10 tested positive in the PAT—an assay that examines heparin-dependent platelet aggregation when patient plasma is tested against (citrate-anticoagulated) platelet-rich plasma obtained from normal platelet donors. This profile of ELISA-negative—but PAT-positive samples—is not consistent with the "iceberg" model of HIT, which states that platelet-activating HIT antibodies (detected in a functional assay) represent a subset of patients who test positive for anti-PF4/heparin antibodies by ELISA.[7] However, it would appear that the iceberg principle is correct, and the PAT results are wrong. This statement is supported by the authors' observation that for the seven ELISA/PAT discrepant samples that were further tested in the other functional assay (the SRA), all seven yielded negative results. This strongly points to the PAT as having given false-positive test results, a problem that was observed in an earlier study of patients in the ICU.[10] Presumably, elevated fibrinogen levels or other acute-phase reactants common in plasma from critically ill patients cause heparin-dependent platelet aggregation in the PAT system for reasons other than HIT antibodies, a phenomenon that is minimized in the SRA, which uses washed platelets. Notably, of the three other ELISA/PAT discrepant patients who, unfortunately, did not undergo SRA testing, the authors classified two as "likely" HIT based upon 4Ts scores of 5 and 6 points and one as HIT "unlikely" based upon a score of only 3 points. However, it seems more probable that neither of the two patients classified as "likely" HIT actually had this diagnosis, given the poor specificity of a positive PAT result in an ICU population and given the authors' uniformly SRA-negative findings in the remaining patients. Although PAT testing has fallen out of favor in the United States, clinicians who continue to use this assay need to be aware of the risk of a false-positive test result, and they should be skeptical about a diagnosis of HIT when the ELISA result is negative and the PAT result is positive.

Although this transatlantic study of HIT—using a drug not available in the United States (danaparoid) and an assay (PAT) that is being used less and less in North America—provides a different perspective of HIT from that of the US physician, it speaks to the challenges of diagnosing and managing HIT, in whichever continent's ICU the patient resides.

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F_2-Isoprostanes

An Emerging Pulmonary Arterial Hypertension Biomarker and Potential Link to the Metabolic Theory of Pulmonary Arterial Hypertension?

Pulmonary arterial hypertension (PAH) remains deadly despite some available treatment options.[1] Diagnosis and therapeutic intervention tend to occur relatively late, particularly because symptoms are nonspecific and become evident when right ventricular

(RV) dysfunction is already advanced. This, along with the fact that there are now groups of patients who are recognized as higher risk of developing the disease (ie, family members of inheritable PAH, patients with scleroderma or AIDS), makes the need for biomarkers pressing. Unfortunately, biomarkers with strong screening and prognostic value are lacking. Of the known circulating biomarkers, brain natriuretic peptide and troponin T are the most accepted,[1] but both are derived from the right ventricle and, therefore, may only be relevant during RV failure, a relatively late stage of the disease. Moreover, both may also be released by the left ventricle, thereby lacking RV specificity, and are not involved in the pathogenesis of the PAH vascular remodeling. Thus, biomarkers that are relevant to disease pathogenesis and measurable at earlier stages and with some specificity for the pulmonary circulation are needed.

In this issue of *CHEST* (see page 869), Cracowski et al[2] present work in which they prospectively measured several serum and urine biomarkers at the time of diagnosis of 110 patients with incident PAH (appropriately diagnosed by right-sided heart catheterization) from six centers. Of the many biomarkers tested, including N-terminal pro-brain natriuretic peptide, endothelin, asymmetric dimethylarginine, creatinine, 7-ketocholesterol, 7β-hydroxycholesterol, troponin T, troponin I, C-reactive protein, and urine F_2-isoprostanes, only F_2-isoprostanes remained independently associated with an increased 3-year hazard of death. These results suggest that urinary F_2-isoprostanes may represent a biomarker with prognostic potential in PAH.

F_2-isoprostane levels are known to be elevated in patients with PAH.[3,4] Although not studied in the discussed article, another group reported that in an admittedly small sample size, F_2-isoprostane levels can be elevated even in asymptomatic patients who are predisposed to PAH because of bone morphogenetic receptor-2 (BMPR2) loss-of-function mutations[4] (Fig 1A). This suggests that F_2-isoprostanes may potentially mark the very early stages of PAH. Furthermore, they are highly stable and can be measured noninvasively in stored urine.[5] The ability to predict survival, to be elevated at early disease stages, and to be stable in urine samples are very attractive features for a potential PAH biomarker, although the complexity of the technique required to measure them (gas chromatography-mass spectroscopy) is a limiting factor.

What Is the Source of F_2-isoprostanes, and Could They Be Involved in the Pathogenesis of PAH?

F_2-isoprostanes, as prostaglandin isomers, may function as signal transduction molecules on prostanoid receptors, which are involved in PAH pathogenesis and serve as major therapeutic targets.[6] In addition to proinflammatory properties, F_2-isoprostanes can directly stimulate smooth muscle cell contraction and promote endothelial cell release of vasoconstrictive and pro-proliferative factors. However, urinary F_2-isoprostanes did not correlate with mean pulmonary artery pressure or pulmonary vascular resistance in this study[2] or in a previous study of patients with PAH.[3] It is possible that at the time of diagnosis, the vascular remodeling in PAH is completed, and beyond that point, the advancement of the disease clinically and hemodynamically is only driven by the progressive failure of the right ventricle.[7] In that case, F_2-isoprostanes, although involved in PAH pathogenesis and may correlate with histologic degree of vascular remodeling at the early stages of the disease, may lose this ability at more-advanced stages. Obviously, more studies need to prove this point and perhaps extend the early evidence of an increased level of F_2-isoprostanes in asymptomatic BMPR2 mutation carriers.

Another potential limitation of their suitability as biomarkers is that elevated levels of F_2-isoprostanes are associated with a plethora of diseases,[8] suggesting lack of specificity. For example, elevated levels of isoprostanes have been associated with metabolic disorders (obesity, hypercholesterolemia, and type 2 diabetes), neurodegenerative diseases (Huntington, Parkinson, and Alzheimer disease), cancer (prostate), or inflammatory diseases (rheumatoid arthritis and systemic sclerosis). Intriguingly, recent evidence suggests that PAH is pathogenetically associated with inflammation[1,9] and characterized by a general metabolic disturbance (including insulin resistance[10] and mitochondrial remodeling[11-13]) that shares similarities with both cancer[14] and neurodegenerative diseases.[15] Mitochondria not only are the major regulators of cellular metabolism but also are able to induce primary inflammatory signaling.[16] Although there is an impressive clinical diversity among these diseases, could this suggest a more fundamental and perhaps unexplored commonality underlying their pathogenesis, potentially pointing to mitochondria?

Isoprostanes are derived from oxidation of arachidonic acid.[5] This enzyme-independent reaction yields an endoperoxide intermediate, which subsequently produces isoprostanes. Once formed, F_2-isoprostanes are cleaved by enzymes, including phospholipase-A_2, circulate, and are eventually excreted in the urine.[8] Alternatively, circulating F_2-isoprostanes may undergo metabolism by prostaglandin dehydrogenase and Δ^{13}-reductase, enzymes highly expressed in the lung, followed by β-oxidation (which occurs in the mitochondria), producing other metabolites that are subsequently excreted in the urine (Fig 1B). Indeed, the

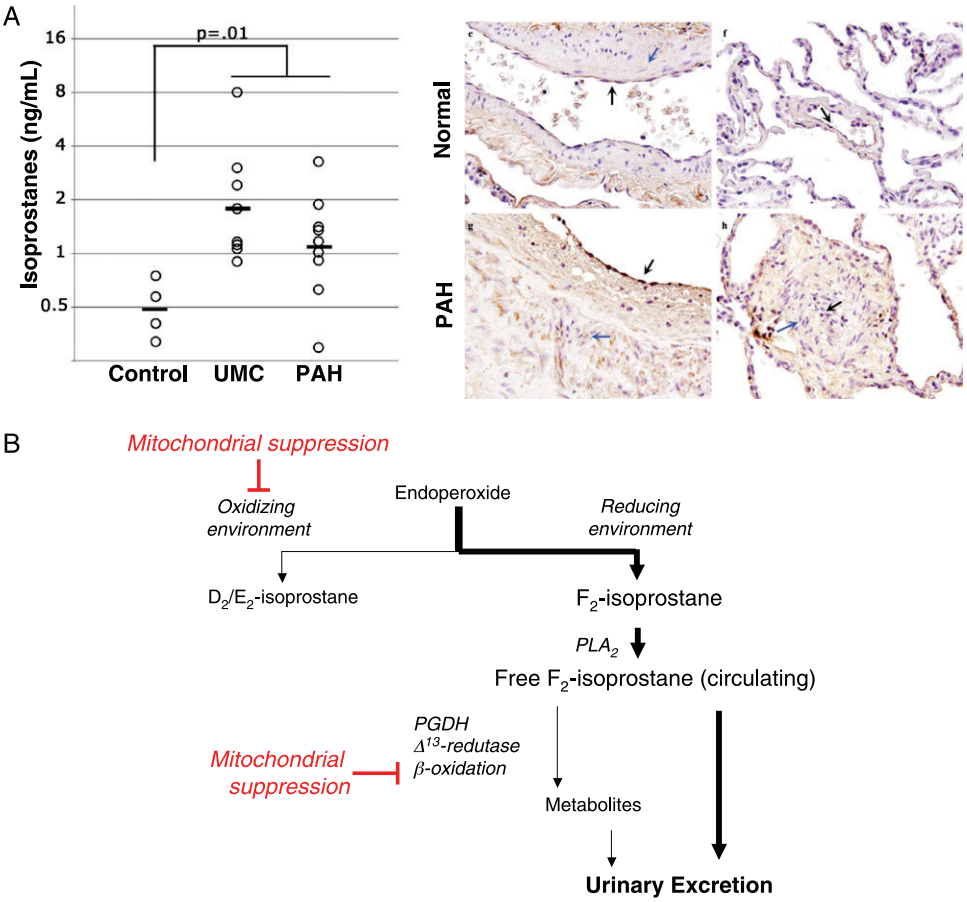

FIGURE 1. F_2-isoprostanes in pulmonary hypertension. A, F_2-isoprostanes are elevated in the urine of patients with idiopathic PAH and UMC compared with control subjects. F_2-isoprostanes (brown) are increased in the pulmonary artery endothelial (black arrows) and smooth muscle cells (blue arrows) in patients with PAH compared with control subjects (magnification ×600). (Modified from Lane et al.[4]) B, Mitochondrial suppression could favor urinary F_2-isoprostanes by reducing mitochondrial reactive oxygen species, promoting a reduced environment that favors F_2-isoprostane synthesis, and suppressing β-oxidation that reduces F_2-isoprostane metabolism. PAH = pulmonary arterial hypertension; PGDH = prostaglandin dehydrogenase; PLA_2 = phospholipase A_2; UMC = unaffected carriers of bone morphogenetic receptor-2 mutations.

majority of infused radiolabled F_2-isoprostanes are excreted as metabolites of β-oxidation within minutes.[17] Because reactive oxygen species (ROS) were initially identified as the trigger for the conversion of arachidonic acid to endoperoxide, F_2-isoprostanes are classically described as an in vivo marker of oxidative stress.[8] Although the term "oxidative stress" is used loosely and remains nonspecific, it tends to be associated with high levels of ROS. Yet, in several animal and human models of PAH, there is an apparent suppression of mitochondrial function and a decreased, not increased, production of mitochondrial ROS in the proliferative and antiapoptotic pulmonary artery smooth muscle cells.[9,11,12] Similar mitochondrial suppression and decrease in ROS is also seen in cancer.[14] Although the initial acute generation of F_2-isoprostanes could be linked to increased ROS production, evidence suggests that excessive and sustained oxidation (where glutathione levels are depleted) favors E_2- and D_2-isoprostanes rather than F_2-isoprostanes.[5,18,19] In other words, F_2-isoprostane production may actually be favored in reduced environments. Thus, the increased F_2-isoprostanes in PAH may be due to both the overall reduced environment and the decreased β-oxidation that result from the suppressed mitochondrial function in the remodeled PAH vasculature (Fig 1B).

A recent report found F_2-isoprostane levels to be elevated in the pulmonary artery wall of patients with inheritable PAH due to BMPR2 mutations (Fig 1A).[4] BMPR2 mutations have been shown to cause cellular mistrafficking,[20] which ignites the endoplasmic reticulum (ER) stress response. ER stress can be caused by many other conditions that trigger PAH, including viral infections, hypoxia, inflammation, or notch signaling.[12] We published evidence for a mechanism by which ER stress induces the reticulin protein Nogo, which causes a disruption of the ER-mitochondria

unit.[12] This results in a decrease in mitochondrial calcium followed by an overall suppression of mitochondrial function, including glucose and β-oxidation. This may be a fundamental mechanism because animals lacking Nogo are completely resistant to pulmonary hypertension.[12] Taken together, these publications show that (1) the remodeled vasculature in PAH can be a source of F_2-isoprostanes, and (2) the suppressed mitochondrial function may explain the increased levels of F_2-isoprostanes in PAH.

Thus, their direct involvement in PAH pathogenesis and their localization in the remodeled pulmonary circulation further improve the profile of F_2-isoprostanes as a potential PAH biomarker. The fact that F_2-isoprostane levels are also increased in other conditions that may coexist in a patient with PAH (ie, cancer, Parkinson disease) on the one hand decreases their specificity but, on the other hand, points to them as a biomarker of a generalized mitochondrial suppression that characterizes these diseases.

Although F_2-isoprostanes were measured at the time of clinical diagnosis, prior to therapeutic intervention, they remained an independent predictor of mortality.[2] We assume that treatment was unbiased and was consistent with the current standard of care because the treating physicians from all six centers were unaware of the biomarker levels. Nevertheless, the type of therapy could, in theory, confound the results; for example, patients receiving more effective therapies, like prostacyclin analogs, could be overrepresented by chance in the low F_2-isoprostane group. Assuming that the type of therapy does not confound the results, this work raises the intriguing, but unfortunate possibility that the currently approved therapies fail to modify the course of the disease. Indeed, there is evidence from recent meta-analysis studies that although these therapies may improve some symptoms, they fail to significantly improve survival.[21] As the authors plan to repeat measurements at a second time point, they will have the opportunity to pursue this possibility. Hopefully, simplifications of the methodology used to measure F_2-isoprostane levels may allow their use in other large and multicenter registries where F_2-isoprostanes levels need to be tested in early stages of the disease.

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Exploring the Adipose Tissue-Lung Interaction in COPD

For many decades, COPD has been approached from a view of respiratory system involvement, and persistent and usually progressive airflow limitation associated with an enhanced chronic inflammatory response in the airways and the lung were characteristic findings. In the last decade, the definition of COPD has evolved to a disease with multicomponent involvement, and comorbidities, which are now assumed to contribute to the overall severity in individual patients.[1] Instead of considering host-related factors to understand the heterogeneity of these comorbid conditions, the unifying concept of spillover of the local inflammation in the respiratory system was launched to understand the development and progression of this spectrum of comorbidities in COPD.

In this issue of *CHEST* (see page 893), Yoon et al[2] analyzed the role of adiponectin on health outcomes in patients with COPD. Adiponectin is almost exclusively synthesized by adipocytes. Adiponectin has a collagen-like domain followed by a globular domain that is similar to complement factor C1q.[3] Adiponectin levels in the plasma and adipose tissue are decreased in obese individuals compared with lean individuals, and the production of adiponectin by adipocytes is inhibited by proinflammatory factors as well as by hypoxia and oxidative stress.[3-5] Yoon et al[2] report that serum adiponectin concentrations are inversely related to hospitalizations and mortality from coronary heart disease and to cardiovascular disease. Serum adiponectin concentrations are not significantly related to total mortality or cancer-related mortality. These data fit with clinical studies reporting an association between low serum levels of adiponectin and coronary artery disease, hypertension, left ventricular hypertrophy, and a greater risk of myocardial infarction.[6-9] Accumulating evidence suggests that adiponectin is a protective adipokine against the development of obesity-linked heart diseases and is a molecular link between adipose and cardiovascular tissues.[10] Furthermore, adiponectin protects the heart from detrimental remodeling and heart failure after myocardial infarction.[11]

More intriguing is the potential link between the adipose tissue and the lungs. Yoon et al[2] report that serum adiponectin levels are positively related to deaths from respiratory causes and that serum adiponectin concentrations are significantly related to increased bronchial reactivity and an accelerated decline in lung function.[2] Their data confirm previous findings that high levels of adiponectin are associated with mortality in patients with respiratory failure.[12] The role of systemic and airway adiponectin in the development and progression of COPD is puzzling. Current data suggest both proinflammatory and antiinflammatory effects of adiponectin. A positive longitudinal association between adiponectin and spirometric lung function has been reported in young healthy adults, possibly by affecting lung growth during early adulthood.[13] In men, systemic adiponectin seems associated with greater COPD severity.[14] Others report no differences in adiponectin levels between patients with COPD and healthy control subjects.[15] Although genetically induced adiponectin-deficient mice demonstrate an abnormal alveolarization, resembling an emphysema-like phenotype,[16] chronic tobacco smoke exposure in wild-type mice increases BAL fluid adiponectin and adiponectin receptor expression on airway epithelial cells.[17] Interestingly, in contrast to subjects with emphysema who have increased levels of BAL adiponectin, current smokers without COPD had reduced BAL adiponectin.[17] Further studies are needed to understand the role of adiponectin in the inflammatory changes in the lungs of patients with COPD.

Reported data by Yoon et al[2] about the relationship between adiponectin and bronchial hyperreactivity require further confirmation. In mouse studies, exogenous adiponectin infusion attenuates bronchial hyperresponsiveness.[18] The role of adiponectin in asthma is still controversial: Some studies demonstrate that serum adiponectin concentrations are inversely associated with asthma prevalence among premenopausal women and peripubertal girls. On the other hand, serum adiponectin concentrations are favorably associated with asthma severity among boys and adversely associated among men.[19,20] Further studies are needed to explore the regulatory processes involved and the effects of certain physiologic conditions on adiponectin modulation of local and systemic inflammation.