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Author(s)	Kato, Eiji; Ibara, Satoshi; Kumazawa, Kazumasa et al.
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Title: Effects of supplemental oxygen on urinary 8-hydroxy-2'-deoxyguanosine levels in extremely low birth weight infants

Short title: Effects of O_2 on urinary 8-OHdG

Eiji Kato 1, Satoshi Ibara 1, Kazumasa Kumazawa 1, Yuko Maruyama 1, Takuya Tokuhisa 1, Takako Matsui 1, Ryuichi Shimono 1, Yoshinobu Maede1, Hisanori Minakami 2

1, Division of Neonatology, Perinatal Medical Center, Kagoshima City Hospital, Kagoshima, Japan

2. Department of Obstetrics, Hokkaido University Graduate School of Medicine

Corresponding author: Eiji Kato, MD

Present address:

TEL: ? , FAX: ? , E-mail: e.kato.funa@funasyusanki.ecnet.jp

Key words: biomarker, oxidative stress, oxygen supplementation, preterm infants, reactive oxygen species

Abstract

As the effects of supplementary oxygen on urinary excretion of 8-hydroxy-2'-deoxyguanosine (8-OHdG) are poorly understood, urinary 8-OHdG levels (ng/mg creatinine) were determined longitudinally on postnatal day (PND) 1, 3, and 30 in 16 neonates with birth weight <1000 g. No supplementary oxygen was required in 9 neonates during the first 24 h of life. Urinary 8-OHdG level on PND 1 was inversely correlated with birth weight in these 9 neonates (P=0.0323) and was higher in four with birth weight <750 g than five with birth weight >750 g (41.0±6.9 vs. 5.6±2.7, respectively, P=0.0200). Median urinary 8-OHdG on PND 1 of these 9 neonates was significantly lower than that of 7 neonates with oxygen (9.3 vs. 60.2, respectively), although there were no significant differences in clinical background, such as birth weight, between the two groups. Five of the 9 did not require supplemental oxygen at all during the first 30 days of life. Median urinary 8-OHdG levels were consistently significantly lower in the 5 neonates than in 11 neonates with oxygen transiently or persistently (9.3 vs. 54.6, 19.1 vs. 61.4, and 28.3 vs. 145 on PND 1, 3, and 30, respectively), although there were no differences in clinical background, such as birth weight, between the two groups. Urinary 8-OHdG on PND 30 was significantly positively correlated with supplemental oxygen dose on PND 30 (P<0.0001), but not with birth weight in the 16 neonates. These results suggested that higher supplemental oxygen tension caused higher urinary 8-OHdG in this population.

Introduction

Newborn infants are vulnerable to oxidative stress because of the immaturity of antioxidant systems [1 – 3], especially in preterm and/or low birth weight (<2500 g) infants [4 – 6]. In addition, as lung function of preterm infants is immature, supplementary oxygen is often administered to such neonates with extremely low birth weight (ELBW) defined as a birth weight <1000 g. These ELBW infants are likely to develop complications, including necrotizing enterocolitis (NEC), chronic lung disease (CLD), retinopathy of prematurity (ROP), and intraventricular hemorrhage (IVH), which may be different manifestations of oxygen radical diseases of prematurity [7 – 10].

Excessive oxidative stress that outweighs the capacity of antioxidant defense mechanisms may result in lipid oxidation, protein dysfunction, and DNA damage. The most extensively studied biomarker is 8-hydroxy-2'-deoxyguanosine (8-OHdG), a stable end product of non-enzymatic DNA oxidation [11]. Urinary 8-OHdG level has been validated as a sensitive biomarker of oxidative stress in an animal model [12]. Although similar validation against a known oxidative stressor in humans has not been conducted, urinary 8-OHdG has been used widely as a marker for oxidative DNA damage in humans.

To our knowledge, there have been eight reports regarding urinary 8-OHdG in infants in the English language literature [13 – 20]. These reports suggested that urinary 8-OHdG levels are higher for neonates (vs. adults), for sick preterm neonates, for neonates with smaller birth weight, and for neonates born at earlier stages of pregnancy. However, in such sick or preterm neonates, supplemental oxygen is often used, but the effects of supplementary oxygen on the urinary 8-OHdG levels are not well understood. The present study addressed this issue by examining ELBW infants with or without supplementary oxygen administration.

Materials and Methods

This study received approval from the institutional review board of Kagoshima City Hospital. Sixteen neonates participated in this study after receiving informed consent from their parents.

The 16 neonates were born before 28 weeks of gestation, admitted to the neonatal intensive care unit of the Kagoshima City Hospital, and had ELBW (Table 1). As all 16 had respiratory distress syndrome as evidenced by chest-X ray, all 16 were given tracheal intubation and surfactant product (Surfactant TA; Mitsubishi Pharma Corporation, Japan) at birth and were mechanically ventilated with varying oxygen concentration (FiO_2) to maintain PaO_2 of 40 – 60 mmHg or SpO_2 of 85% – 91%. Nine (56%), 4 (25%), and 3 (19%) of the 16 infants required no (FiO_2 of 21%), 23% – 29%, and 30% or more supplemental oxygen during the first 24 h of life, respectively, while 5 (31%), 6 (38%), and 5 (31%) required no, 23% – 29%, and 30% or more supplemental oxygen at the age of 30 days, respectively. Fourteen complications, including IVH, CLD, and ROP, developed in 10 of the 16 infants (four infants had two complications).

The morning spot urine samples on postnatal day (PND) 1, 3, and 30 were stored at –40°C in polypropylene tubes until they were assayed. Urinary 8-OHdG concentrations were measured using high performance liquid chromatography (CoulArray, ESA Inc., Chelmsford, MA, USA) and corrected by urinary creatinine concentration expressing as value of ng/mg creatinine (ng/mg Cr). Creatinine concentration in the urine was determined using enzyme assay (CRE II, Kainos Co. Ltd., Tokyo) with autoanalyzer (TBA C-16000, Toshiba Co. Ltd., Tokyo). Inter-assay coefficient variation is less than 2.0% for 8-OHdG concentration.

Data are presented as the median (range) or mean ± standard deviation. Statistical analyses were performed using the JMP10© statistical software package (SAS, Cary, NC). ANOVA and Tukey–Kramer HSD (honestly significant difference) tests were used for comparison of means. Fisher's exact probability test was used for comparison of categorical variables. The Wilcoxon/Kruskal–Wallis method was used for comparison of medians. In all analyses, $P<0.05$ was taken to indicate statistical significance.

Results

The urinary 8-OHdG levels on PND 1, 3, and 30 did not differ significantly between two groups divided by various clinical factors, including birth weight, gestational age at birth, Apgar score, umbilical arterial blood pH, or the presence of complications (Table 2). However, these differed significantly between two groups divided by FiO_2 on PND 0 – 1 and 30. Nine neonates did not require supplemental oxygen during the first 24 h of life even at resuscitation (Table 2), but four of these neonates required supplemental oxygen later and the remaining five did not require supplemental oxygen at all during the first 30 days of life.

Median 8-OHdG levels (ng/mg Cr) on PND 1, 3, and 30 for 7 neonates that required supplemental oxygen during the first 24 h of life were significantly higher than those for the 9 neonates without oxygen, although median values of gestational age (26 [23 – 27] vs. 25 [23 – 27] weeks), birth weight (760 [525 – 984] vs. 758 [597 – 934] g), 1-min Apgar score (6 [4 – 8] vs. 4 [1 – 8]), 5-min Apgar score (9 [7 – 9] vs. 7 [5 – 9]), and the umbilical arterial blood pH (7.30 [7.23 – 7.36] vs. 7.33 [6.97 – 7.42]) did not differ significantly between the two groups. However, among the 9 neonates that did not require supplemental oxygen during the first 24 h of life, the urinary 8-OHdG levels were significantly inversely correlated with birth weight (Fig. 1) and four smaller neonates with birth weight <750 g (mean±SD, 667±68 g) exhibited significantly higher urinary 8-OHdG level on PND 1 (41.0±6.9 vs. 5.6±2.7 ng/mg Cr, P=0.0200) than the remaining five larger neonates with birth weight >750 g (mean birth weight, 829±62 g). Thus, urinary 8-OHdG was significantly higher in smaller neonates when the effect of oxygen was absent.

The median 8-OHdG levels (ng/mg Cr) on PND 1, 3, and 30 for 11 neonates that required supplemental oxygen transiently or persistently were significantly higher than those for the five neonates without oxygen (Table 2, Fig. 2), although there were no significant differences in clinical backgrounds, including birth weight, gestational age, Apgar scores, and umbilical cord arterial blood pH, between the two groups (see legend for Fig. 2). The 8-OHdG level increased significantly over the first 30 days of life in the presence of supplemental oxygen, while did not in the absence of supplemental oxygen (Fig. 2).

Demand for an increase in FiO_2 did and did not occur in 8 and 8 neonates during the first 30 days of life (Table 2). The urinary 8-OHdG levels on PND 3 and 30 were significantly higher in the 8 neonates who required increased FiO_2 compared to those that did not, although there were no significant differences in clinical backgrounds, including birth weight, gestational age, Apgar scores, and umbilical cord arterial blood pH, between the two groups (data not shown).

We analyzed the correlations between urinary 8-OHdG level and various clinical parameters. No correlations of urinary 8-OHdG levels on PND 1, 3, and 30 were observed with birth weight, gestational age, 1-min Apgar score, 5-min Apgar score, or umbilical cord arterial blood pH (data not shown). However, a statistically significant correlation was seen between the urinary 8-OHdG level on PND 1 and FiO_2 on PND 1 and between the urinary 8-OHdG level on PND 30 and FiO_2 on PND 30, with higher FiO_2 associated with higher urinary 8-OHdG level (Fig. 3).

Discussion

In comparison of two groups with similar clinical backgrounds except for supplemental oxygen, this study demonstrated that infants given higher concentrations of supplemental oxygen were likely to exhibit higher urinary 8-OHdG levels. These results suggested that supplemental oxygen may have increased the oxidative stress causing DNA damage and increased urinary excretion of 8-OHdG in these small infants requiring supplemental oxygen. In addition, this study confirmed that, in the absence of supplemental oxygen, birth weight was a determinant factor for urinary 8-OHdG level at the age of 1 day.

Although suggested by the results of two previous studies by Tsukahara et al. [20] and Joung et al. [15], it has not been demonstrated previously that the dose of supplemental oxygen is associated with urinary 8-OHdG level. In the study by Tsukahara et al. [20] examining urinary 8-OHdG levels in two groups of preterm infants at the ages of 1 week and 1 month, 12 sick preterm neonates requiring supplemental oxygen indeed exhibited significantly higher urinary 8-OHdG levels (112±63 vs. 67±50 ng/mg Cr, respectively) at 1 month compared to 27 clinically stable preterm infants not requiring supplemental oxygen. However, the 12 sick neonates, in comparison with the 27 control neonates, were born at significantly earlier stages of pregnancy (28.5±2.8 vs. 32.2±1.8 weeks, respectively) with significantly smaller birth weight (1094±378 vs. 1693±383 g, respectively) and lower 1- and 5-min Apgar scores (5±2 vs. 8±1 for 1-min and 7±2 vs. 9±1 for 5-min, respectively). In the study by Joung et al. examining neonates with birth weight raging from 430 to 1380 g [15], the urinary 8-OHdG levels at the age of 3 days were positively correlated with the duration (days) of mechanical ventilation. However, smaller infants with a significant longer duration of mechanical ventilation were born at significantly earlier stages of pregnancy and had significantly lower 5-min Apgar scores than their counterparts [15]. The present study, which included only ELBW infants, allowed us to determine the effects of supplemental oxygen on urinary 8-OHdG levels between the two groups with comparable clinical backgrounds and strongly suggested that supplemental oxygen was causally associated with the increased level of urinary 8-OHdG.

The urinary 8-OHdG levels vary according to age [23], body weight, and postconceptional age (days) of infants at the time of determination of 8-OHdG [16]. In the 9 ELBW neonates without supplemental oxygen during the first 24 h of life in the present study, the median urinary 8-OHdG level was 9.3 ng/mg Cr at the age of 1 day, and that of healthy infants aged 50 – 53 days (13.4 ng/mg Cr) was higher than that of children aged 3 – 9 years (4.6 ng/mg Cr) and of adults (3.7 and 4.0 ng/mg Cr for males and females, respectively) [23]. Matsubasa et al. [16] examined 66 infants with varying birth weight (500 – 3798 g), including 16 infants that required mechanical ventilation, and reported that the urinary 8-OHdG levels decreased with either increasing body weight or increasing postconceptional days at the time of determination of urinary 8-OHdG level. Thus, younger (smaller) human subjects are likely to exhibit higher urinary 8-OHdG levels. Indeed, among 9 ELBW infants without supplemental oxygen during the first 24 h of life, the urinary 8-

OHdG level at the age of 1 day was 41.0±6.9 ng/mg Cr for four smaller neonates with birth weight <750 g, while it was 5.6±2.7 ng/mg Cr for the remaining five larger neonates with birth weight >750 g in this study.

Although not shown in the 5 neonates without supplemental oxygen in this study (Fig. 2), the urinary 8-OHdG level increased during the first 1 month of life in two previous studies [13, 20], but not in two other reports [14, 17]; the urinary 8-OHdG levels at 14 and 28 days of age were significantly lower or similar to those determined within 7 days of age among breast- or formula-fed neonates, respectively [17], and in another study on 20 full-term neonates by Dziaman et al. [14], the urinary 8-OHdG level continued to increase until the age of 14 days and declined thereafter. Although the reason for these discrepancies between studies is unknown, Drury et al. [13] observed a gradual increase in urinary 8-OHdG over the first month of life and speculated that the reason may be as follows: as the growth velocity curve for neonates increases steadily until a peak is reached between 4 and 6 weeks of age, with a gradual decrease thereafter until about 6 months when the velocity is comparatively stable [24], and as neonates that are growing rapidly are likely to have a larger pool of free nucleotides, which are much more prone to oxidative damage than nucleotides incorporated into DNA, in addition to the high rate of cell division resulting in the removal of the nuclear membrane and histones, DNA may be exposed to a much higher concentration of oxygen-derived free radicals and thus be more vulnerable to oxidative damage [13].

Although oxidative stress may be involved in the pathogenesis of four complications, i.e., IVH, NEC, CLD, and ROP [7 – 10], the 8-OHdG levels on PND 1, 3, and 30 did not differ between neonates with and without these complications in this study. A larger number of subjects may have been required to disclose significant associations between the 8-OHdG levels and these complications if present.

In conclusion, the present study examined longitudinal changes in urinary 8-OHdG levels of 16 neonates with birth weight ranging from 552 to 984 g over the first 1 month of life. Neonates requiring supplemental oxygen, but having similar clinical backgrounds to those not requiring supplemental oxygen with regard to gestational age at birth, birth weight, 1- and 5-min Apgar scores, and umbilical arterial blood pH, exhibited significant higher urinary 8-OHdG levels than those not requiring supplemental oxygen. In addition, the urinary 8-OHdG levels at ages of 1 and 30 days were positively correlated with the corresponding supplemental oxygen concentrations used at that time in the 16 neonates. These results strongly suggested that supplemental oxygen may have increased the oxidative stress causing DNA damage and increased urinary excretion of 8-OHdG in those small neonates requiring supplemental oxygen.

Declarations of Interest

All authors declare that they have no financial relationships with biotechnology manufacturers, pharmaceutical companies, or other commercial entities with an interest in the subject matter or materials discussed in the manuscript.

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Figure legends

Fig. 1: Association between birth weight and urinary 8-OHdG levels at the age of 1 day in 9 neonates without supplemental oxygen

Urinary 8-OHdG level increased with decreasing birth weight.

Fig. 2: Serial changes in urinary 8-OHdG levels in two groups with and without oxygen supplementation

Data are presented as medians. Closed and open circles indicate 11 and 5 neonates that did and did not require supplemental oxygen transiently or persistently during 30 days of life, respectively. *, $P<0.05$ between two groups; †, $P<0.05$ vs. values on postnatal days 1 and 3 within a group. Neither gestational age (25 [23 – 27] vs. 26 [25 – 27] weeks), birth weight (758 [525 – 984] vs. 803 [597 – 934] g), 1-min Apgar score (5 [1 – 8] vs. 4 [3 – 8]), 5-min Apgar score (8 [5 – 9] vs. 9 [6 – 9]), nor umbilical cord arterial blood pH (7.30 [6.97 – 7.36] vs. 7.33 [7.26 – 7.42]) differed significantly between the two groups with and without supplemental oxygen, respectively.

Fig. 3: Association between supplemental oxygen concentrations (FiO_2) and urinary 8-OHdG level

Left and right panels indicate values for postnatal days 1 and 30, respectively. Closed and open circles indicate neonates that did and did not require increased supplemental oxygen concentration during the first 30 days of life, respectively. The urinary 8-OHdG levels increased significantly with increasing supplemental oxygen concentration.

Table 1. Demographic characteristics of 16 neonates

Birth weight (g)		759 (525 – 984)
Gestational age (weeks)		25 (23 – 27)
Apgar score (1-min)	5 (1 – 8)	
Apgar score (5-min)	8 (5 – 9)	
Umbilical cord arterial blood pH	7.33 (6.97 – 7.42)	
Supplemental oxygen (FiO_2,%)		
Day 0 – 1		21 (21 – 50)
Room air (21%)	9 [56%]	
23% – 29%		4 [25%]
30% or more		3 [19%]
Day 30	25 (21 – 45)	
Room air (21%)	5 [31%]	
23% – 29%		6 [38%]
30% or more		5 [31%]
Mechanical ventilation (days)		37 (8 – 121)
Following complications		10 [63%]
Intraventricular hemorrhage (IVH)		4 [25%]
Chronic lung disease (CLD)	7 [44%]	
Retinopathy of prematurity (ROP)		3 [19%]
Necrotizing enterocolitis (NEC)	0 [0.0%]	

Data are presented as the median (range) or number of infants [%]. IVH was diagnosed when grade II and more severe IVH [21] was present. CLD was diagnosed when supplemental oxygen was needed at corrected gestational age of 36 weeks. ROP was diagnosed when laser photocoagulation therapy was required. NEC was diagnosed in the presence of stage III B NEC [22].

Table 2. Urinary 8-OHdG levels in two groups divided by various factors

	Urinary 8-OHdG levels (ng/mg creatinine)		
Postnatal day	1	3	30
Birth weight (g)			
<750 (*n*=7)	48.2 (32.2 – 169)	52.0 (34.3 – 284)	145 (15.0 – 339)
≥750 (*n*=9)	9.3 (1.8 – 78.2)	28.1 (9.5-190)	75.3 (8.6 – 388)
Gestational age (weeks)			
<26 (*n*=9)	47.3 (1.8 – 81.1)	47.7 (14.2 – 284)	99.3 (28.3 – 339)
≥26 (*n*=7)	55.0 (7.1 – 169)	52.0 (9.5 – 190)	88.9 (8.6 – 388)
Apgar score (1-min)			
<5 (*n*=7)	36.3 (3.5 – 81.1)	47.7 (9.5 – 284)	60.9 (8.6 – 339)
≥5 (*n*=9)	55.0 (1.8 – 169)	39.5 (10.6 – 190)	146 (28.3 – 388)
Apgar score (5-min)			
<9 (*n*=9)	47.3 (3.5 – 81.1)	41.2 (9.5 – 284)	75.3 (8.6 – 339)
≥9 (*n*=7)	55.0 (1.8– 169)	52.0 (10.6 – 190)	88.9 (15.0 – 388)
Umbilical arterial blood pH			
<7.32 (*n*=7)	55.0 (3.5 – 169)	52.0 (14.2 – 190)	75.3 (15.0 – 388)
≥7.32 (*n*=9)	36.3 (1.8 – 81.1)	34.3 (9.5 – 284)	145 (8.6 – 339)
Supplemental oxygen (Day 0 – 1)			
No (*n*=9)	9.3 (1.8 – 48.3)	28.1 (9.5 – 77.7)	44.8 (8.6 – 146)
Yes (*n*=7)	60.2 (52.7– 169)*	158.2 (34.3 – 284)*	239 (39.8 – 388)*
Supplemental oxygen (Day 30)			
No (*n*=5†)	9.3 (1.8 – 36.3)	19.1 (9.5 – 52.0)	28.3 (8.6 – 146)
Yes (*n*=11)	54.6 (3.5 – 169)*	61.4 (14.2 – 284)*	145 (39.8 – 388)*
Increase in O_2 concentration‡			
No (*n*=8)	34.3 (1.8– 60.2)	36.9 (9.5 – 61.4)	42.3 (8.6 – 147)
Yes (*n*=8)	50.5 (3.5 – 169)	118.0 (14.2 – 284)*	192 (42.5 – 388*
Any complication¶			
No (*n*=6)	34.3 (1.8 – 57.9)	40.3 (10.6 – 61.4)	42.3 (15.0 – 146)
Yes (*n*=10)	50.5 (3.5 – 169)	62.7 (9.5 – 284)	146 (8.6 – 388)

Data are presented as the median (range). †, These five neonates did not require supplemental oxygen at all during the first postnatal 1 month; ‡, Yes when FiO_2 on postnatal day 30 was higher than that on postnatal day 1; ¶, complications included IVH, CLD, and ROP shown in Table 1; *, $P<0.05$ vs. counterpart.

Fig. 1 Kato

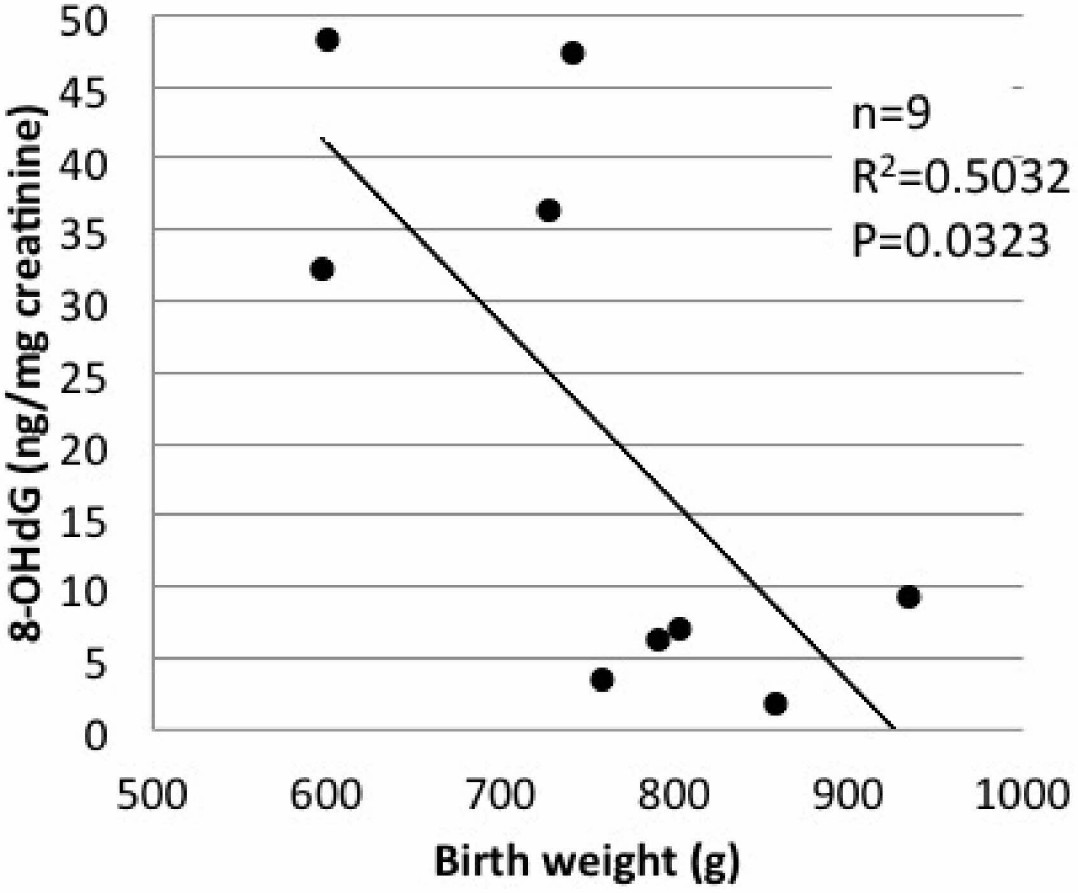
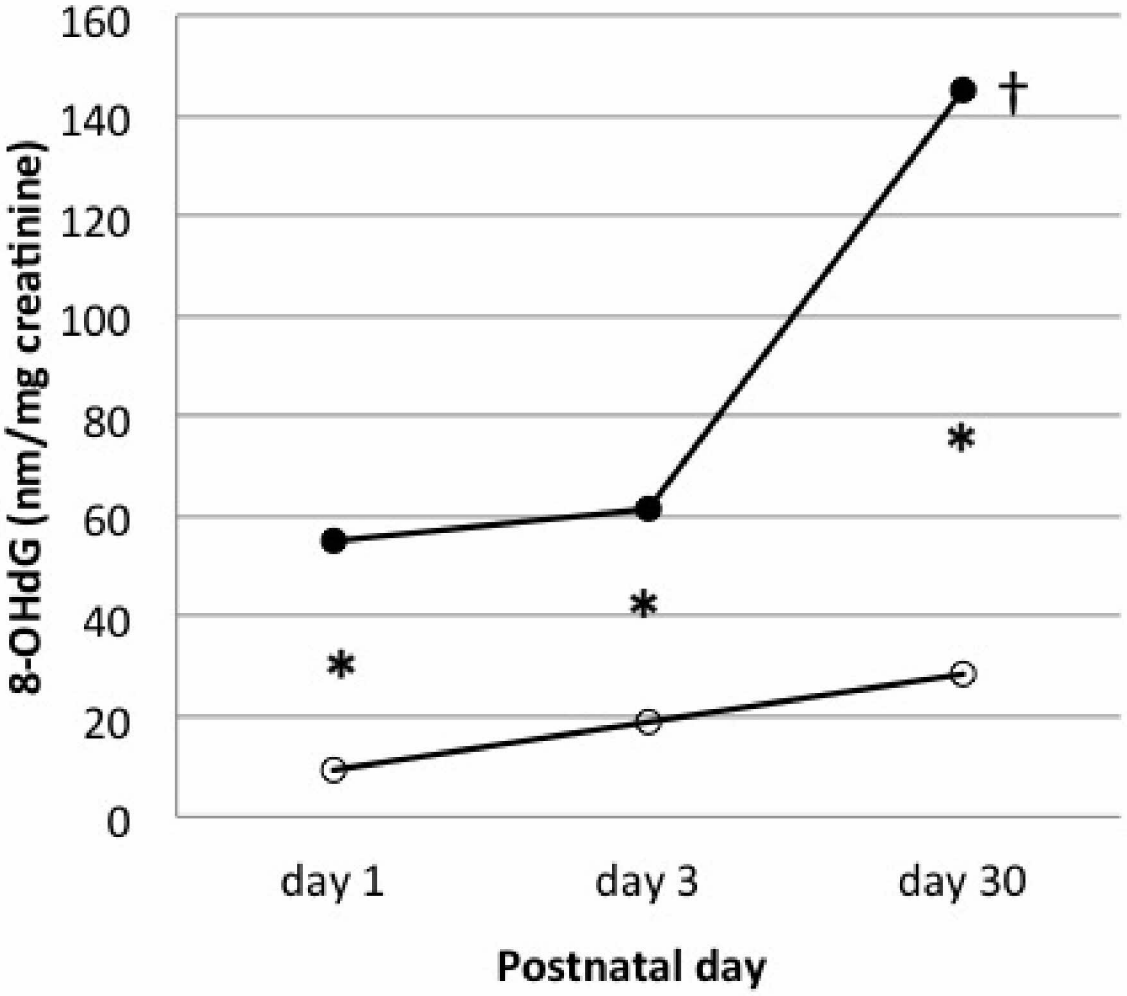
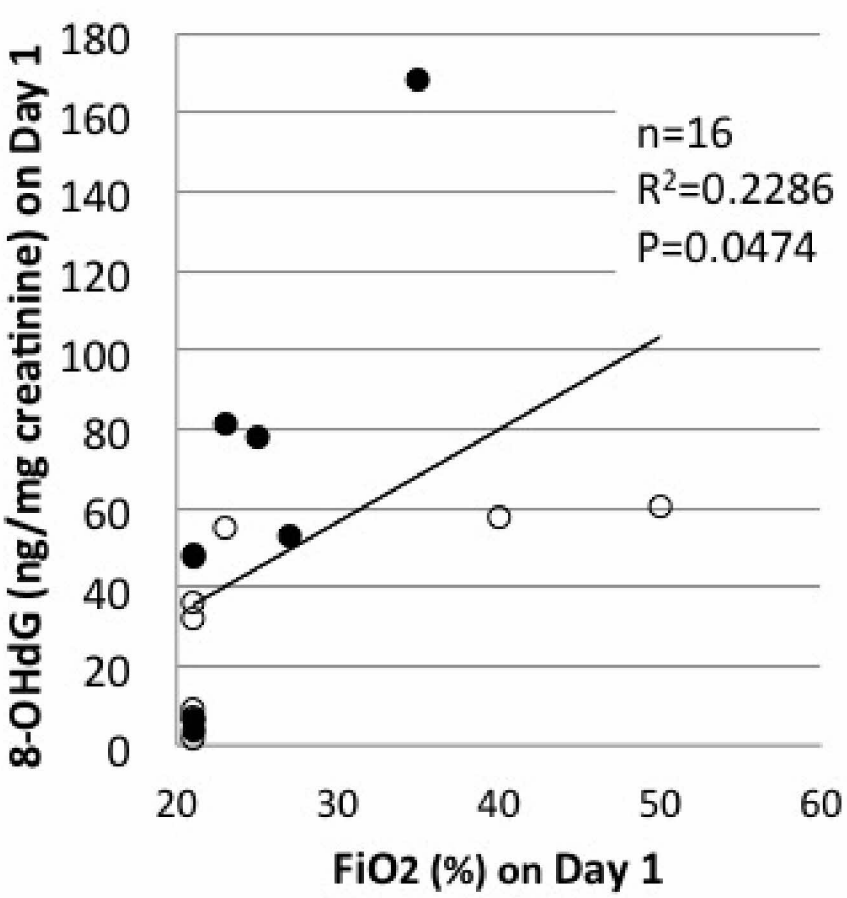
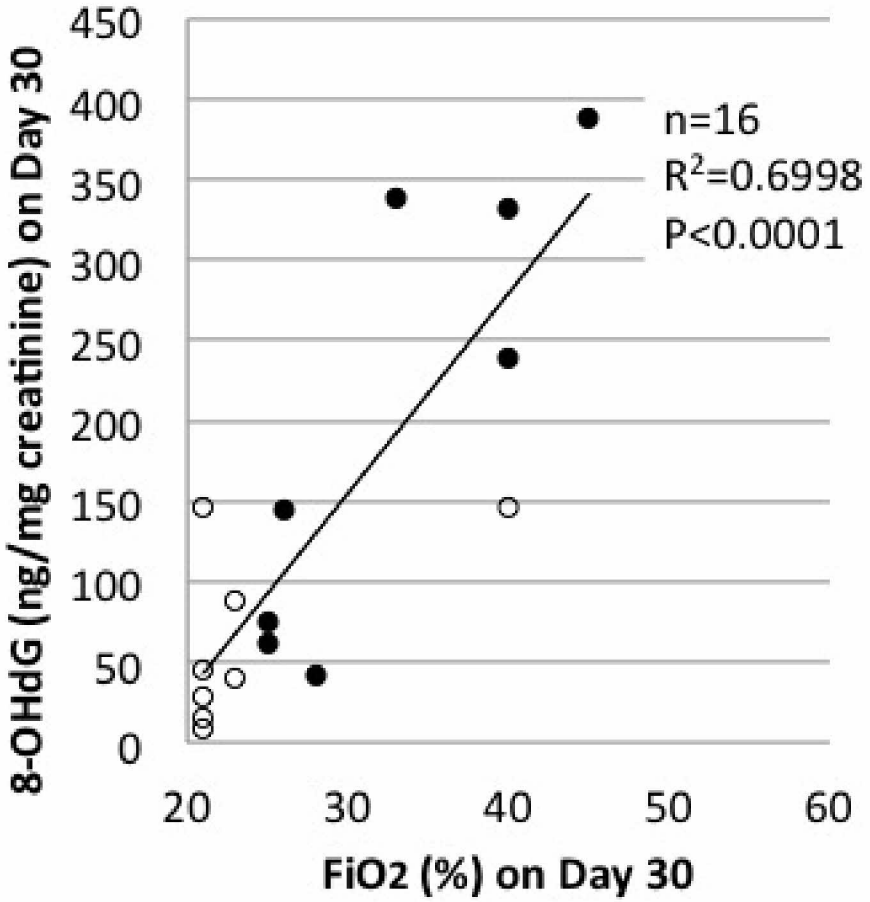

50
45
40
35
30
25
20
15
10
5
0
8-OHdG (ng/mg creatinine)
500
600
700
800
900
1000
Birth weight (g)
n=9
R2=0.5032
P=0.0323

Fig. 2 Kato


160
140
120
100
80
60
40
20
0
8-OHdG (nm/mg creatinine)
†
*
*
*
day 1
day 3
day 30
Postnatal day


8-OHdG (ng/mg creatinine) on Day 1
180
160
140
120
100
80
60
40
20
0
20
30
40
50
60
FiO2 (%) on Day 1
n=16
R2=0.2286
P=0.0474


8-OHdG (ng/mg creatinine) on Day 30
450
400
350
300
250
200
150
100
50
0
20
30
40
50
60
FiO2 (%) on Day 30
n=16
R2=0.6998
P<0.0001