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# Changes in urinary dinor dihydro $F_2$-isoprostane metabolite concentrations, a marker of oxidative stress, during and following asthma exacerbations

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**Abstract**

To investigate changes in oxidant stress during and following acute asthma exacerbations, this stidy measured 2,3-dinor-5,6-dihydro-15-$F_{2t}$-IsoP ($F_2$-IsoP-M), the major urinary metabolite of 15-$F_{2t}$-IsoP, in eight asthmatic adults, during and following an asthma hospitalization. $F_2$-IsoP-M concentrations at admission and follow-up were significantly higher than discharge (admission median: 4.12 ng/Cr mg, range 1.89–7.8; follow-up: 2.47 ng/Cr mg (1.56–6.86); discharge: 1.42 ng/Cr mg (0.7–4.44); both $p < 0.01$), but not significantly different between admission and follow-up. $F_2$-IsoP-M concentrations at follow-up were higher than a control group with stable asthma (0.68 ng/Cr mg (0.31–1.5), $p = 0.0008$). In conclusion, asthma exacerbations requiring hospitalization are associated with 6-fold higher urinary $F_2$-IsoP-M concentrations compared to stable asthmatics. $F_2$-IsoP-M concentrations decreased significantly during hospitalization, but significant elevations 3 months following hospitalization suggest ongoing oxidative stress despite clinical improvement. Urinary $F_2$-IsoP-M may be a clinically useful, simple non-invasive systemic measure of oxidative stress in asthmatics, providing information not captured by spirometry or symptoms.

**Keywords:** *$F_2$-isoprostanes, antioxidant, asthma*

## Introduction

Asthma is a chronic inflammatory disease of the airways which involves oxidant injury to the lung. There is enhanced oxidative stress in stable asthma and also during asthma exacerbations, both systemically and locally [1–6]. Inflammation, which involves recruitment and activation of inflammatory cells, results in excessive oxygen free radicals, overwhelming host antioxidant defenses and leading to oxidant stress. $F_2$-isoprostanes ($F_2$-IsoPs), the stable prostaglandin $F_2$-like compounds that are produced by free radical catalysed peroxidation of arachidonic acid, have emerged as a valuable tool for assessing oxidant status *in vitro* and *in vivo* in animals and humans [7]. The major urinary metabolite of 15-$F_{2t}$-IsoP, 2,3-dinor-5,6-dihydro-15-$F_{2t}$-IsoP ($F_2$-IsoP-M), is a non-invasive marker of oxidant stress which assesses total endogenous production of $F_2$-IsoPs in humans [8]. It provides a reliable index of total systemic production of IsoPs because the metabolism of eicosanoids occurs predominantly in extrarenal tissues [9]. Urinary $F_2$-IsoP-M and $F_2$-IsoPs in both urine and bronchoalveolar lavage fluid have been

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reported to be elevated in atopic asthmatics after allergen challenge, but not after inhalation challenge of methacholine [3,8]. Urinary $F_2$-IsoP-M has not been evaluated in patients with asthma during naturally occurring acute disease exacerbations. We sought to determine the changes of oxidant stress during and following acute asthma exacerbations by measuring urinary $F_2$-IsoP-M in a group of hospitalized adults with asthma at hospital admission, discharge and a 3-month follow-up convalescent visit.

## Methods

### *Patient identification*

Eight subjects were recruited during an acute asthma hospitalization**.** Review of hospital charts, prior medical records and consultation with the currently treating hospital physician(s) were used to confirm the diagnosis of asthma and to exclude other diagnoses. Patients were excluded if they had concurrent congestive heart failure or other chronic pulmonary disease that could account for the acute illness or if they were previously enrolled. Criteria for the diagnosis of acute asthma included confirmation of asthma diagnosis by review of prior records and current admission consistent with an asthma exacerbation including admitting diagnosis of asthma, presence of dyspnea, wheezing and respiratory distress not attributable to another cardiopulmonary processes. Second-hand smoke exposure and current smoking were defined and quantified by personal report and/or elevated urinary cotinine level, as determined by gas chromatography (National Medical Services, Willow Grove, PA). In addition to self-report, patients with urine cotinine levels $>200$ ng/ml were categorized as current smokers regardless of self-reported smoking status and those non-smokers with levels of 1–199 ng/ml were categorized as exposed to environmental tobacco smoke [10–12]. Asthma severity categories were determined by daily and nocturnal symptom reporting based on Global Initiative for Asthma (GINA) criteria [13]. The John Hopkins Asthma and Allergy Center Severity Score (JHAAC), which reflects disease severity over the year prior to hospitalization, was used to determine subjects' asthma severity over the year prior to hospitalization (Table I) [14].

To serve as a comparison group, eight subjects with asthma in the stable state were also recruited. The diagnosis of asthma was confirmed by chart and prior medical record review, as well as consultation with their primary physicians. These subjects had not had an asthma disease exacerbation requiring hospitalization or rescue corticosteroid in the last 6 months by chart review and spirometry was measured during their visit to confirm that they were being testing in their stable state.

### *Study design*

Eight subjects were recruited during an acute asthma hospitalization and were followed longitudinally. In addition, a comparison outpatient group with stable asthma was studied at a single time point. Treatment during the acute asthma hospitalization was determined by the treating physician and subjects were treated as per standard of care. All hospitalized patients initially received between 60–125 mg intravenous methyl prednisolone dosed every 6–12 h ($n=5$) or prednisone 50–60 mg orally once or twice daily ($n=3$). Beta-agonists were administered to all hospitalized subjects by metred dose inhalers or nebulizations at least every 4 h during the first 24 h of admission. All hospitalized subjects were prescribed a corticosteroid taper at hospital discharge. Urine samples were collected at the time of study enrollment on the first day of hospitalization and on the day of hospital discharge. Urinary $F_2$-IsoP-M concentrations were analysed at the time of hospital admission, hospital discharge and at a 3-month outpatient follow-up visit. Spirometry was obtained at each time point. The eight subjects with stable asthma provided urine samples for comparison urinary $F_2$-IsoP-M measurements. The study was approved by the Vanderbilt University School of Medicine Institutional Review Board and all subjects gave informed consent.

### *Urinary $F_2$-IsoP-M analyses*

$F_2$-IsoP-M concentrations were measured in urine samples after purification and derivatization by a gas chromatographic/mass spectrometric assay and adjusted for creatinine, as has been previously described [15]. The assay has a high degree of precision ($\pm 4\%$) and accuracy (97%) [15].

### *Statistical analysis*

Demographic data are reported as median (range) or mean $\pm$ standard deviation (SD) according to their distributions. For categorical variables, proportions were used. Differences in demographic variables between the hospitalized and stable asthma groups were compared with Mann-Whitney U-test for continuous variables or Fisher exact test for categorical variables. In order to assess differences in $F_2$-IsoP-M between hospital admission, hospital discharge and 3-month follow-up, a linear mixed effect model was used with time as a categorical variable. When the overall test for the effect of time was rejected, pairwise difference among the three time points was further assessed with Bonferroni corrected two-sided significance levels of 0.05. Differences in $F_2$-IsoP-M concentrations over time were analysed with and without the current smoker included. Change in $FEV_1$ (% predicted) over the three time points was

Table I. Clinical characteristics of study subjects recruited during acute asthma exacerbations requiring hospitalization and a comparison group of subjects with stable asthma.

| | Asthma exacerbation requiring hospitalization ($n=8$) | Stable asthma ($n=8$) |
|---|---|---|
| Age (mean ± SD) | 46 ± 11 | 47 ± 8 |
| Race (%) | | |
| White | 5 (63) | 7 (88) |
| Black | 2 (25) | 1 (12) |
| Hispanic | 1 (12) | 0 (0) |
| Female (%) | 8 (100) | 5 (63) |
| Body mass index* | 33 (23–64) | 25 (22–36) |
| Use of inhaled corticosteroid (%) | 8 (100) | 7 (88) |
| Regular user | 5 (63) | 4 (57) |
| Non-regular user | 3 (37) | 3 (43) |
| Smoking status (%) | | |
| Current smoker | 1 (12) | 0 (0) |
| Pack year (packs per year) | 22.5 | |
| Cotinine level (ng/ml) | 1500 | |
| Environmental/former smoker | 3 (38) | 3 (37) |
| Never smoker | 4 (50) | 5 (63) |
| Asthma severity (%) | | |
| Severe persistent | 2 (25) | 4 (50) |
| Moderate persistent | 5 (63) | 3 (38) |
| Mild persistent | 1 (12) | 1 (12) |
| Mild intermittent | 0 (0) | 0 (0) |
| Chronic asthma severity score*† | 56 (15–87.5) | 34 (15–87.5) |
| IgE* (IU/ml) | 81.25 (5.5–546) | 39.5 (9.5–546) |
| Atopy (%) | 5 (63) | 6 (75) |
| $FEV_1$ (% predicted) (mean ± SD) | | |
| Hospital admission | 58.5 ± 16.01 | N/A |
| Hospital discharge | 64.3 ± 18.73 | N/A |
| 3-month follow-up visit | 75.1 ± 22.31 | N/A |
| Stable asthma visit | N/A | 73.1 ± 15.75 |
| $F_2$-IsoP-M (ng/Cr mg) (median (range); mean ± SD) | | |
| Hospital admission | 4.12 (1.89–7.8)<br>4.25 ± 2.01 | N/A |
| Hospital discharge | 1.42 (0.7–4.44)<br>2.11 ± 1.41 | N/A |
| 3-month follow-up visit | 2.47 (1.56–6.86)<br>3.83 ± 2.23 | N/A |
| Stable asthma visit | N/A | 0.68 (0.31–1.5)<br>0.75 ± 0.44 |
| Urinary creatinine (mg/ml) (mean ± SD) | | |
| Hospital admission | 0.96 ± 0.62 | N/A |
| Hospital discharge | 1.58 ± 0.57 | N/A |
| 3-month follow-up visit | 0.86 ± 0.28 | N/A |
| Stable asthma visit | N/A | 0.72 ± 0.69 |

* median (range).
† This chronic asthma severity score (The John Hopkins Asthma and Allergy Center Severity Score) reflects chronic disease severity over the year prior to assessment, with higher scores indicating more symptomatic disease.



analysed in a similar manner as with $F_2$-IsoP-M change. Normality of residuals of both linear mixed models was diagnosed and transformation on the dependent variable was done to correct non-normal residuals if needed. $F_2$-IsoP-M concentrations of acute asthmatics at hospital admission and at 3-month follow-up were compared to $F_2$-IsoP-M concentrations of asthmatic subjects in the stable state with Mann-Whitney U-test. SAS (SAS™ version 9, Cary, NC) was used with within subject correlation being considered.

## Results

### *Subject characteristics*

*Hospitalized subjects.* The mean age of the eight hospitalized adults with asthma was 46 ± 11 years. Five subjects (63%) were white, two (25%) were black and one (12%) was Hispanic. All of the subjects studied were female. All of them used inhaled corticosteroids. Among them, five (63%) were regular users of inhaled corticosteroids and three (37%) were prescribed, but not regular users. One (12%)

was a current smoker, three (38%) had environmental tobacco smoke exposure and/or were former smokers and four (50%) were never smokers. Among these eight subjects, two (25%) had severe persistent asthma, five (63%) had moderate persistent asthma and one (12%) had mild persistent asthma based on the GINA guidelines [13]. The median chronic asthma severity score, JHAAC, was 56 (range: 15–87.5) [7]. The median length of hospital stay was 3.25 days (range: 2.5–4.5). Five subjects (63%) were atopic by prick skin testing performed during the convalescent follow-up visit. The median IgE level was 81.25 IU/ml (range: 5.5–546) (Table I). None of the subjects had an identified bacterial infection during their asthma hospitalization; however, six of them were prescribed antibiotics.

*Control subjects.* Among the eight control subjects, the $FEV_1$ (% predicted) at the time of the study was $73.1 \pm 15.75$. The mean age of these eight asthmatics was $47 \pm 8$ years. Seven (88%) subjects were white and one (12%) was black. Five (63%) subjects were female. Seven of eight subjects used inhaled corticosteroids (88%). Among these seven users of inhaled corticosteroids, four (57%) were regular users and three (43%) were not regular users. Three (37%) had environmental tobacco smoke exposure and/or were former smokers, five (63%) were never smokers, none were current smokers. Four (50%) had severe persistent asthma, three (38%) moderate persistent and one (12%) mild persistent based on the GINA guidelines. The median chronic asthma severity score was 34 (range: 15–87.5). All subjects were prick skin tested and six (75%) were atopic. Their median IgE level was 39.5 IU/ml (range: 9.5–546) (Table I). All demographic characteristics were compared between the hospitalized and stable asthma group and there were no significant differences between the two groups ($p > 0.05$).

*Urinary $F_2$-IsoP-M analysis.* During asthma exacerbations requiring hospitalization, subjects had elevated $F_2$-IsoP-M concentrations at hospital admission, with a median of 4.12 ng/Cr mg (range: 1.89–7.8), which is significantly higher than that of the stable asthmatics (0.68 ng/Cr mg (range: 0.31–1.5), $p = 0.0002$) (Figure 1) and the known mean values ($\pm 2$ SD) for persons without asthma ($0.39 \pm 0.18$) [15]. The $F_2$-IsoP-M concentration decreased significantly during hospitalization to 1.42 ng/Cr mg (range: 0.7–4.44) at hospital discharge ($p = 0.0036$). Urinary $F_2$-IsoP-M concentrations significantly increased again to 2.47 ng/Cr mg (range: 1.56–6.86) at the 3-month convalescent follow-up visit ($p = 0.0002$). Although urinary $F_2$-IsoP-M concentrations tended to be lower at 3-month follow-up, there was no significant difference between admission and follow-up urinary $F_2$-IsoP-M concentrations

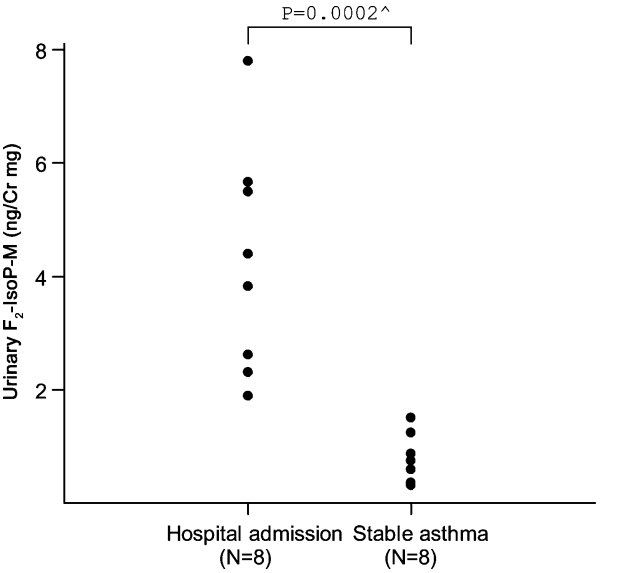


Figure 1. Scatter plot of urinary $F_2$-IsoP-M concentrations in eight adult subjects with asthma at hospital admission for an asthma exacerbation and in eight adult subjects with stable asthma and no recent exacerbation. ^ $p$-value was obtained from Mann-Whitney U-test.

($p = 0.32$) (Figure 2). There were significant differences between both hospital admission and 3-month follow-up urinary $F_2$-IsoP-M concentrations compared with the group of stable asthmatics ($p = 0.0002$ and 0.0008, respectively). We also analysed $F_2$-IsoP-M change over time among non-smokers and the results did not change.

The mean $FEV_1$ (% predicted) was $58.5 \pm 16.01$, $64.3 \pm 18.73$ and $75.1 \pm 22.31$, at hospital admission,

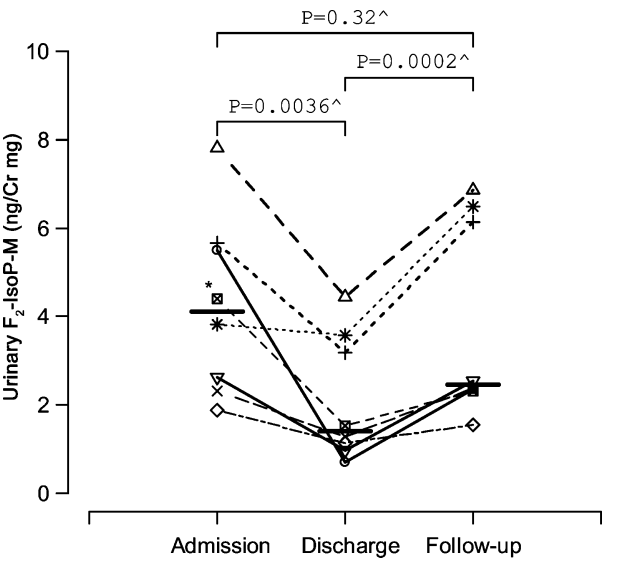


Figure 2. Scatter plot of urinary $F_2$-IsoP-M concentrations in eight adult subjects with asthma at hospital admission, hospital discharge and 3-month convalescent follow-up. Bold bar at each time point represents the median urinary $F_2$-IsoP-M concentration for subjects within each panel. The single subject who was a current smoker is labelled with an asterisk. ^ $p$-values were obtained from a linear mixed effect model.

discharge and 3-month follow-up, respectively (Table I). Subject's $FEV_1$ was significantly improved at the 3-month convalescent follow-up visit compared with hospital discharge ($p = 0.024$). There was no significant correlation between $FEV_1$ (% predicted) and $F_2$-IsoP-M concentrations.

Since our control group also included men and our hospitalized study group included only women, $F_2$-IsoP-M concentrations between men and women with stable asthma were compared to identify potential $F_2$-IsoP-M differences due to gender. There were no $F_2$-IsoP-M differences by gender ($p > 0.05$), which is also supported by analyses of $F_2$-IsoP-M concentrations from normal subjects in which no gender differences have been reported [15].

## Discussion

This study demonstrates the changes in oxidant stress during and following acute asthma exacerbations. Oxidant stress as measured by urinary $F_2$-IsoP-M concentrations was approximately 6-fold higher during an acute asthma exacerbation compared to subjects without recent asthma exacerbations. We observed a significant reduction in oxidant stress during asthma hospitalization. We hypothesized that one of the potential mechanisms for the acute decrease in urinary $F_2$-IsoP-M concentrations observed during hospitalization could be the known anti-oxidant effect of high dose systemic corticosteroids. Corticosteroids can reduce the number of activated eosinophils in the airways, thereby reducing the oxidant burden and resulting in an improvement in oxidative stress biomarkers. In addition, systemic corticosteroids have been reported to significantly reduce malondialdehyde levels and increase glutathione levels in exhaled breath condensate of children with asthma exacerbations, indicating that systemic corticosteroids may have an antioxidant effect during asthma exacerbations [16]. Inhaled corticosteroids have also been observed to result in increases in markers of antioxidants in subjects with asthma and other chronic obstructive pulmonary diseases [17,18]. A potential antioxidant effect of corticosteroids is supported by the known antioxidant effect of lazaroids, a group of 21-aminosteroids possessing the membrane stabilizing effects of the glucocorticoids without the receptor dependent side effects. Lazaroids have been shown to inhibit lipid peroxidation through free radical scavenging and membrane stabilization which in turn leads to reduction of $F_2$-IsoP generation [19].

The 3-month timepoint for the convalescent follow-up was chosen as we expected urinary $F_2$-IsoP-M concentrations to normalize and that this timepoint would represent a return to baseline, as suggested by investigations of measures of airway inflammation [2]. Unexpectedly, urinary $F_2$-IsoP-M concentrations were higher at the 3-month convalescent follow-up visit compared with hospital discharge, despite improved spirometry and clinical symptoms that were comparable to those in subjects with stable asthma. We speculate that the persistently elevated $F_2$-IsoP-M concentrations at this timepoint might be an indication of ongoing systemic oxidant stress, despite clinical improvement; and the significant decline in urinary $F_2$-IsoP-M concentrations seen during hospitalization could be due to systemic corticosteroid administration during hospital admission [20].

We also found no significant correlation between $FEV_1$ (% predicted) and $F_2$-IsoP-M concentrations, nor did changes in oxidant stress, as reflected by urinary $F_2$-IsoP-M, correlate with spirometric improvement over time. The implications of this are that clinical improvement, as measured by spirometry, does not fully correlate with systemic oxidant stress in subjects with asthma.

It is certainly feasible that there are compartmentalized differences, systemic vs lung, that could account for these differences, as well as the likelihood that oxidative measures do not necessarily correlate with measures of airway function. In a study examining the effect of daily vs as-needed corticosteroids for mild persistent asthma, Boushey et al. [21] reported that daily budesonide improved markers of airway oxidant stress, such as bronchial reactivity, the percentage of eosinophils in sputum and exhaled nitric oxide, while no difference in the change in post-bronchodilator $FEV_1$ was detected. Change in $FEV_1$ after fluticasone and montelukast treatments also did not correlate with changes in exhaled nitric oxide, blood total eosinophil counts, serum eosinophil cationic protein levels, serum IgE, nor urinary leukotriene $E_4$ levels [22]. This data also supports that our objective clinical measures may not provide information that correlates with airway and/or systemic oxidative stress. As new strategies to measure airway oxidative stress evolve and the need for objective measures by which to titrate anti-oxidative and/or anti-inflammatory therapy for chronic asthma is recognized, simple non-invasive measures such as urinary $F_2$-IsoP-M measurements, for which there is now a throughput assay available, may be useful [23–27].

There are several potential limitations of this study that should be considered. We did not determine the reason for the significant drop in oxidant stress measures during hospitalizations for asthma exacerbations, as all subjects received standard therapy including intravenous corticosteroids and $\beta$-agonists. Thus, the dramatic decrease in urinary $F_2$-IsoP-M we observed during hospitalization could be meditated by intravenous corticosteroids as we have hypothesized, but could also be mediated by $\beta$-agonists, the interaction of $\beta$-agonists and corticosteroids or another mechanism [28,29]. Additionally,

we only measured urinary $F_2$-IsoP-M at three time points; additional measurements throughout the period of hospitalization and longitudinally during the follow-up period would help us better understand oxidant stress changes over time during and following an exacerbation. Smoking might additionally affect urinary $F_2$-IsoP-M levels, which we could not detect due to the small sample size and the limited number of smokers in the study. However, analysis excluding the smoker indicates that our finding is not affected by smoking status. The small sample size and the large among subject variation also limits our ability to detect moderate changes in $F_2$-IsoP-M concentration at measured time points during asthma hospitalizations. Several other factors, including diet, vitamins, hypercholesterolemia and iron supplements, are potential unmeasured factors influencing $F_2$-IsoP-M concentrations.

We studied, and report for the first time, the changes of urinary $F_2$-IsoP-M, a non-invasive marker of oxidant stress, and report acute and significant decreases in this biomarker during asthma hospitalizations. However, urinary $F_2$-IsoP-M concentrations 3 months after the asthma exacerbation were significantly higher than at the time of hospital discharge, despite clinical and spirometric improvement. The presence of clinically 'silent' increased and persistent oxidant stress following an acute disease exacerbation suggests there is ongoing oxidant stress and perhaps inflammation which is not measured by routine spirometry nor symptoms. As we seek to find biomarkers for airway inflammation and remodelling, urinary $F_2$-IsoP-M may be a useful measure for future studies, providing information that spirometry and symptoms, and perhaps even compartmentalized airway inflammatory measures, do not.



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