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Urine Gastrin Releasing Peptide in the First Week Correlates with BPD and Post-Prematurity Respiratory Disease

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Summary

Rationale: Bronchopulmonary dysplasia (BPD) is associated with post-prematurity respiratory disease (PRD) in survivors of extreme preterm birth. Identifying early biomarkers that correlate with later development of BPD and PRD may provide insights for intervention. In a preterm baboon model, elevated gastrin-releasing peptide (GRP) is associated with BPD, and GRP inhibition mitigates BPD occurrence.

Objective: We performed a prospective cohort study to investigate whether urine GRP levels obtained in the first postnatal week were associated with BPD, PRD, and with other urinary biomarkers of oxidative stress.

Methods: Extremely low gestational age infants (23-28 completed weeks) were enrolled in a US multicenter observational study, The Prematurity and Respiratory Outcomes Program (PROP,

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http://clinicaltrials.gov/ct2/show/NCT01435187). We used multivariable logistic regression to examine the association between urine GRP in the first postnatal week and multiple respiratory outcomes: BPD, defined as supplemental oxygen use at 36+0 weeks postmenstrual age, and post-prematurity respiratory disease, defined by positive quarterly surveys for increased medical utilization over the first year (PRD score).

Results: A total of 109/257 (42%) infants had BPD, and 120/217 (55%) had PRD. On adjusted analysis, GRP level >80 was associated with BPD (adjusted odds ratio (aOR) 1.83, 95% confidence interval (CI): [1.03-3.25]) and positive PRD score (aOR 2.46, 95% CI: [1.35-4.48]). Urine GRP levels correlated with duration of NICU ventilatory and oxygen support and with biomarkers of oxidative stress: allantoin and 8-hydroxydeoxyguanosine.

Conclusions: Urine GRP in the first post-natal week was associated with concurrent urine biomarkers of oxidative stress and with later diagnoses of BPD and PRD.

Keywords

Bronchopulmonary dysplasia; Gastrin releasing peptide; reactive oxygen species

Introduction:

Bronchopulmonary dysplasia (BPD) is a major cause of chronic lung disease in survivors of extreme preterm birth (1) and is associated with respiratory disability throughout the patient's lifespan (2, 3). BPD is a clinical diagnosis, most commonly based on use of supplemental oxygen at 36 weeks post-menstrual age (PMA) (4, 5). BPD has been demonstrated to be a predictor of post-prematurity respiratory disease (PRD) at one year of age (6) and has been a milestone when evaluating potential biomarkers and therapies for PRD. Several perinatal factors are associated with the later diagnosis of BPD including early gestational age, male sex, intrauterine growth retardation, maternal smoking, chorioamnionitis, preeclampsia, early cumulative oxygen requirement, race, and early respiratory failure (5–9). However, identification of these perinatal risk factors is not sufficient to predict risk for BPD accurately and most cannot serve as therapeutic targets or be avoided. Therefore, there is a pressing need to identify physiologic, genetic, or biochemical biomarkers that are associated with BPD, that may also contribute to pathogenesis; thereby, serving as targets for interventions ameliorating lung injury and optimizing lung development in the extrauterine environment.

Gastrin releasing peptide (GRP) has been previously examined for its role in regulating lung development and for its association with BPD in very early gestation baboons as well as human infants. GRP, a product of pulmonary neuroendocrine cells, peaks during fetal development and then progressively decreases to low adult levels at term (10). This spatial and temporal regulation of GRP is critical for normal lung development. GRP activates epithelial and mesenchymal cell receptors during murine (11) and rhesus monkey (12, 13) embryogenesis required for airway branching and epithelial proliferation. However, in contrast to the normal decline in the neuroendocrine cell population during later fetal development, infants who develop BPD have sustained numbers of neuroendocrine cells in the bronchioles that produce GRP (14). GRP is a bombesin-like peptide that is increased in

urine of infants who develop BPD compared to case controls (15). Baboon infants delivered at 125 days gestation (early preterm), treated with supplemental oxygen “as needed,” have elevated GRP, increased numbers of pulmonary neuroendocrine cells, and arrested alveolar-capillary development that mimics human BPD histologically and functionally (16). Importantly, preterm baboons, treated with an anti-GRP antibody at 2-4 hours post-birth and on postnatal days 3 and 6, have a significant decrease in pulmonary neuroendocrine cells, improved alveolar septation, decreased alveolar wall thickness, and increased capillary formation at post-natal day 14, compared to placebo treated animals (17). This study raised the possibility that downregulation of GRP immediately following preterm delivery, may permit increased alveolar-capillary development, mitigating BPD and later post-prematurity respiratory disease.

Several studies have reported that pulmonary neuroendocrine cell hyperplasia is associated with oxidative stress and reactive oxygen species (ROS) (14, 18, 19). Treatment with 100% supplemental oxygen in a preterm baboon model results in inflammation, interstitial fibrosis, decreased alveolar septation, and increased numbers of neuroendocrine cells and urine GRP levels (20). Importantly, treatment with a catalytic antioxidant mitigates the pathologic findings including significantly decreasing the number of neuroendocrine cells and GRP levels (20). Furthermore, the association of ROS in human airway surface liquid (21), urine, and plasma biospecimens (22, 23) with BPD (20, 21, 24) strengthens the potential connection between ROS exposure and regulation of neuroendocrine cell numbers and GRP production. Based on these reports, we hypothesized that increased urine GRP in the first post-natal week would be associated with ROS and later development of BPD and/or PRD. We tested this hypothesis in a well-characterized prospective observational cohort. This cohort was followed over the first-year (age corrected for prematurity) by quarterly surveys for medical utilization, thus, we were also able to test whether urine GRP in the first post-natal week was associated with presence of PRD at one-year corrected age. Our aims were first to test whether urine GRP in the first week of life was associated with later BPD and/or PRD, and second, whether GRP levels were associated concurrently with other urinary measures of ROS.

Materials and Methods

Subjects.

Infants born between 23 $^{0/7}$ and 28 $^{6/7}$ were recruited by post-natal day 7 after obtaining IRB-approved, informed written consent from a parent for a US multicenter observational study, The NHLBI Prematurity and Respiratory Outcomes Program (PROP, http://clinicaltrials.gov/ct2/show/NCT01435187). Enrollment for “GRP and BPD,” our single site study within PROP, closed February 2016, two years after enrollment closure for PROP (December 31, 2013). The subjects (n=331) included 180 infants enrolled in PROP and an additional 151 infants enrolled for the GRP and BPD study only (Figure 1).

Study Design.

Clinical data were recorded including antenatal demographics and weekly clinical data during the NICU course as previously reported (25). Urine was collected from research

diapers in the first postnatal week (n=281) and at 36 weeks post-menstrual age (PMA) in a subset of GRP and BPD infants (n=167), aliquoted and frozen at −80°C until analysis. Utilization of supplemental oxygen, invasive ventilation, or non-invasive ventilation (nasal CPAP, nasal noninvasive positive pressure ventilation, or high flow nasal cannula) was assigned by the chronologic age in weeks PMA for the date of the last day of use. Small for gestational age was defined as <10th percentile for age (26).

BPD Assignment.

The diagnosis of BPD, our primary outcome, (4, 27) was assigned to infants on supplemental oxygen (>21% FiO_2) at 36 weeks PMA. Infants on no supplemental oxygen, or on room-air flow, or discharged before 36 weeks PMA on room air were assigned as no BPD (4).

Post-Prematurity Respiratory Disease Assignment.

The diagnosis of post-prematurity respiratory disease at one-year corrected gestational age (corrected for prematurity), was assigned by using results from quarterly surveys that interrogated three domains: 1) respiratory medication administration, 2) hospitalization for a respiratory indication, 3) home supplemental oxygen or ventilatory support (6). An infant was assigned a positive PRD score if there was at least one positive response on at least two surveys.

Methods to measure urine GRP, reactive oxygen species, and urine creatinine.

Urine samples were de-identified and coded. Urine GRP was measured by an enzymatic immunoassay (Phoenix Pharmaceuticals, Burlingame, CA), and urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) was measured by a competitive ELISA (Trevigen, Gaithersburg, MD) per manufacturer's instructions. 2,3-dinor-iPF2α–III, a urinary metabolite of 8-iso-prostaglandin-F2α (28), allantoin (29), and creatinine (Cr) were analyzed by using ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) at Duke University. Detailed methods are included in the on-line supplement.

Statistical Methods.

We used standard summary statistics for infant demographics and reported median (25th-75th percentiles) for continuous variables and counts (percentages) for categorical variables. We compared demographic and maternal factors between infants with and without GRP levels in the first postnatal week using Fisher's exact test or the Wilcoxon rank sum test, where appropriate. We evaluated different GRP levels in the first postnatal week by receiver operating characteristic analysis to differentiate infants with and without BPD and used the threshold with the highest area under the curve (AUC). We evaluated bivariate relationships among demographic factors, GRP level above threshold in the first postnatal week, and BPD using Fisher's exact tests. We assessed the unadjusted association between urine GRP levels in the first postnatal week and at 36 weeks PMA and the diagnosis of BPD using the Wilcoxon rank sum test. We performed multivariable logistic regression to assess the relationship between urine GRP above threshold and BPD, BPD or death, and PRD with adjustment for gestational age, sex, race, and maternal prenatal smoking. We evaluated the

relationship between GRP levels to ROS biomarkers, including allantoin, 8-hydroxydeoxyguanosine, and 2,3-dinor-iPF2α–III using linear regression. We evaluated the association between urine ROS markers and BPD using the Wilcoxon rank sum test. P-values <0.05 were considered significant. Analyses were conducted using Stata 15.1 (StataCorp LLC, College Station, TX).

Results

Of the 331 infants recruited, urine biospecimens obtained in the first post-natal week were available for 281 (85%) (Figure 1). For a subset of infants in the GRP and BPD study (n=198), urine was also obtained from many infants at 36 weeks PMA (n=167; 84%). Infant demographic and clinical characteristics are shown in Table 1. There were no significant differences in the cohort with urine available and the subjects with no urine available, with the exceptions that infants with urine available were more likely to have a maternal prenatal history of smoking (P=0.01). The infants recruited across our centers were 49% Caucasian infants and 54% males. Maternal prenatal smoking was present for 23% of the infants. A total of 257 infants were evaluable for BPD, and 109/257 (42%) had the diagnosis. A total of 217 infants were evaluable for PRD, and 120/217 (55%) had PRD. On bivariate analysis, BPD was associated with lower gestational age, and Caucasian race (Table 2), but we did not observe an association between maternal prenatal smoking and increased BPD incidence.

In bivariate analysis, urinary GRP concentration in the first post-natal week was positively associated with the diagnosis of BPD at 36 weeks PMA (median (IQR) 90 (56-136) vs. 67 (40-112), p=0.005; Figure 2). In contrast, urine GRP at 36 weeks PMA was not associated with BPD (p=0.63). The AUC of candidate GRP thresholds varied little and were as follows: 0.575 for 80 pmol/mg Cr; 0.562 for 90 pmol/mg Cr; 0.568 for 100 pmol/mg Cr; and 0.552 for 200 pmol/mg Cr. The 80 pmol/mg threshold for higher GRP level vs. lower GRP level comparisons was chosen because it had the highest AUC value. Demographic characteristics were similar between infants with GRP values above and below the chosen threshold value of 80 pmol/mg Cr (Table 2). GRP levels greater than 80 pmol/mg Cr were associated with BPD (Table 2), and with BPD or death (Supplementary Table 1). Consistent with the association with the primary outcome, first-week urine GRP levels were associated with the PMA on the last day of invasive ventilatory support, non-invasive ventilatory support, and supplemental oxygen support (Figure 3). Urine GRP > 80 pmol/mg Cr in the first postnatal week was not associated with maternal demographic factors or antenatal or neonatal factors at the time of delivery (Supplementary Table 2).On adjusted analysis, a GRP value > 80 pmol/mg Cr in the first postnatal week was associated with increased odds of BPD (adjusted odds ratio=1.83, 95% confidence interval 1.03-3.25) (Table 3), increased odds of BPD or death (adjusted odds ratio=1.99, 95% confidence interval 1.14-3.48), and increased odds of PRD (adjusted odds ratio=2.46, 95% confidence interval 1.35-4.48) (Table 3).

In *in vitro* and *in vivo* models, pulmonary neuroendocrine cells and their biological product, GRP, are sustained by oxidative stress. Therefore, we tested whether urine biomarkers of ROS were associated with GRP levels (Figure 4). We found that urinary GRP was associated with concurrent urinary allantoin (P=0.003) (22), a uric acid oxidation product, and 8-hydroxy-2'-deoxyguanosine (P=0.01) (23), reflecting oxidized nucleic acids, but not with

urine 2,3-dinor-iPF2α–III, a urinary metabolite of the isoprostane, 8-iso-PGF2α (P=0.87) (30), a non-enzymatic oxidation product of arachidonic acid. Although BPD has been associated with oxidative stress (31), in our study cohort, urine biomarkers of ROS in the first post-natal week were not associated with BPD (Supplementary Table 3).

Discussion:

Increased urine GRP levels in the first postnatal week were associated with later BPD diagnosis at 36 weeks PMA in a multicenter cohort of extremely premature infants. Higher urine GRP levels were also associated with a greater PMA at the last day of invasive ventilatory support, non-invasive ventilatory support, and supplemental oxygen support, consistent with the requirement for longer duration of respiratory support for these infants with this elevated marker of lung neuroendocrine cells. The association between urine GRP in the first week and later BPD diagnosis remained significant on multivariate analysis controlling for gestational age, male sex and white race. This supports a potential mechanistic role for GRP in mediating BPD, possibly as an oxidant sensor. Finally, increased urine GRP in the first post-natal week was associated with later PRD as indicated by increased medical utilization for respiratory disease at one-year corrected age (a positive PRD score), supporting the importance of urine GRP as an indicator for chronic lung disease.

GRP is one of several biomarkers associated with ROS. Pulmonary neuroendocrine cell density and GRP concentrations are increased following oxygen therapy (20), smoking (18, 32), ozone exposure (33), and chronic lung diseases such as cystic fibrosis (32, 34). Oxidation of proteins, DNA, and lipids generate biomarkers that indicate ROS exposure. We demonstrate that urinary GRP was positively associated with urinary allantoin and 8 hydroxy-2-deoxyguanosine, both established markers of oxidative stress in preterm infants. Interestingly, GRP (15), 8 hydroxy-2’-deoxyguanosine (23), and allantoin (35) concentrations in the urine reflect levels found in airway surface liquid and in blood. Other ROS biomarkers such as 8-iso-PGF2α are present in the airway surface liquid and plasma but are not concentrated in the urine (30) and therefore may not be useful as urinary biomarkers for BPD. Thus, although plasma F_2-isoprostane early post-birth is associated with BPD (36) or with increased oxygen requirement at 40 weeks PMA (37), urinary 8-iso-PGF2α is not associated with BPD (30) or, in our cohort study, with levels of urinary GRP. It is notable that although the selected urine ROS markers were not associated with BPD, GRP, a potential oxidant sensor, was associated with BPD.

Results from our study validate and extend findings from a previous prospective, single center, observational study by Cullen et. al. (15) of 132 preterm infants born at 28 weeks gestation or earlier, that demonstrates that urinary GRP is elevated in the first postnatal week in the infants who go on to develop BPD. The Cullen report measured urinary bombesin-like peptide by a radioimmunoassay (RIA) and values were normalized to urinary creatinine. GRP is one mammalian homologue of bombesin-like peptide; they share a conserved seven amino acid C-terminus, the epitope for immunogenicity assays, and bind the same receptors resulting in the same physiologic effects (32, 38). The cohort for the Cullen study matched the gestational ages of subjects and the percentage of positive BPD infants in our report, but

differed in the racial distribution of subjects with fewer African American infants than included in our cohort. Importantly our study extended beyond the diagnosis of BPD to demonstrate an association of urine GRP in the first postnatal week with PRD at one-year corrected age. To our knowledge this is the first urine biomarker that is associated with both outcomes of preterm lung disease.

Our study has several limitations. First, the definition of BPD was selected pragmatically to minimize the number of unclassified infants (4). Although more recent diagnostic criteria for BPD, for example oxygen requirement at 40 weeks (4, 37, 39) may be a more robust indicator for chronic lung disease, the data required to apply these diagnoses was not available for our study subjects. Second, nine percent of infants died before 36 weeks PMA and were excluded from the BPD classification. Therefore, we analyzed the association of urine GRP in the first week of life with the combined outcome of BPD or death at 36 weeks PMA and determined whether these infants would have significantly contributed to the association between GRP and severe chronic lung disease (Supplementary Table 1 and Table 3). We observed that there was a greater association of urinary GRP in the first post-natal week and the combined outcome of BPD or death. Third, there were 15% (50 out of 331 subjects) missing urine biospecimens in the first 7 days due to failure to collect urine, improper labeling, samples obtained after 7 days, and death of the infant. We demonstrated that there was no significant difference in demographics between these two groups: infants with urine samples and infants without urine samples (Table 1) with the exception of maternal smoking, which was lower among infants without urine samples. Fourth, though we demonstrated a significant association between GRP levels greater than 80 pmol/mg creatinine and outcomes of BPD and PRD, the urine GRP level in the first post-natal week alone may not be sufficiently sensitive as a biomarker to predict individual risk for BPD and PRD. However, our results merit further study of GRP as part of a panel to predict risk for these respiratory outcomes.

Although there is evidence in a preterm baboon lung injury model that inhibition of GRP permits more normal alveolar development, the mechanisms by which excessive GRP alter normal lung development are not known. In a murine model, perinatal administration of GRP inhibits alveolarization, increases alveolar wall thickness, and increases smooth muscle actin deposition, resulting in lung remodeling (40). Furthermore, the arrest of alveolar development, is partially mitigated in GRP receptor-null mice treated with GRP (40). In other mouse models relevant to environmentally induced lung injury, GRP inhibition rescues normal lung structure and increases survival. Mouse exposure to radiation upregulates urine GRP within the first 24 h and is associated with radiation induced pulmonary fibrosis at 15 weeks; blockade of GRP using one dose of an anti-GRP monoclonal antibody administered intraperitoneally at 24 h post-radiation significantly inhibits pulmonary fibrosis at 15 weeks (41). Murine exposure to influenza stimulates increased GRP in lungs and serum at 4-8 days. When influenza-infected mice are treated with anti-GRP antibodies or inhibitors of GRP or GRP receptor at days 2-4, the mice have better survival, less inflammation, and diminished cytokine production (42). These preclinical model systems demonstrate that blockade of GRP plays an important role in environmentally induced lung injury to sustain normal morphology and reduce inflammation.

We propose that GRP is a multifunctional product of neuroendocrine cells that requires a spatial and temporal pattern during fetal development to permit normal lung parenchymal development. Our results are the first to demonstrate an association between urinary GRP and ROS measures in infants with BPD supporting a potential role for neuroendocrine cells and GRP to respond to ROS and disrupt the normal developmental program. This report and previously published results in humans and animal models cited above support further investigation to evaluate whether GRP is associated with post-prematurity respiratory disease in later childhood and whether modifications of early GRP and GRP receptor interactions can safely reduce the risk of BPD and PRD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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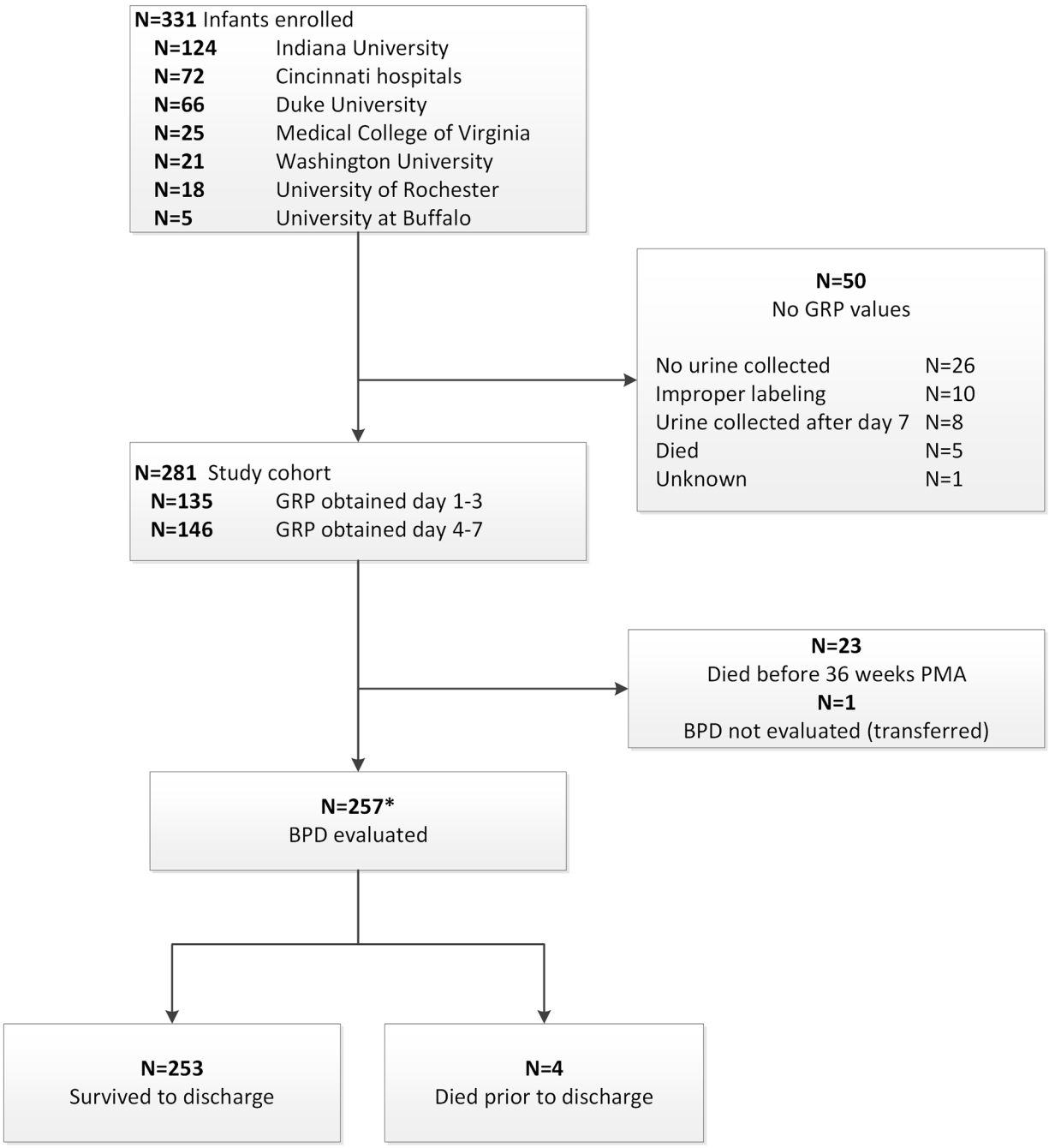


Figure 1.

Study flow diagram. *Eligible infants in the GRP and BPD study who had urine GRP levels determined in the first week, and for infants only in the GRP and BPD study (167 of 198), at 36 weeks postmenstrual age (PMA): 44/55 from Duke University, 91/97 infants from Indiana University, 11/18 infants from Medical College of Virginia, and 21/28 infants from Cincinnati hospitals.

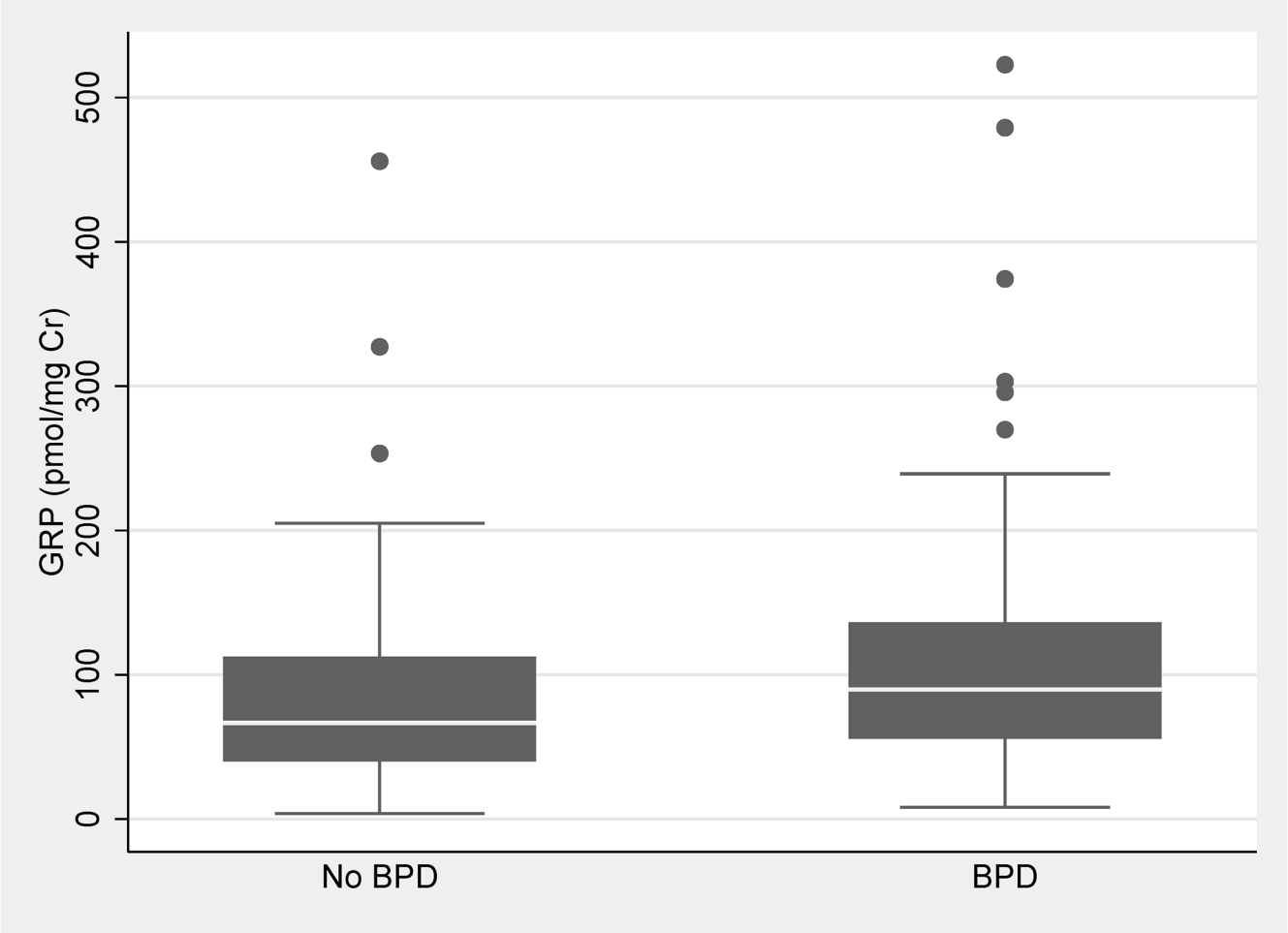
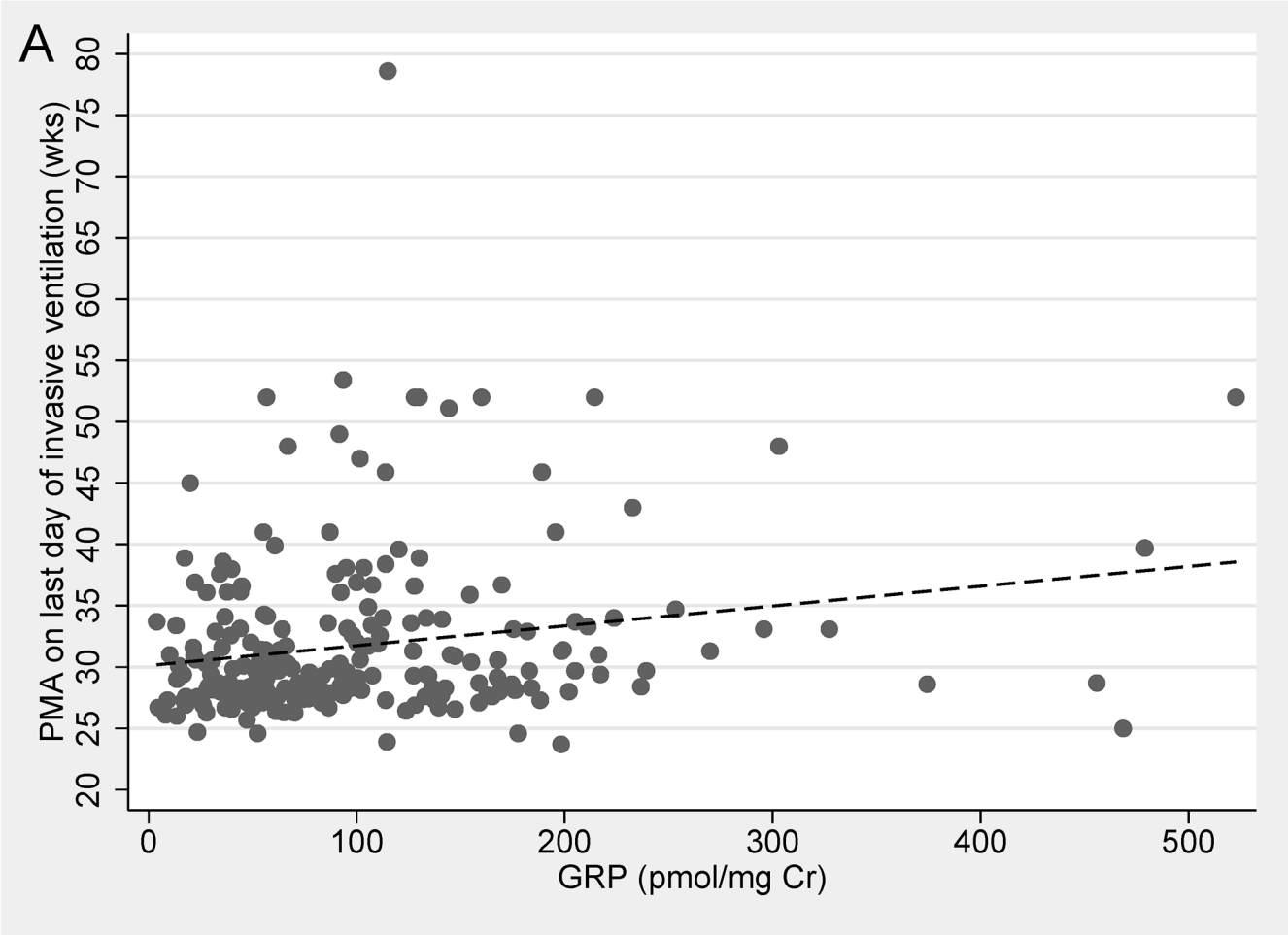
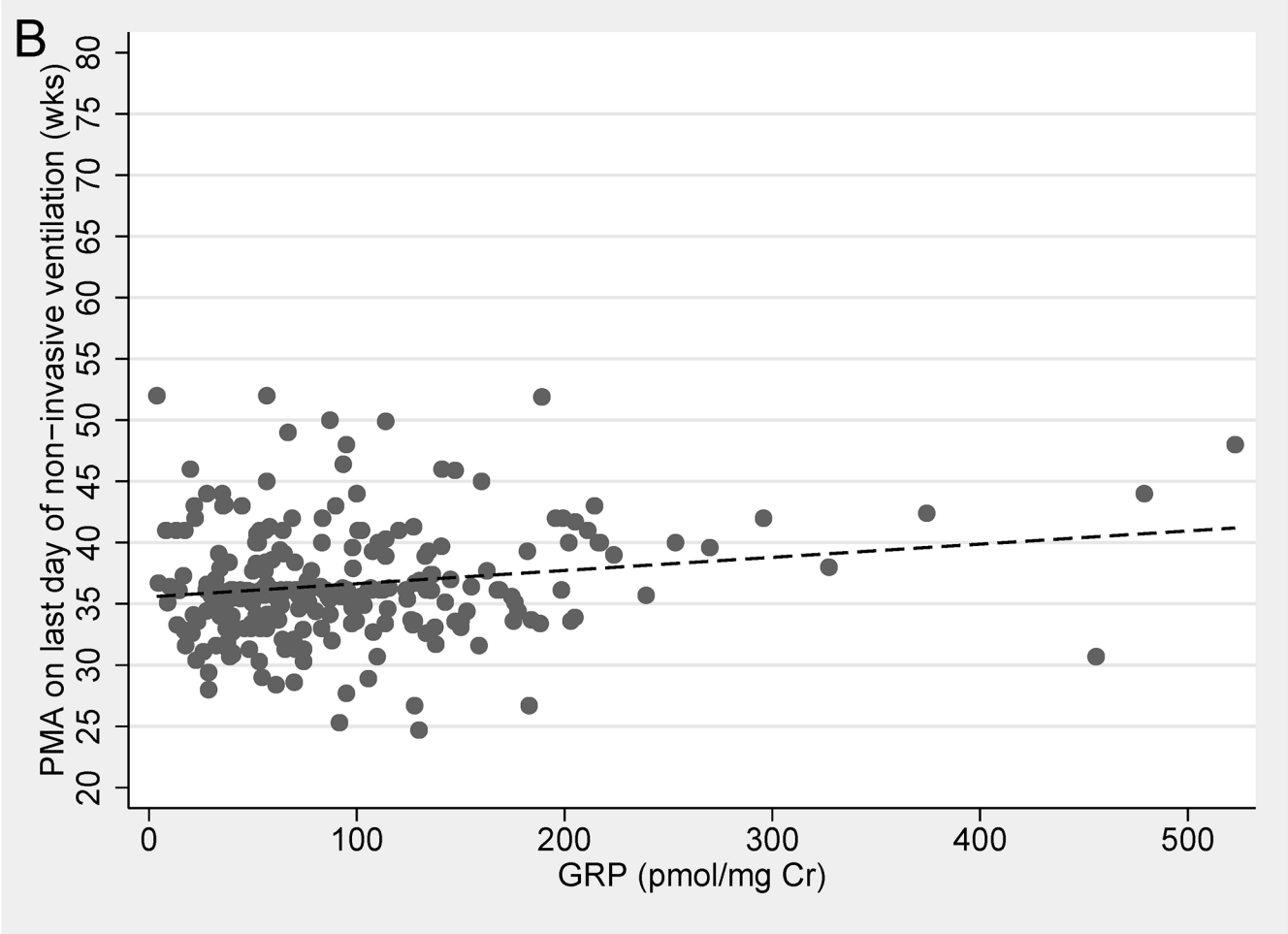


Figure 2.

GRP levels (median (25^{th}-75^{th} percentile) pmol/mg Creatinine) are 90 (56-136) for bronchopulmonary dysplasia (BPD) (n= 109) and 67 (40-112) for No BPD (n= 148) status. Comparison by Wilcoxon rank sum test. *P=0.005





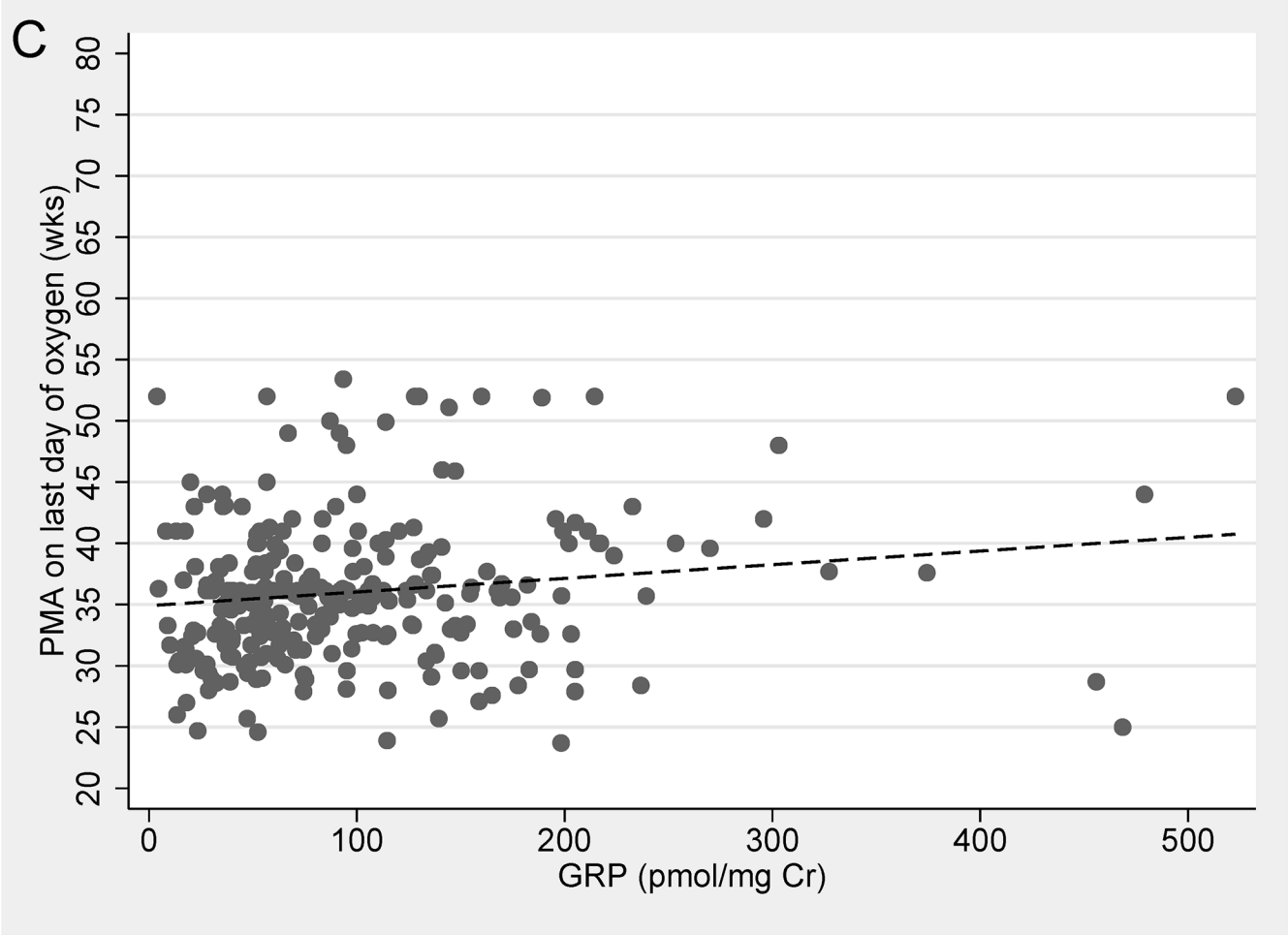
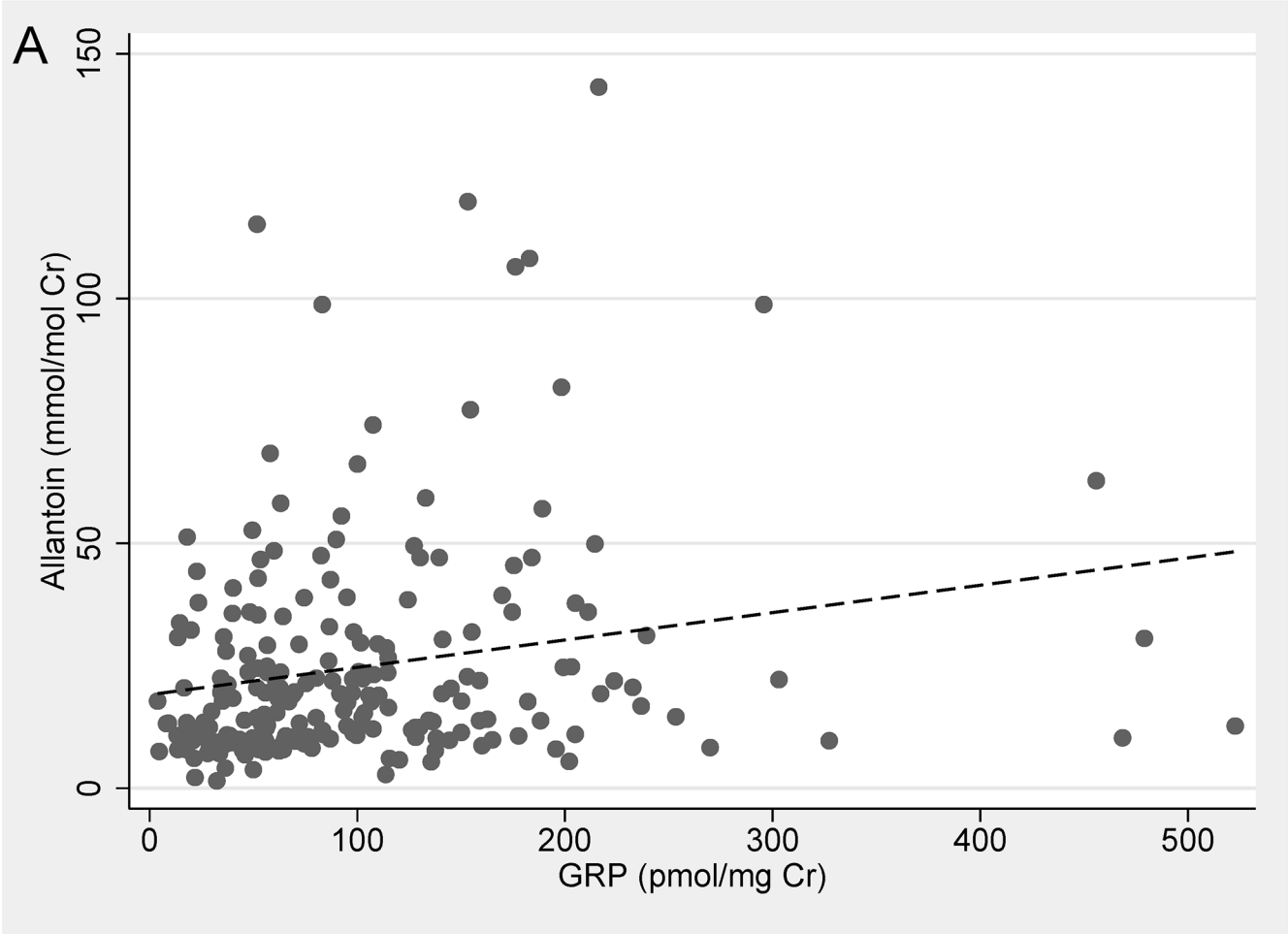
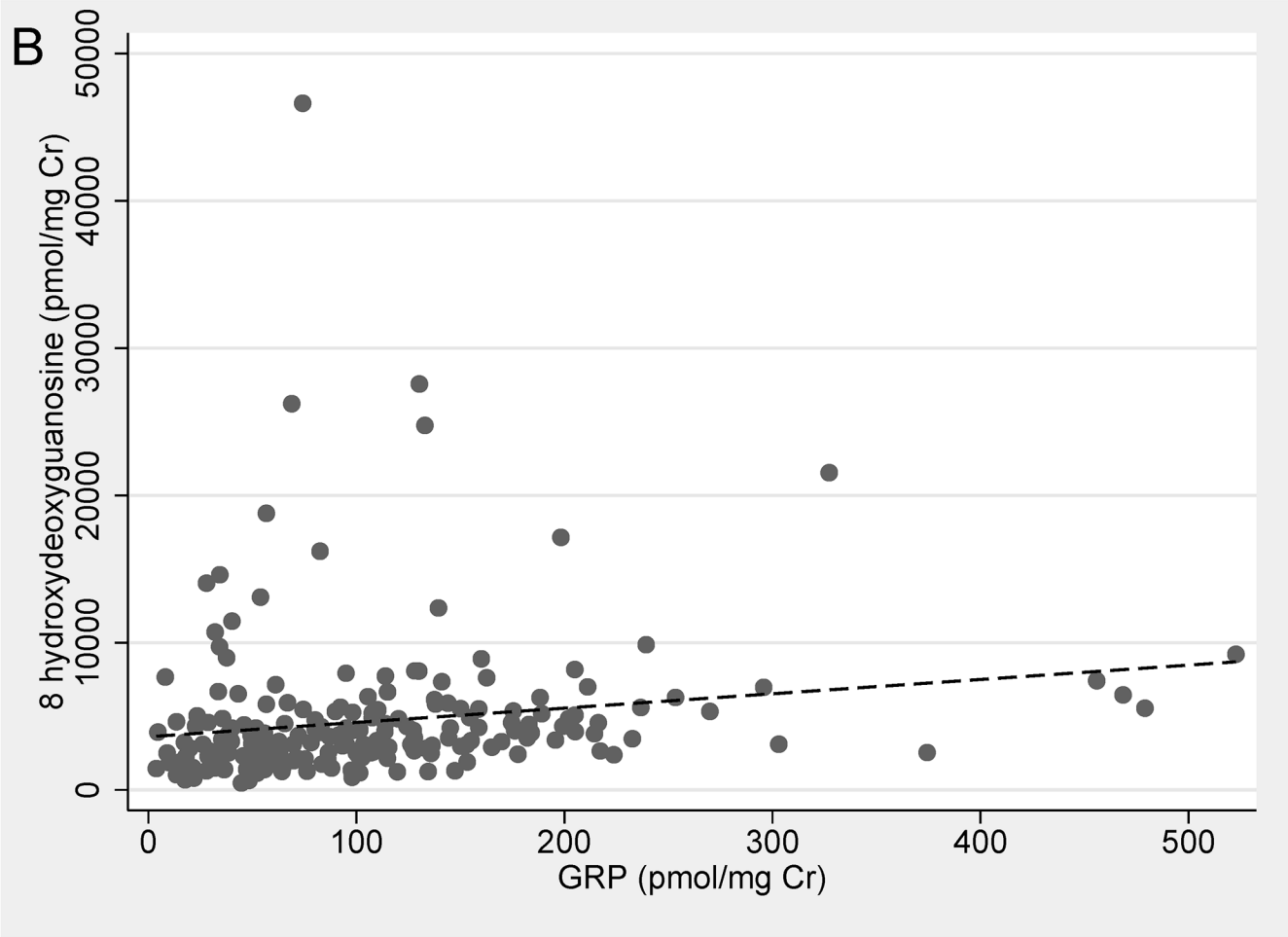


Figure 3.

Postmenstrual age (PMA) in weeks on (A) the last day of invasive ventilation (n=240; R^2=0.04; P=0.002), (B) the last day of non-invasive ventilation (n=258; R^2=0.03; P=0.005), and (C) the last day of oxygen support (n=272; R^2=0.02; P=0.012) by GRP level (pmol/mg Creatinine). Significance testing by linear regression.





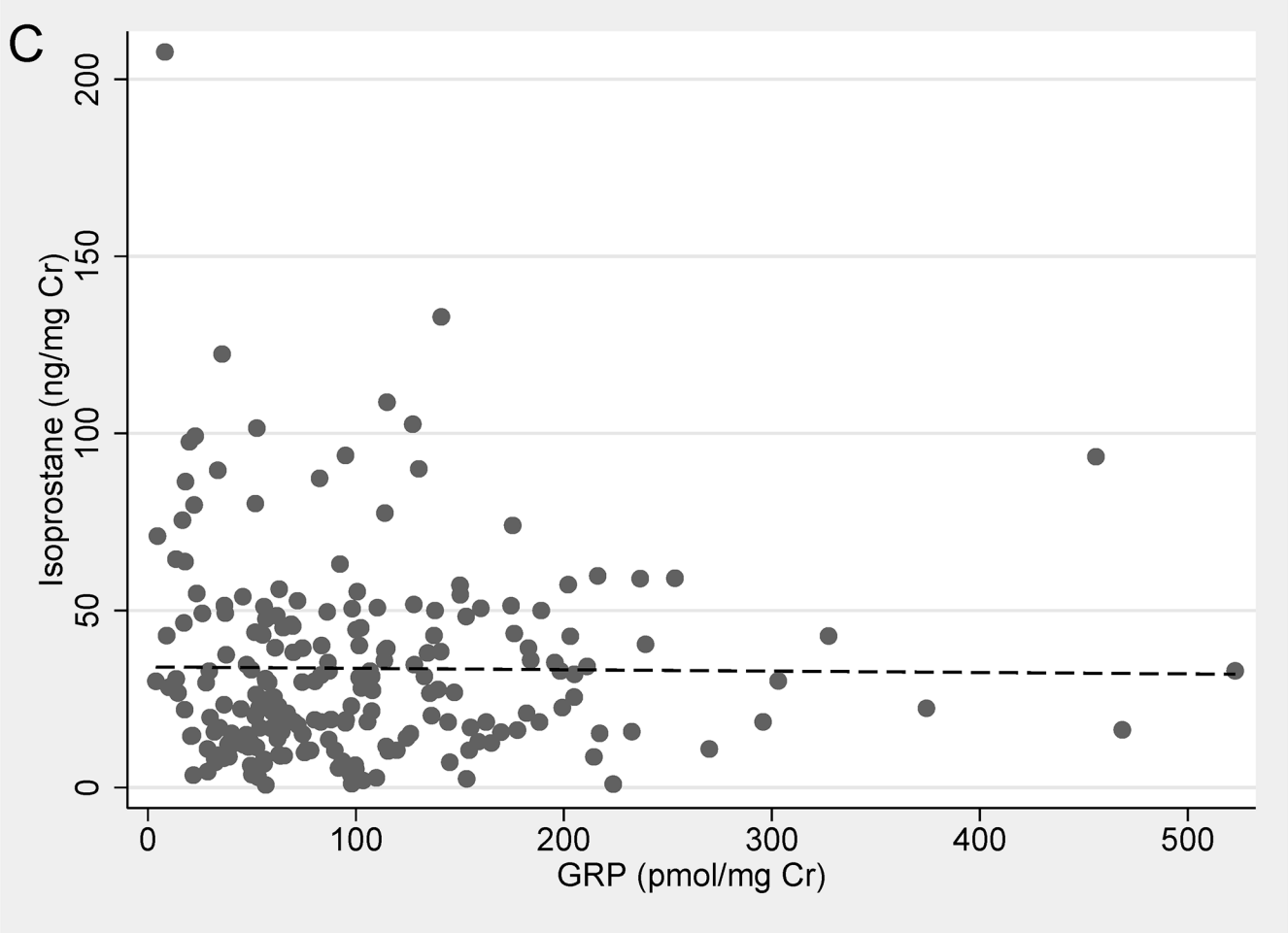


Figure 4.

Relationship of other laboratory values to GRP (pmol/mg Creatinine). Significance testing by linear regression for (A) allantoin (n=214; R^2=0.04; P=0.003), (B) isoprostane (n=208; R^2<0.01; P=0.87), and (C) 8-hydroxydeoxyguanosine (n=222; R^2=0.02; P=0.014).

Table 1.

Participant Characteristics

Characteristic	Infants with urine GRP days 1-7 Median (25^{th}-75^{th} percentiles) or n/N (%)	Infants without urine GRP days 1-7 Median (25^{th}-75^{th} percentiles) or n/N (%)	P-value
Gestational age (wks)	26.6 (25.3-27.7)	26.4 (25.1-27.7)	0.89
Male	151/281 (54%)	23/50 (46%)	0.36
Race			0.19
White	138/281 (49%)	32/50 (64%)	
Black	127/281 (45%)	15/50 (30%)	
Asian	1/281 (0%)	1/50 (2%)	
Multi-racial	7/281 (2%)	1/50 (2%)	
Other	7/281 (2%)	1/50 (2%)	
Hispanic	28/281 (10%)	6/50 (12%)	0.62
Birth weight (g)	878 (689-1080)	839 (650-1020)	0.50
Died	23/281 (8%)	8/50 (16%)	0.11
BPD	109/257 (42%)	20/41 (49%)	0.50
BPD or death at 36 weeks PMA	132/280 (47%)	28/49 (57%)	0.22
PMA at last invasive ventilation (wks)	29.6 (27.9-33.1)	28.6 (27.6-31.9)	0.21
PMA at last non-invasive ventilation (wks) *	36.1 (33.7-39.1)	36.1 (34.0-36.9)	0.42
PMA at last oxygen (wks)	36.0 (32.4-38.8)	35.6 (30.1-36.9)	0.18
Maternal prenatal smoking history †	65/277 (23%)	4/50 (8%)	0.01

*Non-invasive ventilation includes nasal CPAP, nasal noninvasive positive pressure ventilation, or high flow nasal cannulae;

†Prenatal smoking history is missing in 3 infants

Table 2.

Percent of infants with and without GRP>80 pmol/mg Cr and with and without bronchopulmonary dysplasia (BPD)

	GRP >80 pmol/mg Cr			BPD		
	Yes (N=124) (%)	**No (N=133) (%)**	**P-value**	**Yes (N=109) (%)**	**No (N=148) (%)**	**P-value**
GRP >80 pmol/mg Cr	--	--	--	57	42	0.02
BPD	50	35	0.02	--	--	--
Male	52	56	0.53	61	50	0.10
Gestational age <26 weeks	40	35	0.44	56	24	<0.001
White	47	50	0.62	57	43	0.03
Black	48	44	0.46	39	51	0.08
Hispanic	10	10	>0.99	10	9	>0.99
Prenatal smoking history *	35	24	0.10	23	22	0.88
GRP >90 pmol/mg Cr	--	--	--	50	37	0.06
GRP >100 pmol/mg Cr	--	--	--	44	30	0.03
GRP >200 pmol/mg Cr	--	--	--	14	3	0.004

* Prenatal smoking history is missing in 3 infants.

Table 3.

Multivariable logistic regression for BPD, BPD or death, and PRD

	BPD		BPD or death		PRD	
	Adjusted odds ratio (95% confidence interval)	**P-value**	**Adjusted odds ratio (95% confidence interval)**	**P-value**	**Adjusted odds ratio (95% confidence interval)**	**P-value**
GRP >80 pmol/mg Cr	1.83 (1.03-3.25)	0.04	1.99 (1.14-3.48)	0.02	2.46 (1.35-4.48)	0.003
Gestational age	0.49 (0.40-0.61)	<0.001	0.47 (0.38-0.58)	<0.001	0.66 (0.53-0.81)	<0.001
Male	1.82 (1.01-3.27)	0.05	1.83 (1.03-3.23)	0.04	2.83 (1.55-5.16)	0.001
White race	2.24 (1.24-4.03)	0.01	2.22 (1.26-3.91)	0.01	1.06 (0.59-1.91)	0.84
Prenatal smoking	1.05 (0.53-2.10)	0.88	1.26 (0.65-2.44)	0.49	0.88 (0.44-1.79)	0.78

PRD, Post-prematurity respiratory disease score was positive if at least 2 quarterly surveys over the first post-natal year were positive for at least one of three domains: respiratory medications, hospitalizations for respiratory disease, home oxygen or ventilation.