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Isoprostanes and Asthma

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Abstract

Isoprostanes are prostaglandin (PG)-like compounds generated *in vivo* following oxidative stress by non-enzymatic peroxidation of polyunsaturated fatty acids, including arachidonic acid. They are named based on their prostane ring structure and by the localization of hydroxyl groups on the carbon side chain; these structural differences result in a broad array of isoprostane molecules with varying biological properties. Generation of specific isoprostanes is also regulated by host cell redox conditions; reducing conditions favor F_2-isoprostane production while under conditions with deficient antioxidant capacity, D_2- and E_2-isoprostanes are formed. F_2-isoprostanes (F_2-isoP) are considered reliable markers of oxidative stress in pulmonary diseases including asthma. Importantly, F_2-isoP and other isoprostanes function as ligands for PG receptors, and potentially other receptors that have not yet been identified. They have been reported to have important biological properties in many organs. In the lung, isoprostanes regulate cellular processes affecting airway smooth muscle tone, neural secretion, epithelial ion flux, endothelial cell adhesion and permeability, and macrophage adhesion and function. In this review, we will summarize the evidence that F_2-isoP functions as a marker of oxidative stress in asthma, and that F_2-isoP and other isoprostanes exert biological effects that contribute to the pathogenesis of asthma.

Keywords

isoprostane; cyclopentenone; oxidative stress; asthma; airway smooth muscle

Introduction

Isoprostanes are PG-like compounds derived from free radical peroxidation of polyunsaturated fatty acids (primarily arachidonic acid) in tissues. They are isomers of enzymatically-derived PGs, but in contrast to PGs, cyclooxygenases (COX-1 and COX-2) are not required for isoprostane formation. Such peroxidation reactions lead to the formation of many different structures named for the prostane ring structure they most closely resemble, and regioisomers are named for the location of a hydroxyl group on the alkyl chain [1]. One class of isoprostanes in particular, the F_2-isoprostanes (F_2-isoP) are stable molecules that are formed *in vivo* under conditions of oxidative stress. F_2-isoPs are considered reliable markers of oxidative stress in disease, but in addition, they have important biological properties in many tissues [2] including the lung [3]. In this review, we

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will summarize the evidence that F_2-isoP functions as a marker of oxidative stress in asthma, and that F_2-isoP and potentially other isoprostanes exert biological effects that contribute to the pathogenesis of asthma.

Isoprostane Biochemistry

The first class of isoprostanes discovered was the F_2-isoprostanes (F_2-isoP) named because they have the same F-type prostane ring as $PGF_{2\alpha}$. Since this discovery, several other classes of isoprostanes were discovered and named for the PG with a common prostane ring structure including D_2-isoP, E_2-isoP, J_2-isoP, and A_2-isoP [1]. There are a few key distinctions between isoprostanes and PGs. Isoprostane side-chains are mainly *cis* to the cyclopentane ring, while PG side chains are *trans* isomers [1, 4]. Isoprostanes are formed from polyunsaturated fatty acids including arachidonic acid *in situ* in lipid membranes and the isoprostanes are then released by phospholipases. In contrast, PGs are formed from free arachidonic acid. Recently, it has been reported that isoprostanes may be formed from docosahexaenoic acid or eicosapentaenoic acid in addition to arachidonic acid [5–7].

Isoprostane structure is dictated not only by the substrate polyunsaturated acid but also by cellular conditions during the peroxidation process. The net formation of F_2-isoP versus D_2-isoP and E_2-isoP is controlled by the reduction-oxidation status of the cell, the presence or absence of glutathione, and by the presence of oxidative stress [8]. Under reducing conditions, F_2-isoP is favored, while under oxidizing conditions, isoprostane formation is shifted to produce D_2- and E_2-isoP. We have recently reported that host genetic factors also regulate F_2-isoP generation in response to ozone. The loss of NADPH quinone oxidoreductase 1 (NQO1), an enzyme that reduces cellular hydroquinones, results in decreased F_2-isoP formation following ozone exposure [9]. Importantly, a shift in isoprostane production from F_2-isoP to D_2- and E_2-isoP may have profound effects on cellular functions. D_2- and E_2-isoP are highly reactive and undergo dehydration to form cyclopentenones, J_2-isoP and A_2-isoP respectively [4] (Figure 1). The cyclopentenone ring is highly reactive and forms Michael adducts with Cys residues in proteins or glutathione; these modifications may affect enzyme activities resulting in several different biological effects including augmenting antioxidant capacity [10] and inhibiting inflammatory cascades [10, 11].

The production and metabolism of isoprostanes have been reported in most detail for 15-F_{2t}-isoP. Arachidonic acid is exposed to oxygen radicals resulting in the abstraction of a bisallylic hydrogen atom, and formation of a lipid peroxide radical and endocyclization. An additional oxygen molecule is added to form an unstable bicyclic endoperoxide intermediate. After reduction, four potential F_2-isoP families are formed, which are designated as 5-, 8-, 12-, or 15-series regioisomers based on the location of the side chain hydroxyl residue [12] (Figure 1). Each regioisomers has 16 stereoisomers yielding a large variety of structures. The esterified isoprostanes are released as free molecules by phospholipase A_2 or platelet activating factor acetylhydrolase activity, and are rapidly released into the circulation, and excreted into the urine. F_2-isoP is metabolized by an enzymatic process similar to the PG degradation pathway. Beta-oxidation and reduction result in F_2-isoP degradation products including 2,3-dinor-8-iso$PF_{2\alpha}$ and 2,3-dinor-5,6-dihydro-8-PG $F_{2\alpha}$; these degradation products and the parent molecule F_2-isoP can be detected and quantified in urine [12, 13]. Of all of the F_2-isoP regioisomers, 15-F_{2t}-isoP (also known as 8-iso-$PGF_{2\alpha}$ or 8-epi-$PGF_{2\alpha}$) has received most attention due to its utility as a biomarker of oxidative stress [12, 14].

15-F_{2t}-isoP can be quantified by EIA, RIA, and by GC-MS or by LC or UPLC followed by tandem MS [12, 15]. Urinary 15-F_{2t}-isoP and its oxidation/ reduction metabolites require

GC-MS or LC-tandem MS for quantitation of all three isoP species. Immunoassays are validated to exclude other isoprostanes or PGs but are less specific and sensitive than MS [12]. GC-MS or LC-MS permits high throughput analysis with little derivatization of samples but is more expensive and requires skilled technicians [12]. Interpretation of F_2-isoP as a biomarker of oxidative stress requires consideration of the disease process to determine whether the timing of sample collection, and the source of the biological sample will provide an accurate reflection of oxidative stress [16].

15-F_{2t}-isoP, a Biomarker of Oxidative Stress

15-F_{2t}-isoP is increased following exposure to environmental pollutants or during exacerbations of inflammatory diseases, and can be quantified in many tissues and body fluids including urine, bronchoalveolar lavage, exhaled breath condensate (EBC), and plasma. Ozone and tobacco smoke increase lung oxidative stress as detected by 15-F_{2t}-isoP even in healthy individuals. In healthy young adults, acute laboratory exposures of ozone (0.40 ppm, 2 h) immediately decreased airflow (FEV_1), and at 4 h post-exposure, increased EBC levels of 15-F_{2t}-isoP [17]. Similar results were obtained by Alfaro and colleagues [18], except that they demonstrated that in addition to the healthy subjects susceptible to ozone, there is another group of healthy subjects who are not sensitive to ozone and thus are resistant to developing airway obstruction or elevated 15-F_{2t}-isoP in EBC. Importantly, in healthy individuals, cumulative ozone exposure (average 0.043 ppm per month) in association with increased particulate matter with an aerodynamic diameter of ≤ 10 microns (PM_{10}) and nitrogen dioxide, is associated with increased plasma F_2-isoP [19]. In healthy individuals, tobacco smoking increases sputum F_2-isoP [20], plasma F_2-isoP, and urinary metabolites of F_2-isoP [21]. Cumulative exposure of non-smokers to second-hand tobacco smoke exposure (30 cigarettes smoked/ h; 1 h exposure per day for 12 days) results in increased plasma F_2-isoP [22].

15-F_{2t}-isoP levels are significantly increased in diverse human disease conditions which share increased oxidative stress as a common pathologic feature including cardiovascular diseases, chronic liver disease, chronic renal disease, neurodegenerative diseases, type 2 diabetes mellitus, obesity, and inflammatory diseases such as arthritis [12]. Elevated 15-F_{2t}-isoP has also been a consistent biomarker of inflammatory pulmonary diseases including ARDS, CF, pulmonary hypertension, chronic obstructive pulmonary disease, interstitial lung disease, and asthma [12].

Asthma and Oxidative Stress

Asthma is one of the most common chronic pulmonary diseases, affecting approximately 10% of the population. It is characterized by reversible airflow obstruction, airway remodeling, and inflammation which is biased toward Th2 cytokine production and involves mast cells, eosinophils, lymphocytes, macrophages and neutrophils [23]. An important pathogenic feature of the disease is the imbalance of oxidative stress and antioxidant capacity resulting in the generation of reactive oxygen species and reactive nitrogen species that are both biomarkers of disease activity and effectors of disease progression [24].

15-F_{2t}-isoP has been consistently elevated in exhaled breath condensate, plasma and urine in asthma compared to healthy controls (Table 1). Importantly, these results have been confirmed by other biomarkers of oxidative stress in respiratory secretions, plasma and urine including nitrotyrosine, 3-bromotyrosine [25], NO [24], and GSH: GSSG ratio [26] and leukotrienes (Table 1). 15-F_{2t}-isoP has been reported to be increased in children with asthma and not affected by inhaled corticosteroid (ICS) therapy [27, 28]. 15-F_{2t}-isoP is increased following allergen challenge [29], following bacterial bronchitis [30], following eosinophilic inflammation [31], and in exercise-induced bronchospasm [32]. 15-F_{2t}-isoP varies by

asthma severity [25]; it increases during exacerbations of asthma [27] and decreases when allergen triggers such as house dust mite (HDM) are avoided [33]. Overall, elevated levels of 15-F_{2t}-isoP in asthma may reflect acute exposures to oxidants that trigger asthma such as pollutants or tobacco smoke. Alternatively, elevated levels of 15-F_{2t}-isoP may directly reflect the inflammation associated with exacerbations of asthma due to infections or allergens. Increased levels of 15-F_{2t}-isoP or the urinary metabolite, 2,3-dinor-8-epi$PGF_{2\alpha}$ are stable markers that reflect oxidative stress. Although there is no evidence that changes in isoprostane levels precede immediate airflow obstruction, the sustained increase in isoprostanes may regulate longer-term airway remodeling or airway smooth muscle function.

Asthma and the Biological Impact of Isoprostanes

Asthma is defined as recurrent exacerbations of reversible airflow obstruction related to bronchospasm in response to allergen exposures, infections, or pollutant exposures. Over time, asthmatic airways undergo remodeling that contributes to impaired physiology; these airway changes are characterized by mucous cell metaplasia and regions of epithelial loss; subepithelial influx of myofibroblasts and collagen deposition resulting in basement membrane thickening; and angiogenesis. During exacerbations of asthma, inflammatory cells in the airway, including eosinophils, Th_2 lymphocytes, neutrophils, macrophages and mast cells, are activated and release mediators that interact with structural cells to trigger airway hyperresponsiveness and remodeling [34]. Interactions between environmental triggers, inflammatory cells and structural cells result in the generation of reactive oxygen species including the generation of isoprostanes. Recent reports suggest that isoprostanes have biologic functions that contribute to pathophysiologic changes in asthma.

Isoprostanes and Airway Smooth Muscle Signaling

Given the structural similarity of isoprostanes to prostanoids, it is predicted that isoprostanes can influence ASM function through activation of prostanoid receptors. 15-F_{2t}-isoP activates thromboxane receptor (TP) [35, 36] and possibly a distinct yet uncharacterized specific receptor [37], while 15-E_{2t}-isoP activates TP, PGF receptor (FP), and $PGE_{1–4}$ receptors ($EP_{1–4}$) [38]. $EP_{1–4}$ receptors compete for ligand binding and stimulate distinct signaling pathways, resulting in a broad range of physiologic effects from bronchoconstriction to bronchial smooth muscle relaxation depending on the concentration of the activating ligand [39].

Isoprostanes regulate human airway smooth muscle (ASM) tone and hyperresponsiveness to stimuli [38, 40]. Both 8-iso-PGE_2 and 8-iso-$PGF_{2\alpha}$ induce airway smooth muscle contractions; although 8-iso-PGE_2 is reported to be 10–100 fold more potent than 8-iso-$PGF_{2\alpha}$. In human and guinea pig airways, ASM contraction to 8-iso-$PGF_{1\alpha}$ (5.9 μM) 8-iso-$PGF_{2\alpha}$ (6.2 μM), and 8-iso-PGE_2 (7.0 μM) is mediated through TP but not through EP receptors [41, 42]. TP receptor activation is functionally coupled to calcium influx [35, 36] and signaling through monomeric G-protein RhoA and its downstream effector RhoA-associated kinase [36, 42, 43]. However, a different isoprostane, 8-iso-$PGF_{3\alpha}$ ($logIC_{50}$=4.9) relaxes human ASM, suggesting that this isoP activates a non-TP receptor [42]. In canine and porcine ASM, activation of specific EP receptors results in bronchodilation [44]. In canine and porcine trachea, E-ring isoprostanes, 8-iso-PGE_1 and 8-iso-PGE_2 with $logIC_{50}$ values of 6.9 and 6.9 respectively, activate EP receptors, and relax ASM following pre-contraction with carbachol [45]. Thus the isoprostane species and the receptor subtypes expressed determine the airway smooth muscle physiologic response.

There are species differences in the action of isoPs to regulate ASM contraction that are regulated by differences in receptor expression. Bovine ASM contracts in response to 8-iso-

PGE_2, but these responses are mediated through a non-TP, non-EP receptor [46]. In canine airways, neither 8-iso-PGE_2 nor 8-iso-$PGF_{2\alpha}$ isoprostanes have an excitatory effect. Neither dog nor porcine airways express TP receptors, therefore none of the isoprostanes evoke constrictor responses in these species [42] [45].

Isoprostanes also regulate ASM tone by regulating prejunctional neuronal acetylcholine secretion. 8-iso-$PGF_{2\alpha}$ acts at the prejunctional neuron to inhibit electrical field stimulated (EFS)- acetylcholine release in guinea pig trachea [47]. E-ring isoprostanes also inhibit EFS-acetylcholine release from prejunctional neurons in guinea pig trachea; this neural effect is inihibited by an EP_3-receptor specific inhibitor [48]. In contrast, in bovine trachea, 15-E_{2t}-isoP augments EFS-acetylcholine release via FP receptors [49]. The effect of isoprotanes on neutrally-mediated ASM contraction has not yet been evaluated in human airway.

Finally, isoprostanes also regulate chemokine production in ASM cells that contribute to the inflammatory cascade. 8-iso-PGE1 and -E_2 augment IL-1β-induced G-CSF in human ASM via cAMP signaling and specific EP_2 and EP_4 receptor activation [50]. This report adds to the growing body of evidence that ASM secrete inflammatory cytokines and contribute to the inflammatory milieu in the airway [51].

Isoprostanes and Inflammatory cells

Macrophages are sentinel immune cells in the lower respiratory tract detecting environmental stress. They play a major role in regulating the innate immune response to environmental pollutants by secreting cytokines to enhance neutrophil chemotaxis [52]. Exposure of human alveolar macrophages to elemental carbon in ultrafine particles induces 8-iso-$PGF_{2\alpha}$ production [53]. Isoprostane (8-iso-$PGF_{2\alpha}$) stimulates human macrophage binding to human venous endothelial cells [54] and increases macrophage IL-8 expression by activating Erk1/2 and p38 MAP kinase signaling [55]. Isoprostanes (8-iso-$PGF_{2\alpha}$ and 8-iso-PGE_2) may further contribute to neutrophilic inflammation by increasing neutrophil adhesion to human venous endothelial cells [56], and by increasing endothelial cell permeability in response to oxidative stress [57].

In contrast to the effects of 8-iso-$PGF_{2\alpha}$ to enhance neutrophilic inflammation in the airway, cyclopentenone isoprostanes blunt NF-κB activated inflammatory cascades. Exposure of macrophages to 15-A_{2t}-isoP inhibits lipopolysaccharide-stimulated NF-κB transcriptional activity, by impairing IκBα degradation [11]. Cyclopentenone isoprostanes inhibit IκK enzyme activity by forming a direct adduct to a susceptible Cys residue [58]. Cyclopentenone isoprostanes may attack susceptible Cys residues on any molecule affecting protein or enzyme function. For example, cyclopentenone isoprostanes modify a Cys domain in Keap1, a protein that sequesters the transcription factor, NF-E2 related factor-2 (Nrf2) in the cytoplasm. Oxidation of Keap1, results in release of Nrf2, translocalization to the nucleus and activation of antioxidant response element (ARE) domains in promoters of Phase II response genes which enhance antioxidant enzyme activities [10]. Together, the mechanisms of cyclopentenone isoprostanes formed under cellular oxidant conditions may stimulate macrophages to respond paradoxically to air pollutants by enhancing antioxidant capacity and inhibiting inflammation [59].

Isoprostanes and Epithelial cells

There are a few reports that isoprostanes regulate anion conductance across epithelial cells. The E-ring isoprostane, 8-iso PGE_2, regulates anion efflux in Calu-3, an epithelial cancer cell line, in part though the activation of the TP receptor [60] and also in part by the activation of the EP_4 prostanoid receptor via PKA and PI3 kinase activation [61]. This conductance is regulated by CFTR [61]. 8-iso PGE_2 regulates chloride transepithelial

conductance in primary bovine tracheal epithelial cells [62] via the EP_4 receptor coupled to adenylate cyclase and soluble guanylate cyclase. In contrast, F-ring isomers do not regulate changes in epithelial short circuit current [62]. Ionic currents at the apical plasma membrane affect airway surface fluid balance and mucus hydration and clearance; all of these factors are important components of the pathophysiology of asthma and CF. Given the potential differences in isoprostane generation and PG receptor expression between these model systems and primary human airway epithelial cells, the function of isoprostanes in primary human airway epithelial cells still needs to be evaluated.

Summary

Isoprostanes are upregulated in asthma under conditions that cause asthma exacerbations such as allergen exposure, infections and air pollutant exposures. It is well established that 8-isoPGF$_{2\alpha}$ is a stable biomarker of oxidative stress that can be detected in BAL, sputum, EBC, blood and urine, and is upregulated in asthma exacerbations following exposures to triggers. However, 8-isoPGF$_{2\alpha}$ is not only a biomarker; this and other isoprostanes have biological functions that have been reported in most airway cells and that potentially contribute to the pathophysiology of asthma. The generation of specific isoprostanes varies depending on the native redox status of the cell and the quantity and duration of environmental oxidative stress. Importantly, different isoprostanes may have opposite functions within a tissue and these functions also vary by the isoprostane receptors present in the tissue. Therefore further investigation is warranted to determine the function of isoprostanes in primary human airway tissues under normal homeostatic conditions and under the stress of triggers that exacerbate asthma.

Research Highlights

- Isoprostanes are non-enzymatic peroxidation products of polyunsaturated fatty acids.
- F2-isoprostane, a stable biomarker of oxidative stress, is increased in asthma.
- Isoprostanes activate receptors to regulate airway and immune functions in asthma.

Abbreviations

ARE	antioxidant response element
ASM	airway smooth muscle
COX	Cyclooxygenase
EFS	electrical field stimulation
F_2-isoP	F_2-isoprostanes
GC	Gas chromatography
HDM	House dust mite
ICS	inhaled corticosteroids
LC	liquid chromatography
MS	mass spectrometry
Nrf2	NF-E2 related factor-2

PG	prostaglandin
RIA	radioimmunoassay
EIA	enzyme-linked immunoassay
UPLC	ultraperformance liquid chromatography

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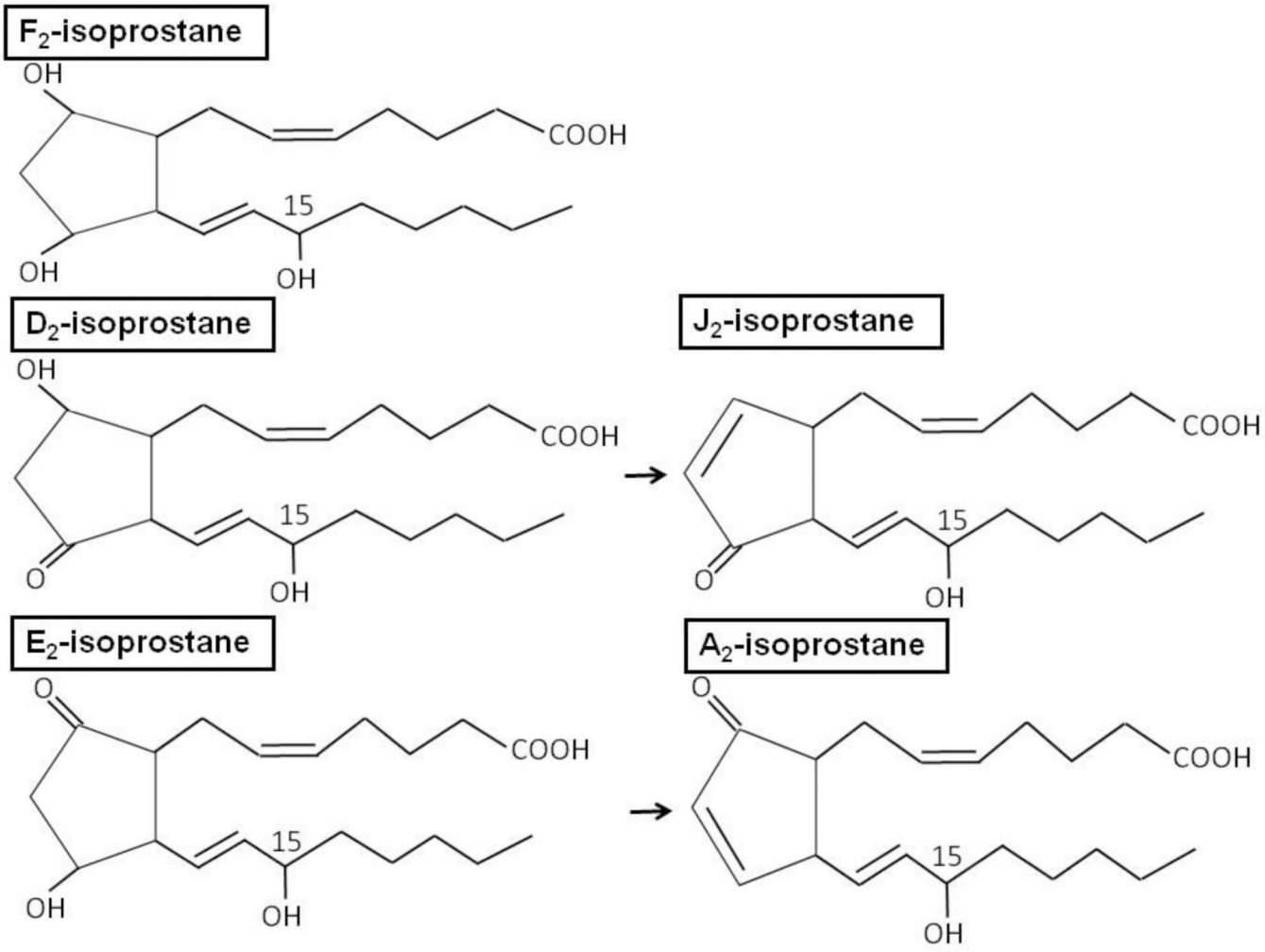

Figure 1.

Isoprostane structure. Four major regioisomers of isoprostanes are formed defined by the location of the hydroxyl group on the alkyl chains. The 15- and 5-hydroxyl forms are the most abundant compared to the 8- and 12- series. The 15- regioisomer is depicted here. F_2- and D_2- and E_2 are all formed from an endoperoxide intermediate derived from peroxidation of arachidonic acid. The D_2 and E_2-isoprostanes have a ketone and hydroxyl group on the cyclopentane ring that are reduced to form the J_2 and A_2-isoprostanes respectively with cyclopentenone rings.

Table 1

F_2-Isoprostane Levels in Asthma

Study Design	F_2-isoP Levels	Reactive oxygen species	Ref.
Adults:			[63]
10 Healthy*	15.8±1.6(pg/ml)[a]	eNO(ppb)[b]:6.5±0.6	
12 Mild Asthma*	33.7±2.8	22.6±2.9	
17 Moderate*	38.3±3.7	11.9±1.3	
15 Severe*	49.1±5.0	10±1.8	
Adults:			[29]
9 Mild Atopic Asthma			
Segmental allergen challenge			
BAL-pre	0.9±0.2 (pg/ml)[c, d]		
24h after	11.4±3		
Urine-pre	1.5±0.2 (ng/mg creat)		
2–8h after	2.6±0.3		
Adults:			[25]
38 Healthy#	23.9 (17.2–48.9)[g, e]	eNO(ppb)[b]:15.9(12–21)	
57 Asthma#	46.9 (28.6–71.5)	41.6(16.4–83.1)	
Children:			[64]
15 Healthy+	139 (109–174)(pg/ml)[e]		
15 Asthma+	213 (122–455)		
Children:			[65]
12 Healthy*	34.2±4.5 (pg/ml)[f]	PGE_2(pg/ml)[f]:46.8±5.3	
12 Asthma/non-steroid*	56.4±7.7	44.6±5.0	
30 Asthma/steroid-treated*	47.2±2.3	51.5±4.0	
Children:		$LTC_4/D_4/E_4$[e]	[27]
10 Healthy*	2.6 (2.1–3.0)(pg/ml)[a,e]	4.3(2–5.7)	
15 Asthma-Exacerbation*	12.0 (9.4–29.5)	12.7 (5.4–15.6)	
Post- prednisone*	8.4 (5.4–11.6)	5.2 (3.9–8.8)	
Children:		$LTC_4/D_4/E_4$[a]	[33]
12 Asthma, + HDM*	17.47±3.18 (pg/ml)[a]	14.24±4.53	
HDM avoidance, 3 months*.	7.36±3.26	4.65±0.68	
Children:		$LTC_4/D_4/E_4$[e]	[66]
19 Healthy*	3.5 (2.6–7.9) (pg/ml)[e]	4.3 (2–8.3)	

Study Design	F_2-isoP Levels	Reactive oxygen species	Ref.
14 Asthma/ non-steroid*	16.2 (11.7–19.1)	10.8 (9.3–13.1)	
13 ICS-treated Asthma*	18.1 (14.8–20.5)	12.7 (9.9–15.1)	
9 Unstable Asthma*	29.7 (4.8–35.1)	106.0 (4.2–218)	
Children:		LTE_4[a,e]	[67]
20 Healthy*	15.5 (14.1–17.5)[f,e]	12.5 (11.6–14.7)	
20 Atopic/non-Asthma*	15.8 (13.9–20.1)	15.9 (12–17.5)	
30 Atopic/Asthma*	29.8 (26–34.3)	28.2 (24.9–35.7)	
25 Atopic/Asthma/ICS*	33 (28.5–35.8)	29 (25–36.6)	
Children:			[28]
11 Healthy*	3.8±0.6 (pg/ml)[a]		
13 Asthma/ non-steroid*	9.3±1.7		
12 ICS-treated Asthma*	6.7±0.7		
Children:			[32]
Asthma	Pre-exercise (pg/ml)[f]		
34 non-EIB*	32.3 (27.6–37)		
12 EIB*	44.9 (38.3–51.5)		

[a] EIA (Cayman Chemical);

[b] Chemiluminescence analyzer;

[c] Gas chromatography/negative-ion chemical ionization mass spectrometry (GC/NICI/MS);

[d] No immediate release of F_2-isoP into BAL after allergen challenge;

[e] median and quartile range;

[f] RIA;

[g] 2,3-dinor-8-epi$PGF_{2\alpha}$, a urinary metabolite of F_2-isoP, determined by HPLC/NICI/MS.

Source of analysis:

* EBC,

+ Plasma,

Urine.