 2 diabetes incidence. Previous studies had a cross-sectional design and consistently reported that subjects with type 2 diabetes had higher concentrations of 8-OHdGuo molecules in urine samples than healthy control subjects (12–17). Regarding 8-isoprostane levels, a cross-sectional study observed that the urinary concentrations of 8-isoprostane and several other F2-isoprostanes were significantly increased in patients with type 2 diabetes (14). The observed null or inverse associations of F2-isoprostanes with type 2 diabetes incidence in previous studies may be explained by the lower statistical power to detect weak associations (n = 138–222) compared with our study (n = 1,328) and a lower mean age of the study population (41–59 years vs. 62 years in our study) (18–20).

The previous studies were too small to stratify by age-groups, and therefore, the observation that the association of oxidative stress biomarkers and type 2 diabetes incidence could only be detected with statistical significance at higher ages (≥60 years for OxGua and ≥65 years for 8-isoprostane levels) is novel. This result was also supported by the observation that no associations were observed with early events in the first 5 years of follow-up. Both biomarkers were only associated with later events when the study participants were older. Usually it is the other way around: biomarkers have the strongest association with early events because their levels could change during follow-up. An explanation of the importance of age in the association of oxidative stress biomarkers and type 2 diabetes incidence may be that redox homeostasis becomes more and more difficult to maintain with advanced age as a result of decreasing antioxidative capacities (29). Our group has shown previously that total thiol levels, which are a proxy for antioxidative capacity (29), were inversely associated with age and that the inverse association of total thiol levels with cardiovascular disease mortality increased in strength with the age of the study participants and was particularly pronounced in the oldest age-group (70–84 years) (30). We believe that there is a similar pattern for type 2 diabetes: whereas oxidative stress can be tolerated well at a younger age, it contributes to the development of type 2 diabetes at an older age. The overarching mechanism might be an accumulation of oxidative damage of cell compartments over time that overwhelms the cellular repair mechanisms in aged cells. Regarding pancreatic β-cells, pancreatic islets are very prone to damage by free radicals because they have a high metabolic activity and a particularly low expression of antioxidant enzymes (6). Thus, oxidative stress in β-cells can lead to impaired glucose-stimulated insulin secretion or even apoptosis of β-cells (6). Furthermore, oxidative stress can cause insulin resistance by disrupting the insulin receptor signaling pathway (7,31). Most importantly, free radicals can activate the proinflammatory signaling pathways nuclear factor κB and c-Jun N-terminal protein kinase, which can induce serine hyperphosphorylation in insulin receptor substrate 1 (7,32). As a result of impaired insulin signaling, GLUT-4 localization in cell membranes is suppressed (33).

Oxidatively damaged DNA lesions are mainly repaired by human 8-oxoguanine glycosylase (hOGG1). Compared with the

Table 3—Associations of 8-isoprostane levels with type 2 diabetes incidence

Modeling (nmol/mmol creatinine)	n_{total}	n_{cases}	Model 1[a]	Model 2[b]	Model 3[c]
Per 1 SD[d]	7,828	1,328	**1.04 (1.00; 1.09)**	**1.05 (1.01; 1.10)**	**1.05 (1.00; 1.10)**
≤0.15	1,567	232	Ref	Ref	Ref
>0.15 to ≤0.18	1,564	257	1.06 (0.89; 1.27)	1.06 (0.89; 1.27)	1.06 (0.89; 1.27)
>0.18 to ≤0.23	1,566	285	1.19 (0.99; 1.42)	1.19 (0.99; 1.42)	1.19 (0.99; 1.42)
>0.23 to ≤0.29	1,566	275	1.14 (0.95; 1.36)	1.14 (0.96; 1.36)	1.13 (0.95; 1.36)
>0.29	1,565	279	1.14 (0.95; 1.36)	1.14 (0.96; 1.37)	1.13 (0.94; 1.36)

Data are HR (95% CI) unless otherwise indicated. Boldface type indicates statistical significance at $P < 0.05$. Ref, reference. [a]Model adjusted for BMI, smoking, alcohol consumption, HbA_{1c}, HDL cholesterol, and eGFR. [b]Model adjusted for BMI, smoking, alcohol consumption, use of antihypertensive medication, systolic blood pressure, HbA_{1c}, triglycerides, HDL cholesterol, eGFR, and family history of diabetes. [c]Model adjusted for all baseline characteristics shown in Table 1. [d]1 SD = 0.29 nmol/mmol creatinine.

Table 4—Associations of OxGua and 8-isoprostane levels with type 2 diabetes incidence in subgroups defined by sex and age

Stratification	Estimate	OxGua levels[a]	8-Isoprostane levels[b]
Sex			
Male	HR (95% CI) per 1 SD	**1.12 (1.02; 1.23)**	1.03 (0.89; 1.19)
Female	HR (95% CI) per 1 SD	1.04 (0.99; 1.10)	1.05 (1.00; 1.09)
Interaction term of sex and continuous biomarker variable (per 1 SD)	β (SE), *P*	0.077 (0.052), 0.138	−0.029 (0.076), 0.699
Age (years)			
<60	HR (95% CI) per 1 SD	1.01 (0.95; 1.09)	1.03 (0.98; 1.10)
60 to <65	HR (95% CI) per 1 SD	**1.12 (1.01; 1.24)**	1.09 (0.86; 1.38)
65 to ≤75	HR (95% CI) per 1 SD	**1.14 (1.05; 1.23)**	**1.22 (1.02; 1.45)**
Interaction term of continuous age (per 1 SD) and biomarker variable (per 1 SD)	β (SE), *P*	0.038 (0.025), 0.123	0.013 (0.034), 0.702

Boldface type indicates statistical significance at $P < 0.05$. [a]1 SD (OxGua levels) = 275 μg/g creatinine. The model for OxGua levels is adjusted for alcohol consumption, triglycerides, and HDL cholesterol. [b]1 SD (8-isoprostane levels) = 0.29 nmol/mmol creatinine. The model for 8-isoprostane levels is adjusted for BMI, smoking, alcohol consumption, HbA_{1c}, HDL cholesterol, and eGFR.

Ser^{326}/Ser and Ser^{326}/Cys hOGG1 genotype, the Cys/Cys genotype has been found to be associated with higher levels of oxidatively damaged DNA in monoclonal blood cells (34) as well as with insulin resistance (35) and type 2 diabetes prevalence (36) in human studies, which strongly supports the hypothesis of oxidative stress being a risk factor for type 2 diabetes. We hypothesize that hOGG1 expression is upregulated under conditions of oxidative stress, which leads to higher urinary OxGua excretion and would explain the observed positive association of high urinary OxGua levels and incident diabetes in the current study.

The important role of oxidative stress in the development of type 2 diabetes raises the question of how this can be prevented. Regarding pharmacological treatments, Rytter et al. (37) reported that supplementation with a combination of antioxidants did not affect F2-isoprostane and 8-OHdGuo levels in individuals with type 2 diabetes. Furthermore, Rasmussen et al. (38) did not observe any alteration in 8-OHdGuo levels after short-term simvastatin treatment in healthy volunteers. Additional clinical studies are required to search for effective pharmacological treatments for high oxidative stress. Regarding nonpharmacological preventive efforts, weight reduction among obese individuals should be named because it is an effective measure for type 2 diabetes prevention and its efficacy is likely mediated by a reduction of oxidative stress (6). At least for 8-isprostane levels, our data support this hypothesis because BMI and 8-isoprostane levels were positively associated with each other.

Our study has several strengths, including the large sample size, long follow-up, and the large number of assessed potential confounders. Another strength is the high validity of incident diabetes case ascertainment, which included consultations with GPs and measurements of HbA_{1c}.

The study also has several limitations. First, the main limitation is the observational nature of this study, and thus, residual confounding cannot be totally excluded. Second, the oxidative stress biomarkers were measured with ELISA assays, and a general limitation of these assays compared with mass spectrometry–based methods is a lower specificity for the target proteins. As outlined in detail in the RESEARCH DESIGN AND METHODS section, the chosen OxGua ELISA measured three different OxGua species from DNA and RNA. Although this better mirrors the overall DNA and RNA damage by reactive oxygen species, future studies should aim for including distinct measurements of all these molecules to assess their individual associations with type 2 diabetes incidence. Third, the OxGua and 8-isoprostane levels were not repeatedly measured during follow-up, which may have biased the results toward weaker effect estimates. Fourth, neither cytokine nor hormone measurements were available to elucidate reasons for increased oxidative stress biomarkers on the molecular level. This should be addressed in future studies. Finally, our results can only be generalized to Caucasian populations aged 50–75 years.

In conclusion, in this large cohort study, an association of high urinary OxGua and 8-isoprostane levels with type 2 diabetes incidence was observed; however, these associations might only be present in individuals aged ≥60 years. These results suggest an important contribution of an imbalanced redox system to the etiology of type 2 diabetes at older age. Additional studies would be desirable to corroborate our findings in aged populations (≥60 years) for urinary OxGua and 8-isoprostane levels and other biomarkers of oxidative stress.

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