



Review Article

Urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine as a biomarker in type 2 diabetes

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ABSTRACT

The increasing prevalence of diabetes together with the associated morbidity and mortality calls for additional preventive and therapeutic strategies. New biomarkers that can be used in therapy control and risk stratification as alternatives to current methods are needed and can facilitate a more individualized and sufficient treatment of diabetes. Evidence derived from both epidemiological and mechanistic studies suggests that oxidative stress has an important role in mediating the pathologies of diabetic complications. A marker of intracellular oxidative stress that potentially could be used as a valuable biomarker in diabetes is the DNA oxidation marker 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), which can be assessed noninvasively in the urine, with minimal discomfort for the patient. In this review the analytical validity of 8-oxodG is addressed by highlighting important methodological issues. The available epidemiological evidence regarding urinary 8-oxodG and type 2 diabetes is presented. A possible role for DNA oxidation in cancer development in type 2 diabetes patients is discussed, followed by an evaluation of the potential of urinary 8-oxodG as a clinical biomarker in type 2 diabetes.

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Introduction

Diabetes mellitus constitutes a major global health problem because of its increasing prevalence and the accompanying risk of serious complications. According to recent estimates from the International Diabetes Federation 285 million people worldwide have diabetes, representing 6.6% of the world population, and it is

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predicted that this figure will increase to 438 million in 2030 [1]. In most countries diabetes is one of the major causes of premature illness and death, and the associated cardiovascular disease causes the death of 50% or more of diabetes patients [1].

The huge morbidity and mortality associated with diabetes together with the increasing prevalence calls for additional preventive and therapeutic strategies. New biomarkers that can be used in therapy control and risk stratification as alternatives to current methods, e.g., HbA1c and urinary albumin excretion, are needed and can facilitate a more individualized and sufficient treatment of diabetes.

In diabetes management the currently available biomarkers are of extracellular origin and are most of all “by-products” of the disease that do not provide information on the intracellular environment under diabetic conditions. The identification of new biomarkers that actually reflect the intracellular pathological processes could improve diabetes care.

Over the past decade there has been an increasing focus on the role of oxidative stress in the pathophysiology of diabetes-related complications. The diabetic state is associated with increased levels of markers of oxidative stress and evidence derived from mechanistic studies suggests that oxidative stress has an important role in mediating the pathologies of diabetic complications [2–5]. A marker of intracellular oxidative stress that potentially could be used as a new biomarker in diabetes is the DNA oxidation marker 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG). This marker can be assessed noninvasively in the urine, with minimal discomfort for the patient, which makes it suitable for use in risk stratification and therapy control.

8-OxodG is a frequently measured urinary marker of oxidative stress, and in this review we discuss the potential role of urinary 8-oxodG as a biomarker in diabetes. Some 90% of diabetic individuals have type 2 diabetes mellitus, and consequently type 2 diabetes represents the focus of our discussion.

Oxidative stress and type 2 diabetes

Despite considerable research, the exact cellular and molecular mechanisms responsible for type 2 diabetes and its complications are still unresolved.

Oxidative stress is defined as an imbalance between production of reactive oxygen and a biological system's ability to detoxify the reactive products or repair the resulting damage. Disturbances in the normal redox state can cause toxic effects through generation of reactive oxygen species (ROS) that can damage all components of the cell, including lipids, proteins, and nucleic acids (nucleic acid oxidation) [6].

ROS are produced in various tissues under diabetic conditions and are possibly involved in both the progression of pancreatic β -cell dysfunction and the insulin resistance found in type 2 diabetes. In addition, ROS seem to play an important role in the progression of atherosclerosis seen in diabetes patients [7].

DNA oxidation

ROS can cause the formation of a large number of pyrimidine- and purine-derived lesions in DNA, and 8-hydroxylation of guanine (8-oxoGua or 8-OHGua) is one of the most widely studied lesions. Oxidized nuclear DNA undergoes repair and the repair products 8-oxoGua and its corresponding deoxyribonucleoside equivalent 8-oxodG are excreted into the urine [8]. Although it is currently unclear exactly which repair process is the most important, observations point to DNA repair as the predominant process in producing urinary 8-oxodG, with only a negligible contribution from cell turnover and diet, and thus the measurement of 8-oxodG, rather than 8-oxoGua, is preferred as a biomarker of oxidative damage to DNA [9].

The contribution from the deoxyribonucleotide pool to the total amount of excreted 8-oxodG remains to be determined but seems to

be negligible owing to the small pool size compared to total DNA [10,11].

In the steady state the amount of excreted 8-oxodG will equal the newly formed 8-oxodG, and the urinary excretion of 8-oxodG will be equal to the rate of oxidative damage to DNA. Hence, urinary excretion of 8-oxodG reflects the average rate of oxidative damage to DNA in the whole body [12].

Many different cell types have shown increased levels of 8-oxodG in the diabetic state in both human and animal models, which indicates that there is a generalized increased level of oxidative stress in diabetes. The majority of the evidence regarding tissue levels of 8-oxodG is derived from animal studies. In rat models of streptozotocin-induced diabetes 8-oxodG has been shown to be higher in several tissues of the diabetic rats, including kidney, liver, heart, retina, and nerve tissue, compared with nondiabetic controls [13–17]. Studies of human cells have shown increased levels of 8-oxodG in the vitreous of patients with diabetic retinopathy [18], in islets of pancreatic tissues of type 2 diabetes patients [19], and in isolated human endothelial cells exposed to high glucose [20].

Methods for measurement of urinary 8-oxodG

The two major analytical approaches that are used for the measurement of urinary 8-oxodG are chromatography, combined with electrochemical detection or mass spectrometry, or direct immunodetection. A few interlaboratory comparisons between some of the chromatography-based techniques and enzyme-linked immunosorbent assay (ELISA) have been made for the quantification of 8-oxodG in urine [21–23]. They showed a reasonable agreement between the chromatography-based techniques, but the ELISA-based techniques gave higher values and showed more variability. Lack of specificity seems to be the main issue in the ELISA method, which presumably is because the epitope (8-hydroxylation in the 8-position) resembles many molecules that are present in biological samples. The greater variability of the ELISA method and measurement of background levels of additional compounds that are recognized by the antibody can reduce the assay's ability to detect differences.

Among the sufficiently sensitive methods to measure urinary 8-oxodG only liquid chromatography with mass spectrometry that includes qualifier and quantifier ion detection [24] and gas chromatography coupled to mass spectrometry meet the recommended requirements for identification and quantification set by the Commission of the European Communities [25].

When determining the urinary excretion of 8-oxodG, 24-h urine collection is preferred. Because 24-h collection can be cumbersome, often resulting in incomplete or improper collection, spot urine samples are frequently used instead. Urinary creatinine concentration is used to adjust for the varying dilution of these spot urine samples, but the interpretation of the results is often difficult because creatinine in itself is closely associated with other important variables such as sex, age, body mass index, and race. In large cohort studies 24-h collection is often not practically possible, and in these situations creatinine-corrected spot urine samples can be used, if the uncertainties associated with this correction are handled appropriately. One way of doing this is to assess the effects of 8-oxodG and creatinine separately in multiple regression models as described by Barr et al. [26]. Although 24-h urine is considered the gold standard in paired and randomized trials, bias from creatinine correction is less likely. In these designs spot urine with creatinine correction can be used without considerable risk of bias.

Urinary 8-oxodG as a marker of oxidative stress in type 2 diabetes

Studies of the associations between urinary 8-oxodG and diabetes or diabetes-related quantitative traits are presented in Table 1.

Table 1
Studies of associations between urinary 8-oxodG and type 2 diabetes.

Design	Method	No. of subjects	Results	Ref.
Case-control	ELISA	81 T2D patients, 100 controls	Increased 24-h excretion in patients compared with controls ($68.2 \pm 39.4 \mu\text{g}$ vs $49.6 \pm 37.7 \mu\text{g}$, $P = 0.001$). Patients with HbA1c higher than 9.6% had higher excretion of 8-oxodG than patients with HbA1c less than 6.8% ($P = 0.009$).	[27]
Case-control	HPLC-EC	53 T2D patients, 39 controls	Increased 8-oxodG excretion in patients compared with controls (352 ± 183 vs $161 \pm 56 \text{ pmol/kg/day}$, $P < 0.001$).	[28]
Case-control	ELISA	25 T2D patients, 20 controls	Increased 8-oxodG excretion in patients compared with controls (11.68 ± 6.21 vs $8.37 \pm 2.26 \mu\text{g/day}$, $P < 0.05$). 8-OxodG excretion increased with the severity of tubulointerstitial injury ($P = 0.03$) and tended to increase with severity of glomerular diffuse lesion ($P = 0.057$).	[29]
Case-control	ELISA	60 T2D patients, 35 controls	Increased 8-oxodG excretion in patients compared with controls (19.6 ± 6.7 vs $11.9 \pm 4.9 \text{ ng/mg creatinine}$, $P < 0.05$). HbA1c correlated with urinary 8-oxodG excretion in the diabetic group ($r = 0.53$, $P < 0.05$). Higher 8-oxodG levels in patients with proliferative retinopathy ($26.2 \pm 4.5 \text{ ng/mg creatinine}$) than in patients with nonproliferative retinopathy ($18.7 \pm 3.6 \text{ ng/mg creatinine}$) or without retinopathy ($12.6 \pm 2.6 \text{ ng/mg creatinine}$).	[30]
Cohort	HPLC-EC	396 T2D patients	Association between 24-h urinary 8-oxodG excretion and average HbA1c ($P < 0.05$). 8-OxodG excretion significantly higher in patients with hypertension ($P < 0.005$).	[31]
Case-control	CE-AD	69 T2D patients, 30 controls	Positive correlation between 24-h urinary excretion of 8-oxodG and HbA1c ($r = 0.287$, $P = 0.022$). Higher 8-oxodG excretion in patients with macroalbuminuria than in patients with normoalbuminuria ($19.2 \pm 16.8 \mu\text{g/24 h}$ vs $8.1 \pm 1.7 \mu\text{g/24 h}$, $P = 0.015$), and higher 8-oxodG excretion in patients with macroalbuminuria than in healthy controls ($5.72 \pm 6.89 \mu\text{mol/mol creatinine}$ vs $2.33 \pm 2.83 \mu\text{mol/mol creatinine}$, $P = 0.018$).	[32]
Cohort	ELISA	96 T2D patients	8-OxodG excretion 2.5-fold increased in patients with increased HbA1c than in those with normal HbA1c (63.6 ± 59.7 vs $26.6 \pm 13.7 \mu\text{g/g creatinine}$, $P < 0.05$). Higher excretion of 8-oxodG in patients with albuminuria than in patients without albuminuria (82.6 ± 64.8 vs $43.5 \pm 45.7 \mu\text{g/g creatinine}$, $P < 0.01$). Higher 8-oxodG excretion in patients with increased ($> 1.1 \text{ mm}$) compared to those with normal intima media thickness of carotid arteries (71.8 ± 63.0 vs $30.7 \pm 25.1 \mu\text{g/g creatinine}$, $P < 0.005$). Significant correlation between 8-oxodG excretion and coronary heart disease risk score ($r = 0.27$, $P < 0.01$).	[33]
Case-control	ELISA	73 diabetic patients, 24 nondiabetic nephropathy patients, 25 controls	24-h 8-oxodG excretion higher in patients with proteinuria compared with control subjects and diabetic patients with normoalbuminuria (32.4 ± 5.5 vs 14.3 ± 2.4 and $15.4 \pm 1.9 \mu\text{g/day}$, $P < 0.05$, respectively).	[34]

T2D, type 2 diabetes; CE-AD, capillary electrophoresis with end-column amperometric detection.

Increased urinary 8-oxodG excretion has been observed in type 2 diabetes patients compared with healthy subjects. In a Finnish study of 81 type 2 diabetes patients and 100 controls the authors found a significantly higher 24-h urinary 8-oxodG excretion (determined with the ELISA method) in patients compared with controls [27]. This association was also shown in two other smaller studies. Hinokio et al. observed that 24-h 8-oxodG excretion (by high-performance liquid chromatography with electrochemical detection (HPLC-EC)) was significantly higher in 53 type 2 diabetic patients compared to 39 age-matched healthy control subjects [28], and Kanauchi et al. reported that 24-h 8-oxodG excretion (ELISA) was significantly increased in 25 type 2 diabetes patients compared with 20 healthy control subjects [29]. In a Chinese study of 60 patients with type 2 diabetes and 35 nondiabetic control subjects using creatinine-corrected spot urine samples the patients with type 2 diabetes had significantly higher concentrations of 8-oxodG (ELISA) compared to the control subjects [30].

Hyperglycemia

Urinary 8-oxodG excretion is increased by hyperglycemia and correlates with glycosylated hemoglobin (HbA1c). Leinonen et al. divided patients into three groups according to their level of HbA1c. The group of patients with the poorest glycemic control (HbA1c higher than 9.6%) had total 24-h urinary excretion of 8-oxodG 56% higher than the group with the best glycemic control (HbA1c less than 6.8%) [27].

Hinokio et al. showed an association between 24-h urinary 8-oxodG excretion (HPLC-EC) and average HbA1c in a study of 396 diabetic patients [31], and Xu et al. observed a positive correlation between 24-h urinary excretion of 8-oxodG (capillary electrophoresis with end-column amperometric detection (CE-AD)) and HbA1c in 69 type 2 diabetic patients ($r = 0.29$) [32].

In a spot urine assay Dong et al. showed that HbA1c values were significantly correlated with urinary 8-oxodG excretion in the diabetic group ($r = 0.53$) [30], and in a study of 96 type 2 diabetic patients Nishikawa et al. found that the urinary 8-oxodG excretion (ELISA) was

2.5-fold increased in patients with increased (HbA1c above 5.8%) compared with those with normal HbA1c [33].

Other diabetes-related variables

Only a few studies have assessed the possible associations between urinary 8-oxodG and other diabetes-related variables, e.g., blood pressure, obesity, and cholesterol.

Hinokio et al. showed that 24-h urinary 8-oxodG excretion was significantly higher in type 2 diabetes with hypertension compared to those without hypertension [31]. In contrast, other studies did not find any significant association between 8-oxodG excretion and blood pressure [32,33]. Furthermore, a lack of association between urinary 8-oxodG and the variables total cholesterol, HDL cholesterol, and body mass index in type 2 diabetes patients has been reported [32,33].

Complications

Increased excretion of 8-oxodG in type 2 diabetic patients with known complications compared to type 2 diabetics without complications have been reported.

Microvascular complications

Type 2 diabetic patients with proliferative retinopathy had significantly higher 8-oxodG levels than type 2 diabetics with nonproliferative retinopathy or without retinopathy [30].

Xu et al. found higher 24-h urinary 8-oxodG excretion in diabetes patients with macroalbuminuria compared with diabetes patients with normoalbuminuria, and higher 8-oxodG excretion in patients with macroalbuminuria than in healthy controls [32].

Nishikawa et al. observed a 1.9-fold higher excretion of 8-oxodG in patients with albuminuria compared to patients without albuminuria [33], and Shimoike et al. showed that 24-h urinary 8-oxodG excretion (ELISA) was significantly higher in diabetic patients with proteinuria compared with control subjects and diabetic patients with normoalbuminuria [34].

Twenty-four-hour urinary 8-oxodG excretion (ELISA) increased with the severity of tubulointerstitial injury determined by semi-quantitative estimate of the space occupied by fibrous tissue and/or interstitial infiltrates in renal biopsies from type 2 diabetic patients. In addition, 8-oxodG excretion tended to increase with the severity of glomerular diffuse lesion (using Gellman's criteria), but this was not significant ($P = 0.057$) [29].

Macrovascular complications

There is only limited evidence to support an association between urinary 8-oxodG excretion and macrovascular complications in diabetes.

Urinary 8-oxodG excretion has been shown to be 2.3-fold higher in type 2 diabetics with increased (>1.1 mm) compared to those with normal intima media thickness of the carotid arteries [33]. In addition, the authors found a significant correlation between 8-oxodG excretion and coronary heart disease risk score ($r = 0.27$) [33].

The data from the studies of urinary 8-oxodG and type 2 diabetes are consistent and include several methodologies and 24-h urine collection. Still, bias from comparing noncomparable groups cannot be excluded and confirmation in prospective cohort studies is needed. Bias due to changes in 8-oxodG excretion in patients with affected kidney function does, on the other hand, not seem to be significant, because in the steady state the excretion rate of 8-oxodG will equal the generation regardless of kidney function.

Urinary 8-oxodG and intervention in type 2 diabetes

Studies have found that various pharmacological treatments reduce urinary excretion of 8-oxodG in patients with type 2 diabetes (Table 2). The results of the intervention studies should be interpreted with caution because most of the mentioned studies measured 8-oxodG in spot urine using the ELISA method and many of the studies were not placebo-controlled.

Ogawa et al. showed that treatment with angiotensin II receptor blockers (ARB) for 8 weeks reduced 8-oxodG excretion (ELISA) significantly in diabetic patients with nephropathy [35], and Miyashita et al. found a significant reduction in 8-oxodG excretion (ELISA) after 12 months ARB treatment in 35 type 2 diabetes patients with hypertension [36]. Addition of the calcium channel blocker azelnipidine to ongoing inhibition of the renin–angiotensin–aldosterone system significantly reduced 8-oxodG excretion in patients with diabetic nephropathy [37,38]. Endo et al. found significantly decreased urinary 8-oxodG excretion (ELISA) in patients with type 2 diabetes and hypercholesterolemia after 3 months treatment with the cholesterol-

lowering agents probucol and atorvastatin [39], and after pitavastatin treatment for 12 months, a significant decrease in urinary 8-oxodG (ELISA) was observed by Miyashita et al. in 45 type 2 diabetes patients with high LDL cholesterol [40].

Only a few studies have been performed to assess the effect of lifestyle intervention on 8-oxodG excretion in type 2 diabetes patients. Nojima et al. found that 12 months of moderate-intensity aerobic exercise reduced 8-oxodG excretion significantly [41], and Nakamura et al. reported that 4 oz red wine daily for 6 months reduced 8-oxodG in type 2 diabetes patients with nephropathy [42].

The predictive value of urinary 8-oxodG in type 2 diabetes

Morbidity

Hinokio et al. found that the incidence of macroalbuminuria was increased in type 2 diabetes patients with higher excretion of 8-oxodG in urine compared with patients with moderate or lower excretion of 8-oxodG ($P < 0.0001$), and multivariate logistic regression analysis suggested that urinary 8-oxodG was the strongest predictor of nephropathy among several known risk factors [31].

Mortality

To the authors' knowledge there are no studies that have assessed the urinary excretion of 8-oxodG as a predictor of mortality, the ultimate outcome variable, in a prospective setting. This emphasizes the need for studies of urinary 8-oxodG in large cohorts with long follow-up.

Urinary 8-oxodG, type 2 diabetes, and cancer

There is evidence that type 2 diabetes is associated with increased risk of several kinds of cancer including liver, pancreas, breast, endometrial, kidney, bladder, and colorectal cancer and non-Hodgkin lymphoma [43–48]. A recent epidemiological study covering approximately one-half of the Swedish type 2 diabetes patients showed an elevated risk for several cancers after hospitalization for type 2 diabetes, and the highest risks were found for liver and pancreatic cancers [49]. The compelling epidemiological evidence suggests that cancer should be numbered among the complications of diabetes.

The exact mechanisms underlying the detrimental association between type 2 diabetes and cancer have not been fully elucidated, but because of the mutagenic properties of oxidatively damaged DNA the possible role of DNA oxidation in this mechanism should be

Table 2
Urinary 8-oxodG excretion in type 2 diabetic patients—intervention trials.

Method	No. of subjects	Results	Ref.
ELISA	66 T2D patients with diabetic nephropathy: ARB, 33 (candesartan 8 mg/day, 11; valsartan 80 mg/day, 22); trichlormethiazide, 33	Treatment with angiotensin II receptor blockers for 8 weeks reduced the 8-oxodG in spot urine significantly.	[35]
ELISA	70 T2D patients with hypertension: olmesartan 10 mg/day, 35; amlodipine 5 mg/day, 35	Significant reduction in 8-oxodG excretion after 12 months ARB treatment (from 9.59 ± 3.9 to 7.4 ± 2.9 ng/mg creatinine).	[36]
ELISA	45 diabetes patients with microalbuminuria already treated with ARB: azelnipine 8 mg/day, 15; azelnipine 16 mg/day, 15; controls, 15	Addition of azelnipidine to ongoing ARB treatment significantly reduced 8-oxodG excretion.	[37]
ELISA	T2D patients with diabetic nephropathy taking RAAS inhibitors: azelnipine 16 mg/day, 21; nifedipine 40 mg/day, 17	Addition of azelnipidine to ongoing RAAS inhibition significantly reduced 8-oxodG excretion.	[38]
ELISA	T2D patients with hypercholesterolemia: probucol 500 mg/day, 18; atorvastatin 10 mg/day, 18	Significantly decreased 8-oxodG excretion when treated with the cholesterol-lowering agents probucol and atorvastatin (probuco, 12.3 ± 8.8 to 6.8 ± 2.6 ng/mg creatinine; atorvastatin, 12.4 ± 7.5 to 8.1 ± 4.2 ng/mg creatinine).	[39]
ELISA	45 T2D patients with high LDL cholesterol: pitavastatin 2 mg/day	Pitavastatin treatment for 12 months decreased urinary 8-oxodG excretion (from 11.3 to 8.4 ng/mg creatinine, $P < 0.05$).	[40]
ELISA	Patients with T2D: aerobic training combined with fitness center, 43; aerobic training only, 44; controls, 16	12 months intervention with moderate-intensity aerobic exercise reduced 8-oxodG excretion significantly.	[41]
ELISA	T2D patients with diabetic nephropathy: red wine, 12; white wine, 12; controls, 12	Red wine reduced 8-oxodG after 3 and 6 months.	[42]

T2D, type 2 diabetes; ARB, angiotensin II receptor blockers; RAAS, renin–angiotensin–aldosterone system.

considered. The literature on DNA oxidation and cancer is too extensive to be covered here, and only evidence regarding urinary 8-oxodG is mentioned.

No studies of the relationship between 8-oxodG excretion and cancer in type 2 diabetes patients were found in our literature search. In general, the possible association between urinary 8-oxodG excretion and cancer has not been thoroughly investigated, although some case–control studies have shown elevated levels of urinary 8-oxodG in patients with various malignancies (Table 3) [50–57]. No published study has assessed the urinary excretion of 8-oxodG or any other oxidized nucleosides or corresponding bases as predictors of cancer in diabetes patients. Cross-sectional studies are likely to be associated with reverse causality, and large prospective cohort studies with long follow-up are required to provide evidence that a high level of urinary 8-oxodG implies an elevated risk of cancer.

Only a few studies have addressed the possible predictive role of urinary 8-oxodG in relation to cancer risk. In a nested case–cohort design Loft et al. examined associations between urinary excretion of 8-oxodG measured in spot urine and risk of lung cancer in a population-based cohort of 25,717 men and 27,972 women ages 50–64 years with 3–7 years follow-up. Overall there was no association between 8-oxodG excretion and lung cancer risk, but among never smokers the authors found a significant association with an incidence rate ratio of 11.8 (1.21–115) per doubling of 8-oxodG excretion [58].

Other indirect studies indicate that urinary 8-oxodG levels might possess predictive information regarding cancer development. In a study of renal transplant patients with ($n = 17$) and without ($n = 17$) squamous cell carcinoma (SCC), urinary 8-oxodG in spot urine was significantly elevated ($P = 0.03$), both pre- and post-tumor development, compared to non-SCC transplant patients [59]. Increased levels of urinary 8-oxodG were found in patients with celiac disease [60], a disease state associated with considerable cancer risk. In hereditary hemochromatosis iron depletion, the treatment known to prevent cirrhosis and development of hepatocellular carcinoma resulted in reduced urinary 8-oxodG excretion, and diminished DNA oxidation is a plausible explanation for the amelioration of the increased liver cancer risk seen in treated patients [61]. Thanan et al. showed that urinary 8-oxodG levels were significantly higher in patients with *Opisthorchis viverrini* infection, a major risk factor for cholangiocarcinoma, compared to healthy subjects ($P < 0.01$) [54].

Although the data of 8-oxodG and cancer at first glance seem quite consistent, they should be interpreted with some caution. In the majority of the studies oxidative stress was measured by the 8-oxodG/creatinine ratio. Creatinine excretion is influenced by muscle mass and because cancer patients may have low muscle mass and therefore

reduced creatinine excretion, the results may be biased toward higher ratios in cancer patients. Prospective studies and studies with 24-h urine collection are needed to verify these findings.

Conclusions

Although the studies of urinary 8-oxodG excretion and type 2 diabetes often involve considerable uncertainties (different methods, publication bias, and difference in the quality of trials), which should be taken into account, the available data provide evidence of consistent associations between 8-oxodG and type 2 diabetes and the complications of the disease.

The available evidence indicates that compared with healthy subjects, type 2 diabetes patients have increased urinary 8-oxodG excretion, and type 2 diabetes patients with complications have increased 8-oxodG excretion compared with those without complications. Urinary 8-oxodG excretion seems to be closely associated with glycemic control in patients, and various kinds of intervention that improve outcome in patients also reduce 8-oxodG excretion.

One possible mechanism by which DNA oxidation could be involved in the development of complications in diabetes is induction of cell senescence via either telomeric or nontelomeric DNA damage [62,63]. Premature endothelial cell senescence leads to vasculopathy and atherosclerosis and eventually the clinical manifestations of the vascular complications seen in diabetes patients.

The demonstrated associations between, on one hand, 8-oxodG excretion and diabetes and, on the other hand, 8-oxodG and cancer, together with the shown overrepresentation of cancer in diabetes patients and the mutagenic properties of the lesion, suggest that 8-oxodG excretion could reflect important pathogenetic mechanisms in the development of cancer in diabetes patients, which again leaves open the possibility that 8-oxodG possesses predictive information regarding development of cancer in diabetes.

An evaluation of the clinical potential of a novel biomarker such as urinary 8-oxodG may be structured around three different types of validation: analytical validity, clinical validity, and clinical utility. The analytical validity has been addressed in several studies and the chromatography-based techniques seem to be superior to ELISA with respect to specificity and reproducibility [21,23,64]. In the assessment of clinical validity consistent associations between urinary 8-oxodG and diabetes-related phenotypes have been shown. When evaluating the clinical utility the potential uses of the biomarker must be considered. Can urinary 8-oxodG be used in risk stratification/selection of therapy or does it have a place in monitoring disease progression or response to therapy? Is 8-oxodG a predictor of mortality or cancer? Does 8-oxodG

Table 3
Case–control studies of urinary 8-oxodG and cancer.

Method	No. of subjects	Results	Ref.
HPLC-EC	18 patients with carcinoma of the female genitalia, 10 controls	Excretion of 8-oxodG in patients with carcinoma of the female genitalia was significantly higher than in patients without carcinoma.	[50]
HPLC-GC-MS	29 breast or ovarian cancer patients with BRCA1 mutations, 70 carriers of BRCA1 mutations without symptoms, 91 controls	Excretion of urinary 8-oxodG was higher in patients with breast or ovarian cancer carrying the BRCA1 mutation than in healthy carriers of the mutation.	[51]
HPLC-EC	60 breast cancer patients, 60 control subjects	In multiple regression analysis urinary 8-oxodG levels were significantly higher among breast cancer patients after adjustments for confounders such as age at menarche, menopause, use of oral contraceptives, smoking, and coffee consumption ($P < 0.001$).	[52]
ELISA	37 lung cancer patients and 52 controls	Patients ($n = 14$) with small-cell carcinoma of the lung had significantly higher 8-oxodG excretion than controls.	[53]
HPLC-EC	55 cholangiocarcinoma patients, 17 healthy controls	Urinary 8-oxodG levels were significantly higher in cholangiocarcinoma patients ($6.83 \pm 1.00 \mu\text{g/g creatinine}$) than in healthy subjects ($3.03 \pm 0.24 \mu\text{g/g creatinine}$; $P < 0.01$).	[54]
ELISA	82 prostate cancer patients, 33 age-matched healthy controls	Urinary excretion of 8-oxodG in patients with prostate cancer was significantly higher than in age-matched healthy controls (20.8 ± 8.9 vs $16.1 \pm 7.8 \text{ ng/mg creatinine}$).	[55]
HPLC-EC	436 cancer patients, 129 healthy controls	8-OxodG in random urinary samples from cancer patients was significantly higher than in those from healthy individuals (2.42 ± 2.28 vs $1.19 \pm 0.48 \mu\text{mol/mol creatinine}$, $P < 0.001$).	[56]
ELISA	116 children with acute leukemia, 51 controls	Urinary 8-oxodG in children with acute leukemia was significantly elevated compared with controls (11.92 ± 15.42 vs $4.03 \pm 4.70 \text{ ng/mg creatinine}$, $P < 0.05$).	[57]

add valuable information to existing tests (e.g., HbA1c, albumin excretion). These questions are yet to be answered.

Because 8-oxodG is a measure of intracellular oxidative stress and, therefore, in contrast to extracellular markers such as HbA1c and albumin, reflects the intracellular environment in diabetic conditions, it could possess valuable information that cannot be obtained from currently available biomarkers.

To answer the above-mentioned and other questions regarding the clinical use of 8-oxodG there is a need for large cohort studies with long follow-up to identify consistent associations between 8-oxodG and outcome, and randomized clinical trials must be initiated to investigate whether outcome is modifiable with specific therapies targeted at oxidative stress.

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