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Research Article

Association between 8-oxo-7,8-dihydro-2′-deoxyguanosine Excretion and Risk of Postmenopausal Breast Cancer: Nested Case–Control Study

Steffen Loft[1], Anja Olsen[2], Peter Møller[1], Henrik E. Poulsen[3], and Anne Tjønneland[2]

Abstract

Background: Oxidative stress may be important in carcinogenesis and a possible risk factor for breast cancer. The urinary excretion of oxidatively generated biomolecules, such as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), represents biomarkers of oxidative stress, reflecting the rate of global damage to DNA in steady state.

Methods: In a nested case–control design, we examined associations between urinary excretion of 8-oxodG and risk of breast cancer in a population-based cohort of 24,697 postmenopausal women aged 50 to 64 years with 3 to 7 years follow-up. The accruing cases of breast cancer were matched to controls by age at diagnosis, baseline age, and hormone replacement therapy (HRT). Spot urine samples collected at entry was analyzed for 8-oxodG by high-performance liquid chromatography with electrochemical detection. Incidence rate ratio (IRR; 95% confidence intervals) based on 336 matched pairs with all information was estimated per unit increase in 8-oxodG divided by creatinine for all and estrogen receptor (ER) positive and negative breast cancers.

Results: There was a borderline significant positive association between 8-oxodG and risk of all breast cancer (IRR: 1.08; 1.00–1.17 per unit increase in nmol/mmol creatinine). This association was significant with respect to the risk of ER-positive cancer (IRR: 1.11; 1.01–1.23) and among women not using HRT (IRR: 1.11; 0.97–1.26) or with low dietary iron intake (IRR: 1.10; 1.06–1.37 per unit increase) for all breast cancer.

Conclusions: We observed positive association between 8-oxodG excretion and risk of especially ER-positive breast cancer.

Impact: Our results suggest that oxidative stress with damage to DNA is important for the development of breast cancer. *Cancer Epidemiol Biomarkers Prev; 22(7); 1289–96. ©2013 AACR.*

Introduction

Oxidative stress is thought to be involved in the etiology of many forms of cancer including breast cancer (1, 2). Estrogens can generate intracellular oxidative stress with both genotoxic and signaling consequences through redox cycling of catechol metabolites and mitochondrial dysfunction, partly dependent on expression of estrogen receptors (ER; ref. 2). Oxidized guanine (8-oxoGua), frequently measured as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), is one of the most abundant oxidative stress-induced lesion in DNA, resulting from direct oxidation or from possibly DNA polymerase-dependent incorporation of 8-oxodGTP from the nucleotide pool (3–5). 8-OxoGua in DNA causes G–T transversion mutations upon replication of DNA (6), unless excised by oxoguanine DNA glycosylase (OGG1; ref. 7 and 8). Experimental studies indicate that estrogens, especially equine and synthetic estrogens, can induce 8-oxodG in cell types expressing ER (2, 9, 10). Moreover, elevated levels of 8-oxodG or oxidized purines have been found in breast cancer tissue (11–14). Accordingly, oxidative stress and DNA damage could play a role in development of ER-positive postmenopausal cancer with hormone replacement therapy (HRT) as possible risk factor (15).

Biomarkers of oxidative stress include oxidized nucleobases in leukocytes and urinary excretion of 8-oxoGua and 8-oxodG, originating from sanitization of the nucleotide pool for oxidized dGTP and possibly alternative repair pathways in DNA (3, 16). Several epidemiological studies have addressed risk of breast cancer associated with these biomarkers in urine samples assayed by chromatographic methods with electrochemical or mass spectrometry–based detection or by ELISA. Large-scale interlaboratory and other validation studies have documented that ELISA-based measurement of 8-oxodG is unspecific and

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imprecise, not discriminating between healthy subjects and patients (17, 18). Indeed, one case–control study with approximately 1,100 cases and one nested-in-cohort prospective case–control study with 326 cases using ELISA-based assay of 8-oxodG found no association between the level and risk of breast cancer (19–21). In contrast, four smaller case–control studies with a total of more than 400 cases and using chromatographic measurement of 8-oxodG all found significantly higher risk of breast cancer associated with high excretion levels (22–25). Accordingly, the more accurate chromatographic assays might be required to detect associations with risk of breast cancer. This is in line with elevated levels of oxidized guanine or similar biomarkers found in leukocytes and plasma from patients with breast cancer compared with controls (25, 26). Alternatively, associations found in the case–control studies might result from reverse causality if breast cancer *per se* causes increased formation or excretion of 8-oxodG (27). This possibility is supported by decreased excretion of 8-oxodG after tumor removal in breast cancer patients (24). Furthermore, associations between 8-oxodG and risk of breast cancer could be modified by intake of fruit and vegetables, which might reduce oxidative stress-induced DNA damage (28), or of iron, which has been suggested to be a risk factor of breast cancer via oxidative stress (1).

In this study, we examined the association between the urinary excretion of 8-oxodG measured by a chromatographic method and later risk of breast cancer in a population-based cohort of 24,697 postmenopausal women aged 50 to 64 years with 3 to 7 years follow-up. We addressed specifically the risk of ER-positive tumors and the possible influence of HRT as well as intake of fruit, vegetables, and iron.

Materials and Methods

Study population

We used a previously described study population addressing risk of postmenopausal breast cancer in the prospective Danish follow-up study, "Diet, Cancer and Health," approved by the ethical committees on human studies in Copenhagen and Aarhus and Danish Data Protection Agency (29, 30). From December 1993 to May 1997, 79,729 women aged 50 to 64 years, born in Denmark, living in the greater Copenhagen or Aarhus areas and without previously registered cancer in the Danish Cancer Registry were invited to participate in the study. A total of 29,875 women accepted the invitation and visited 1 of 2 established study centers where spot urinary samples were collected. The women completed a food-frequency questionnaire and a lifestyle questionnaire, including questions about reproductive factors, health status, social factors, and lifestyle habits as described previously (30). From this, we obtained information about years of school education (short: $\leq$7 years, medium: 8–10 years, or long: $\geq$10 years), parity (parous/nulliparous, number of births, and age at first birth), use (never, past, current in relation to urine sampling) and duration of HRT. Health professionals obtained anthropometrical measurements and body mass index (BMI: weight/height^2 in kg/m^2).

Of the 29,875 women enrolled in the study, 326 were reported to the Danish Cancer Registry with a cancer diagnosed before their baseline visit and therefore excluded. Eight women were excluded from the study because they did not complete the lifestyle questionnaire. Furthermore, 4,844 women were not considered postmenopausal and excluded; 4,798, who were considered premenopausal, with at least 1 menstruation within the 12 months before study entry and no use of HRT; 9 with no lifetime history of menstruation; and 37 who did not answer the questions about current or previous use of HRT. Known postmenopausal women were either (i) nonhysterectomized and reporting no menstruation during the 12 months before inclusion, (ii) reporting bilateral oophorectomy, or (iii) reporting age at last menstruation lower than age at hysterectomy. Probable postmenopausal women were either (i) reporting menstruation during the 12 months before inclusion and current use of HRT (we assumed the bleeding were caused by HRT), (ii) reported hysterectomy with a unilateral oophorectomy or an oophorectomy of unknown laterality, or (iii) reported last menstruation at the same age as that at hysterectomy. Accordingly, 24,697 postmenopausal women remained in the cohort. Cohort members were identified by a personal identification number allocated to every Danish citizen and information on vital status and emigration was retrieved by linkage to the Central Population Registry, whereas information of cancer occurrence was obtained by linkage to the Danish Cancer Registry. Follow-up for breast cancer was done on each woman from the date of entry (date of visit to the study center) until diagnosis of cancer (all except nonmelanoma skin cancer), date of death, date of emigration or December 31, 2000. During the follow-up period, 434 women from the cohort were diagnosed with breast cancer, of these 84 were diagnosed within the first year of follow-up. The median (5–95 percentiles) period from collection of the urinary samples to diagnosis was 2.4 (0.2–4.9) years.

In addition to the Danish Cancer Registry, the Danish Breast Cancer Co-operative Group (DBCG) registry has information about approximately 90% of all Danish breast cancer cases, and includes information on ER status (31). A standardized immunohistochemical method is used with a cut-off level used to define positive receptor status as 10%, or more, positive cells.

Matching of cases and controls

It was not feasible to determine urinary levels of 8-oxodG for all cohort members and we used a nested case–control design. For each of the 434 cases, one control was selected as cancer-free at the exact age at diagnosis of the case and match of certainty of postmenopausal status (known/probably menopausal), use of HRT on inclusion into the cohort (current/former/never), and age on entry into the cohort (6 month intervals) by incidence density sampling.

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Of the 434 pairs (866 women: 434 cases; and 434 controls, including 2 cases), 58 pairs were excluded due to the lack of a urine sample or to failure of the analysis to resolve 8-oxodG sufficiently for quantification in one or both samples from a pair. Furthermore, 40 pairs were excluded because information was missing in either case or control on one or more of the potential confounding factors, including number of births or age at first birth (15 pairs), education (1 pair), duration of HRT use (22 pairs), alcohol intake (1 pair), and BMI (1 pair), thus leaving 336 case–control pairs for study.

Urine sample storage and analysis

Urine samples collected at entry in the study were frozen at −20°C within 2 hours and from the end of the day stored at −150°C until analysis with short storage at −80°C.

The urinary concentrations of 8-oxodG were determined by column-switching high-performance liquid chromatography with electrochemical detection as described elsewhere (32). The intrabatch and interbatch coefficients of variations were below 6%. The assay has been validated by almost identical results from randomly selected urine samples dG in 2 other laboratories using chromatography methods with electrochemical detection ($r = 0.95$; ref. 33) or tandem mass spectrometry detection ($r = 0.99$; ref. 34). We have stored urine samples at −20°C and repeatedly measured for 8-oxodG during 15 years without decline in concentrations achieving values within 10% of the original measurements. The urinary concentration of creatinine was determined by a standard colorimetric method (17).

Statistical methods

Because of the design with match on age at cancer diagnosis, we used conditional logistic regression analyses to estimate the breast cancer incidence rate ratios (IRR).

We evaluated the potentially confounding effects of a set of baseline values of established risk factors for breast cancer: parity (yes/no), number of births (linear), age at first birth (linear), length of school education (short, medium, long), duration of HRT (linear), BMI (linear), and alcohol intake (linear) as well as smoking status (never, former, current), duration (years), and intensity (g/day), which has a known effect on 8-oxodG excretion (35). The choice of potential confounders was based on the information obtainable from the questionnaire and a literature-based assessment of the most important variables.

All quantitative variables were entered linearly in the model (36). The linearity of the associations was evaluated graphically using linear splines with 3 boundaries placed at the quartile cut points according to the exposure distribution among cases (36). None of the associations showed signs of inflection or threshold values.

The association between excretion of 8-oxodG and the investigated confounders as well as dietary intake of fruits and vegetables was assessed by categorized analysis as well as linear regression in univariate and mutually adjusted analysis. SAS, release 6.12 (SAS Institute, Inc.) was used for the analyses.

Results

Information about ER status of the tumors was obtained for 393 (92%) cases of breast cancer, with 302 tumors reported to be ER-positive and 91 tumors ER-negative. Information about ER status was not obtained for the remaining 33 cases; it was not possible to determine ER status on 9 *in situ* tumors, and 24 tumors could not be found in the DBCG registry.

Baseline characteristics of the study population are presented in Table 1. Cases had a longer duration of HRT, a higher alcohol intake, and more often a longer school education than controls, although none of these factors were significantly associated with the risk of breast cancer. Age at baseline and use of HRT was identical among cases and controls due to the matching.

The level of 8-oxodG was successfully measured in 402 samples from cases and 402 samples from controls. This allowed 336 matched pairs with complete information on potential confounders for data analysis. Table 2 shows the association between 8-oxodG excretion and lifestyle and potential risk factors for cancer. A high intake of fruit was associated with high excretion of 8-oxodG among controls. If intake of fruit was expressed per energy intake, this difference in 8-oxodG excretion was still present among controls and reached statistical significance among cases as well [1.67; 95% confidence interval (CI), 0.48–6.16 nmol/mmol creatinine vs. 2.01; 95% CI, 0.55–7.15 nmol/mmol creatinine]. Expression of intake of vegetables or iron per energy intake had no effect on the difference related to high or low intake with respect to 8-oxodG excretion. The excretion of 8-oxodG was 30% higher in active smokers as compared with never smokers among cases and controls combined. There were no significant associations between the excretion of 8-oxodG and alcohol consumption, intake of iron and vegetables, BMI, education, use of HRT, or reproductive history.

Table 3 shows the association between 8-oxodG excretion and the subsequent risk of breast cancer. In the whole population, there was a borderline significant positive association between 8-oxodG and risk of breast cancer in adjusted analysis. This association was more pronounced and significant with respect to the risk of ER-positive cancer.

Table 4 shows the associations between the risk of breast cancer and excretion of 8-oxodG according to HRT use, smoking, intake of fruit, vegetables, and iron, and use of iron supplement. The association between breast cancer risk and 8-oxodG excretion seemed to be confined to women with a low dietary intake of iron and a similar but not significant pattern for use of iron supplements, whereas none of the other factors showed any signs of interactions.

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Table 1. Baseline characteristics and associated IRR of postmenopausal breast cancer among cases and controls in the Danish "Diet, Cancer and Health" study

	Cases ($N = 336$)		Controls ($N = 336$)		
	Median	(5%, 95%)	Median	(5%, 95%)	IRR (95% CI)[a]
Duration of HRT use in years[b]	6	(1–20)	5	(1–21)	1.00 (0.96–1.04)
Age at first birth[c]	23	(18–32)	23	(18–31)	1.04 (0.83–1.30)
Number of births[d]	2	(1–4)	2	(1–4)	0.87 (0.71–1.06)
BMI (IRR per 5 units[e])	25	(20–34)	25	(20–34)	1.09 (0.90–1.32)
Alcohol intake g/day (IRR per 10 g/day)	11	(0–44)	10	(1–44)	1.12 (1.00–1.24)
Smoking duration (years)[f]	30	(3–45)	32	(4–46)	0.98 (0.95–1.02)
Amount (g tobacco/day)[g]	15	(3–25)	15	(4–30)	0.98 (0.96–1.01)
Smoking status at inclusion					
Never	43%		39%		1
Former	24%		24%		1.15 (0.65–2.02)
Current	32%		36%		1.87 (0.75–4.66)
School education					
$\leq$7 years	29%		34%		1
8–10 years	47%		47%		1.08 (0.73–1.59)
$\geq$11 years	24%		18%		1.47 (0.91–2.39)
Nulliparous[h]	13%		13%		0.66 (0.34–1.29)

[a]Estimates are mutually adjusted.
[b]Among ever users of HRT, per additional year of use.
[c]Among parous women, per 5 year increment in age at first birth.
[d]Among parous women, rate ratio per additional birth.
[e]Body mass index kg/m^2 (BMI).
[f]Among ever smokers.
[g]Among current smokers.
[h]Rate ratio for nulliparous vs. one birth at age 35 years.

Discussion

In this study, we found a positive association between urinary excretion of 8-oxodG, a biomarker of oxidative stress-induced damage to DNA, and subsequent risk of breast cancer in a large cohort of postmenopausal women. The association was borderline significant in the whole population and significantly enhanced for women with ER-positive cancer and in women with low iron intake.

Our results are consistent with smaller case–control studies, showing elevated levels of chromatographically measured 8-oxodG in urine among breast cancer cases compared to healthy controls (22–25). However, these studies lack details on menopause, HRT, and receptor status, relative risk was not estimated and reverse causality is possible because of the case–control design. Indeed, 1 case–control study reported that the high levels of 8-oxodG measured in the urine of the 150 cases decreased after surgical treatment of the tumor to the levels found in controls, suggesting that the tumor presence could give rise to high urinary 8-oxodG excretion levels (24). A larger case–control study with 1,066 cases and a prospective study with 350 cases split in pre- and postmenopausal cancer found no association between 8-oxodG excretion and breast cancer risk in logistic regression analysis (19–21). Nevertheless, these studies used ELISA-based measurement of 8-oxodG, which show substantially less precision, accuracy, and ability to discriminate patients and healthy subjects compared with the chromatographic assays (17, 18). This could increase measurement error in exposure assessment biasing risk toward null. Furthermore, the studies do not report on separate analysis of postmenopausal women, HRT users, dietary interactions, or for ER-positive cancer.

This study on a homogenous postmenopausal population of women was prospective and effects of cancer on the excretion of 8-oxodG can thus be excluded. Indeed, exclusion of cases diagnosed within 1 year of sample collection did not change the results. Although we had limited power for subgroup analysis, the excretion of 8-oxodG was mainly associated with the risk of ER-positive cancer, suggesting a specific role of oxidative stress for this form. This could be in consistence with cell culture experiments, showing estrogen-induced DNA oxidation to be ER dependent (2, 9, 10). However, the risk of breast cancer associated with high excretion of 8-oxodG in this study did not seem related to the high level of estrogens from HRT use because the association was slightly less pronounced in women using HRT, although there was no significant interaction. The 8-oxodG excreted into urine can originate from all cells in the body. The excretion

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Table 2. Excretion of 8-oxodG according to ER status, use of HRT, age, BMI, fruit, vegetable and iron intake, and use of iron supplement among subsequent cases of breast cancer and matched controls

	N	8-OxodG [nmol/mmol creatinine; median (5–95%)]			
	Cases/controls	Cases	*P*[a]	Controls	*P*[a]
All	336/336	1.86 (0.52–6.59)		1.75 (0.47–6.28)	
Receptor status			0.38		
ER-positive	239	1.86 (0.55–6.58)			
ER-negative	73	1.86 (0.50–5.74)			
HRT use at inclusion			0.34		0.47
Never	120/120	2.02 (0.56–7.03)		1.82 (0.62–6.07)	
Former	46/46	1.91 (0.52–6.58)		1.64 (0.48–4.85)	
Current	170/170	1.73 (0.52–6.56)		1.73 (0.42–6.63)	
Parity			0.95		0.85
Nulliparous	42/43	1.76 (0.55–5.76)		1.77 (0.62–6.29)	
Parous	294/293	1.87 (0.52–6.59)		1.74 (0.46–6.17)	
Smoking status at inclusion			0.10		0.02
Never	146/132	1.65 (0.66–6.16)		1.62 (0.59–5.35)	
Former	81/82	1.86 (0.54–5.52)		1.58 (0.33–4.85)	
Current	109/122	2.22 (0.48–10.54)		1.98 (0.57–7.58)	
Alcohol consumption			0.64		0.60
<10.6 g/day	159/177	1.83 (0.54–6.56)		1.74 (0.50–5.97)	
≥10.6 g/day	177/159	1.87 (0.51–7.47)		1.76 (0.45–7.38)	
BMI			0.44		0.62
<25	176/180	1.82 (0.50–7.04)		1.73 (0.48–6.39)	
≥25	160/156	1.91 (0.62–6.57)		1.77 (0.46–5.77)	
Intake of fruit			0.11		0.002
<175 g/day	154/182	1.76 (0.51–6.56)		1.58 (0.40–5.35)	
≥175 g/day	182/154	1.87 (0.55–6.91)		1.89 (0.59–7.38	
Intake of vegetables			0.68		0.79
<164 g/day	166/171	1.82 (0.54–6.91)		1.74 (0.42–6.51)	
≥164 g/day	170/165	1.87 (0.52–6.56)		1.76 (0.51–6.17)	
Intake of iron			0.65		0.78
<11.47 mg/day	153/183	1.87 (0.48–7.04)		1.75 (0.47–5.35)	
≥11.47 mg/day	183/153	1.80 (0.55–5.87)		1.71 (0.48–6.49)	
Use of iron supplement			0.44		0.16
Yes	171/164	1.87 (0.62–7.25)		1.88 (0.50–6.29)	
No	165/172	1.80 (0.52–6.56)		1.62 (0.46–6.17)	

[a]*P* values for equal levels in categories by Kruskal–Wallis test.

Table 3. IRRs with 95% CIs of all and ER-specific breast cancer for one unit increase and for quartiles in 8-oxodG in nmol/mmol creatinine excretion in urine samples collected up to 5 years before diagnosis

	Linear estimates		Quartiles of 8-oxodG in nmol per mmol creatinine				
	Per 1 unit increase						
Cases (336)	Crude	Adjusted[a]	0.12–1.15	1.15–1.78	1.78–2.84	2.84–25.42	*P* trend[a]
All (336)	1.06 (0.99–1.15)	1.08 (1.00–1.17)	1	0.78 (0.50–1.22)	0.88 (0.54–1.43)	1.42 (0.86–2.34)	0.06
All ER+ (239)	1.09 (0.99–1.19)	1.11 (1.01–1.23)	1	0.80 (0.47–1.36)	0.93 (0.51–1.70)	1.62 (0.90–2.90)	0.04
All ER− (73)	0.95 (0.83–1.10)	0.96 (0.82–1.13)	1	0.25 (0.06–1.01)	0.35 (0.09–1.27)	0.51 (0.13–1.95)	0.65

[a]Adjusted for baseline levels of smoking status (never, former, current), amount (g/day) and duration (years) of smoking, parity (age at first birth and number of births), education, intake of alcohol, BMI, and duration of HRT use.

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Table 4. IRR of breast cancer in relation to excretion of 8-oxodG according to use of HRT, smoking, intake of fruit, vegetables, and iron and use of iron supplement

		Per 1 nmol 8-oxodG/mmol creatinine unadjusted			Per 1 nmol 8-oxodG/mmol creatinine adjusted[a]		
	Cases/controls	IRR	95% CI	P^b	IRR	95% CI	P^b
HRT use							
Never	120/120	1.09	0.96–1.24	0.45	1.11	0.97–1.26	0.41
Current	170/170	1.03	0.94–1.12		1.03	0.94–1.14	
Smoking status at inclusion							
Never	142/132	1.07	0.92–1.25	0.82	1.07	0.91–1.25	0.94
Current	109/122	1.05	0.96–1.14		1.06	0.97–1.16	
Intake of fruit							
<175 g/day	153/183	1.08	0.97–1.20	0.54	1.09	0.97–1.21	0.68
≥175 g/day	183/153	1.04	0.94–1.14		1.06	0.95–1.17	
Intake of vegetables							
<164 g/day	166/171	1.10	0.98–1.23	0.47	1.12	0.99–1.26	0.40
≥164 g/day	170/165	1.04	0.95–1.14		1.05	0.96–1.16	
Intake of iron							
<11.47 mg/day	153/183	1.17	1.04–1.33	0.05	1.21	(1.07–1.38)	0.02
≥11.47 mg/day	183/153	1.01	0.93–1.10		1.01	(0.93–1.11)	
Use of iron supplement							
Yes	171/164	1.03	(0.94–1.14)	0.35	1.04	0.94–1.15	0.31
No	165/172	1.10	(0.99–1.22)		1.12	1.00–1.26	

[a]Adjusted for baseline levels of: smoking status (never, former, current), amount (g/day) and duration (years), parity (age at first birth and number of births), education, intake of alcohol, BMI, and duration of HRT use.
[b]*P*-value for interaction.

of 8-oxodG represents in steady state an average rate of oxidative damage to guanine in dGTP and probably DNA although repair pathways for the latter resulting in 8-oxodG have yet to be showed (3). The contribution from the breast tissue to urinary 8-oxodG is unknown, but likely to be low, and at present the excretion can be considered as a biomarker of general or global oxidative stress levels. That is also consistent with our previous finding of associations between risk of lung cancer and high excretion of 8-oxodG and 8-oxoGua as well as mRNA expression of the related base excision repair enzyme OGG1 among nonsmokers (33, 37, 38).

In general smokers excrete more 8-oxodG than nonsmokers do in accordance with the present results (32, 35, 39, 40). The risk estimates obtained after adjustment for smoking can thus be interpreted as an association between the variation in 8-oxodG, which is not attributed to smoking, and breast cancer. A number of other factors are known to influence the 8-oxodG excretion, but none are likely to be particularly relevant in this study. Exposure to ambient air pollution, several occupational hazards, high altitude, and exhaustive exercise have been associated with increased 8-oxodG excretion (41–44). The present positive association between intake of fruit and excretion of 8-oxodG among controls is not easily explained. In another subset for a nested case–cohort-based study of the risk of lung cancer in the same cohort we found no association between fruit and vegetable intake and 8-oxodG excretion (33). Moreover, studies of a high intake or intervention with fruits and vegetables in particular with high diversity or supplements with putatively active compounds collectively suggest decreased DNA oxidation in leukocytes and 8-oxodG excretion (28, 45, 46). Nevertheless, neither smoking nor intake of fruit or vegetables seemed to modify the associations between 8-oxodG excretion and risk of breast cancer. In contrast, the association between 8-oxodG and risk of breast cancer seemed confined to women with a low dietary intake of iron and a similar pattern was seen for use of iron supplements, although the latter interaction was not statistically significant. Accordingly, the association is not likely to be related to oxidative stress caused by a high iron intake, consistent with the lack of association between iron intake and risk of postmenopausal breast cancer in American cohort studies (47, 48), although other indices of body iron storage might support an association between iron load and risk (1).

In urine, 8-oxodG is stable at only −20°C, far beyond the storage time of this study. Excellent interlaboratory agreement between different chromatographic methods and a low analytical coefficient of variation indicate that measurement error was limited. However, only one spot sample of urine was collected at enrolment and this may not be representative for the long period at risk.

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Nevertheless, the urinary excretion of 8-oxodG is relatively constant within an individual showing coefficients of variations less than 20% over prolonged periods of time (18, 49, 50).

The concentrations of 8-oxodG had to be adjusted by creatinine concentration (18). Creatinine excretion is affected by muscle mass possibly explaining higher levels of 8-oxodG per creatinine with increasing age, whereas 24-hour urinary collection show decreased excretion with age (32, 50). However, age-associated differences in 8-oxodG excretion are not likely to affect our results about breast cancer risk because the controls were matched for age and the subjects had a narrow age span. Furthermore, the cases and controls had similar BMI at enrollment.

Unfortunately, prospective study of the levels of 8-oxodG in leukocytes, or even better in breast or other tissues, in relation to risk of cancer faces great challenges. This is due to the laborious sampling, tissue or cell preparation, and assays required for measurement in high numbers at entry in cohorts or if applied in a nested design based on biobank material, the likely spurious oxidation of DNA during storage in cells without very demanding preparation (27).

In conclusion, this case–control study nested in a population-based cohort indicates that the risk of ER-positive breast cancer is associated with urinary excretion of 8-oxodG, a biomarker of oxidative stress. This supports the hypothesis that oxidative stress is involved in the pathogenesis, whereas this was not related to use of HRT or a high iron intake in our study. Our results appear at odds with a similar cohort study that assessed 8-oxodG by an ELISA assay with limited accuracy and precision. Accordingly, the excretion of 8-oxodG and similar products of oxidatively damaged DNA assayed by accurate and precise methods as risk factors for breast cancer warrants study in larger prospective settings, which is feasible due to the stability of the compounds in urine.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Loft, A. Olsen, H.E. Poulsen, A. Tjønneland
Development of methodology: S. Loft, H.E. Poulsen
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Loft, A. Tjønneland
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Loft, A. Olsen, H.E. Poulsen
Writing, review, and/or revision of the manuscript: S. Loft, A. Olsen, Peter Møller, H.E. Poulsen, A. Tjønneland

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