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The relationship between plasma lipid peroxidation products and primary graft dysfunction after lung transplantation is modified by donor smoking and reperfusion hyperoxia

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Abstract

Purpose—Donor smoking history and higher FiO₂ at reperfusion are associated with primary graft dysfunction (PGD) after lung transplantation. We hypothesized that oxidative injury biomarkers would be elevated in PGD, with higher levels associated with donor exposure to cigarette smoke and recipient hyperoxia at reperfusion.

Methods—We performed a nested case control study of 72 lung transplant recipients from the Lung Transplant Outcomes Group cohort. F₂-isoprostanes and isofurans were measured by mass spectroscopy in plasma collected after transplantation. Cases were defined in two ways: grade 3 PGD present at day 2 or day 3 after reperfusion (severe PGD), or any grade 3 PGD (any PGD).

Results—There were 31 severe PGD cases with 41 controls and 35 any PGD cases with 37 controls. Plasma F₂-isoprostane levels were higher in severe PGD compared to controls (28.6 pg/ml vs. 19.8 pg/ml, p=0.03). Plasma F₂-isoprostane levels were higher in severe PGD compared to controls (29.6 pg/ml vs. 19.0 pg/ml, p=0.03) among patients reperfused with FiO₂>40%. Among recipients of lungs from donors with smoke exposure, plasma F₂-isoprostane (38.2 pg/ml vs. 22.5 pg/ml, p=0.046) and isofuran (66.9 pg/ml vs. 34.6 pg/ml, p=0.046) levels were higher in severe PGD compared with controls.

Conclusions—Plasma levels of lipid peroxidation products are higher in patients with severe PGD, in recipients of lungs from donors with smoke exposure, and recipients exposed to higher FiO₂ at reperfusion. Oxidative injury is an important mechanism of PGD and may be magnified by donor exposure to cigarette smoke and hyperoxia at reperfusion.

Introduction

Primary graft dysfunction (PGD) is a form of acute lung injury predominantly resulting from severe ischemia reperfusion injury (IRI) in the allograft in the setting of lung transplantation¹⁻³. We have previously reported that receipt of a lung from a donor with smoking exposure history as well as higher fraction of inspired oxygen (FiO₂) at the time of organ reperfusion are independent risk factors for the development of PGD⁴. The mechanisms leading to these associations are unclear.

Exposure to both cigarette smoke and hyperoxia can induce oxidative injury in the lung^{5,6}. Although the role of reactive oxygen species (ROS) in signaling and lung homeostasis is complex, during lung transplantation there may be an excess of ROS production, leading to lipid peroxidation⁷. F₂-isoprostanes are a group of prostaglandin-like compounds formed via nonenzymatic free radical-induced peroxidation of arachidonic acid that serve as robust markers of oxidative lipid peroxidation⁸. Some of these compounds have other physiologic effects in the lung including vasoconstriction of the pulmonary vasculature⁹. F₂-isoprostane

levels are higher in smokers compared to non-smokers and decrease in response to smoking cessation and antioxidant therapy^{10,11}. In animal models of ischemia and reperfusion of the heart and liver, F₂-isoprostanes are released in response to reperfusion¹²⁻¹⁴. In rabbit lungs, hyperoxia and anoxia induced release of F₂-isoprostanes¹⁵. Plasma F₂-isoprostane levels have also been associated with organ dysfunction in patients with severe sepsis¹⁶.

Isofurans are also formed as a result of lipid peroxidation of arachidonic acid, but differ from F₂-isoprostanes by the presence of a tetrahydrofuran ring¹⁷. The formation of isofurans and F₂-isoprostanes share an intermediate step that is dependent on oxygen concentration. In the presence of high oxygen tension, isofuran formation is favored^{18,19}. Isofurans may thus play a role in hyperoxia-induced oxidant injury¹⁸.

Because oxidant injury and lipid peroxidation may be important mechanistic pathways in PGD, we hypothesized that higher systemic F₂-isoprostane and isofuran concentrations would be associated with PGD. Furthermore, based on the association of F₂-isoprostanes and isofurans with hyperoxia and cigarette smoke exposure, we hypothesized that the association of F₂-isoprostanes and isofurans with PGD would be impacted by donor smoking history and higher FiO₂ at reperfusion.

Materials and Methods

Subject selection and study design

A nested case control study was chosen for efficiency based on the cost of performing the bioassays of lipid peroxidation. Subjects were selected from lung transplant recipients enrolled in the prospective multicenter Lung Transplant Outcomes Group (LTOG) cohort between 2002 and 2012^{4,20}. PGD cases were defined utilizing the International Society for Heart and Lung Transplantation guidelines in two ways: 1) grade 3 PGD present at day 2 or day 3 after reperfusion (severe PGD); 2) any grade 3 PGD in the first 72 hours after allograft reperfusion (any PGD)^{2,4,21-24}. The severe PGD definition is thought to represent a more severe phenotype, associated with worse mortality, and has been utilized in previous studies⁴. Cases and controls were selected to ensure inclusion of subjects who had received allografts from both donors with and without a history of cigarette smoke exposure as well as a broad range of fraction of inspired oxygen (FiO₂) at the time of allograft reperfusion. Donor smoke exposure was defined as any reported current or former history of donor cigarette use, collected prospectively from multiple sources at the time of transplant, including UNet data. FiO₂ at reperfusion was defined as the value recorded in the anesthesia clinical record at the time of allograft reperfusion⁴. The number of controls was limited by the small number of non-PGD patients receiving an allograft from a previously non-smoking donor in the overall LTOG cohort. Subjects had blood samples collected 6 hours after allograft reperfusion in citrated tubes; samples were processed within 30 minutes and then stored at -80°C as previously described²⁵⁻²⁸. Clinical information regarding donor, recipient, and perioperative characteristics and events were prospectively recorded on standardized case report forms^{4,20}. The institutional review boards at all participating centers approved the study.

Plasma F₂-isoprostane and isofuran measurement

Plasma F₂-isoprostane and isofuran levels were measured in duplicate by stable isotope dilution negative ion chemical ionization gas chromatography mass spectrometry²⁹. There was no association between sample storage time and plasma concentration measurements. All laboratory personnel were blinded to the PGD status of the recipients.

Statistical Analysis

Donor, recipient, and peri-operative demographics were compared utilizing Student's t-test or Chi-squared analysis as appropriate. Plasma F₂-isoprostane and isofuran concentrations were log transformed for statistical analyses in order to account for non-normal distributions. Differences in mean transformed concentrations were evaluated using two-sided Student's t-test. Reported plasma concentrations correspond to the mean transformed concentrations. There was >90% power to detect a 1 standard deviation difference in transformed plasma concentration. We utilized a priori subgroup analysis in order to evaluate the impact of reperfusion FiO₂ and donor smoke exposure on the association of plasma F₂-isoprostanes and isofurans with PGD. All statistical analysis was performed using STATA 13.1 software (STATA Corp., College Station, TX); GraphPad Prism 6 (GraphPad Software, La Jolla, CA) was used for generating graphs. A p-value <0.05 was used to determine significance.

Results

Severe PGD case definition

There were 31 severe PGD cases and 41 non-PGD controls. Table 1 describes the donor, recipient, and perioperative characteristics. Recipients with severe PGD had significantly higher mean body mass index (BMI) compared to controls (27.4 vs. 24.0, p<0.001), and other risk factor variables were similarly distributed as in prior studies⁴. Plasma F₂-isoprostane levels were significantly higher in patients with severe PGD compared to those without PGD (28.6 pg/ml vs. 19.8 pg/ml, p=0.03). There was no significant difference in plasma isofuran levels according to PGD status (39.7 pg/ml vs. 31.9 pg/ml, p=0.2) (Figure 1).

The impact of reperfusion FiO₂ and donor smoke exposure history on the association between lipid peroxidation products and severe PGD was assessed using subgroup analyses. In patients reperfused with FiO₂>40%, plasma F₂-isoprostane levels were significantly higher in patients with severe PGD compared to those without PGD (29.6 pg/ml vs. 19.0 pg/ml, p=0.03). In contrast, there was no association between plasma F₂-isoprostane levels and severe PGD in subjects reperfused at FiO₂ 40% (24.9 pg/ml vs. 21.5 pg/ml, p=0.6). There was no detected association of plasma isofuran levels with severe PGD in subjects reperfused with either FiO₂>40% (39.3 pg/ml vs. 34.0 pg/ml, p=0.5) or FiO₂ 40% (41.2 pg/ml vs. 28.2 pg/ml, p=0.3) (Figure 2).

Among patients receiving a lung from a donor with cigarette smoke exposure, plasma F₂-isoprostane levels were significantly higher in patients with severe PGD compared to controls (38.2 pg/ml vs. 22.5 pg/ml, p=0.046). There was no association between plasma F₂-

isoprostane levels and severe PGD among patients receiving a lung from a non-smoking donor (23.2 pg/ml vs. 18.1 pg/ml, $p=0.3$). In subjects receiving a lung from a previously smoking donor, isofuran levels were significantly higher in patients with severe PGD compared to controls (66.9 pg/ml vs. 34.6 pg/ml, $p=0.046$). In subjects receiving a lung from a non-smoking donor, there was no difference in isofuran levels between patients with and without severe PGD (27.2 pg/ml vs. 30.2 pg/ml, $p=0.5$) (Figure 3).

Alternate PGD case definition

The demographics of the patients utilizing any PGD as the case definition are presented in Supplemental Table 1. There was an association between plasma F_2 -isoprostanes with any PGD (28.8 vs. 19.1, $p=0.01$) but no association between isofurans (40.1 pg/ml vs. 31.1 pg/ml, $p=0.1$) and any PGD (Supplemental Figure 1). Among patients reperfused with $FiO_2 > 40\%$, F_2 -isoprostane levels were significantly higher in patients with any PGD compared to control subjects (30.2 pg/ml vs. 18.3 pg/ml, $p=0.02$). In patients reperfused at lower FiO_2 , there was no difference in F_2 -isoprostane levels (24.7 pg/ml vs. 21.0 pg/ml, $p=0.6$). There was no association between isofuran levels and any PGD when evaluating subgroups defined by reperfusion FiO_2 . In contrast to the analysis of severe PGD, there was no association between F_2 -isoprostane or isofuran levels and any PGD when evaluating subgroups defined by previous donor smoking status.

Discussion

We found that post-reperfusion plasma levels of lipid peroxidation products are associated with severe PGD after lung transplantation. Furthermore, the association between plasma F_2 -isoprostanes and PGD differed according to donor smoking history and reperfusion FiO_2 . These findings provide possible evidence that previously identified associations between receipt of a lung from a donor with smoke exposure or higher reperfusion FiO_2 and development of PGD involve potentiation of oxidant injury by these pre- and peri-operative exposures⁴.

Oxidative injury may be an important mechanism of acute lung injury in PGD that may be potentiated by donor exposure to cigarette smoke. There is a well-documented association between lipid peroxidation products and cigarette exposure^{11,30}. For example, thiobarbituric acid reactive substances (TBARS), biomarkers of lipid peroxidation, are doubled in the bronchoalveolar lavage (BAL) fluid of chronic smokers and increased 6-fold in acute smokers compared to non-smoking controls³¹. Thus, the allograft procured from a donor with smoke exposure may be inherently prone to injury due to the accumulation of lipid peroxidation products, and less likely to be able to recover from IRI in the setting of oxidative damage. In support of this, donor smoking history is strongly associated with risk of PGD in both risk factor as well as predictive models^{4,32}. Furthermore, donor smoking is associated with increased pulmonary edema, impaired gas exchange, and higher BAL levels of inflammatory chemokines in explanted lungs not used for transplantation³³. The current finding of association of PGD with high levels of lipid peroxidation products secondary to donor smoke exposure further supports the importance of oxidative injury pathways in PGD. However, given that our results cannot rule out that lipid peroxidation is an epiphenomenon

rather than the underlying cause of lung injury, model systems such as the ex vivo human lung will need to be employed to provide further evidence of causality. Our study provides proof-of-concept to justify such studies.

Higher FiO_2 at reperfusion was also associated with increased lipid peroxidation. Hyperoxia is associated with increased reactive oxygen species production and neutrophil recruitment³⁴. In prior studies, both isofuran and isoprostane formation increase with hyperoxia. Isofuran formation increases with increasing oxygen tension in rat lung models and in vitro using arachidonic acid oxidation models¹⁷. Increasing FiO_2 from 21% to 28% for one hour in healthy human subjects and subjects with COPD resulted in a significant increase in exhaled breath condensate levels of isoprostanes in both groups³⁵. Mice exposed to hyperoxia have increased production of pulmonary F_2 -isoprostanes and isofurans compared to mice maintained at room air³⁶. We previously demonstrated that compared to a reperfusion FiO_2 less than 0.40, reperfusion FiO_2 0.40 was associated with a 6% absolute risk increase for the development of PGD⁴. While the increased risk of PGD with higher reperfusion FiO_2 may simply be a marker of poor allograft function at the time of reperfusion and a need for higher FiO_2 in order to maintain reasonable PaO_2 levels, we have previously demonstrated that there is wide variability of reperfusion FiO_2 across transplant centers and that centers with the lowest PGD incidence reported the lowest mean reperfusion FiO_2 ⁴. Increased lipid peroxidation in the setting of higher FiO_2 may provide an explanation for the association of hyperoxia at reperfusion with PGD. When combined with previous animal and human studies, the suggestion of differing relationships between lipid peroxidation products and PGD based on reperfusion FiO_2 highlights the need to focus on reducing reperfusion FiO_2 as a possible preventative therapy for patients undergoing lung transplantation.

Lipid peroxidation pathways may be an attractive therapeutic target for decreasing PGD risk. Dietary flaxseed contains lignans, specifically secoisolariciresinol diglucoside, that have been shown to scavenge hydroxyl radicals and inhibit lipid peroxidation³⁷. Pre-treatment of mice with dietary flaxseed containing lignans decreased lung tissue production of F_2 -isoprostane after IRI in a hilar crossclamp model³⁸. Urinary F_2 -isoprostane levels decreased in patients with cystic fibrosis after ingestion of dietary flaxseed compared to baseline³⁹. Increases in bronchoalveolar lavage isoprostane levels after lung ischemia-reperfusion were attenuated in mice pre-treated with 14-days of azithromycin, suggesting that azithromycin may have an anti-oxidant effect⁴⁰. Maternal tobacco smoke exposure significantly decreases tissue glutathione levels in the lungs of fetal rats. Maternal administration of the anti-oxidant n-acetylcysteine protected rat fetal lungs from oxidative damage and prevented the smoke exposure-related decline in glutathione levels⁴¹. In a feline cigarette exposure model, tiotropium administration was associated with reduction in serum, lung, and tracheal lipid peroxides and abrogated reductions in antioxidant levels⁴². Tiotropium may increase anti-inflammatory signaling through modification of histone deacetylases (HDAC) and modulation of nitric oxide production⁴³. Similarly, treatment with valproic acid, an HDAC inhibitor, during hypoxia resulted in decreased levels of lipid peroxidation biomarkers and enhanced anti-oxidant enzymatic function in newborn rats⁴⁴. Ex vivo lung perfusion technology allows for evaluating therapies in the allograft prior to organ implantation^{45,46}. Therefore, lipid peroxidation product levels may be useful biomarkers of therapeutic

efficacy for preliminary trials of dietary flaxseed or lignans, azithromycin, n-acetylcysteine, valproic acid, and tiotropium, therapies with known safety profiles in human studies.

This study has some limitations. The overall sample size is small because measurements of plasma F₂-isoprostanes and isofurans are costly and labor intensive. In order to maximize the power of our study design using small numbers of subjects, we enriched the sample for patients receiving an organ from a previous smoker as well as a wide range of reperfusion FiO₂. The small sample size limits our ability to perform formal tests for interaction, e.g. using multiplicative terms in logistic regression models. Despite the small size and lack of power, a priori sub-group analyses suggest potential effect modification by both donor smoke exposure and reperfusion FiO₂. Given the post-transplant collection of plasma, we are also unable to make definitive conclusions regarding the causal relationship between lipid peroxidation products and PGD. Future studies evaluating longitudinal changes from recipient pre-transplant levels of these products as well as the impact of donor plasma and BAL levels of lipid peroxidation products prior to allograft procurement combined with model systems will be needed to further define the relationship between these products of oxidant stress and PGD.

In summary, plasma levels of lipid peroxidation products are significantly higher in patients with PGD after lung transplantation compared to those who do not develop PGD and there are significant differences within groups defined by donor smoking history and reperfusion FiO₂. This study further highlights the importance of oxidative injury in the development of lung injury after lung transplantation. Future studies should be aimed at evaluating the causal relationship of lipid peroxidation with PGD after lung transplantation and therapies targeting lipid peroxidation pathways and products in order to prevent the development of PGD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Christie JD, Bavaria JE, Palevsky HI, et al. Primary Graft Failure Following Lung Transplantation. *Chest*. Jul; 1998 114(1):51–60. 1998. [PubMed: 9674447]
2. Christie JD, Carby M, Bag R, et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part II: definition. A consensus statement of the International Society for Heart and Lung Transplantation. *The Journal of heart and lung transplantation : the official publication of the International Society for Heart Transplantation*. Oct; 2005 24(10):1454–1459.
3. Lee JC, Christie JD. Primary graft dysfunction. *Clinics in chest medicine*. Jun; 2011 32(2):279–293. [PubMed: 21511090]
4. Diamond JM, Lee JC, Kawut SM, et al. Clinical risk factors for primary graft dysfunction after lung transplantation. *American journal of respiratory and critical care medicine*. Mar 1; 2013 187(5): 527–534. [PubMed: 23306540]

5. Porzionato A, Sfriso MM, Mazzatenta A, Macchi V, De Caro R, Di Giulio C. Effects of hyperoxic exposure on signal transduction pathways in the lung. *Respiratory physiology & neurobiology*. Apr. 2015 209:106–114. [PubMed: 25485998]
6. Schweitzer KS, Hatoum H, Brown MB, et al. Mechanisms of lung endothelial barrier disruption induced by cigarette smoke: role of oxidative stress and ceramides. *Am J Physiol Lung Cell Mol Physiol*. Dec; 2011 301(6):L836–846. [PubMed: 21873444]
7. Nathan C, Cunningham-Bussell A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nature reviews. Immunology*. May; 2013 13(5):349–361.
8. Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ 2nd. A series of prostaglandin F₂-like compounds are produced in vivo in humans by a noncyclooxygenase, free radical-catalyzed mechanism. *Proc Natl Acad Sci U S A*. Dec; 1990 87(23):9383–9387. [PubMed: 2123555]
9. Banerjee M, Kang KH, Morrow JD, Roberts LJ, Newman JH. Effects of a novel prostaglandin, 8-epi-PGF₂ alpha, in rabbit lung in situ. *The American journal of physiology*. Sep; 1992 263(3 Pt 2):H660–663. [PubMed: 1415588]
10. Morrow JD, Frei B, Longmire AW, et al. Increase in circulating products of lipid peroxidation (F₂-isoprostanes) in smokers. Smoking as a cause of oxidative damage. *The New England journal of medicine*. May 4; 1995 332(18):1198–1203. [PubMed: 7700313]
11. Reilly M, Delanty N, Lawson JA, FitzGerald GA. Modulation of oxidant stress in vivo in chronic cigarette smokers. *Circulation*. Jul 1; 1996 94(1):19–25. [PubMed: 8964113]
12. Mathews WR, Guido DM, Fisher MA, Jaeschke H. Lipid peroxidation as molecular mechanism of liver cell injury during reperfusion after ischemia. *Free radical biology & medicine*. Jun; 1994 16(6):763–770. [PubMed: 8070679]
13. Mober J, Becker BF. Cyclooxygenase inhibition aggravates ischemia-reperfusion injury in the perfused guinea pig heart: involvement of isoprostanes. *Journal of the American College of Cardiology*. Jun; 1998 31(7):1687–1694. [PubMed: 9626852]
14. Reilly MP, Delanty N, Roy L, et al. Increased formation of the isoprostanes IPF₂alpha-I and 8-epi-prostaglandin F₂alpha in acute coronary angioplasty: evidence for oxidant stress during coronary reperfusion in humans. *Circulation*. Nov 18; 1997 96(10):3314–3320. [PubMed: 9396422]
15. Schmidt R, Schafer C, Luboinski T, et al. Increase in alveolar antioxidant levels in hyperoxic and anoxic ventilated rabbit lungs during ischemia. *Free radical biology & medicine*. Jan 1; 2004 36(1):78–89. [PubMed: 14732292]
16. Ware LB, Fessel JP, May AK, Roberts LJ 2nd. Plasma biomarkers of oxidant stress and development of organ failure in severe sepsis. *Shock*. Jul; 2011 36(1):12–17. [PubMed: 21372753]
17. Fessel JP, Porter NA, Moore KP, Sheller JR, Roberts LJ 2nd. Discovery of lipid peroxidation products formed in vivo with a substituted tetrahydrofuran ring (isofurans) that are favored by increased oxygen tension. *Proc Natl Acad Sci U S A*. Dec 24; 2002 99(26):16713–16718. [PubMed: 12482927]
18. Fessel JP, Jackson Roberts L. Isofurans: novel products of lipid peroxidation that define the occurrence of oxidant injury in settings of elevated oxygen tension. *Antioxidants & redox signaling*. Jan-Feb; 2005 7(1-2):202–209. [PubMed: 15650408]
19. Morrow JD, Roberts LJ, Daniel VC, et al. Comparison of formation of D₂/E₂-isoprostanes and F₂-isoprostanes in vitro and in vivo--effects of oxygen tension and glutathione. *Archives of biochemistry and biophysics*. May 1; 1998 353(1):160–171. [PubMed: 9578611]
20. Diamond JM, Akimova T, Kazi A, et al. Genetic variation in the prostaglandin E₂ pathway is associated with primary graft dysfunction. *Am J Respir Crit Care Med*. Mar 1; 2014 189(5):567–575. [PubMed: 24467603]
21. Christie JD, Van Raemdonck D, de Perrot M, et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part I: introduction and methods. *J Heart Lung Transplant*. Oct; 2005 24(10):1451–1453. [PubMed: 16210115]
22. de Perrot M, Bonser RS, Dark J, et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part III: donor-related risk factors and markers. *J Heart Lung Transplant*. Oct; 2005 24(10):1460–1467. [PubMed: 16210117]

23. Barr ML, Kawut SM, Whelan TP, et al. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part IV: recipient-related risk factors and markers. *J Heart Lung Transplant.* Oct; 2005 24(10):1468–1482. [PubMed: 16210118]
24. Arcasoy SM, Fisher A, Hachem RR, Scavuzzo M, Ware LB. Report of the ISHLT Working Group on Primary Lung Graft Dysfunction part V: predictors and outcomes. *J Heart Lung Transplant.* Oct; 2005 24(10):1483–1488. [PubMed: 16210119]
25. Diamond JM, Kawut SM, Lederer DJ, et al. Elevated plasma clara cell secretory protein concentration is associated with high-grade primary graft dysfunction. *Am J Transplant.* Mar; 2011 11(3):561–567. [PubMed: 21299834]
26. Diamond JM, Lederer DJ, Kawut SM, et al. Elevated Plasma Long Pentraxin-3 Levels and Primary Graft Dysfunction After Lung Transplantation for Idiopathic Pulmonary Fibrosis. *Am J Transplant.* Nov; 2011 11(11):2517–2522. [PubMed: 21883907]
27. Shah RJ, Diamond JM, Lederer DJ, et al. Plasma monocyte chemotactic protein-1 levels at 24 hours are a biomarker of primary graft dysfunction after lung transplantation. *Translational research : the journal of laboratory and clinical medicine.* Dec; 2012 160(6):435–442. [PubMed: 22989614]
28. Shah RJ, Emtiazjoo AM, Diamond JM, et al. Plasma complement levels are associated with primary graft dysfunction and mortality after lung transplantation. *Am J Respir Crit Care Med.* Jun 15; 2014 189(12):1564–1567. [PubMed: 24930532]
29. Milne GL, Yin H, Brooks JD, Sanchez S, Jackson Roberts L 2nd, Morrow JD. Quantification of F2-isoprostanes in biological fluids and tissues as a measure of oxidant stress. *Methods in enzymology.* 2007; 433:113–126. [PubMed: 17954231]
30. Ho E, Karimi Galougahi K, Liu CC, Bhindi R, Figtree GA. Biological markers of oxidative stress: Applications to cardiovascular research and practice. *Redox biology.* 2013; 1:483–491. [PubMed: 24251116]
31. Morrison D, Rahman I, Lannan S, MacNee W. Epithelial permeability, inflammation, and oxidant stress in the air spaces of smokers. *Am J Respir Crit Care Med.* Feb; 1999 159(2):473–479. [PubMed: 9927360]
32. Shah RJ, Diamond JM, Cantu E, et al. Objective Estimates Improve Risk Stratification for Primary Graft Dysfunction after Lung Transplantation. *Am J Transplant.* Aug; 2015 15(8):2188–2196. [PubMed: 25877792]
33. Ware LB, Lee JW, Wickersham N, et al. Donor smoking is associated with pulmonary edema, inflammation and epithelial dysfunction in ex vivo human donor lungs. *Am J Transplant.* Oct; 2014 14(10):2295–2302. [PubMed: 25146497]
34. Repine JE, Cheronis JC, Rodell TC, Linas SL, Patt A. Pulmonary oxygen toxicity and ischemia-reperfusion injury. A mechanism in common involving xanthine oxidase and neutrophils. *The American review of respiratory disease.* Aug; 1987 136(2):483–485. [PubMed: 3619213]
35. Carpagnano GE, Kharitonov SA, Foschino-Barbaro MP, Resta O, Gramiccioni E, Barnes PJ. Supplementary oxygen in healthy subjects and those with COPD increases oxidative stress and airway inflammation. *Thorax.* Dec; 2004 59(12):1016–1019. [PubMed: 15563698]
36. Lingappan K, Jiang W, Wang L, et al. Mice deficient in the gene for cytochrome P450 (CYP)1A1 are more susceptible than wild-type to hyperoxic lung injury: evidence for protective role of CYP1A1 against oxidative stress. *Toxicological sciences : an official journal of the Society of Toxicology.* Sep; 2014 141(1):68–77. [PubMed: 24893714]
37. Prasad K. Hydroxyl radical-scavenging property of secoisolariciresinol diglucoside (SDG) isolated from flax-seed. *Molecular and cellular biochemistry.* Mar; 1997 168(1-2):117–123. [PubMed: 9062900]
38. Lee JC, Bhora F, Sun J, et al. Dietary flaxseed enhances antioxidant defenses and is protective in a mouse model of lung ischemia-reperfusion injury. *Am J Physiol Lung Cell Mol Physiol.* Feb; 2008 294(2):L255–265. [PubMed: 18083772]
39. Turowski JB, Pietrofesa RA, Lawson JA, Christofidou-Solomidou M, Hadjiliadis D. Flaxseed modulates inflammatory and oxidative stress biomarkers in cystic fibrosis: a pilot study. *BMC complementary and alternative medicine.* 2015; 15:148. [PubMed: 25963404]

40. Geudens N, Timmermans L, Vanhooren H, et al. Azithromycin reduces airway inflammation in a murine model of lung ischaemia reperfusion injury. *Transplant international : official journal of the European Society for Organ Transplantation*. Jul; 2008 21(7):688–695. [PubMed: 18435685]
41. Basyigit I, Tugay M, Dilioglulil MO, Yildiz F, Maral H, Sozubir S. Protective effects of N-acetylcysteine on peroxidative changes of the fetal rat lungs whose mothers were exposed to cigarette smoke. *Human & experimental toxicology*. Feb; 2007 26(2):99–103. [PubMed: 17370867]
42. Kolahian S, Shahbazfar AA, Tayefi-Nasrabadi H, et al. Tiotropium effects on airway inflammatory events in the cat as an animal model for acute cigarette smoke-induced lung inflammation. *Experimental lung research*. Aug; 2014 40(6):272–287. [PubMed: 24784973]
43. Holownia A, Mroz RM, Wielgat P, et al. Altered histone deacetylase activity and iNOS expression in cells isolated from induced sputum of COPD patients treated with tiotropium. *Advances in experimental medicine and biology*. 2013; 788:1–6. [PubMed: 23835951]
44. Cetinkaya M, Cansev M, Cekmez F, et al. Protective Effects of Valproic Acid, a Histone Deacetylase Inhibitor, against Hyperoxic Lung Injury in a Neonatal Rat Model. *PLoS One*. 2015; 10(5):e0126028. [PubMed: 25938838]
45. Cypel M, Keshavjee S. Extracorporeal lung perfusion. *Curr Opin Organ Transplant*. Oct; 2011 16(5):469–475. [PubMed: 21857514]
46. Cypel M, Yeung JC, Liu M, et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. *The New England journal of medicine*. Apr 14; 2011 364(15):1431–1440. [PubMed: 21488765]

Figure 1.

Box plots of lipid peroxidation product plasma levels. Box represents the non-transformed plasma levels corresponding to the mean and 95% confidence intervals of the transformed values. Black boxes are patients with severe PGD and grey boxes are patients without PGD. A: Comparison of F₂-isoprostane levels in patients with and without severe PGD; B: Comparison of isofuran levels in patients with and without severe PGD. P-value represents result of t-test of log-transformed values.

Figure 2.

Box plots of lipid peroxidation product plasma levels with subgroups defined by reperfusion FiO₂ 40% vs. FiO₂>40%. Box represents the non-transformed plasma levels corresponding to the mean and 95% confidence intervals of the transformed values. Black boxes are patients with severe PGD and grey boxes are patients without PGD. A: Comparison of F₂-isoprostane levels in patients with and without severe PGD; B: Comparison of isofuran levels in patients with and without severe PGD. P-value represents result of t-test of log-transformed values.

Figure 3.

Box plots of lipid peroxidation product plasma levels with subgroups defined by donor smoking history. Box represents the non-transformed plasma levels corresponding to the mean and 95% confidence intervals of the transformed values. Black boxes are patients with severe PGD and grey boxes are patients without PGD. A: Comparison of F₂-isoprostane levels in patients with and without severe PGD; B: Comparison of isoflurane levels in patients with and without severe PGD. P-value represents result of t-test of log-transformed values.

Table 1

Subject Demographics. PGD is defined as grade 3 PGD at day 2 or 3.

Covariate	PGD (n=31)	Non-PGD (n=41)	p-value
Donor Variables			
Male Gender, n (%)	19 (61)	25 (61)	0.9
Age, mean	35.2	35.1	0.9
Race, n (%)			0.4
Caucasian	16 (52)	29 (71)	
African American	9 (29)	6 (15)	
Other	6 (19)	6 (15)	
Any smoking, yes	13 (42)	17 (41)	0.1
Recipient Variables			
Male Gender, n (%)	16 (52)	22 (54)	0.9
Age, mean	53.9	53.9	0.9
BMI, mean	27.4	24.0	0.0008
Pulmonary Diagnosis, n (%)			0.3
Chronic obstructive pulmonary disease	9 (29)	19 (39)	
Idiopathic pulmonary fibrosis	13 (42)	14 (34)	
Cystic Fibrosis	1 (3)	6 (15)	
Other	8 (26)	5 (12)	
mPAP	50.5	44.2	0.2
Race, n (%)			0.2
Caucasian	22 (71)	35 (85)	
African American	8 (26)	4 (10)	
Other	1 (3)	2 (5)	
Operative Variables			
Ischemic time, min	324	332	0.7
Transplant type, single, n (%)	10 (32)	13 (32)	0.9
PRBC volume, ml	1282	1015	0.5
Reperfusion FiO ₂ , %	73	64	0.2
Reperfusion FiO ₂ category, n (%)			0.2
21-40%	6 (19)	14 (34)	
>40%	25 (81)	27 (66)	
Cardiopulmonary bypass use, yes, n (%)	18 (58)	17 (41)	0.2

BMI: Body mass index

mPAP: mean pulmonary artery pressure

FiO₂: Fraction of inspired oxygen

Percentages may not exactly equal 100% because of rounding.