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Response of vascular endothelial growth factor and angiogenesis-related genes to stepwise increases in inspired oxygen in neonatal rat lungs

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BACKGROUND: Bronchopulmonary dysplasia is an inflammatory lung disease that afflicts preterm infants requiring supplemental oxygen and is associated with impaired pulmonary angiogenesis. We tested the hypothesis that there is a critical threshold of inspired O_2 (FiO_2) that alters pulmonary angiogenesis.

METHODS: Within 2–6 h of birth, rat pups were exposed to 10%, 21%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% FiO_2 for 2 h. Mixed arterial–venous blood gases, serum and pulmonary levels of vascular endothelial growth factor (VEGF) and soluble VEGF receptor-1, and pulmonary angiogenesis gene profiles were determined.

RESULTS: Po_2 increased with hyperoxia from 35.6 ± 5.0 (range: 31.5–39.8) at 10% O_2 to 108.5 ± 25.0 (range: 82.2–134.8) at 100% O_2. Po_2 at 21% O_2 was 42.4 ± 7.3 (range: 36.8–48.1). Lung VEGF levels declined at 40%–100%. The critical Po_2 associated with decreased lung VEGF was 66 mm Hg, achieved with a FiO_2 of 0.4. Po_2 was inversely correlated with VEGF levels in the lungs ($R = -0.377$; $P < 0.008$). Antiangiogenesis genes were robustly upregulated at 70%, predominantly in males. Data are reported as mean ± SD.

CONCLUSIONS: A critical threshold of FiO_2 affecting angiogenesis exists in immature lungs. Exposure of preterm lungs to >40% inspired O_2, even for 2 h, may result in abnormal expression of biomarkers regulating lung angiogenesis.

Bronchopulmonary dysplasia (BPD), a chronic lung disease in extremely low gestational–age neonates, is a major cause of long-term hospitalization, slow growth, recurrent respiratory illness, or death (1,2). The cause of BPD is complex and involves oxidant injury, barotrauma/volutrauma from mechanical ventilation, chronic inflammation, and disordered repair in immature lungs (3–5). The pathology of BPD includes inflammation, abnormal alveolarization, fibrosis, and vascular abnormalities (6–11). Pulmonary development occurs by two main processes, alveolarization and microvascular maturation. Microvascular development, which is essential for efficient gas exchange, is regulated by vascular endothelial growth factor (VEGF), which has been shown to be disrupted in BPD (12–14). Exposure to high concentrations of oxygen during lung development results in lung injury characterized by alterations in capillary density, endothelial cell destruction, pulmonary inflammation, and inhibition of the process of alveolarization (15–19).

VEGF is a potent, endothelial cell mitogen that stimulates vessel proliferation, migration, and tube formation leading to angiogenic growth of new blood vessels (15–19). It is essential for angiogenesis during development; the deletion of a single allele arrests angiogenesis and causes embryonic lethality (20–22). VEGF is highly regulated by oxygen. VEGF gene expression increases through several different mechanisms during hypoxia and decreases during hyperoxia. Tambunting *et al.* (23) have shown that VEGF and VEGF receptor mRNA expression are impaired in lungs of extremely preterm baboons developing BPD, contributing to the dysmorphic microvasculature and disrupted alveolarization. Furthermore, Hasan *et al.* (24) showed that VEGF levels are low and soluble VEGF receptor (sVEGFR)-1 levels are high at birth in the tracheal aspirates of preterm newborn infants who developed BPD later, suggesting that sVEGFR-1, the endogenous inhibitor of VEGF, may be a biological marker for BPD.

Infants dying from BPD have decreased VEGF levels in their lungs, particularly in the thickened septae (15). This finding suggests that exposure to oxygen prevents VEGF signaling in the lungs, leading to arrested microvascular maturation and BPD. Studies have examined many combinations of oxygen exposure and the effects of hyperoxia and hypoxia on vasculogenesis. Most commonly, a fraction of inspired O_2 (FiO_2) of >0.95 was used with "recovery" from hyperoxia at 0.21 to 0.5 to mimic the oxygen fluctuations experienced by extremely low gestational–age neonates with acute hyperoxia exposure (25,26). However, there are no studies examining the minimum FiO_2 level that results in the suppression of VEGF. We therefore proposed the hypothesis that there exists a critical threshold of hyperoxia that suppresses regulators of

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angiogenesis in immature lungs. To test this hypothesis, we determined the response of systemic and pulmonary levels of VEGF and sVEGFR-1, as well as pulmonary angiogenesis gene profiles during graded oxygen exposure in newborn rat pups. Because male infants are at higher risk for respiratory complications (27), we examined gender differences in response to graded oxygen.

RESULTS

Pooling of the pups for expanding litter sizes resulted in equal mean body weight and linear growth (crown to rump length) in all groups. There were no differences in mean lung wet weight or lung-to-body weight ratio after oxygen exposure. Table 1 lists the blood gas parameters (mixed venoarterial sample) in response to graded O_2 exposure. As expected, Po_2 levels increased significantly from 40% to 100% as compared with 21%, as did oxygen saturation (SaO_2), which increased earlier from 30% to 100% and was lower at 10%. Pco_2 was lower at 10%, 60%, 80%, 90%, and 100%. pH was lower at 10% and higher at 60%, 80%, and 100%. Base excess was lower at 10% and higher at 100%, and bicarbonate was lower only at 10%. The critical Po_2 associated with decreased lung VEGF was 103 mm Hg achieved with inspired O_2 of 60% (Figure 1a). Po_2 was inversely correlated with lung VEGF levels ($R = -0.377$; $P < 0.008$).

VEGF Levels

VEGF levels (pg/ml) in the serum are presented in Figure 1a (all groups) and Figure 1b (by gender). Serum VEGF was significantly increased at 70% inspired O_2 vs. 60% (264.3 ± 30.8; $P < 0.01$) and vs. 21% (136.8 ± 4.5). A similar increase at 70% was noted in females only (298.4 ± 46.0; $P < 0.01$) as compared with 21% (144.8 ± 3.2). Males had higher VEGF levels at 40% (184.3 ± 44.0; $P < 0.01$) as compared with females (149.9 ± 6.7), but lower levels at 70% (230.2 ± 38.3 vs. 298.4 ± 46.0; $P < 0.05$), 80% (122.3 ± 1.4 vs. 148.4 ± 4.8; $P < 0.01$), and 100% (146.1 ± 7.7 vs. 177.3 ± 5.7; $P < 0.01$). VEGF levels (pg/mg protein) in the lungs are presented in Figure 2a (all groups) and Figure 2b (by gender). As compared with 21% (178.2 ± 55.8), lung VEGF levels were lower at 10% (42.0 ± 4.2; $P < 0.01$), 60% (27.7 ± 6.3; $P < 0.01$), 70% (32.5 ± 7.3; $P < 0.01$), 80% (22.6 ± 4.6; $P < 0.01$), 90% (29.6 ± 5.3; $P < 0.01$), and 100% (26.0 ± 5.1; $P < 0.01$). No gender effect was noted because both genders responded similarly, with increased lung VEGF levels at 30% (male: 282.9 ± 123.2, $P < 0.01$; and female: 288.5 ± 85.6, $P < 0.01$) as compared with 21% (male: 90.6 ± 25.0, and female: 165.8 ± 45.4), followed by decreases at 60%–100%.

sVEGFR-1 Levels

In contrast to VEGF serum levels, sVEGFR-1 levels (pg/ml) in the serum are gender dependent, as shown in Figure 3a (all groups) and Figure 3b (by gender). Serum sVEGFR-1 levels declined at 30% (171.0 ± 61.4; $P < 0.01$) as compared with 21% (427.2 ± 19.7). A similar phenomenon occurred in males at 30% (146.6 ± 30.4; $P < 0.01$) as compared with 21% (443.3 ± 12.2), but not in females. Hypoxia (10% O_2) resulted in higher sVEGFR-1 levels in males (463.8 ± 56.4; $P < 0.05$) than in females (244.1 ± 51.2). Similarly, hyperoxia at 50% resulted in higher sVEGFR-1 levels in males (506.2 ± 97.8; $P < 0.05$) than in females (142.5 ± 79.5). Lung sVEGFR-1 levels (pg/mg protein) are presented in Figure 4a (all groups) and Figure 4b (by gender). Lung sVEGFR-1 levels declined at 70% (3243.8 ± 230.0; $P < 0.01$) as compared with 21% (7571.1 ± 230.6). In males, lung sVEGFR-1 levels were lower at 10% (4840.3 ± 214.9; $P < 0.01$), 30% (5454.5 ± 449.8; $P < 0.05$), 50% (5602.5 ± 385.0; $P < 0.05$), 70% (2885.5 ± 326.7; $P < 0.01$), and 80% (4036.7 ± 214.6; $P < 0.01$) than at 21% (7431.3 ± 291.4). In females, lung sVEGFR-1 levels were lower at 10% (6201.7 ± 127.5; $P < 0.05$), 70% (3602.2 ± 309.5; $P < 0.01$), and 80% (4597.2 ± 321.0; $P < 0.05$) than at 21% (7710.9 ± 245.0). Consistent with serum levels, sVEGFR-1 levels were lower in males at 10% ($P < 0.002$), but higher at 60% (6561.3 ± 355 vs. 4769.4 ± 173.4; $P < 0.003$) than in females.

Angiogenesis-Related Gene mRNA Expression

Tables 2–4 show the fold difference in lung angiogenesis genes from 21%. Table 2 represents the combined male and female data ($n = 4$ samples per group), and Tables 3 and 4 depict male and female responses, respectively. For the combined data, the genes that responded to changes in inspired O_2 from 10% to 100% with modest to robust upregulation were the antiangiogenesis-related *collagen type XVIII α-1* (4- to 59-fold), *endoglin* (4- to 21-fold), *matrix metalloproteinase-9* (2- to 5-fold), *platelet-derived growth factor-α* (6- to 18-fold), *tissue inhibitor of metalloproteinase (TIMP)-3* (7- to 49-fold), and *tumor necrosis factor-α* (2- to 36-fold). Upregulation of most genes peaked at 70%, with the exception of endoglin and TIMP-3. Other genes that responded with upregulation

Table 1. Blood gas parameters

	10%	21%	30%	40%	50%	60%	70%	80%	90%	100%
Po_2	35.6 ± 1.8	42.4 ± 2.4	50.3 ± 2.6	65.8 ± 6.1**	80.0 ± 8.8**	103.3 ± 16.2**	87.3 ± 6.9**	110.7 ± 15.7**	83.2 ± 24.0**	108.5 ± 10.2**
Pco_2	31.8 ± 4.9**	45.5 ± 5.6	42.1 ± 6.3	41.4 ± 4.4	41.3 ± 4.7	34.9 ± 3.0**	41.1 ± 1.2	32.7 ± 3.7**	38.7 ± 4.8**	32.7 ± 7.2**
pH	7.37 ± 0.1*	7.44 ± 0.07	7.48 ± 0.05	7.49 ± 0.05	7.48 ± 0.07	7.52 ± 0.06**	7.45 ± 0.015	7.55 ± 0.05**	7.49 ± 0.07	7.59 ± 0.07**
BE	−5.9 ± 6.6**	5.63 ± 3.33	7.2 ± 1.17	7.4 ± 2.3	6.7 ± 2.9	5.0 ± 2.6	4.7 ± 0.58	7.0 ± 2.1	5.4 ± 2.3	8.5 ± 1.05*
HCO_3	18.8 ± 5.7**	30.5 ± 2.8	31.2 ± 1.9	31.3 ± 2.0	31.1 ± 2.2	28.3 ± 1.7	29.0 ± 0.06	29.1 ± 1.9	29.4 ± 1.5	30.6 ± 1.9
SaO_2	68.9 ± 3.6**	74.7 ± 4.0	86.5 ± 2.5**	93.2 ± 1.6**	95.0 ± 1.4**	98.0 ± 1.0**	89.0 ± 3.6**	98.2 ± 0.75**	93.6 ± 1.8**	98.8 ± 0.3**

Data are expressed as means ± SD (*$P < 0.05$; **$P < 0.01$ vs. 21%).

HCO_3, bicarbonate.

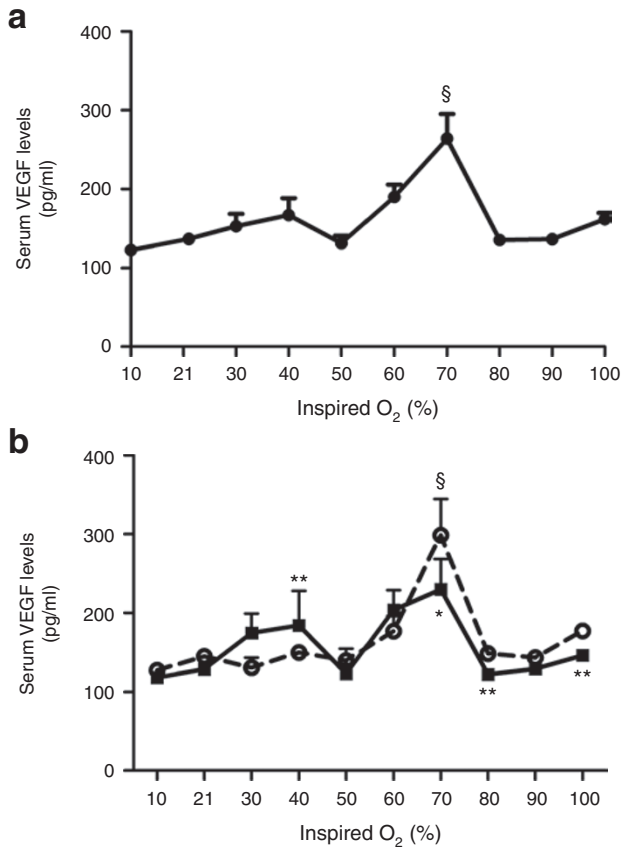

Figure 1. Effects of graded inspired O_2 on systemic VEGF levels (**a**) in all groups ($n = 8$ samples per group) and (**b**) by gender ($n = 4$ samples per group). In **b**, the solid square represents males and the open circle represents females. Data are expressed as mean ± SEM. $^{§}P < 0.01$ vs. 21%; $^{*}P < 0.05$; $^{**}P < 0.01$ vs. females. VEGF, vascular endothelial growth factor.

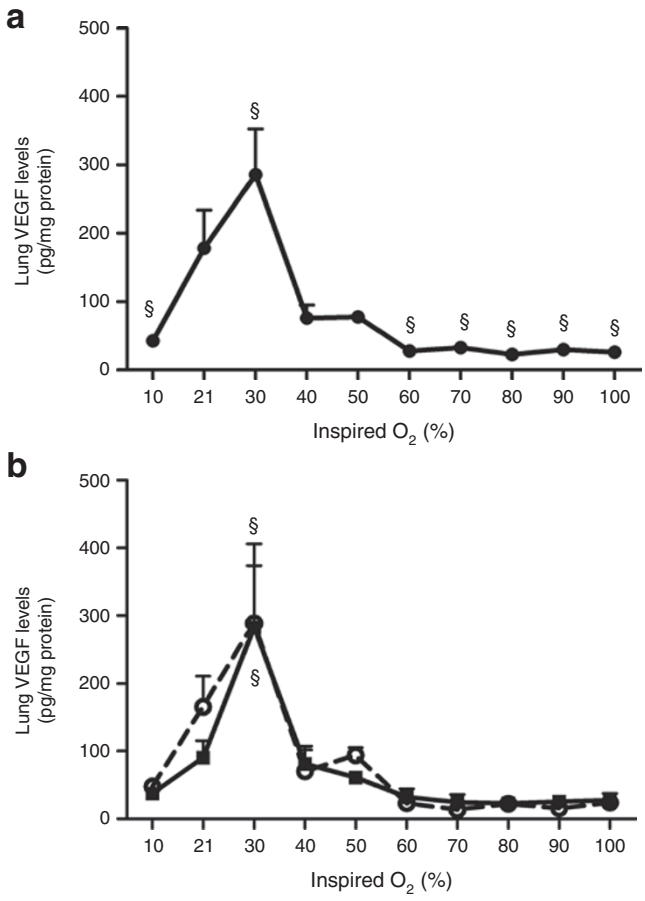

Figure 2. Effects of graded inspired O_2 on pulmonary VEGF levels (**a**) in all groups ($n = 8$ samples per group) and (**b**) by gender ($n = 4$ samples per group). In **b**, the solid square represents males and the open circle represents females. Data are expressed as mean ± SEM. $^{§}P < 0.01$ vs. 21%. VEGF, vascular endothelial growth factor.

at 70% were *angiopoietin-1* (9-fold), *angiopoietin-2* (15-fold), *collagen type IV* (9-fold), *insulin-like growth factor-1* (4-fold), *leptin* (25-fold), and *VEGF-B* (15-fold). Interleukin-1β was upregulated 6-, 9-, and 5-fold at 70%, 80%, and 90%, respectively (Table 2). Males exhibited a similar gene profile from 10% to 100%, but the effect was more robust. The most robust upregulation occurred at 70%, particularly for *collagen type XVIII α-1* (1,068-fold), *leptin* (387-fold), and *tumor necrosis factor-α* (171-fold) (Table 3). By contrast, the angiogenesis gene profile differed in females, with moderate upregulation of most genes at 80%. These genes included *cyclooxygenase-1* (15-fold), *epidermal growth factor* (18-fold), *insulin-like growth factor-1* (15-fold), *interleukin-1β* (47-fold), *interleukin-6* (8-fold), *leptin* (13-fold), *matrix metalloproteinase-2* (18-fold), *platelet-derived growth factor-α* (23-fold), *TIMP-1* (19-fold), *TIMP-2* (20-fold), *TIMP-3* (14-fold), *tumor necrosis factor-α* (13-fold), and *VEGFR-1* (27-fold) (Table 4).

DISCUSSION

The current study was conducted to test the hypothesis that there exists a critical threshold of hyperoxia that alters the factors regulating lung angiogenesis. Our study is the first to demonstrate a response of systemic and pulmonary angiogenesis biomarkers and genes to graded inspired O_2 and Po_2 levels. Despite the short (2 h) exposure, we demonstrated that Po_2 and SaO_2 were directly correlated with hyperoxia and inversely correlated with VEGF levels in the lungs but not in the systemic circulation. Nonlinear regression analysis revealed that the critical Po_2 associated with decreased lung VEGF (57% from normoxia) was 66 mm Hg achieved with a FiO_2 of 0.4. sVEGFR-1, the endogenous negative regulator of VEGF action, did not change as a function of Po_2 or SaO_2. We did not establish a relationship between systemic or pulmonary VEGF and sVEGFR-1. There was a robust elevation of mRNA expression for all angiogenesis genes at 70% O_2, which was predominant in males. Taken together, these findings prove our hypothesis and enable us to conclude that exposure of immature lungs to even short periods of inspired O_2 >40% will alter biomarkers and genes that regulate lung angiogenesis.

Rats born at term are in the late cannalicular to saccular stage of lung development (28). This corresponds to a gestational age of 24–30 wk in human lung development. BPD primarily occurs in this group of neonates (29). Therefore, the newborn rat is a good model to study the effects of graded hyperoxia on lung angiogenesis. The first test of our hypothesis demonstrated that serum levels tended to increase as a function of increasing

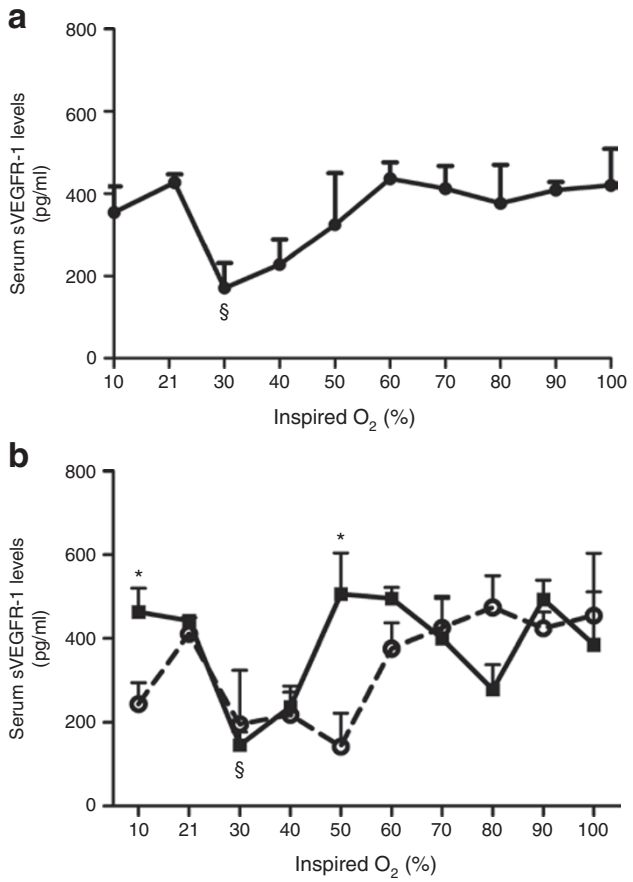

Figure 3. Effects of graded inspired O_2 on systemic sVEGFR-1 levels (**a**) in all groups ($n = 8$ samples per group) and (**b**) by gender ($n = 4$ samples per group). In **b**, the solid square represents males and the open circle represents females. Data are expressed as mean ± SEM. $^{\S}P < 0.01$ vs. 21%; $^{*}P < 0.05$ vs. females. sVEGFR-1, soluble VEGF receptor-1.

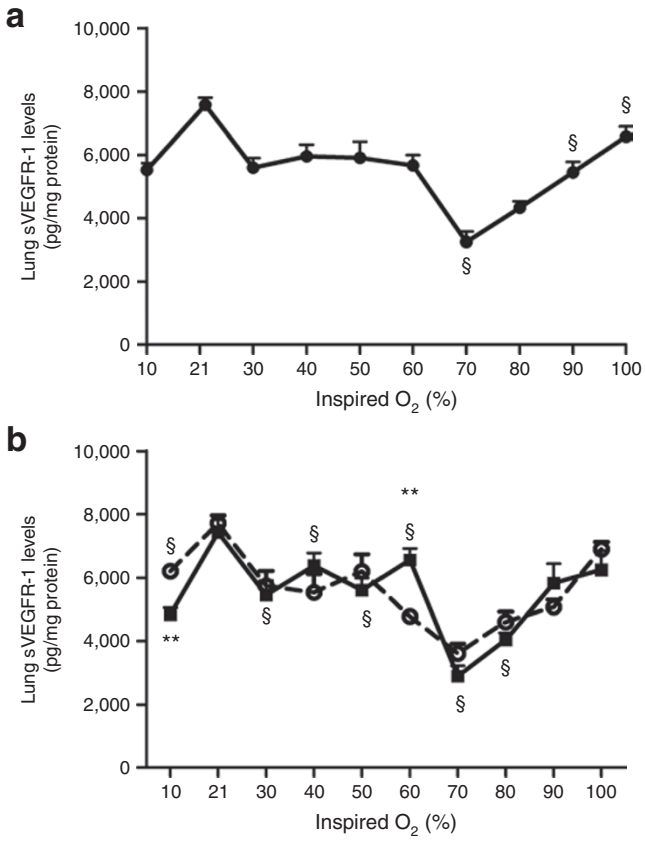

Figure 4. Effects of graded inspired O_2 on pulmonary sVEGFR-1 levels (**a**) in all groups ($n = 8$ samples per group) and (**b**) by gender ($n = 4$ samples per group). In **b**, the solid square represents males and the open circle represents females. Data are expressed as mean ± SEM. $^{\S}P < 0.01$ vs. 21%; $^{**}P < 0.01$ vs. females. sVEGFR-1, soluble VEGF receptor-1.

inspired O_2, with maximum levels at 70%. Based on the knowledge that VEGF is suppressed with hyperoxia, our results were surprising. It is important to note that because of the age and size of the pups, we collected mixed arterial–venous blood samples after decapitation. Therefore, three possible explanations could account for the VEGF response in serum. (i) First, short-term exposure to hyperoxia may cause an initial increase in systemic VEGF as a compensatory mechanism. This result has been demonstrated by Sheikh *et al.* (30) using intermittent hyperoxia for wound healing. A longer exposure may have resulted in lower levels. A duration of 2 h was chosen based on the phenotypic/posttranslational value of VEGF after hypoxia (25). (ii) A second explanation might involve cleavage of the higher VEGF membrane-bound isoforms to the lower soluble isoform, $VEGF_{120}$, because our assay measures both $VEGF_{164}$ and $VEGF_{120}$. Cleavage of VEGF can be mediated by matrix metalloproteinase-9 (31). Indeed, we saw higher expression of matrix metalloproteinase-9, which peaked at 70%, coinciding with the peak of serum VEGF. (iii) A third possible explanation revolves around the developmental stage of the lungs. Studies by Bhandari *et al.* (32) showed that in tracheal aspirates of premature infants with respiratory distress syndrome, VEGF levels increased early during the first 12 h of life and followed a pattern in which there was an initial spike, followed by a decline, and then a subsequent rise, by days 21–28, in those neonates with an adverse outcome. Our serum data seem to have a similar biphasic response, with minor peaks at 30% and 40%, a decline at 50% and 60%, and a second peak at 70%.

The serum VEGF profile across the range of inspired O_2 was consistent between genders. One interesting finding was the response of lung VEGF, which peaked at 30% and then declined at 40% to reach significance at 60%–100% O_2. This finding demonstrates differences between the systemic and pulmonary responses to short-term oxygen exposure, with initial pulmonary compensatory elevations in VEGF occurring at a much lower FiO_2. This result is important because it leads to speculation that biomarkers of angiogenesis in the systemic circulation may not be entirely representative of pulmonary responses. The differences in responses between the lungs and the serum may occur because the lungs are in direct contact with oxygen and, thus, the peaks occur earlier. Nevertheless, O_2 exposure of >30%, even after only 2 h, leads to the suppression of pulmonary VEGF, which may ultimately result in aberrant angiogenesis and implies the need for further consideration of neonatal resuscitation.

VEGFR-1 is the respective tyrosine kinase receptor needed for VEGF to act physiologically (20,23,24,33). sVEGFR-1 is the splice variant of the VEGFR-1, which acts as a VEGF "trap" (21).

Table 2. Up- (+) or downregulation (–) of angiogenic growth factors in the lungs of neonatal rats exposed to incremental inspired O_2 percentages on the first day of life for 2 h

Gene	10%	30%	40%	50%	60%	70%	80%	90%	100%
Ang-1	−1.8 ± 0.05	−1.1 ± 0.46	−1.4 ± 0.08	−1.1 ± 0.36	−1.5 ± 0.05	9.0 ± 0.36	−1.2 ± 0.39	1.3 ± 0.92	−1.8 ± 0.02
Ang-2	−1.5 ± 0.18	1.9 ± 0.02	1.5 ± 0.11	1.33 ± 0.4	−1.1 ± 0.59	15.0 ± 0.36	1.2 ± 0.66	1.3 ± 0.45	1.1 ± 0.92
ColIV	1.3 ± 0.67	1.1 ± 0.68	1.6 ± 0.52	1.4 ± 0.69	−1.9 ± 0.27	9.2 ± 0.35	1.6 ± 0.44	1.6 ± 0.28	1.7 ± 0.48
ColVIII	6.7 ± 0.27	6.2 ± 0.33	7.4 ± 0.19	8.0 ± 0.15	7.0 ± 0.36	59.4 ± 0.36	8.6 ± 0.12	4.4 ± 0.84	4.3 ± 0.78
COX-1	2.1 ± 0.34	−1.8 ± 0.11	−1.4 ± 0.15	−1.5 ± 0.07	−2.3 ± 0.04	3.5 ± 0.36	3.6 ± 0.36	−1.2 ± 0.53	−1.2 ± 0.32
EGF	−2.3 ± 0.05	−1.3 ± 0.57	−1.2 ± 0.61	−1.4 ± 0.25	1.1 ± 0.7	6.4 ± 0.36	3.6 ± 0.36	−1.3 ± 0.1	−1.6 ± 0.1
ENG	14.6 ± 0.22	17.9 ± 0.01	21.3 ± 0.01	16.2 ± 0.01	10.9 ± 0.14	4.4 ± 0.37	7.1 ± 0.22	15.7 ± 0.05	16.3 ± 0.07
HIF1α	1.2 ± 0.46	1.1 ± 0.79	−1.1 ± 0.7	−1.1 ± 0.02	−1.3 ± 0.01	−1.5 ± 0.34	−1.6 ± 0.26	−1.1 ± 0.65	−1.2 ± 0.3
IGF-1	1.5 ± 0.4	1.1 ± 0.8	−1.4 ± 0.25	−1.3 ± 0.19	−1.5 ± 0.26	4.2 ± 0.34	3.3 ± 0.35	1.2 ± 0.52	−1.2 ± 0.49
IL-1β	1.3 ± 0.43	3.5 ± 0.05	2.4 ± 0.22	3.0 ± 0.04	1.1 ± 0.74	5.7 ± 0.33	8.6 ± 0.001	4.7 ± 0.03	3.4 ± 0.03
Il-6	1.2 ± 0.42	1.7 ± 0.26	1.7 ± 0.23	2.0 ± 0.19	2.7 ± 0.02	2.4 ± 0.36	2.7 ± 0.36	2.7 ± 0.2	3.1 ± 0.07
Jag1	1.6 ± 0.38	1.4 ± 0.05	1.5 ± 0.08	1.5 ± 0.06	−1.5 ± 0.12	2.9 ± 0.31	−1.1 ± 63	1.5 ± 0.07	1.2 ± 0.32
Lep	−1.2 ± 0.61	1.5 ± 0.17	1.2 ± 0.36	1.1 ± 0.7	1.5 ± 0.1	24.7 ± 0.36	3.9 ± 0.35	1.7 ± 0.08	1.3 ± 0.34
MMP-2	1.8 ± 0.35	1.1 ± 0.62	−1.0 ± 0.92	−1.3 ± 0.06	−1.6 ± 0.03	2.1 ± 0.35	4.0 ± 0.35	−1.0 ± 0.9	−1.2 ± 0.21
MMP-3	−1.4 ± 0.76	1.3 ± 0.32	−1.2 ± 0.86	−1.0 ± 0.99	1.3 ± 0.31	2.8 ± 0.35	2.8 ± 0.35	1.0 ± 0.8	1.1 ± 0.7
MMP-9	5.2 ± 0.35	3.2 ± 0.36	2.8 ± 0.6	2.5 ± 0.78	1.5 ± 0.52	4.8 ± 0.36	4.4 ± 0.33	3.1 ± 0.41	3.0 ± 0.51
Nrp-1	−1.2 ± 0.96	1.3 ± 0.7	1.3 ± 0.75	1.4 ± 0.57	−1.9 ± 0.23	−1.4 ± 0.68	−1.7 ± 0.4	1.5 ± 0.47	1.1 ± 0.94
Nrp-2	1.8 ± 0.38	−1.1 ± 0.57	1.1 ± 0.99	1.3 ± 0.66	−1.8 ± 0.13	−1.2 ± 0.9	−1.2 ± 0.6	1.3 ± 0.55	1.0 ± 0.99
PECAM-1	1.6 ± 0.27	−1.0 ± 0.83	1.1 ± 0.52	1.4 ± 0.07	1.1 ± 0.5	2.5 ± 0.35	1.1 ± 0.51	−1.0 ± 0.84	−1.1 ± 0.96
PDGF-α	6.3 ± 0.3	6.8 ± 0.07	8.3 ± 0.04	12.4 ± 0.03	5.7 ± 0.02	10.6 ± 0.02	18.3 ± 0.32	8.3 ± 0.02	8.0 ± 0.18
PDGF-β	1.2 ± 0.48	2.3 ± 0.11	2.3 ± 0.06	2.4 ± 0.004	2.3 ± 0.02	7.7 ± 0.35	1.7 ± 0.06	1.6 ± 0.01	1.4 ± 0.36
TIMP-1	2.3 ± 0.33	1.3 ± 0.42	1.2 ± 0.52	1.1 ± 0.82	−2.2 ± 0.02	−1.6 ± 0.14	4.3 ± 0.36	1.4 ± 0.09	1.4 ± 0.09
TIMP-2	2.8 ± 0.33	1.1 ± 0.58	1.3 ± 0.27	2.0 ± 0.005	1.6 ± 0.03	3.2 ± 0.28	4.7 ± 0.35	1.2 ± 0.28	1.0 ± 0.79
TIMP-3	28.0 ± 0.32	9.5 ± 0.5	10.8 ± 0.31	14.4 ± 0.05	14.5 ± 0.06	7.0 ± 0.96	49.1 ± 0.35	8.7 ± 0.63	11.9 ± 0.22
TNF-α	1.2 ± 0.52	6.3 ± 0.02	4.5 ± 0.09	4.0 ± 0.07	2.3 ± 0.45	35.9 ± 0.35	4.4 ± 0.34	5.5 ± 0.04	−1.5 ± 0.32
VEGF-A	1.8 ± 0.37	1.4 ± 0.11	1.3 ± 0.2	1.2 ± 0.26	1.3 ± 0.22	2.1 ± 0.05	−2.4 ± 0.71	−1.1 ± 0.9	−1.5 ± 0.42
VEGF-B	2.5 ± 0.25	1.5 ± 0.46	2.6 ± 0.14	1.9 ± 0.13	−1.0 ± 0.75	14.8 ± 0.3	2.9 ± 0.19	1.9 ± 0.2	1.4 ± 0.56
VEGF-C	2.4 ± 0.36	3.0 ± 0.08	3.2 ± 0.05	2.3 ± 0.19	1.8 ± 0.58	2.1 ± 0.31	2.0 ± 0.41	2.5 ± 0.2	2.4 ± 0.19
VEGFR-1	2.1 ± 0.34	1.4 ± 0.3	1.5 ± 0.16	1.6 ± 0.06	1.7 ± 0.02	1.4 ± 0.25	1.4 ± 0.35	5.8 ± 0.74	1.1 ± 0.67
VEGFR-2	1.1 ± 0.62	1.9 ± 0.22	1.4 ± 0.44	1.1 ± 0.83	−2.1 ± 0.06	−2.2 ± 0.9	−2.1 ± 0.39	1.7 ± 0.14	1.4 ± 0.34

The data are presented as fold expression difference as compared with pups exposed to 21% oxygen. All data were corrected using five different housekeeping genes. Genes are selected from a profile of 84 genes. In alphabetical order, the genes of interest are: Ang (angiopoietin) -1 and -2; Col (collagen) type IV and -type XVIII; COX (cyclooxygenase) -1; EGF (epidermal growth factor); ENG (endoglin); HIF (hypoxia inducible factor) -1α; IGF-1 (insulin-like growth factor) -1; IL (interleukin) -1β and -6; Lep (leptin); MMP (matrix metalloproteinase) -2, -3, and -9; Nrp (neuropilin) -1 and -2; PDGF (platelet-derived growth factor) -α and -β; PECAM (platelet/endothelial cell adhesion molecule) -1; TIMP (tissue inhibitor of metalloproteinases) -1, -2, and -3; TNF (tumor necrosis factor) -α; VEGF (vascular endothelial growth factor) -A, -B, and -C; VEGFR (vascular endothelial growth factor receptor) -1 and -2.

The next test of our hypothesis involved the response of serum and pulmonary sVEGFR-1 to graded inspired O_2. Of note, the systemic levels declined with 30%, whereas the pulmonary levels declined at 70%, although the response was not sustained. This result was in contrast to systemic and pulmonary VEGF, which increased at 70% and 30%, respectively. This finding strengthens the concept that sVEGFR-1 is antagonistic to VEGF. It is possible that a longer exposure to increased oxygen concentrations would have resulted in lower levels, as demonstrated by Feng *et al.* (34). Resumption of baseline levels would further inhibit the function of VEGF. The final test of our hypothesis was the response of pulmonary angiogenesis genes to inspired O_2. The data revealed that a majority of genes were upregulated at 70%, but the most predominant effect was noted in males. At 70%, the antiangiogenic *collagen type XVIII* was robustly upregulated, whereas *endoglin*, an endothelial cell specification gene, was downregulated. In males, the negative regulators of angiogenesis were mostly upregulated. These included *angiopoietin 1 and 2*, which control blood vessel development and stability (35), *collagen type XVIII*, and *TIMP-3*, thus providing evidence for one of the mechanisms explaining why male infants are at higher risk for respiratory complications. In females, the response of angiogenesis genes occurred at a higher FiO_2 of 0.8 and was significantly less

Table 3. Up- (+) or downregulation (–) of angiogenic growth factors in the lungs of male neonatal rats exposed to incremental inspired O_2 percentages on the first day of life for 2 h

Gene	10%	30%	40%	50%	60%	70%	80%	90%	100%
Angpt1	−2.8	−1.5	−1.7	−1.4	−1.8	100.5	−1.2	−2.0	−2.2
Angpt2	−1.3	2.2	1.9	1.9	−1.0	193.4	1.3	1.4	1.3
ColIV	3.0	1.5	1.6	1.4	−1.8	49.3	3.1	2.4	2.0
ColVIII	24.1	9.3	17.2	21.0	16.0	1067.5	22.0	11.7	8.3
COX-1	5.6	−1.1	−1.1	−1.2	−1.9	26.2	−1.1	−1.2	1.2
EGF	−2.7	−1.9	−1.9	−1.6	−1.6	39.7	−1.4	−1.8	−2.0
ENG	147.1	152.4	149.5	116.9	59.8	10.7	123.4	144.5	85.7
HIF1-α	2.5	1.5	1.2	−1.0	−1.0	2.3	1.3	1.2	1.0
IGF-1	2.9	1.1	−1.0	−1.0	1.1	11.4	−1.3	−1.2	−1.1
IL-1β	2.7	3.3	2.2	1.9	−1.6	11.0	1.6	3.8	1.9
IL-6	1.4	1.9	2.2	1.7	2.5	3.2	−1.1	3.9	3.2
Jag1	3.0	1.3	1.3	1.4	−1.3	5.7	1.6	1.4	1.2
Lep	1.3	2.2	1.7	−1.3	1.2	387.4	1.2	1.7	1.2
MMP-2	3.3	1.5	1.2	−1.2	−1.8	4.1	−1.1	−1.3	−1.1
MMP-3	1.3	2.3	1.3	−1.1	1.2	12.0	1.1	1.0	1.3
MMP-9	31.3	11.1	8.5	5.0	3.6	30.9	6.1	8.3	5.9
Nrp-1	1.9	1.3	1.8	1.8	1.1	−2.9	1.4	1.4	1.1
Nrp-2	3.0	−1.2	1.1	1.1	−1.6	−2.1	1.1	1.0	−1.2
PECAM-1	1.8	−1.2	−1.3	1.3	−1.1	5.5	1.4	−1.2	−1.5
PDGF-α	21.6	5.7	7.7	19.8	10.6	11.8	14.7	10.7	7.5
PDGF-β	1.2	1.2	1.5	2.3	3.1	25.3	2.2	1.6	−1.2
TIMP-1	5.6	1.8	1.8	1.1	−1.9	−2.1	−1.0	1.5	1.9
TIMP-2	4.7	−1.4	−1.2	1.9	1.3	4.8	1.1	1.0	−1.4
TIMP-3	619.6	104.1	87.0	151.5	154.5	59.1	168.5	130.0	99.5
TNF-α	1.9	6.4	4.9	3.1	1.5	171.2	1.5	2.8	−1.3
VEGF-A	3.3	1.2	1.3	1.3	1.5	2.4	1.4	−1.1	−2.0
VEGF-B	7.7	3.9	3.6	2.7	1.7	37.2	3.7	2.3	1.5
VEGF-C	8.4	5.6	6.9	4.5	3.5	3.5	3.5	3.5	5.4
VEGFR-1	2.8	−1.4	−1.1	−1.5	−1.4	−1.4	−1.3	−1.2	−1.8
VEGFR-2	2.3	2.9	2.7	1.5	−1.2	−4.6	−1.2	−1.6	−2.0

The data are presented as fold expression difference as compared with pups exposed to 21% oxygen. All data were corrected using five different housekeeping genes. Genes are as described in **Table 2**.

robust, suggesting that females are more resistant to the effects of hyperoxia. Gender dimorphism may suggest a key role for gender hormones. Estrogen has been shown to be a potent stimulator of VEGF and angiogenesis, rendering a protective effect in females (36).

Despite the important clinical implications of this study, there were some limitations, including the use of mixed arterial–venous blood to determine blood gases. Arterial blood sampling in the neonatal rat is difficult; therefore, we opted to collect mixed venous–arterial blood immediately after killing the pups by decapitation. Better results would have been obtained with pure arterial sampling. Nevertheless, our study showed that short-term exposure to graded inspired O_2 may result in compensatory increases in angiogenic and antiangiogenic factors, which are mostly associated with the suppression of vessel sprouting. Exposure of the immature lungs to even short periods of inspired O_2 >40% will have a negative impact on lung angiogenesis and may increase the risk for the development of BPD.

METHODS

This study was approved by the State University of New York, Downstate Medical Center Animal Care and Use Committee (Brooklyn, NY). Animals were cared for and handled according to the guidelines of the U.S. Department of Agriculture. The animals were killed according to the guidelines of the American Veterinary Medical Association Panel for Euthanasia.

Experimental Design

Certified infection-free timed-pregnant Sprague Dawley rats (200–300 g) carrying fetuses (9–15) of known gestational age (18 d) were purchased from Charles River Laboratories (Wilmington, MA).

Table 4. Up- (+) or downregulation (–) of angiogenic growth factors in the lungs of female neonatal rats exposed to incremental inspired O_2 percentages on the first day of life for 2 h

Gene	10%	30%	40%	50%	60%	70%	80%	90%	100%
Angpt1	−1.2	1.2	−1.2	−1.1	−1.2	−1.2	−1.2	−1.4	−1.4
Angpt2	−1.7	1.7	1.2	−1.1	−1.2	1.2	1.1	1.1	−1.1
ColIV	−1.6	−1.3	1.7	1.4	−1.9	1.7	1.2	1.7	1.4
ColVIII	1.9	4.2	3.2	3.0	3.1	3.3	3.4	1.7	2.2
COX-1	−1.3	−3.0	−2.0	−1.8	−2.8	−2.1	14.7	−1.2	−1.7
EGF	−2.0	1.2	1.3	−1.2	1.9	1.0	17.7	1.1	−1.2
ENG	1.4	2.1	3.0	2.3	2.0	1.8	−2.4	1.7	3.1
HIF1-α	−1.7	−1.3	−1.5	−1.2	−1.7	1.0	−3.6	1.0	−1.4
IGF-1	−1.3	1.0	−1.8	−1.5	−2.3	1.5	14.3	1.6	−1.2
IL-1β	−1.6	3.7	2.6	4.8	2.0	3.0	47.3	5.7	6.3
IL-6	1.0	2.4	1.3	2.3	3.0	1.8	7.7	1.9	3.0
Jag1	−1.2	1.5	1.7	1.5	−1.8	1.5	−1.9	1.5	1.3
Lep	−1.7	1.1	−1.1	1.5	1.9	1.6	13.2	1.8	1.3
MMP-2	1.0	−1.4	−1.2	−1.4	−1.4	1.0	17.6	1.2	−1.4
MMP-3	−2.4	−1.4	−2.0	1.0	1.3	−1.5	7.1	1.0	−1.1
MMP-9	−1.1	−1.1	−1.1	1.2	−1.6	−1.4	3.2	1.2	1.5
Nrp-1	−2.6	1.3	−1.0	1.1	−4.0	1.5	−4.2	1.5	1.0
Nrp-2	1.0	1.0	1.2	1.4	−2.1	1.5	−1.5	1.7	1.3
PECAM-1	1.4	1.1	1.6	1.5	1.3	1.1	−1.1	1.1	1.3
PDGF-α	1.9	8.0	8.9	7.7	3.2	9.5	22.6	6.5	8.5
PDGF-β	1.1	4.3	3.3	2.5	1.7	2.3	1.3	1.5	2.5
TIMP-1	−1.0	−1.1	−1.3	−1.0	−2.7	−1.3	18.9	1.4	1.1
TIMP-2	1.6	1.6	2.0	2.1	1.9	2.1	20.3	1.5	1.5
TIMP-3	1.3	−1.2	1.3	1.4	1.4	−1.2	14.3	−1.7	1.4
TNF-α	−1.3	6.2	4.2	5.3	3.5	7.5	12.9	10.9	4.3
VEGF-A	−1.1	1.7	1.3	1.2	1.1	1.8	−8.0	−1.0	−1.1
VEGF-B	−1.3	−1.8	1.9	1.3	−1.8	5.9	2.3	1.5	1.3
VEGF-C	−1.5	1.6	1.5	1.2	−1.0	1.3	1.2	1.7	1.1
VEGFR-1	1.5	2.5	2.5	1.7	2.0	2.6	26.7	1.4	2.1
VEGFR-2	−1.9	1.2	−1.4	−1.2	−3.7	−1.0	−5.3	−1.7	−1.1

The data are presented as fold expression difference as compared with pups exposed to 21% oxygen. All data were corrected using five different housekeeping genes. Genes are as described in **Table 2**.

The pregnant rats were placed in cages compliant with the U.S. Department of Agriculture guidelines and allowed to stabilize for 48 h; they then remained undisturbed until the birth of their pups. The animals were housed in a 12-h day/12-h night cycle and were provided standard laboratory diet and water *ad libitum*. Within 2–6 h of birth, newborn pups delivered on the same day were pooled and randomly assigned to an expanded litter of 16 pups per litter to simulate the nutritional status of extremely low gestational–age neonates. Each pup was weighed and measured for linear growth (crown to rump length) and randomly assigned to 10%, 21%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% oxygen exposure for 2 h (n = 16 pups per group). For hypoxia and hyperoxia exposure, the newborn rat pups and dams were placed in a specialized oxygen chamber, and the oxygen environment was maintained using an oxycycler (BioSpherix, Lacona, NY). At the end of the hypoxic, normoxic, or hyperoxic episode, the rat pups were immediately killed by decapitation. Blood samples (from 6 males and 6 females, respectively) were analyzed for blood gases and SaO_2 using an i-Stat portable blood gas instrument (Abbot Laboratories, Abbot Park, IL). Blood samples (pooled for a total of 4 males and 4 females) and lung samples (from 6 males and 6 females, respectively) were analyzed for VEGF and sVEGFR-1 levels using enzyme immunoassay. Biopsy of the lung samples was carried out from the left lobe, rinsed in ice-cold phosphate-buffered saline, and snap-frozen in liquid nitrogen for mRNA expression of genes regulating angiogenesis using quantitative real-time PCR arrays (2 males and 2 females).

Assay of VEGF and sVEGFR-1

To determine the levels of VEGF and sVEGFR-1 in serum, blood samples were collected in sterile polypropylene tubes and allowed to clot on ice for 30 min before centrifugation at 3,000 rpm at 4 °C. The resulting serum was transferred to a clean, sterile polypropylene tube and frozen at −20 °C until assay. Immediately after the animals were killed, the whole lungs were removed and the wet weight was determined. Tissue samples were excised from the left lower lobe, rinsed several times in ice-cold phosphate-buffered saline until all evidence of blood elements was removed, snap-frozen in liquid nitrogen, and stored at −80 °C before assay. For VEGF and sVEGFR-1 levels, lung samples were placed in 2 ml of sterile

normal saline and homogenized in microtubes containing ceramic beads using the FastPrep-24 instrument (MP Biomedicals, Solon, OH). The samples were centrifuged at 8,000 rpm for 20 min at 4 °C and filtered before assay. A portion of the filtrate (10 µl) was used for total cellular protein levels to standardize the levels of VEGF and sVEGFR-1 in lung homogenates. VEGF and sVEGFR-1 levels in serum and lung homogenates were determined using commercially available rat sandwich immunoassay kits (R&D Systems, Minneapolis, MN) according to the manufacturer's protocol. VEGF and sVEGFR-1 concentrations in the sample were determined from a linear standard curve ranging from 0 to 2,000 pg/ml. The coefficient of variation from inter- and intra-assay precision assessment was <10% for all assays.

Total Cellular Protein Assay

On the day of the assay, lung homogenates were assayed for total protein levels using the dye-binding Bio-Rad protein assay (Bio-Rad, Hercules, CA) with bovine serum albumin as a standard. The standard curve was linear from 0.05 to 1.45 mg/ml of protein.

Real-Time PCR

Total RNA was extracted from frozen lung samples using RNA Pro solution (MP Bio, Solon, OH) as previously described (37). Cleanup of the RNA was performed using RNEasy mini cleanup kits (Qiagen, Valencia, CA) followed by on-column treatment with DNase I (Qiagen). Reverse transcription was performed using a RT^2 First Strand kit purchased from SABiosciences (Frederick, MD). The real-time PCR arrays were carried out with the resulting cDNA using the Rat Angiogenesis PCR array system (n = 4 per group) on 96-well plates precoated with 84 genes regulating angiogenesis using a Bio-Rad IQ5 real-time instrument (Bio-Rad). The PCR cycle and analyses were carried out as previously described (28).

Statistical Analyses

Two-way ANOVA was used to determine the differences among the oxygen groups for normally distributed data, and the Kruskal–Wallis test was used for non–normally distributed data following Bartlett's test for equality of variances. *Post hoc* analysis was performed using Dunnett's test for significance. An unpaired t test was used for comparisons between gender for normal distribution, and an unpaired t test with Welch correction was used for nonnormal data. Nonlinear regression analysis was used to determine the relationships between Po_2 and VEGF or sVEGFR-1. Significance was set at $P < 0.05$, and data are reported as means ± SD. All analyses were two tailed and performed using SPSS (SPSS Chicago, IL).

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REFERENCES

1. Doyle LW, Ford G, Davis N. Health and hospitalistions after discharge in extremely low birth weight infants. Semin Neonatol 2003;8:137–45.
2. Bhandari A, Bhandari V. Pathogenesis, pathology and pathophysiology of pulmonary sequelae of bronchopulmonary dysplasia in premature infants. Front Biosci 2003;8:e370–80.
3. Bhandari A, Bhandari V. Bronchopulmonary dysplasia: an update. Indian J Pediatr 2007;74:73–7.
4. Coalson JJ. Pathology of bronchopulmonary dysplasia. Semin Perinatol 2006;30:179–84.
5. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med 2001;163:1723–9.
6. Erickson AM, de la Monte SM, Moore GW, Hutchins GM. The progression of morphologic changes in bronchopulmonary dysplasia. Am J Pathol 1987;127:474–84.
7. Bonikos DS, Bensch KG, NORTHWAY WH Jr, Edwards DK. Bronchopulmonary dysplasia: the pulmonary pathologic sequel of necrotizing bronchiolitis and pulmonary fibrosis. Hum Pathol 1976;7:643–66.
8. Husain AN, Siddiqui NH, Stocker JT. Pathology of arrested acinar development in postsurfactant bronchopulmonary dysplasia. Hum Pathol 1998;29:710–7.
9. Jobe AJ. The new BPD: an arrest of lung development. Pediatr Res 1999;46:641–3.
10. Coalson JJ. *Pathology of chronic lung disease in early infancy*. In: Bland RD, Coalson JJ, eds. Chronic Lung Disease in Early Infancy. New York, USA: Marcel Dekker, 2000:85–124.
11. Coalson JJ, Winter VT, Siler-Khodr T, Yoder BA. Neonatal chronic lung disease in extremely immature baboons. Am J Respir Crit Care Med 1999;160:1333–46.
12. D'Angio CT, Maniscalco WM. The role of vascular growth factors in hyperoxia-induced injury to the developing lung. Front Biosci 2002;7:d1609–23.
13. Bhatt AJ, Pryhuber GS, Huyck H, Watkins RH, Metlay LA, Maniscalco WM. Disrupted pulmonary vasculature and decreased vascular endothelial growth factor, Flt-1, and TIE-2 in human infants dying with bronchopulmonary dysplasia. Am J Respir Crit Care Med 2001;164(10 Pt 1):1971–80.
14. Maniscalco WM, Watkins RH, Pryhuber GS, Bhatt A, Shea C, Huyck H. Angiogenic factors and alveolar vasculature: development and alterations by injury in very premature baboons. Am J Physiol Lung Cell Mol Physiol 2002;282:L811–23.
15. Jakkula M, Le Cras TD, Gebb S, et al. Inhibition of angiogenesis decreases alveolarization in the developing rat lung. Am J Physiol Lung Cell Mol Physiol 2000;279:L600–7.
16. Le Cras TD, Markham NE, Tuder RM, Voelkel NF, Abman SH. Treatment of newborn rats with a VEGF receptor inhibitor causes pulmonary hypertension and abnormal lung structure. Am J Physiol Lung Cell Mol Physiol 2002;283:L555–62.
17. Gerber HP, Hillan KJ, Ryan AM, et al. VEGF is required for growth and survival in neonatal mice. Development 1999;126:1149–59.
18. Galambos C, Ng YS, Ali A, et al. Defective pulmonary development in the absence of heparin-binding vascular endothelial growth factor isoforms. Am J Respir Cell Mol Biol 2002;27:194–203.
19. Kumar VH, Ryan RM. Growth factors in the fetal and neonatal lung. Front Biosci 2004;9:464–80.
20. Shibuya M. Differential roles of vascular endothelial growth factor receptor-1 and receptor-2 in angiogenesis. J Biochem Mol Biol 2006;39:469–78.
21. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc Natl Acad Sci USA 1993;90:10705–9.
22. Shibuya M, Yamaguchi S, Yamane A, et al. Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (flt) closely related to the fms family. Oncogene 1990;5:519–24.
23. Tambunting F, Beharry KDA, Waltzman J, Modanlou HD. Lung vascular endothelial growth factor signaling is impaired in extremely premature baboons developing bronchopulmonary dysplasia/chronic lung disease. J Investig Med 2005;53:1–11.
24. Hasan J, Beharry KD, Valencia AM, Strauss A, Modanlou HD. Soluble vascular endothelial growth factor receptor 1 in tracheal aspirate fluid of preterm neonates at birth may be predictive of bronchopulmonary dysplasia/chronic lung disease. Pediatrics 2009;123:1541–7.
25. Remesal A, Pedraz C, San Feliciano L, Ludeña D. Pulmonary expression of vascular endothelial growth factor (VEGF) and alveolar septation in a newborn rat model exposed to acute hypoxia and recovered under conditions of air or hyperoxia. Histol Histopathol 2009;24:325–30.
26. Watkins RH, D'Angio CT, Ryan RM, Patel A, Maniscalco WM. Differential expression of VEGF mRNA splice variants in newborn and adult hyperoxic lung injury. Am J Physiol 1999;276(5 Pt 1):L858–67.
27. Binet ME, Bujold E, Lefebvre F, Tremblay Y, Piedboeuf B; Canadian Neonatal Network™. Role of gender in morbidity and mortality of extremely premature neonates. Am J Perinatol 2012;29:159–66.
28. Pringle KC. Human fetal lung development and related animal models. Clin Obstet Gynecol 1986;29:502–13.

29. Langston C, Kida K, Reed M, Thurlbeck WM. Human lung growth in late gestation and in the neonate. Am Rev Respir Dis 1984;129:607–13.
30. Sheikh AY, Gibson JJ, Rollins MD, Hopf HW, Hussain Z, Hunt TK. Effect of hyperoxia on vascular endothelial growth factor levels in a wound model. Arch Surg 2000;135:1293–7.
31. Hawinkels LJ, Zuidwijk K, Verspaget HW, et al. VEGF release by MMP-9 mediated heparan sulphate cleavage induces colorectal cancer angiogenesis. Eur J Cancer 2008;44:1904–13.
32. Bhandari V, Choo-Wing R, Lee CG, et al. Developmental regulation of NO-mediated VEGF-induced effects in the lung. Am J Respir Cell Mol Biol 2008;39:420–30.
33. Tuder RM, Flook BE, Voelkel NF. Increased gene expression for VEGF and the VEGF receptors KDR/Flk and Flt in lungs exposed to acute or to chronic hypoxia. Modulation of gene expression by nitric oxide. J Clin Invest 1995;95:1798–807.
34. Feng HY, Lu AZ, Zhang XB, Wang LB, Chen C. [Effects of prolonged exposure of high concentration of oxygen on expression of vascular endothelial growth factor and its receptors in neonatal rat lungs]. Zhongguo Dang Dai Er Ke Za Zhi 2009;11:927–30.
35. Singh H, Tahir TA, Alawo DO, Issa E, Brindle NP. Molecular control of angiopoietin signalling. Biochem Soc Trans 2011;39:1592–6.
36. Losordo DW, Isner JM. Estrogen and angiogenesis: A review. Arterioscler Thromb Vasc Biol 2001;21:6–12.
37. Brock RS, Gebrekristos BH, Kuniyoshi KM, Modanlou HD, Falcao MC, Beharry KD. Biomolecular effects of JB1 (an IGF-I peptide analog) in a rat model of oxygen-induced retinopathy. Pediatr Res 2011;69:135–41.