Free Radical Research, October 2008; 42(10): 841–848

informa
healthcare

Oxidative stress early in pregnancy and pregnancy outcome

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Accepted by Professor F. Kelly

(Received 7 July 2008; revised 26 September 2008)

Abstract
The objectives of this study were to determine whether oxidative stress early in pregnancy influenced pregnancy outcome. A combination of assays were used for exogenous and endogenous anti-oxidants together with two well accepted biomarkers for oxidative stress, the urinary excretion of 8-iso-$PGF_{2\alpha}$ (a biomarker marker for lipid oxidation, $n = 508$) and 8-oxo-7,8 dihydro-2 deoxyguanosine (8-OHdG, a biomarker for DNA oxidation, $n = 487$). The two biomarkers tracked different pregnancy outcomes. Isoprostanes were associated with an increased risk of pre-eclampsia and a decreased proportion of female births. In contrast, 8-OHdG tracked lower infant birthweight and shortened gestation duration. Birth defects were associated with low levels of 8-OHdG.

Keywords: *Oxidative stress, pregnancy, 8-hydroxy-2-deoxyguanosine, isoprostane, SOD, GPx, dietary antioxidants*

Introduction

Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS), anti-oxidants and repair processes. Proteins, lipids and DNA are all vulnerable to oxidative damage which has been implicated in the aetiology of a wide variety of chronic diseases and acute pathological states. Anti-oxidants defenses can be of either an endogenous (e.g. Glutathione peroxidase (GPx), Superoxide dismutase (SOD)) or exogenous nature (e.g. the anti-oxidant vitamins [1,2]). Pro-oxidants include smoking, iron and numerous environmental agents.

Many studies, particularly animal models, have documented the influence of maternal oxidative stress on pregnancy outcome. Increased oxidative stress in human pregnancy has been associated with pre-eclampsia [3–6], hypertension and childhood insulin resistance [7,8]. Because biomarkers usually are measured late in pregnancy, it has been difficult to separate the antecedents of oxidative stress from consequences. There is little data on the influence of maternal oxidative stress early in pregnancy when antioxidant activity of the conceptus is minimal and embryonic and foetal susceptibility likely to be maximized [9,10].

The objectives of this study were to determine whether oxidative stress early in pregnancy influenced pregnancy outcome in a cohort of urban, low income and minority pregnant women. We used a combination of assays for exogenous and endogenous anti-oxidants together with two well accepted biomarkers for oxidative stress. These are the urinary excretion of 8-iso-$PGF_{2\alpha}$ (isoprostane) and 8-oxo-7,8 dihydro-2-deoxyguanosine (8-OHdG). 8-OHdG is a marker for oxidative damage to DNA [11,12] and isoprostane a marker for the oxidative damage of lipids [13,14]

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ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.1080/10715760802510069

generated by the auto-oxidation of arachidonic acid [15].

Materials and methods

The samples used for this study were obtained from a prospective study on the effects of maternal nutrition and growth in generally healthy pregnant women from Camden, NJ. Camden is one of the poorest cities in the US. Participants include young (≤ 18 years) and more mature (19–45 years) women enrolling for prenatal care in Camden clinics. Gravidas with serious non-obstetric problems (6e.g. lupus, diabetes mellitus Type 1 or Type 2 and seizure disorders, malignancies, drug or alcohol abuse) were excluded. Approximately 80% of gravidae who were eligible agreed to participate. We focused on samples and data from 989 gravidas enrolling in the study between January 1998 and April 2005. The Institutional Review Board of the University of Medicine and Dentistry of New Jersey (Stratford, NJ) approved the study.

Socioeconomic, demographic, lifestyle and dietary data were obtained by interview at entry to prenatal care (12.1 ± 0.1 weeks (mean, standard error) for women entering by week 16, the group of interest in this report). Urine and blood specimens were collected at entry to care, the blood was centrifuged and aliquots of the serum and plasma stored at -70°C until analysed. Metaphosphoric acid (0.5 ml of 10% metaphosphoric acid) was added to a 0.5 ml aliquot of plasma for vitamin C analysis. Dietary information was obtained by recall of the previous days' intake for the entry to care visit and for two additional visits (weeks ~ 20 and ~ 28 gestation). The resultant data was processed with databases from the Campbell Institute of Research and Technology (Campbell Soup Company) in Camden, NJ, and the resultant nutrient values averaged across the pregnancy.

Pregravid weight was determined by recall. BMI was computed as pregravid weight (in kg) divided by height^2 (m). Information on current and past pregnancy outcomes, complications and infant abnormalities, infant birth weight and birth defects were abstracted from the prenatal record, the delivery record, delivery logbooks and the infant's chart. Gestation duration was based upon the gravidas' last normal menstrual period (LMP) confirmed or modified by ultrasound. Preterm delivery was defined by a delivery occurring at less than 37 completed weeks gestation. Small-for-gestational age (SGA) was defined by a birth weight for gestation below the 10th percentile of a standard which adjusts for maternal ethnicity, parity and foetal sex [16].

The diagnosis of pre-eclampsia was based upon high maternal blood pressure (systolic blood pressure ≥ 140 mm Hg systolic or diastolic blood pressure $\geq$ 90 mm Hg) and proteinuria ($\geq 1+$ by dipstick) after week 20 in a previously normotensive woman [4]. Data on infant birth weight, birth defects and all other measures related to the outcome of the pregnancy were abstracted from patient records.

Commercially available kits were used to measure erythrocyte concentrations of glutathione peroxidase (GPx, Oxford Biomedical research, Oxford, MI) and superoxide dismutase (SOD, Oxis Research, Foster City, CA). Plasma ferritin, serum iron and unsaturated iron binding capacity (UIBC) were determined with kits obtained from Bio-Rad (Hercules, CA). Total iron binding capacity was calculated from the sum of serum iron and UIBC. Plasma vitamin C was assayed colourimetrically [17] and vitamin E_α and E_γ by HPLC [18]. Cholesterol was determined by the Lieberman-Burchardt method using a kit marketed by Pointe Scientific (Canton, MI). Inter and intra coefficients of variation were as follows: SOD 6.3 and 3.5%, GPx 5.9 and 3.1%, ferritin 5.0 and 2.6%, serum iron 5.0 and 2.6%, unsaturated iron binding capacity 3.0 and 2.6%, Vitamin E_α 1.0 and 8%, vitamin E_γ 0.8 and 8%, vitamin C 7.8 and 5.2% and cholesterol 2.0 and 2.7%. Data on circulating levels of vitamin E_α and vitamin E_γ were adjusted for cholesterol.

8-OHdG in the urine was analysed by isotope dilution gas chromatography-mass spectrometry (gc-ms) with selective ion monitoring (SIM [19–21]; ^{18}O labelled 8-OHdG was used as the internal standard [19]). 8-[^{18}O] hydroxyl-2'-deoxyguanosine was prepared by using a modification of the method of Hermanns et al. [22] by brominating 2'-Deoxyguanosine (Aldrich, Milwaukee, WI) with N-Bromosuccinimide in Acetonitrile/Water (4:1) followed by hydrolysis with $H_2^{18}O$ (99%, Cambridge Isotopes, Cambridge, MA) [23]. The purity was $> 99\%$ as determined by HPLC/UV analysis and the isotopic purity as determined by MS analysis was $> 93\%$.

Similarly the urinary 8-iso-$PGF_{2\alpha}$ isoprostane concentration was measured by GCMS with isotope dilution and selective ion monitoring. ^{2}H 8-iso-$PGF_{2\alpha}$ was used as the internal standard (Cayman Chemicals, Ann Arbor, MI). Partially resolved peaks ($\sim$ 10%) were resolved using the Peakfit deconvolution software program (Systat Inc., Chicago, IL). Isoprostane and 8-OHdG values were normalized to creatinine. Inter and intra coefficients of variation were 5.2 and 13.4% for isoprostane and 4.8 and 12.7% for 8-OHdG.

Data analyses

The significance of the association of 8-OHdG and isoprostane with maternal characteristics was assessed using analysis of variance (ANOVA), Chi

Square and *t*-test. The *p* for trend was computed and, when appropriate, the highest and lowest quintiles of 8-OHdG or isoprostane were contrasted.

Potential confounding variables associated in Camden with infant birth weight (age, parity, smoking, ethnicity, pregravid BMI) were included in multivariable models. Separate models were fitted for birth weight, gestation duration, birth weight adjusted for gestation and other outcomes of interest (pre-eclampsia, preterm delivery, small for gestation births). Separate models were fit as well for individual pro-oxidants (serum ferritin, iron, TIBC, UIBC), endogenous antioxidants (GPx, SOD) and circulating levels of exogenous anti-oxidants (vitamins E_α, E_γ and C) by ANOVA, multiple logistic or multiple linear regression, after control for potential confounding variables. Confounding was assessed by comparing crude and adjusted odds ratios or regression coefficients. Adjusted odds ratios (AOR) and their 95% confidence intervals (95% CI) were computed from the logistic regression coefficients and their corresponding covariance matrices. Data were analysed with SAS version 9.0 (SAS Institute, Cary, NC). Since both creatinine adjusted and unadjusted data gave similar results, the adjusted data are presented. Data are presented by quintile or as the mean $\pm$ standard error (SEM).

Although we aimed to obtain data early in pregnancy, actual gestation at entry ranged from 5–28 weeks ($n = 989$). After eliminating subjects who miscarried, had a foetal demise or delivered their pregnancy elsewhere, outcome data from 922 gravidae remained. To have at least 500 subjects for the isoprostane analyses (100 per quintile) we cut at 16 weeks to give 508 cases with a mean entry to care time of 12.1 ± 0.1 weeks. For 8-OHdG the corresponding numbers amounted to $n = 487$ because of a contaminating peak in the mass spectrum which we were unable to resolve. Comparison of outcomes data sets from these subjects with the main cohort did not reveal any differences or trends towards differences.

Results

Both 8-OHdG and 8-iso-$PGF_{2\alpha}$ are markers for oxidative stress, but in this study they did not correlate with each other for either the total group ($r^2 = 0.002$, $p = 0.989$) or the sub-set of women with any poor pregnancy outcomes.

Maternal characteristics

Gravidae in the early entry cohort had a mean age of 23.3 ± 0.2 years (mean $\pm$ standard error, SEM) and the majority were parous (61%) and non-smokers (84.5%). Most were Hispanic (45.3%) or African American (37.4%). Pregravid BMI averaged 26.4 ± 0.3 kg/m^2. Mean gestation duration was 38.6 ± 0.1 completed weeks and the mean birth weight 3199 ± 24 g.

Tables I and II show the relationships between the creatinine adjusted 8-OHdG (Table I) and isoprostane (Table II) and relevant maternal characteristics for the early entry cohort. Data on both markers suggested that smoking was associated with increased oxidative stress. When mean levels of 8 OHdG were examined

Table I. Creatinine adjusted 8-OHdG quintile and maternal characteristics.

Quintile	1	2	3	4	5	*p* for trend
n	97	97	97	99	97	
8-OHdG (ng/mg Creat)	<17.6	17.6–22.6	22.6–29.9	29.9–38.9	> 38.9	
Age (years)	24.0±0.5	22.4±0.5	23.3±0.5	23.5±0.5	23.3±0.5	0.931
Pre-gravid BMI (kg/m^2)	27.2±0.7	27.6±0.7	27.2±0.7	26.4±0.7	24.0±0.7[a]	0.001
Diet						
Protein (g/d)	90.8±3.8	83.8±3.8	88.8±3.6	90.9±3.6	89.8±3.8	0.677
Energy (kcal/d)	2227±74	2222±74	2147±71	2187±70	2207±75	0.759
Carbohydrate (g/d)	279±10	293±10	260±10	268±10	282±10	0.584
Fat (g/d)	90.8±3.8	83.7±3.8	88.8±2.6	90.8±3.6	89.8±3.8	0.875
Vitamin C (mg/d)	154±12	211±21	144±11	165±20	164±13	0.758
Vitamin E (μg/d)	8.8±1.0	6.9±0.3	6.7±0.3	6.8±0.3	7.4±0.4	0.110
Parous%	66 (68.0)	60 (61.9)	60 (61.9)	65 (65.7)	49 (50.5)	
Nulliparous%	31 (32.0)	37 (38.1)	37 (38.1)	34 (34.3)	48 (49.5)	0.046
Cigarette smoking%	10 (10.4)	14 (14.4)	14 (14.4)	17 (17.2)	21 (21.7)	0.031
Ethnicity%						
Hispanic	37 (38.1)	33 (34.0)	51 (52.6)	51 (51.5)	48 (49.5)	
African American	54 (55.7)	49 (50.5)	29 (29.9)	27 (27.3)	23 (23.7)	
White & other	6 (6.2)	15 (15.5)	17 (17.5)	21 (21.2)	26 (26.8)	0.777
Medicaid%	96 (99.0)	96 (99.0)	96 (99.0)	95 (96.0)	89 (91.8)	0.002

Data are unadjusted means $\pm$SE or proportions, trends computed by ANOVA or Mantel Haenszel Chi-Square.
[a] vs quintile 1, $p = 0.001$.

Table II. Creatinine adjusted isoprostane and maternal characteristics.

Quintile	1	2	3	4	5	*p* for trend
n	102	103	101	100	102	
Isoprostane (ng/mg Creat)	<4.49	4.49–7.49	7.50–12.36	12.37–21.39	>21.39	
Age (years)	23.7±0.5	23.2±0.5	23.4±0.5	22.1±0.5	24.2±0.5	0.100
Pre-gravid BMI (kg/m^2)	26.1±0.7	25.6±0.6	26.5±0.7	26.3±0.7	28.1±0.7[a]	0.021
Diet						
Protein (g/d)	85.8±3.6	92.8±3.6	86.4±3.6	88.7±3.7	89.2±3.6	0.817
Energy (kcal/d)	2177±71	2270±70	2189±71	2171±71	2113±70	0.316
Carbohydrate (g/d)	285±9	283±9	276±9	262±9.3	259±9.3	0.015
Fat (g/d)	79.7±3.7	87.3±3.7	84.1±3.7	87.3±3.7	81.9±3.7	0.714
Vitamin C (mg/d)	169±12	155±11	185±23	155±14	151±10	0.418
Vitamin E (μg/d)	7.2±0.6	8.3±0.7	7.0±0.3	7.0±0.3	6.7±0.4	0.133
Parous%	62 (60.8)	65 (63.1)	70 (69.3)	53 (53.0)	63 (61.8)	
Nulliparous%	40 (39.2)	38 (36.9)	31 (30.7)	47 (47.0)	39 (38.2)	0.599
Cigarette smoking%	13 (12.8)	16 (15.5)	15 (15.0)	15 (15.0)	22 (21.6)	0.135
Ethnicity%						
Hispanic	40 (39.2)	38 (36.9)	53 (52.5)	46 (46.0)	55 (53.9)	
African American	44 (43.1)	45 (43.7)	33 (32.7)	42 (42.0)	25 (24.5)	
White & other	18 (17.6)	20 (19.4)	15 (14.9)	12 (12.0)	22 (21.6)	0.100
Medicaid%	96 (94.1)	97 (94.2)	100 99.0)	99 (99.0)	101 (99.0)	0.006

Data are unadjusted means±SE or proportions, trends computed by ANOVA or Mantel Haenszel Chi-Square.
[a] vs quintile 1, $p=0.030$.

by maternal smoking status the difference remained significant (38.8 ± 3.5 ng/mg Creat for smokers vs 29.5 ± 1.5 ng/mg Creat. for non-smokers, $p = 0.016$); for isoprostane, levels approached significance (18.6 ± 2.1 ng/mg Creat for smokers, 15.0 ± 0.9 mg/ng creatinine non-smokers, $p = 0.12$).

A difference in the pattern of association was noted for the biomarkers. For example, pre-gravid BMI was significantly higher for gravidae in the highest quintile of maternal urinary isoprostane excretion. The opposite was found for 8-OHdG where higher excretion was associated with significantly lower pre-gravid BMI (Tables I and II). Low-income gravidae (pregnancy care was financed by Medicaid) had lower levels of 8-OHdG but higher levels of isoprostane compared to Medicaid non-recipients. Parous gravidae tended to have lower excretion of 8-OHdG when compared to nulliparae, but similar excretion of isoprostane. Other relationships are discussed in the paragraphs that follow.

Blood pro-oxidants and anti-oxidants

Serum levels of UIBC and TIBC were significantly decreased and serum ferritin levels significantly increased with higher 8-OHdG excretion. In contrast UIBC and TIBC tended to rise as levels of isoprostane fell, a trend which approached but did not attain significance; serum ferritin was unrelated to isoprostane excretion (Tables III and IV).

Dietary intake of vitamins E and C was unrelated to excretion of isoprostane and 8-OHdG (Tables I and II). While higher plasma levels of vitamin E_γ were associated with increased isoprostane, there was no

Table III. Adjusted OHdG by quintile and antioxidant/oxidant concentrations (mean±SE).

OHdG Quintile	1	2	3	4	5	*p* for trend
Serum Iron (mg/dl)	73.9±4.8	77.9±4.5	81.2±4.5	67.7±5.2	75.7±5.1	0.723
UIBC (μg/dl)	269.0±9.5	248.1±8.9	223.3±8.8	244.02±10.3	232.2±10.19[a]	0.015
TIBC (μg/dl)	339.3±8.1	327.7±7.6	304.4±7.4	312.2±8.7	308.9±8.5[b]	0.005
Ferritin (ng/ml)	33.5±4.8	45.3±4.7	52.2±4.7	53.4±4.6	56.2±4.9[c]	0.001
GPX (μU/mg Hb)	26.3±0.7	23.7±0.7	24.3±0.7	24.8±0.7	25.2±0.8	0.594
SOD (μU/mg Hb)	2.65±0.05	2.33±0.05	2.37±0.05	2.31±0.05	2.38±0.05[d]	0.023
Vitamin C (ng/ml)	16.5±0.4	16.5±0.4	16.6±0.3	16.8±0.3	16.7±0.4	0.528
Vitamin E_α (ng/ml)	10.3±0.3	10.6±0.3	10.0±0.3	10.1±0.3	10.2±0.3	0.313
Vitamin E_γ (ng/ml)	1.91±0.07	1.93±0.07	1.83±0.07	1.81±0.07	1.89±0.07	0.497

Models were adjusted for age, BMI, smoking, ethnicity and parity. Vitamin E_γ and E_α concentrations were also adjusted for serum cholesterol.
[a] vs quintile 1, $p=0.011$; [b] vs quintile 1, $p=0.013$; [c] vs quintile 1, $p=0.001$; [d] vs quintile 1, $p=0.018$.

Table IV. Adjusted isoprostanes by quintile and antioxidant/oxidant concentrations (mean ± SE).

Isoprostane Quintile	1	2	3	4	5	*p* for trend
Serum Iron (mg/dl)	70.9 ± 5.1	75.8 ± 4.7	83.4 ± 5.1	78.1 ± 4.5	71.2 ± 4.8	0.788
UIBC (μg/dl)	238.1 ± 10.4	247.1 ± 9.5	226.4 ± 10.3	239.6 ± 9.1	263.7 ± 9.7	0.185
TIBC (μg/dl)	308.5 ± 8.8	320.3 ± 8.1	306.9 ± 8.7	319.9 ± 7.7	335.5 ± 8.3[a]	0.053
Ferritin (ng/ml)	43.3 ± 4.7	47.1 ± 4.6	56.5 ± 4.8	47.8 ± 4.6	44.0 ± 4.8	0.896
GPX (μU/mg Hb)	27.0 ± 0.8	24.1 ± 0.8	25.7 ± 0.8	25.4 ± 0.8	24.0 ± 0.8[b]	0.064
SOD (μU/mg Hb)	2.47 ± 0.05	2.41 ± 0.05	2.37 ± 0.05	2.38 ± 0.05	2.33 ± 0.05[c]	0.044
Vitamin C (ng/ml)	16.7 ± 0.4	17.1 ± 0.3	16.4 ± 0.4	16.6 ± 0.4	16.3 ± 0.4	0.295
Vitamin E_α (ng/ml)	10.0 ± 0.3	10.5 ± 0.2	10.2 ± 0.2	10.1 ± 0.2	10.1 ± 0.2	0.015
Vitamin E_γ (ng/ml)	1.93 ± 0.07	1.84 ± 0.07	1.83 ± 0.07	1.85 ± 0.07	1.90 ± 0.07	0.886

Models were adjusted for age, BMI, smoking, ethnicity and parity. Vitamin E_γ and E_α concentrations were also adjusted for cholesterol. [a] vs quintile 1, $p = 0.031$; [b] vs quintile 1, $p = 0.008$; [c] vs quintile 1, $p = 0.042$.

relationships between plasma levels of vitamins E_α or C and either isoprostane or 8-OHdG excretion (Tables III and IV).

Both biomarkers showed similar associations with SOD; activity was reduced in the highest quintile of isoprostane or 8-OHdG compared to the lowest quintile (Tables III and IV). GPX activity was reduced for the highest quintile of isoprostane but was unrelated to 8-OHdG excretion (Tables III and IV).

8-OHdG and outcome

Mean gestation duration and birth weight decreased with increasing 8-OHdG (Tables V and VI). At delivery there was a −208 g difference ($p = 0.007$) in birth weight for women in the highest quintile of 8-OHdG at entry compared to women in the lowest quintile. The difference was associated with shortened gestation duration, which was 0.9 weeks less for women in the highest quintile (Tables V and VI). When infant birth weight was adjusted for gestation duration as well as other potential confounding variables included in previous models, the difference was no longer significant (−48 g birth weight, comparing highest and lowest quintiles).

For the 19 babies diagnosed with (minor) birth defects; the maternal 8-OHdG levels were significantly lower than those of the controls (24.0 ± 1.9 vs 29.1 ± 0.7 ng/mg Creat, $p = 0.046$). Levels of GPx also tended to be lower in these women (21.7 ± 1.9 vs 25.2 ± 0.7 μM/mg Creat, $p = 0.033$) and SOD (2.26 ± 0.08 vs 2.39 ± 0.02 μM/mg Creat, $p = 0.084$).

Isoprostane 8-iso-PGF$_{2\alpha}$ and outcome

Like 8-OHdG, high isoprostane excretion was associated with poor pregnancy outcomes, but the nature of the risk differed. While there was no relationship between isoprostane and infant birthweight (Table VI), there was a significant trend for risk of pre-eclampsia to increase with increasing isoprostane ($p = 0.029$); pre-term delivery ($p = 0.085$) tended to be increased as well (Table VI). For pre-eclampsia, risk was nearly 5-times higher when highest and lowest isoprostane quintiles were compared and potential confounding variables were controlled (AOR = 4.96, 95% CI 1.54, 15.95). In contrast, there was no relationships between 8-OHdG excretion and pre-eclampsia or pre-term delivery (Table V).

There was a gender effect associated with isoprostane 8-iso-PGF$_{2\alpha}$. Elevated isoprostane excretion was associated with an increased proportion of male births (57% vs 40% for the highest and lowest

Table V. Adjusted mean gestation duration, infant birthweight, proportion with SGA, preterm delivery and foetal gender for OHdG.

Quintile	1	2	3	4	5	*p* for trend
n	97	97	97	99	97	
8-OHdG (ng/mg Creat)	<17.6	17.6–22.6	22.6–29.9	29.9–38.9	>38.9	
Gestation duration (wks)	39.0 ± 0.2	38.8 ± 0.2	38.8 ± 0.2	38.6 ± 0.2	38.1 ± 0.2[a]	0.009
Birthweight (g)	3262 ± 58	3262 ± 58	3223 ± 56	3201 ± 56	3053 ± 59[b]	0.014
SGA%	3 (3.2)	8 (8.7)	7 (7.3)	5 (5.1)	9 (9.9)	0.369
Pre-eclampsia%	10 (10.3)	9 (9.4)	8 (8.3)	5 (5.1)	5 (5.3)	0.217
Preterm delivery%	7 (7.3)	10 (10.8)	9 (9.4)	9 (9.1)	11 (11.8)	0.244
Gender%						
Male	46 (47.9)	53 (57.0)	45 (46.9)	55 (55.6)	46 (49.5)	
Female	50 (52.1)	40 (43.1)	51 (53.1)	44 (44.4)	47 (50.5)	0.659

All models were adjusted for maternal age, BMI, ethnicity, parity and cigarette smoking by multiple linear or logistic regression. [a] vs quintile 1, $p = 0.014$; [b] vs quintile 1, $p = 0.007$.

Table VI. Adjusted mean gestation duration, infant birthweight, proportion with SGA, preterm delivery and foetal gender for Isoprostane.

Quintile	1	2	3	4	5	*p* for trend
n	102	103	101	100	102	
Isoprostane (ng/mg Creat)	<4.49	4.49–7.49	7.50–12.36	12.37–21.39	>21.39	
Gestational duration (wks)	39.0±0.2	39.0±0.2	38.4±0.2	38.6±0.2	38.5±0.2	0.030
Birthweight (g)	3268.1±55	3201±54	3150±55	3212±55	3189±56	0.403
SGA%	6 (6.1)	9 (8.8)	6 (6.1)	6 (6.3)	5 (5.1)	0.636
Pre-eclampsia%	5 (5.0)	7 (6.8)	4 (4.0)	4 (4.0)	15 (15.2)[a]	0.029
Preterm delivery%	5 (5.1)	6 (5.9)	15 (15.3)	10 (10.1)	12 (12.0)	0.085
Gender%						
Male	40 (40.4)	45 (44.1)	53 (54.1)	58 (58.6)	57 (57.0)	
Female	59 (59.6)	57 (55.9)	45 (45.9)	41 (41.4)	43 (43.0)	0.007

All models were adjusted for maternal age, BMI, ethnicity, parity and cigarette smoking by multiple linear or logistic regression.
a vs quintile 1, Odds Ratio (OR) =3.39 (95% Confidence Interval (CI) 1.18, 9.73); Adjusted Odds Ratio (AOR) =4.96 (95% CI 1.54, 15.95).

isoprostane quintiles, Tables V and VI). In contrast to the situation with 8-OHdG, there was no association between maternal isoprostane excretion and birth defects.

Discussion

Early in pregnancy, urinary excretion of oxidative stress markers probably reflects maternal rather than foetal redox status. However what happens to the mother is likely to happen to the foetus since foetal anti-oxidant defenses are poor early in gestation [9,10]. Numerous studies have shown that foetal development and gestation duration are sensitive to the maternal biochemical environment [24,25].

8-OHdG and isoprostane are uncorrelated markers for different aspects of oxidative stress (DNA, lipids) that track different routes whereby oxidative stress impacts pregnancy outcome. This is not too surprising; oxidative stress can damage most biomolecules, there is no reason for oxidative damage to a lipid (measured by isoprostane) or to DNA (8-OHdG) to lead to the same phenotypic outcome. Because the origins of the two markers are known, our observations provide information about routes whereby oxidative stress has a potential impact on phenotypic expression.

8-OHdG

Low maternal adiposity, as measured by BMI, was associated with a high level of oxidative stress as measured by 8-OHdG excretion and a low level of oxidative stress when measured by isoprostane excretion. Other investigators have also found an inverse relationship between BMI and urinary 8-OHdG excretion in non-pregnant subjects [26–28]. A number of explanations have been proposed including a nutritional deficiency, an artifact from the normalization of the urinary data to creatinine and increased oxidative metabolism in subjects with low BMI [26–28].

Tables I and II shows that the subjects with high 8-OHdG were not nutritionally deficient. When we examined the data without normalization to creatinine, the relationship between high urinary 8-OHdG (in ng/ml) and lower birth weight remained (3062± 65 g vs 3229±55 g, $p<0.05$). Loft et al. [29] suggested that the higher rate of 8-OHdG excretion in lean subjects was due to a higher rate of oxidative metabolism resulting in an increased availability of reactive oxygen species. Overweight and obese subjects tend to be less active than their leaner counterparts [30–32].

Elevated levels of maternal urinary 8-OHdG were associated with reduced birth weight and shortened gestation as well as with lower BMI (Tables V and VI). Thus, it is plausible that increased 8-OHdG excretion may be a factor underlying the consistent but unexplained observation that an increased risk of poor pregnancy outcome is associated with low maternal BMI or weight [33]. In our study, the highest quintile by 8-OHdG was associated with a birth weight difference of more than 200 g and a 0.9 week reduction in gestation duration. Other studies have found that women with high levels of urinary 8-OHdG measured closer to delivery had a predisposition to low (<2500 g) or lower infant birth weight [34,35].

We suggest that chronically elevated oxidative stress to maternal DNA may lead to increased risk of altered gene expression. The perturbations will not be permanent if normally operating DNA repair mechanisms mend the damage. However, the cumulative effect of numerous (minor) transient perturbations of maternal gene expression could adversely impact the maternal/foetal environment and lead to shortened gestation [36].

Counter-intuitively, mothers of offspring with birth defects ($n=19$) had lower urinary 8-OHdG excretion than the remainder of the cohort. This is the opposite of what was expected from a simple model of

oxidative stress increasing DNA oxidation and leading to foetal damage. The incidence of birth defects is related to increased oxidative stress, since maternal GPx and SOD are lower in mothers of offspring with birth defects, but levels of the marker for oxidative stress, 8-OHdG, are decreased rather than increased.

Oxidative damage to DNA is repaired by the base excision pathway; defects in this pathway have been shown to increase DNA 8-OHdG content and mutagenesis [37,38]. Thus the simplest explanation is that there may have been a deficit in foetal ability to repair damaged DNA. The products of oxidative damage to the DNA remain in the DNA; they are not 'repaired' and fewer of the damaged nucleotides are excreted in the urine. However, DNA repair mechanisms are heritable, so if the maternal repair mechanisms are compromised, the same processes are likely compromised in the developing foetus. Unrepaired damage to DNA can result in developmental disorders; if genes are altered there may be ensuing perturbations in metabolism leading to sub-optimal intrauterine growth or gestation.

Isoprostane

The outcomes associated with high isoprostane excretion were primarily maternal and secondarily foetal. They relate to a serious complication of pregnancy, pre-eclampsia, and to medically indicated preterm delivery [4]. In contrast to 8-OHdG, increased oxidative stress, as measured by higher isoprostane excretion, was associated with a higher, as opposed to a lower maternal BMI.

The $F_{2\alpha}$ isoprostanes are derived from the free radical oxidation of arachidonic acid, a polyunsaturated fatty acid that is part of the adipose tissue store [13,14]. Isoprostane excretion is positively related to maternal adiposity [39] and to the adiposity measure used in this study, higher BMI (Tables I and II), A high BMI is a well-known risk factor for pre-eclampsia [33]. Studies have demonstrated that isoprostane levels are higher in women who subsequently develop pre-eclampsia [6,39,40]. As we have demonstrated, this effect apparently is detectable early in pregnancy.

Isoprostanes are potent vasoconstrictors [41,42]. The $F_{2\alpha}$ isoprostanes constrict blood flow throughout the body [42,43]. Vasoconstriction underlies pre-eclampsia, thus providing a plausible link between maternal oxidative stress, high BMI and the rise in blood pressure that characterizes pre-eclampsia. Therefore it seems reasonable that increased isoprostane excretion could underlie the well-know relationship between high maternal BMI and serious complications of pregnancy like pre-eclampsia [33].

In conclusion: (1) Both 8-OHdG and isoprostane are biomarkers for oxidative stress early in pregnancy. They are uncorrelated with each other, track different aspects of oxidative stress and are related to different levels of pregravid BMI. 8 OH-dG is related to lower and isoprostane to higher maternal BMI. (2) Maternal oxidative stress measured early in pregnancy (12 weeks gestation on average) was associated with pre-eclampsia, shortened gestation duration, lower infant birth weight, the occurrence of minor congenital defects and a distortion in the sex ratio at birth suggests that oxidative stress is antecedent to the pathophysiology of adverse pregnancy outcomes rather than a consequence of their occurrence. (3) Thus, this research supports the prudence of paying attention to maternal redox status early in pregnancy.

Acknowledgements

This work was supported by a grant from the National Institute of Child Health and Human Development, HD38329). We thank the staff of the Osborn Family Health Center, Our Lady of Lourdes Hospital for providing access to patients.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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