Exhaled Breath Condensate Isoprostanes Are Elevated in Patients With Acute Lung Injury or ARDS*

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Background: Oxidant stress is a purported mechanism of tissue damage in patients with ARDS and acute lung injury (ALI). Isoprostanes, prostanoid compounds primarily formed nonenzymatically via lipid peroxidation, are precise markers of in vivo oxidant stress. Plasma levels of metabolites of 8-iso-prostaglandin-F$_{2\alpha}$ (8-iso-PGF$_{2\alpha}$) correlate with outcome in patients with ARDS.

Objective: To examine exhaled breath condensate levels of 8-iso-PGF$_{2\alpha}$ as a noninvasive quantification of pulmonary oxidant stress in patients with, or at risk for, ARDS/ALI.

Methods: Breath condensate was collected from 22 patients with, or at risk for, ARDS/ALI by placing Tygon tubing submerged in an ice bath in line with the expiratory limb of the ventilator circuit. Ten patients without lung disease, who were intubated while undergoing minor surgical procedures, served as control subjects. Between 1 and 3 mL of condensate was collected over a 30- to 60-min period, then immediately frozen and stored at $-70^\circ$C until analysis. The 8-iso-PGF$_{2\alpha}$ was purified and derivatized, then quantified by stable isotope dilution in conjunction with gas chromatography/mass spectrometry.

Results: The mean level of exhaled 8-iso-PGF$_{2\alpha}$ in the patients with ALI/ARDS, 87 ± 28 pg/mL, was significantly higher than the mean in the normal group, 7 ± 4 pg/mL (p = 0.007). The 8-iso-PGF$_{2\alpha}$ levels were greater than two standard deviations above the mean of the normal group in 12 of 22 patients with or at risk for ARDS/ALI.

Conclusions: These results provide further evidence that lipid peroxidation does occur in patients with ARDS/ALI. The measurement of exhaled isoprostanes provides a novel, noninvasive method to quantify oxidant stress in such patients.

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Key words: ARDS; breath condensate; isoprostanes; lipid peroxidation; lung injury; oxidant stress; prostaglandins

Abbreviations: ALI = acute lung injury; F$_{I02}$ = fraction of inspired oxygen; F$_{2}$-isop-M = F$_{2}$-isoprostane-M; PGE$_{2}$ = prostaglandin E$_{2}$; 8-iso-PGF$_{2\alpha}$ = 8-iso-prostaglandin-F$_{2\alpha}$; PG C/h S-2 = prostaglandin endoperoxide synthase; VE = minute ventilation

The presence of endothelial cell injury leading to the high-permeability pulmonary edema seen in ARDS has been firmly established. The mechanism of endothelial damage is not certain; however, oxidant stress is suspected to play a central role. In the setting of inflammation, reactive oxygen species are produced both within endothelial cells and from other sources, such as activated neutrophils. These free radicals may interact with endothelial cell membranes via lipid peroxidation causing altered membrane fluidity and function.\textsuperscript{2}

Quantitation of oxidant stress might provide insight into the mechanisms of lung injury. In addition, in individual patients, such data might provide prognostic information or identify a subset of patients who could possibly benefit from antioxidant therapy. Isoprostanes, prostanoid compounds primarily formed nonenzymatically by free radical-catalyzed peroxidation of membrane phospholipids, are excellent markers of in vivo oxidant stress.\textsuperscript{3} One of the isoprostanes, 8-iso-PGF$_{2\alpha}$ (8-iso-PGF$_{2\alpha}$), and its metabolite, F$_{2}$-isoprostane-M (F$_{2}$-isop-M), are present in the plasma and urine of normal humans.\textsuperscript{4–6} Both of these compounds are elevated in response to oxidant stress in animal models, and the formation of

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the metabolite parallels the production of the parent compound. In addition, elevated concentrations of 8-iso-PGF$_{2\alpha}$ have been demonstrated in a variety of clinical syndromes thought to be associated with oxidant stress, including acetaminophen and paraquat poisoning, coronary reperfusion with thromboytic agents, and alcohol ingestion in cirrhotic patients. More recently, elevated plasma levels of F$_2$-isop-M have been shown to be predictive of mortality in patients with ARDS.

Breath condensate, formed by the cooling of exhaled gas, may provide organ-specific information about pulmonary oxidant stress. Collection of breath condensate has several advantages over the more traditional method of sampling the pulmonary airspaces, BAL. It is noninvasive, is less expensive in terms of both equipment and personnel costs, and potentially provides more concentrated samples as airway fluid is diluted by the use of saline in BAL. This study was designed to assess the feasibility of using breath condensate samples to quantitate oxidant stress in patients with or at risk for acute lung injury (ALI) or ARDS. We hypothesized that (1) isoprostanes would be detectable in breath condensate; and (2) isoprostane concentrations would be elevated in patients with or at risk for ALI or ARDS compared with normal control subjects.

**Materials and Methods**

**Patients**

Patients with or at risk for ALI/ARDS due to severe sepsis were studied. Specific inclusion criteria included the presence of the systemic inflammatory response syndrome and at least one acute organ failure. To meet criteria for systemic inflammatory response syndrome, three or more of the following were demonstrated: core temperature $\geq 38^\circ$C (100.4°F) or $\leq 36^\circ$C (96.8°F); heart rate $\geq 90$/min (in the absence of $\beta$-blocker therapy or complete heart block); respiratory rate $\geq 20$/min or minute ventilation (Ve) $\geq 10$ L/min; and WBC count $\geq 12,000$ or $\leq 4,000$, or the presence of $\geq 10\%$ immature neutrophils (bands). Organ failures were defined as follows: (1) hypotension—systolic BP $\leq 90$ mm Hg for at least 1 h in the face of pulmonary artery wedge pressure $\geq 12$ mm Hg or unresponsive to saline infusion ($\geq 500$ Ml during 2 h); (2) renal insufficiency—urine output $\leq 30$ mL/h or $\leq 0.5$ mL/kg/h for at least 2 h; (3) acute lung injury—PaO$_2$/fraction of inspired oxygen (FIO$_2$) $\leq 300$, diffuse bilateral infiltrates on chest radiograph, and pulmonary artery wedge pressure $\leq 18$ mm Hg; and (4) coagulation dysfunction—platelet count $\leq 80,000$ within 24 h of inclusion. Patients were excluded if not intubated and receiving mechanical ventilation. Nonsmoking patients without lung disease, who were intubated while undergoing minor surgical procedures, served as control subjects. The study was approved by the Institutional Review Board/Committee for Protection of Human Subjects of Vanderbilt University Medical Center. Data collection included temperature, heart rate, BP, respiratory rate, WBC count, Ve, FIO$_2$, tidal volume, arterial blood gas values, and presence or absence of organ failures as outlined above.

**Sample Collection**

Breath condensate was collected by placing a 100-cm length of Teflon-lined tubing (Tygon; Norton Performance Plastics; Akron, OH) in line with the expiratory limb of the ventilator circuit. The collection tubing was submerged in an ice bath. Prior to collection, humidification was removed from the inspiratory side of the ventilator circuit. Between 1 and 3 mm of condensate was collected over a 30- to 60-min period. A urine sample was simultaneously obtained from a subset of patients with ALI/ARDS. Samples were frozen immediately at $-70^\circ$C and stored until analysis.

To rule out ex vivo production of isoprostanes in the collection system, three concentrations (50, 250, and 500 pg/mL) of 8-iso-PGF$_{2\alpha}$ were instilled in Tygon tubing submerged in ice and allowed to dwell for 30 min. The concentrations of 8-iso-PGF$_{2\alpha}$ in the recovered fluid were assayed. Recovery of 8-iso-PGF$_{2\alpha}$ was also assessed after nebulization of the same three concentrations of standard into the collection system using a Collison nebulizer (BGI; Waltham, MA).

To determine whether duration of collection influenced the concentration of breath condensate 8-iso-PGF$_{2\alpha}$, samples were collected from two normal volunteers breathing through a mouthpiece connected to the collection system for 15 min, and then for 30 min. A noseclip was worn during collection. The 8-iso-PGF$_{2\alpha}$ concentrations did not differ with longer collection intervals. To evaluate the effect of differing FIO$_2$ values on breath condensate 8-iso-PGF$_{2\alpha}$, samples were collected from two normal volunteers breathing first room air, then 50% FIO$_2$. Concentrations of 8-iso-PGF$_{2\alpha}$ did not vary with the change in inspired oxygen content.

Finally, prostanoid production in inflammatory states is thought to be upregulated by prostaglandin endoperoxide synthase-2 (PG G/H S-2). To assure that breath condensate 8-iso-PGF$_{2\alpha}$ was formed primarily by lipid peroxidation, rather than enzymatically through this pathway, levels of prostaglandin E$_2$ (PGE$_2$), a major product of PG G/H S-2, were measured in 10 study patients and 10 normal control subjects.

**Analytical Methods**

Breath condensate 8-iso-PGF$_{2\alpha}$ was quantified by modification of previously published methods using stable isotope dilution methods in conjunction with gas chromatography/mass spectrometry. Analogous techniques were used to quantify isoprostane metabolites in the urine of selected patients.

**Statistical Analysis**

Results are reported as the mean $\pm$ SEM. Levels of 8-iso-PGF$_{2\alpha}$ and PGE$_2$ in breath condensate from study patients and control subjects were compared using Student’s $t$ test. Pearson’s linear correlation coefficient was used to test the relationship between breath condensate 8-iso-PGF$_{2\alpha}$ and urine F$_2$-isop-M, Ve, FIO$_2$, and PaO$_2$/FIO$_2$ ratio. We considered $p$ values of $\leq 0.05$ to indicate statistical significance. Sensitivity, specificity, and positive and negative predictive values were derived from $2 \times 2$ table analysis using standard methods.

**Results**

Twenty-two patients with or at risk for ALI/ARDS were studied. All patients met the criteria for severe sepsis as outlined in the Materials and Methods...
section, and all were mechanically ventilated. Twenty of the 22 met criteria for either ALI or ARDS. The mean age was 52 ± 3 years. Twelve were male and 10 were female. Seven patients also met criteria for shock, five for acute renal insufficiency, and three for coagulation dysfunction. The overall mortality of the patients with or at risk for ALI/ARDS was 36%.

Samples were collected from 10 normal control subjects. The mean age for this group was 35 ± 5 years. Three were male and seven were female. All of the control patients were intubated for minor surgical procedures.

A representative selected ion current profile used for quantification of 8-iso-PGF$_{2\alpha}$ is shown in Figure 1. The concentrations of 8-iso-PGF$_{2\alpha}$ recovered after the 30-min dwell of 50, 250, and 500 pg/mL of standard 8-iso-PGF$_{2\alpha}$ were 37 pg/mL (74%), 176 pg/mL (70%), and 383 pg/mL (76%), respectively. This experiment demonstrated linear recovery of 8-iso-PGF$_{2\alpha}$ (despite a constant, fractional loss of yield, likely due to lipid adsorption to the tubing) and absence of ex vivo lipid peroxidation within the collection system. After nebulization of the same three concentrations of standard, the recovered samples again demonstrated a linear increase in concentration with a greater loss in yield, presumably both due to lipid adsorption to the nebulizer apparatus, and incomplete condensation of the airborne droplets.

The distribution of 8-iso-PGF$_{2\alpha}$ values in the study and control populations is illustrated in Figure

![Figure 1](https://example.com/fig1.png)

**Figure 1.** Selected ion current profile used for the quantification of 8-iso-PGF$_{2\alpha}$. In the lower tracing (m/z 573), the peak represents the derivatized internal standard introduced for sample measurement. The upper tracing (m/z 569) shows a peak co-eluting with the internal standard. This represents the endogenously generated 8-iso-PGF$_{2\alpha}$, m/z = mass to charge ratio.
levels, and patients with ALI/ARDS. Of the three patients with a history of recent smoking, isoprostane was detectable in breath condensate in two (mean value, 100 pg/mL).

Additional analyses were performed in patients with ALI/ARDS who did not have detectable isoprostane in breath condensate (n = 8). Such patients tended to be younger, with a difference in group means of 11 years; seven of eight were nonsmokers. Mortality rate did not differ between study patients with detectable 8-iso-PGF$_{2\alpha}$ and those with non-detectable levels.

Levels of the metabolite of 8-iso-PGF$_{2\alpha}$ in urine were assayed in a subset of five patients with ALI/ARDS. Concentrations of the urinary metabolite, F$_{2\alpha}$-isop-M, correlated with levels of 8-iso-PGF$_{2\alpha}$ in the breath condensate of these patients (r = 0.97; p = 0.007) (Fig 4). The mean VE in the study patients was 12.5 ± 0.2 L/min. Concentrations of 8-iso-PGF$_{2\alpha}$ in breath condensate roughly correlated with VE (r = 0.44; p = 0.07), but did not correlate with P$\mathrm{Fio}_2$ (r = 0.025; p > 0.05), or P$\mathrm{PaO}_2$/P$\mathrm{Fio}_2$ ratio (r = 0.07; p > 0.05) in the patients with or at risk for ALI/ARDS. Mean PGE$_2$ levels in 10 study patients (13.9 ± 0.7 pg/mL) and 10 normal controls (11.5 ± 1.0 pg/mL) were not significantly different (p = 0.47).

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Figure 3. a 2 x 2 analysis demonstrating the sensitivity, specificity, and predictive values of breath condensate 8-iso-PGF$_{2\alpha}$ levels > 25 pg/mL for detecting ARDS.
The oxidant stress theory of ARDS proposes that an insult, such as severe sepsis, leads to activation of neutrophils and macrophages that subsequently release reactive oxygen species. These oxygen free radicals, while clearly beneficial in host defense, may result in lipid peroxidation of endothelial and epithelial cell membrane phospholipids, thereby altering the structure and function of the cell membrane. Such alterations could significantly compromise barrier function.

Attempts to quantify oxidant stress or lipid peroxidation, specifically, have been numerous. Conceptually, the measurement of markers of oxidant stress in exhaled breath is attractive because of the potential to provide organ-specific information in the setting of lung injury. Exhaled hydrocarbons, especially pentane, have been studied extensively. However, the measurement of exhaled pentane is fraught with technical difficulties, as evidenced by the 1,000-fold differences in values reported by various investigators. Potential confounders are failure to account for pentane concentrations in ambient air, differences in rates of pentane excretion among individuals due to varying body fat content, and variability in storage techniques of exhaled gas prior to measurement.

Sznajder et al and Baldwin et al have measured concentrations of hydrogen peroxide in the exhaled breath condensate of patients with ARDS as a means of quantifying oxidant stress. Despite a large range in the distribution of hydrogen peroxide concentrations, both of these groups of investigators found that the mean concentration was higher in patients with ARDS than in control patients. In these studies, hydrogen peroxide was assayed by spectrophotometry after the samples were reacted with horseradish peroxidase. Although these studies were conceptual breakthroughs, the concentrations of hydrogen peroxide were not precisely quantitative.

In the current study, we measured concentrations of one of the isoprostanes, 8-iso-PGF\textsubscript{2α}, in exhaled breath condensate. Measurement of these prostanoid compounds by gas chromatography/mass spectrometry provides the advantage of being rigorously quantitative. The isoprostanes are a novel class of compounds formed by in vitro lipid peroxidation of membrane arachidonic acid. In contrast to the prostaglandins, the isoprostanes are predominantly formed independently of cyclooxygenase. In addition to serving as markers of oxidant stress, the isoprostanes also have important biological activities. 8-iso-PGF\textsubscript{2α} is a potent renal and pulmonary vasoconstrictor that interacts with the thromboxane

![Graph showing correlation between breath condensate 8-iso-PGF\textsubscript{2α} and urinary F\textsubscript{2}-iso-M. The coefficient of correlation is r = 0.97 (p = 0.007).](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21893/)

**FIGURE 4.** Correlation between breath condensate 8-iso-PGF\textsubscript{2α} and urinary F\textsubscript{2}-iso-M. The coefficient of correlation is r = 0.97 (p = 0.007).
receptor on platelets and possibly with a similar, but distinct, receptor on vascular smooth muscle. Recently, intratracheal instillation of 8-iso-PGF$_2\alpha$ has been shown to induce airway plasma exudation in an animal model in addition to causing airway obstruction.20

Morrow and Awad have demonstrated that isoprostanes and their metabolites are present in the plasma and urine of normal humans and are increased in animal models of oxidant stress.5,6,21 As previously mentioned, concentrations of 8-iso-PGF$_2\alpha$ have been found to be increased in humans with clinical syndromes associated with oxidant stress, including acetaminophen and paraquat poisoning and coronary reperfusion after thrombolysis.8 In addition, elevation of plasma isoprostanes and urinary isoprostane metabolites in smokers compared with nonsmokers has been described.22,23 This finding supports the theory that smoking results in oxidative damage via free radical–catalyzed lipid peroxidation. Finally, elevated concentrations of F$_2$-isop-M in plasma drawn within 48 h of onset of ARDS are predictive of increased mortality.8

FitzGerald and coworkers have elegantly shown that 8-iso-PGF$_2\alpha$ is produced in a cyclooxygenase-dependent pathway by platelets in trivial amounts, and in greater amounts by monocytes via a PG G/H synthase-2 pathway.23-25 Breath condensate isoprostane levels would not be affected by the former as platelets are excluded from the airway. In addition, maximal 8-iso-PGF$_2\alpha$ production by monocytes stimulated in vitro is one sixth the corresponding production of PGE$_2$.24 PGE$_2$ levels in the breath condensate of patients with or at risk for ALI/ARDS were not increased in comparison with normal subjects. Therefore, lipid peroxidation appears to be the mechanism for the formation of most, if not all, of the 8-iso-PGF$_2\alpha$ in these patients.

We found that the mean level of breath condensate 8-iso-PGF$_2\alpha$ was significantly elevated in patients with or at risk for ALI/ARDS compared with normal control subjects. Similar to the scatter found in the values of breath condensate hydrogen peroxide, we observed a wide distribution of 8-iso-PGF$_2\alpha$ concentrations in the study patients. However, the breath condensate 8-iso-PGF$_2\alpha$ levels were greater than two SDs above the mean of the control group in 12 of 22 study patients (55%). There was excellent correlation between breath condensate 8-iso-PGF$_2\alpha$ and its urinary metabolite, F$_2$-isop-M. Urinary F$_2$-isop-M has been shown to rise in parallel with plasma levels of 8-iso-PGF$_2\alpha$ in response to oxidant stress. Our findings provide further evidence that lipid peroxidation does occur in patients with or at risk for ALI/ARDS.

The mean age in the control group was significantly lower than that of the study population. However, in a previous study of 46 patients with ARDS, age did not correlate with plasma F$_2$-isop-M.8 The measurement of breath condensate 8-iso-PGF$_2\alpha$ is not confounded by the FIO$_2$ or VE. The concern that higher levels of inspired oxygen would provide more substrate for the formation of oxygen free radicals and thus lead to increased lipid peroxidation is not supported, based on the lack of correlation between FIO$_2$ and breath condensate 8-iso-PGF$_2\alpha$. Similarly, there is only a rough correlation between VE and concentrations of breath condensate 8-iso-PGF$_2\alpha$. We theorize that as air moves over the epithelial lining fluid during exhalation, droplets are aerosolized, pass through the expiratory limb of the ventilator in the exhaled gas, and condense again as the gas is cooled in the collection system. Therefore, we expect that increased VE will increase the rate of accumulation of breath condensate, but will not affect the concentration of isoprostanes. The rough correlation between minute ventilation and breath isoprostanes suggests a not unexpected relationship between severity of oxidant stress and one parameter reflecting extent of lung injury.

The question of site of production of the breath condensate 8-iso-PGF$_2\alpha$ is not answered by this study. Whether this method provides organ-specific quantification of pulmonary lipid peroxidation or reflects overall systemic production remains uncertain. It is possible that isoprostanes, produced at sites remote from the lung, could circulate in plasma and enter the pulmonary epithelial lining fluid, especially during times of increased permeability. The well-described rapid metabolism of prostanoioids in the pulmonary microcirculation would tend to argue against this.

The clinical utility of this technique should be studied prospectively. No predictive value of breath condensate 8-iso-PGF$_2\alpha$ with regard to mortality was seen in patients with ALI/ARDS. However, the study was not designed to examine mortality and was lacked sufficient power to do so effectively. In addition, given the clinical complexity of patients with severe sepsis and ARDS, it is difficult to control for all the variables that might affect isoprostane concentrations. For example, administration of medications with antioxidant properties is a potential confounder that should be considered.

In conclusion, measurement of breath condensate isoprostanes is a novel, noninvasive quantitative technique to evaluate oxidant stress in patients with or at risk for lung injury. Future directions include the application of this technique to other inflammatory lung disease and determination of the specificity of such measurements for pulmonary lipid peroxidation.
REFERENCES


