**Article title:** Impaired endothelium-dependent vasodilator response in patients with pulmonary fibrosis

**Running title:** Digital reactive hyperemia in pulmonary fibrosis

**Authors:** Kensaku Aihara[a], Tomohiro Handa[a,b], Sonoko Nagai[c], Kiminobu Tanizawa[a], Kohei Ikezoe[a], Kizuku Watanabe[a], Yuichi Chihara[a], Yuka Harada[a], Chikara Yoshimura[d], Toru Oga[d], Neiko Ozasa[e], Kazuko Uno[f], Kazuo Chin[d], Michiaki Mishima[a]

[a]Department of Respiratory Medicine, Kyoto University Graduate School of Medicine

[b]Department of Rehabilitation Medicine, Kyoto University Hospital

[c]Kyoto Central Clinic/Clinical Research Center

[d]Department of Respiratory Care and Sleep Control Medicine, Kyoto University Graduate School of Medicine

[e]Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine

[f]Louis Pasteur Center for Medical Research

**Correspondence to:** Tomohiro Handa

Department of Respiratory Medicine, Kyoto University Graduate School of Medicine,

54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto, 606-8507, Japan

Tel: +81-75-751-3830 Fax: +81-75-751-4643 Email: hanta@kuhp.kyoto-u.ac.jp

**Abstract**

*Background:* Recent epidemiological evidence indicates an association between cardiovascular diseases and pulmonary fibrosis. The vascular endothelium acts to maintain vascular homeostasis through multiple mechanisms and impaired endothelial function can contribute to the development, progression and clinical expression of atherosclerosis.

*Methods:* We consecutively recruited 39 newly-diagnosed chronic interstitial pneumonitis/fibrosis patients without any specific etiology. We assessed endothelium-dependent vasodilator response of patients using digital pulse amplitude tonometry and compared the reactive hyperemia index (RHI) with age-, sex- and body mass index-matched control subjects (n=30). We further investigated the relationships between RHI and clinical characteristics, laboratory cardiovascular risk factors, disease-related factors and circulating levels of inflammatory biomarkers.

*Results:* RHI was significantly lower in patients with chronic interstitial pneumonitis/fibrosis than in control subjects ($p$=0.02). While circulating levels of total cholesterol, triglycerides, HbA1c and fasting glucose did not differ significantly between groups, patients with chronic interstitial pneumonitis/fibrosis had significantly lower high density lipoprotein levels and higher low density lipoprotein levels as

compared with control subjects. Regarding disease-related factors, RHI was significantly associated with the diffusing capacity for carbon monoxide, alveolar-arterial oxygen pressure difference, 6-minute walk distance and end-exercise oxygen saturation. Additionally, circulating levels of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 were inversely correlated with RHI.

*Conclusions:* We confirmed a possible link between pulmonary fibrosis and cardiovascular disease by demonstrating an impairment of endothelium-dependent vasodilator response, which was significantly associated with the severity of pulmonary fibrosis and circulating levels of adhesion molecules.

**Keywords:** digital pulse amplitude tonometry, endothelial function, pulmonary fibrosis, reactive hyperemia

## Introduction

A growing body of epidemiological evidence indicates an association between cardiovascular diseases and pulmonary fibrosis.[1-4] Although most deaths in idiopathic pulmonary fibrosis (IPF) are due to a respiratory cause, cardiovascular events are also reported to be an important cause of mortality.[5,6] There are several possible mechanisms linking pulmonary fibrosis to cardiovascular disease. For example, several cytokines including interleukin-1β (IL-1β), IL-8, tumor necrosis factor-α (TNF-α) and intercellular adhesion molecule-1 (ICAM-1), all of which have been reported to be involved in the pathogenesis of pulmonary fibrosis,[7-9] also play a key role in the process of atherosclerosis.[10] In addition, the clotting cascade is activated in pulmonary fibrosis and has been implicated in its pathogenesis.[11] Furthermore, chronic hypoxic stress, to which patients with pulmonary fibrosis are often exposed, results in irreversible remodeling of the vasculature and surrounding tissues, characterized by smooth muscles proliferation and fibrosis.[12]

The vascular endothelium acts to maintain vascular homeostasis through multiple mechanisms, and alteration in its function precedes the development of morphological atherosclerotic changes.[13] Endothelial dysfunction results in impaired regulation of vascular tone, a prothrombotic state, and increased production of inflammatory

cytokines and adhesion molecules, and hence, it is associated with the risk of future cardiovascular events.[13] Endothelial function has been assessed based on the endothelium-dependent vasodilator response,[13] and measurements of this response using digital pulse volume amplitude has emerged as a noninvasive, automated quantitative test for endothelial function.[14,15]

Based on the epidemiological association between pulmonary fibrosis and cardiovascular disease, we hypothesized that endothelial function would be more impaired among patients with pulmonary fibrosis and that the impairment would not be explained by the presence of classical cardiovascular risk factors. In the present study, we assessed endothelium-dependent vasodilator response in patients with pulmonary fibrosis using digital pulse amplitude tonometry (PAT) and evaluated the relationship between disease-related factors and endothelium-dependent vasodilator response.

## Methods

### *Study subjects*

We consecutively recruited 39 newly-diagnosed idiopathic chronic interstitial pneumonitis/fibrosis patients who visited Kyoto University Hospital. Patients diagnosed

as having any collagen vascular disease or vasculitis, and patients whose lung diseases were potentially caused by drug or occupational-environmental exposures were excluded. None of the patients had other pulmonary diseases or a history of cardiovascular events such as myocardial infarction or stroke, and none were receiving insulin, corticosteroids or supplementary oxygen therapy. IPF was diagnosed on the basis of the current official joint statement on IPF from the American Thoracic Society (ATS), European Respiratory Society (ERS), Japanese Respiratory Society and Latin American Thoracic Association.[16] Age-, sex- and body mass index (BMI)-matched subjects without lung disease or any previous history of cardiovascular events were also recruited as controls. This study was approved by the Ethics Committee of Kyoto University and informed consent was obtained from all patients.

Arterial blood gas analysis, including arterial partial pressure of oxygen ($PaO_2$) and arterial partial pressure of carbon dioxide ($PaCO_2$), was performed while patients were breathing room air at rest in the supine position. The alveolar-arterial oxygen pressure difference ($A\text{-}aDO_2$) was calculated according to the standard formula, using a respiratory exchange ratio of 0.8. The degree of nocturnal hypoxemia was assessed by oxygen desaturation index (ODI) using a pulse oximeter (Pulsox-300i, Konica Minolta Inc., Osaka, Japan). ODI was calculated by dividing the total number of oxygen

desaturations by the total recording time, with desaturation defined as a decrease in $SpO_2$ to 4% or more below the baseline level. Six-minute walk testing was performed as recommended by ATS guidelines.[17]

***Digital PAT***

Endothelium-dependent vasodilator response was assessed by digital PAT in the fasting state, the principle of which has been described previously.[14,15] Briefly, digital pulse amplitude was continuously recorded with the Endo-PAT 2000 device (Itamar Medical Inc., Caesarea, Israel) placed on the tip of each index finger and a blood pressure cuff was placed on one upper arm (study arm), while the contralateral arm served as a control (control arm). After a 5-min equilibration period, the cuff was inflated to 60 mmHg above the systolic blood pressure or 200 mmHg for 5-min and then deflated to induce reactive hyperemia. Pulse amplitude was recorded electronically in both fingers and analyzed by a computerized, automated algorithm that provided the ratio of the average amplitude over a 1-min time interval starting 1.5-min after cuff deflation divided by the average amplitude of a 2.5-min time period before cuff inflation. The calculated ratio reflects the reactive hyperemia index (RHI), with a higher index indicating a higher flow-mediated hyperemic response.

***Blood sample collection and laboratory assessments***

Samples of peripheral venous blood were collected in the morning after an overnight fast. Total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, HbA1c and fasting glucose were measured as classical cardiovascular risk factors. C-reactive protein (CRP) and fibrinogen were also measured as conventional systemic inflammatory biomarkers.

Serum concentrations of IL-1β, IL-6, IL-8, TNF-α, ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) in patients with chronic interstitial pneumonitis/fibrosis were determined using the Bio-Plex Pro Human Cytokine Assay (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions.[18] Cytokine-specific antibody-coated beads were used for these experiments. Beads were read on the Bio-Plex 200 suspension array system, and cytokine concentrations were automatically calculated with Bio-Plex Manager Software by a standard curve derived from a recombinant cytokine standard.

***Pulmonary function tests***

Pulmonary function tests were performed using a CHESTAC system (Chest M.I.

Inc., Tokyo, Japan). The diffusing capacity for carbon monoxide ($DL_{CO}$) was measured using the single-breath technique. Percent-predicted values were used for analyses.

***Visual scoring of high-resolution computed tomography (HRCT)***

Two independent observers (AK and TK) reviewed the three HRCT images taken at the level of the aortic arch, the carina and right inferior pulmonary venous confluence. Each lobe of the lung was scored on a scale of 0-5 for the extents of ground glass opacity (ground-glass score) and fibrotic opacity (fibrosis score).[19] The scores for each lobe were averaged for the two observers for data analyses.

***Statistics***

All statistical analyses were performed using JMP version 9 software (SAS Institute, Cary, NC, USA). Continuous variables are expressed as mean±standard deviation (SD). Comparisons of variables between two groups were made by chi-square tests or unpaired *t*-tests. Relationships between pairs of variables were analyzed by Pearson's correlation coefficient tests. A *p* value less than 0.05 was considered to indicate statistical significance.

## Results

### *Characteristics of patients and controls*

The clinical characteristics of all subjects are summarized in Table 1. Among 39 patients with idiopathic chronic interstitial pneumonitis/fibrosis, 11 had a typical usual interstitial pneumonia (UIP) pattern on HRCT scan. In the remaining 28 patients with HRCT results that were atypical for UIP, 12 patients underwent surgical lung biopsy: 6 had UIP, 1 had nonspecific interstitial pneumonia, and 5 did not meet ATS/ERS histopathologic criteria for a specific diagnosis of idiopathic interstitial pneumonias.[20] There was no statistically significant difference between groups with respect to sex, age, BMI, smoking, blood pressure, heart rate, ODI, prevalence of diabetes and usage rate of antihypertensive agents or statins.

Table 2 shows pulmonary function, arterial blood gas data and laboratory cardiovascular risk factors in patients and control subjects. Forced vital capacity (FVC) and $DL_{CO}$ were significantly lower in chronic interstitial pneumonitis/fibrosis patients than in control subjects (both $p<0.001$) but there were no significant differences in $PaO_2$, $PaCO_2$ or $A\text{-}aDO_2$. Although total cholesterol and triglycerides levels were similar in both groups, HDL levels were significantly lower and LDL levels were significantly

higher in idiopathic chronic interstitial pneumonitis/fibrosis patients than in control subjects ($p$=0.002 and 0.01, respectively). With regard to indices of diabetes, HbA1c and fasting glucose levels were comparable between groups.

***RHI and its related factors***

As shown in Figure 1, RHI of idiopathic chronic interstitial pneumonitis/fibrosis patients was significantly lower than that of control subjects (1.8±0.4 vs 2.1±0.6, $p$=0.02). To identify predisposing factors for impaired endothelium-dependent vasodilator response in idiopathic chronic interstitial pneumonitis/fibrosis patients, we investigated the associations of RHI with clinical characteristics, laboratory cardiovascular risk factors and disease-related factors such as pulmonary function, arterial blood gas data, 6-minute walk testing data and HRCT visual scores. In the entire study population (n=69), RHI was significantly correlated with BMI [$r$ (correlation coefficient) =-0.24, $p$=0.045], but not with other variables (Table 3). Within the patient group (n=39), statistically significant relationship with RHI was not found in age, BMI, smoking, ODI or indices of dyslipidemia and diabetes. Regarding disease-related factors, RHI was significantly correlated with $DL_{CO}$ ($r$=0.42, $p$=0.008), A-a$DO_2$ ($r$=-0.34, $p$=0.04), 6-minute walk distance ($r$=0.38, $p$=0.02), and end-exercise oxygen

saturation ($r$=0.37, $p$=0.03) (Figure 2), but not with $PaCO_2$ ($r$=0.26, $p$=0.13), $PaO_2$ ($r$=0.27, $p$=0.11), FVC ($r$=0.17, $p$=0.29), HRCT ground-glass score ($r$=-0.27, $p$=0.10) or HRCT fibrosis score ($r$=-0.02, $p$=0.89).

Next, we assessed whether significant relationships between RHI and disease severity are also observed in the homogeneous subgroup of IPF-confirmed patients. Pulmonary function, arterial blood gas data, 6-minute walk distance and end-exercise oxygen saturation were not significantly different between IPF-confirmed patients and others. IPF-confirmed patients had less ground-glass and more fibrosis scores on HRCT (Supplementary Table 1). Among IPF-confirmed patients, RHI was significantly correlated with FVC ($r$=0.58, $p$=0.02), $DL_{CO}$ ($r$=0.64, $p$=0.006), A-$aDO_2$ ($r$=-0.50, $p$=0.04), end-exercise oxygen saturation ($r$=0.53, $p$=0.03) and HRCT fibrosis score ($r$=-0.54, $p$=0.03), but not with $PaCO_2$ ($r$=0.41, $p$=0.10), $PaO_2$ ($r$=0.40, $p$=0.12), 6-minute walk distance ($r$=0.36, $p$=0.16) or HRCT ground-glass score ($r$=-0.37, $p$=0.14) (Supplementary Table 2).

***Relationships between RHI and circulating inflammatory biomarkers***

To assess the possible role of systemic inflammation in the impairment of endothelium-dependent vasodilator response in patients with idiopathic chronic

interstitial pneumonitis/fibrosis, we investigated the associations of RHI with circulating levels of inflammatory biomarkers. Since the concentrations of IL-1β, IL-6 and TNF-α were below detectable levels (<1 pg/ml) in more than half of the samples, we conducted further analyses only for CRP (0.4±0.7 mg/dl), fibrinogen (344.9±73.0 mg/dl), IL-8 (5.4±2.6 pg/ml), ICAM-1 (237.3±60.8 ng/ml) and VCAM-1 (135.7±25.3 ng/ml) levels. Significant and near significant negative correlations were found between RHI and serum levels of ICAM-1 ($r$=-0.30, $p$=0.07) and VCAM-1 ($r$=-0.42, $p$=0.008), whereas CRP ($r$=-0.27, $p$=0.10), IL-8 ($r$=0.01, $p$=0.94) and fibrinogen ($r$=0.02, $p$=0.92) levels were not significantly associated with RHI.

**Discussion**

The present study demonstrated a significant impairment of digitally-recorded endothelium-dependent vasodilator response in patients with idiopathic chronic interstitial pneumonitis/fibrosis. This impairment was significantly related to $DL_{CO}$, A-a$DO_2$, 6-minute walk distance and end-exercise oxygen saturation, all of which were major physiologic indices for the severity of pulmonary fibrosis. Additionally, circulating levels of ICAM-1 and VCAM-1 correlated inversely with RHI.

The impairment of endothelium-dependent vasodilator response in patients with pulmonary fibrosis and its correlation with several functional parameters of disease status not only support a possible link between pulmonary fibrosis and cardiovascular disease[1-4] but also imply potential mechanisms for the impairment of vascular function in pulmonary fibrosis. Reduced exercise performance and systemic oxygen desaturation with exercise may lead to sedentary lifestyle or chronic exposures to hypoxia. Suvorava *et al.* showed that forced physical inactivity in young healthy mice induces reduction of endothelium-dependent vasorelaxation and vascular endothelial nitric oxide synthase (eNOS) expression.[21] In addition, diminished eNOS expression and NO release in chronic hypoxic human endothelial cells were also noted.[22] Thus, reduced exercise performance and systemic oxygen desaturation with exercise in pulmonary fibrosis can potentially elicit the impairment of endothelium-dependent vasodilator response via reduced expression of eNOS in the absence of other cardiovascular risk factors.

Dysfunctional endothelial cells can induce increased local production of endothelin-1,[23] angiotensin-II[24] and plasminogen activator inhibitor-1,[25] all of which are involved in the pathogenesis of pulmonary fibrosis.[26-28] Hence, conversely, local endothelial dysfunction in the lung could be associated with the development or progression of fibrosis, thereby possibly resulting in gas change derangement. Although

we evaluated endothelial function in the extrapulmonary systemic circulation, which does not necessarily reflect changes in pulmonary capillaries, it is plausible that, in patients with pulmonary fibrosis, local endothelial dysfunction in the pulmonary microcirculation is present and perpetuates the progression of fibrosis via mediators released by injured endothelial cells.

The recruitment, adhesion and subsequent transendothelial migration of circulating leukocytes are important processes involved in atherosclerosis.[29] These processes are mediated by inflammatory cytokines and adhesion molecules,[10] the expression of which is upregulated in dysfunctional endothelial cells.[13] In our population, the concentrations of IL-1β, IL-6 and TNF-α were below detectable levels in more than half of the samples and circulating levels of CRP, fibrinogen and IL-8 were not significantly associated with RHI. These results suggest that systemic inflammation is less prominent and may play a minor role in the impairment of endothelium-dependent vasodilator response in patients with pulmonary fibrosis. However, we found significant and near significant negative correlations between circulating adhesion molecules and endothelial function, indicating the presence and association of dysfunction and inflammation in the vascular endothelium, although the magnitude might not be great.

Notably, while circulating levels of total cholesterol, triglycerides, HbA1c and

fasting glucose were similar, patients with chronic interstitial pneumonitis/fibrosis had significantly lower HDL levels and higher LDL levels than did control subjects. Although these factors were not significantly related to RHI, they might contribute at least in part to the development of endothelial dysfunction. It is unclear whether impaired lipid metabolism is an outcome of pulmonary fibrosis itself or of comorbid conditions associated with pulmonary fibrosis. Local alterations in the lipid composition of bronchoalveolar lavage fluid were reported in animal models of bleomycin-induced pulmonary fibrosis,[30] as well as in patients with IPF.[31,32] In addition, recent studies showed that obstructive sleep apnea was prevalent in patients with IPF[33] and that chronic intermittent hypoxia can cause circulating lipid levels to increase in relation to the severity of the hypoxic stimulus.[34] Thus, pulmonary fibrosis, comorbid conditions and metabolic alterations may be interrelated, but the underlying mechanisms remain to be elucidated and further studies are needed.

We should mention some of the limitations of the present study. Firstly, our cohort of patients was relatively heterogeneous because it also included some patients other than IPF or those who could not be diagnosed as IPF due to the lack of surgical lung biopsy. Although further studies with more samples of specific lung disease populations are necessary, RHI was also significantly correlated with disease severity including

FVC, $DL_{CO}$, A-a$DO_2$, end-exercise oxygen saturation and HRCT fibrosis score even in the subgroup of IPF-confirmed patients. Secondly, the sample size was small and the impact of pulmonary hypertension, which commonly complicates the course of IPF,[6] was not investigated. Although the relationships of RHI with several classical cardiovascular risk factors were not statistically significant in the present study, there also remains a need for further studies with larger samples to demonstrate that impaired endothelium-dependent vasodilator response in pulmonary fibrosis patients was unlikely to be explained by confounders.

In conclusion, we confirmed a possible link between pulmonary fibrosis and cardiovascular disease by demonstrating an impairment of endothelium-dependent vasodilator response in patients with pulmonary fibrosis. Further knowledge of the presence and pathophysiological relevance of endothelial dysfunction in pulmonary capillaries may provide a more integrated understanding of the mechanisms of pulmonary fibrosis.

**References**

1 Hubbard RB, Smith C, Le Jeune I, Gribbin J, Fogarty AW. The association between idiopathic pulmonary fibrosis and vascular disease: a population-based study. *Am J Respir Crit Care Med* 2008; **178**: 1257-1261.

2 Ponnuswamy A, Manikandan R, Sabetpour A, Keeping IM, Finnerty JP. Association between ischaemic heart disease and interstitial lung disease: a case-control study. *Respir Med* 2009; **103**: 503-507.

3 Izbicki G, Ben-Dor I, Shitrit D, Bendayan D, Aldrich TK, Kornowski R et al. The prevalence of coronary artery disease in end-stage pulmonary disease: is pulmonary fibrosis a risk factor? *Respir Med* 2009; **103**: 1346-1349.

4 Nathan SD, Basavaraj A, Reichner C, Shlobin OA, Ahmad S, Kiernan J et al. Prevalence and impact of coronary artery disease in idiopathic pulmonary fibrosis. *Respir Med* 2010; **104**: 1035-1041.

5 Panos RJ, Mortenson RL, Niccoli SA, King Jr TE. Clinical deterioration in patients with idiopathic pulmonary fibrosis: causes and assessment. *Am J Med* 1990; **88**: 396-404.

6 Hamada K, Nagai S, Tanaka S, Handa T, Shigematsu M, Nagao T et al. Significance of pulmonary arterial pressure and diffusion capacity of the lung as

prognosticator in patients with idiopathic pulmonary fibrosis. *Chest* 2007; **131**: 650-656.

7 Wynn TA. Integrating mechanisms of pulmonary fibrosis. *J Exp Med* 2011; **208**: 1339-1350.

8 Ziegenhagen MW, Zabel P, Zissel G, Schlaak M, Muller-Quernheim J. Serum level of interleukin 8 is elevated in idiopathic pulmonary fibrosis and indicates disease activity. *Am J Respir Crit Care Med* 1998; **157**: 762-768.

9 Nakao A, Hasegawa Y, Tsuchiya Y, Shimokata K. Expression of cell adhesion molecules in the lungs of patients with idiopathic pulmonary fibrosis. *Chest* 1995; **108**: 233-239.

10 Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. *Physiol Rev* 2006; **86**: 515-581.

11 Chambers RC. Role of coagulation cascade proteases in lung repair and fibrosis. *Eur Respir J Suppl* 2003; **44**: 33s-35s.

12 Faller DV. Endothelial cell responses to hypoxic stress. *Clin Exp Pharmacol Physiol* 1999; **26**: 74-84.

13 Widlansky ME, Gokce N, Keaney Jr JF, Vita JA. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol* 2003; **42**: 1149-1160.

14 Matsuzawa Y, Sugiyama S, Sugamura K, Nozaki T, Ohba K, Konishi M et al. Digital assessment of endothelial function and ischemic heart disease in women. *J Am Coll Cardiol* 2010; **55**: 1688-1696.

15 Hamburg NM, Benjamin EJ. Assessment of endothelial function using digital pulse amplitude tonometry. *Trends in Cardiovascular Medicine* 2009; **19**: 6-11.

16 Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011; **183**: 788-824.

17 ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 2002; **166**: 111-117.

18 Kokkonen H, Soderstrom I, Rocklov J, Hallmans G, Lejon K, Rantapaa Dahlqvist S. Up-regulation of cytokines and chemokines predates the onset of rheumatoid arthritis. *Arthritis Rheum* 2010; **62**: 383-391.

19 Kazerooni EA, Martinez FJ, Flint A, Jamadar DA, Gross BH, Spizarny DL et al. Thin-section CT obtained at 10-mm increments versus limited three-level thin-section CT for idiopathic pulmonary fibrosis: correlation with pathologic scoring. *AJR Am J Roentgenol* 1997; **169**: 977-983.

20 American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. *Am J Respir Crit Care Med* 2002; **165**: 277-304.

21 Suvorava T, Lauer N, Kojda G. Physical inactivity causes endothelial dysfunction in healthy young mice. *J Am Coll Cardiol* 2004; **44**: 1320-1327.

22 Ostergaard L, Stankevicius E, Andersen MR, Eskildsen-Helmond Y, Ledet T, Mulvany MJ et al. Diminished NO release in chronic hypoxic human endothelial cells. *American Journal of Physiology Heart and Circulatory Physiology* 2007; **293**: H2894-H2903.

23 Kinlay S, Behrendt D, Wainstein M, Beltrame J, Fang JC, Creager MA et al. Role of endothelin-1 in the active constriction of human atherosclerotic coronary arteries. *Circulation* 2001; **104**: 1114-1118.

24 Dzau VJ. Theodore Cooper Lecture: Tissue angiotensin and pathobiology of vascular disease: a unifying hypothesis. *Hypertension* 2001; **37**: 1047-1052.

25 Kohler HP, Grant PJ. Plasminogen-activator inhibitor type 1 and coronary artery

disease. *N Engl J Med* 2000; **342**: 1792-1801.

26 Park SH, Saleh D, Giaid A, Michel RP. Increased endothelin-1 in bleomycin-induced pulmonary fibrosis and the effect of an endothelin receptor antagonist. *Am J Respir Crit Care Med* 1997; **156**: 600-608.

27 Otsuka M, Takahashi H, Shiratori M, Chiba H, Abe S. Reduction of bleomycin induced lung fibrosis by candesartan cilexetil, an angiotensin II type 1 receptor antagonist. *Thorax* 2004; **59**: 31-38.

28 Senoo T, Hattori N, Tanimoto T, Furonaka M, Ishikawa N, Fujitaka K et al. Suppression of plasminogen activator inhibitor-1 by RNA interference attenuates pulmonary fibrosis. *Thorax* 2010; **65**: 334-340.

29 Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med* 1999; **340**: 115-126.

30 Low RB, Adler KB, Woodcock-Mitchell J, Giancola MS, Vacek PM. Bronchoalveolar lavage lipids during development of bleomycin-induced fibrosis in rats. Relationship to altered epithelial cell morphology. *Am Rev Respir Dis* 1988; **138**: 709-713.

31 Robinson PC, Watters LC, King TE, Mason RJ. Idiopathic pulmonary fibrosis. Abnormalities in bronchoalveolar lavage fluid phospholipids. *Am Rev Respir Dis*

1988; **137**: 585-591.

32 Fireman E, Spitzer S, Grief J, Kivity S, Topilsky M. Cholesterol crystals in BAL fluid from patients with idiopathic pulmonary fibrosis. *Respir Med* 1996; **90**: 361-363.

33 Lancaster LH, Mason WR, Parnell JA, Rice TW, Loyd JE, Milstone AP et al. Obstructive sleep apnea is common in idiopathic pulmonary fibrosis. *Chest* 2009; **136**: 772-778.

34 Li J, Savransky V, Nanayakkara A, Smith PL, O'Donnell CP, Polotsky VY. Hyperlipidemia and lipid peroxidation are dependent on the severity of chronic intermittent hypoxia. *J Appl Physiol* 2007; **102**: 557-563.

**Figure legends**

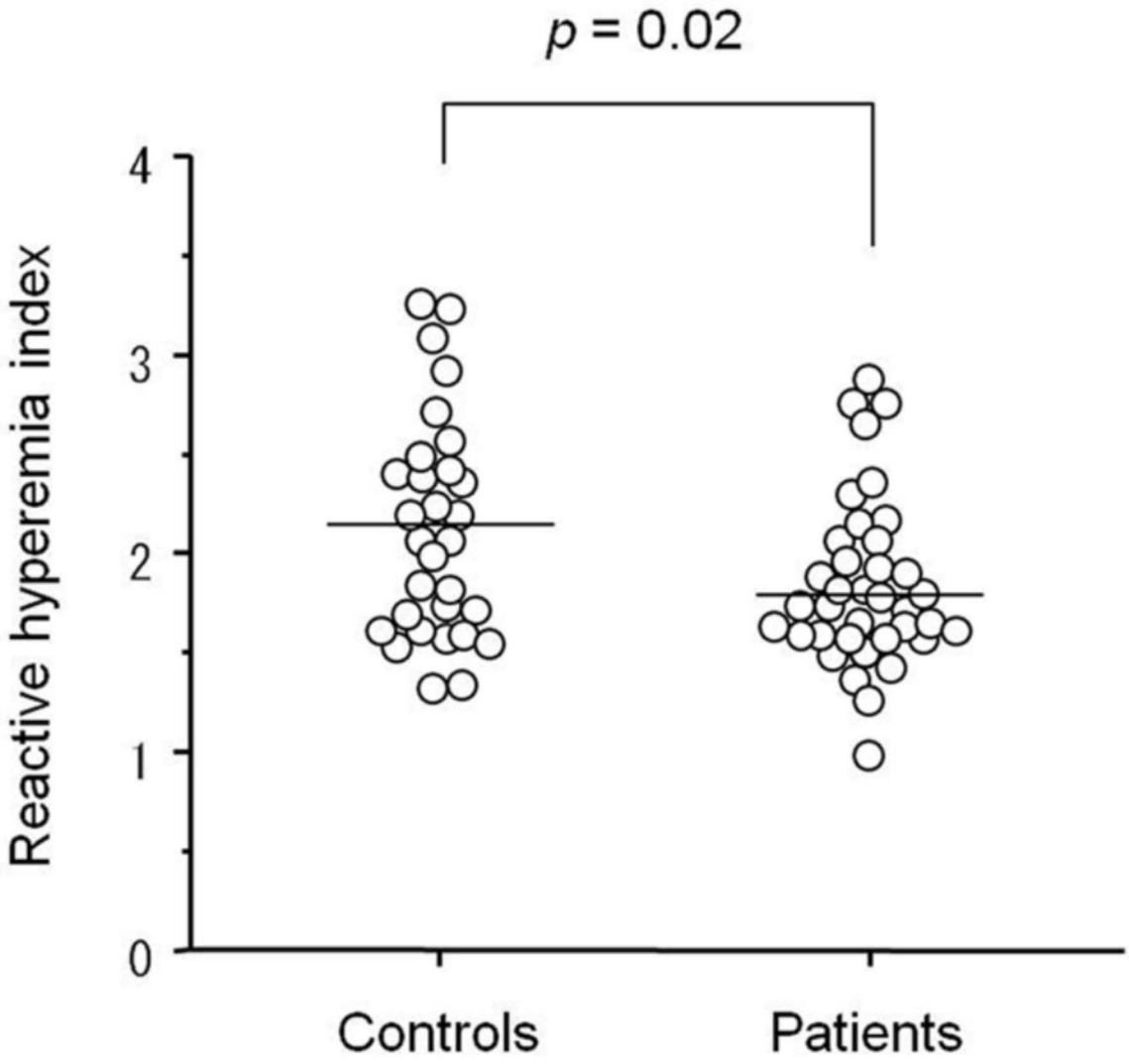
Figure 1.

Comparison of reactive hyperemia index in controls (2.1±0.6) and patients with idiopathic chronic interstitial pneumonitis/fibrosis (1.8±0.4). Horizontal bars indicate mean values.

Figure 2.

Scatter diagrams showing the correlation of reactive hyperemia index with $DL_{CO}$ (a), A-a$DO_2$ (b), 6-minute walk distance (c) and end-exercise oxygen saturation (d) in patients with idiopathic chronic interstitial pneumonitis/fibrosis. The *r* value indicates the correlation coefficient. Lines indicate regression lines.

Figure 1.



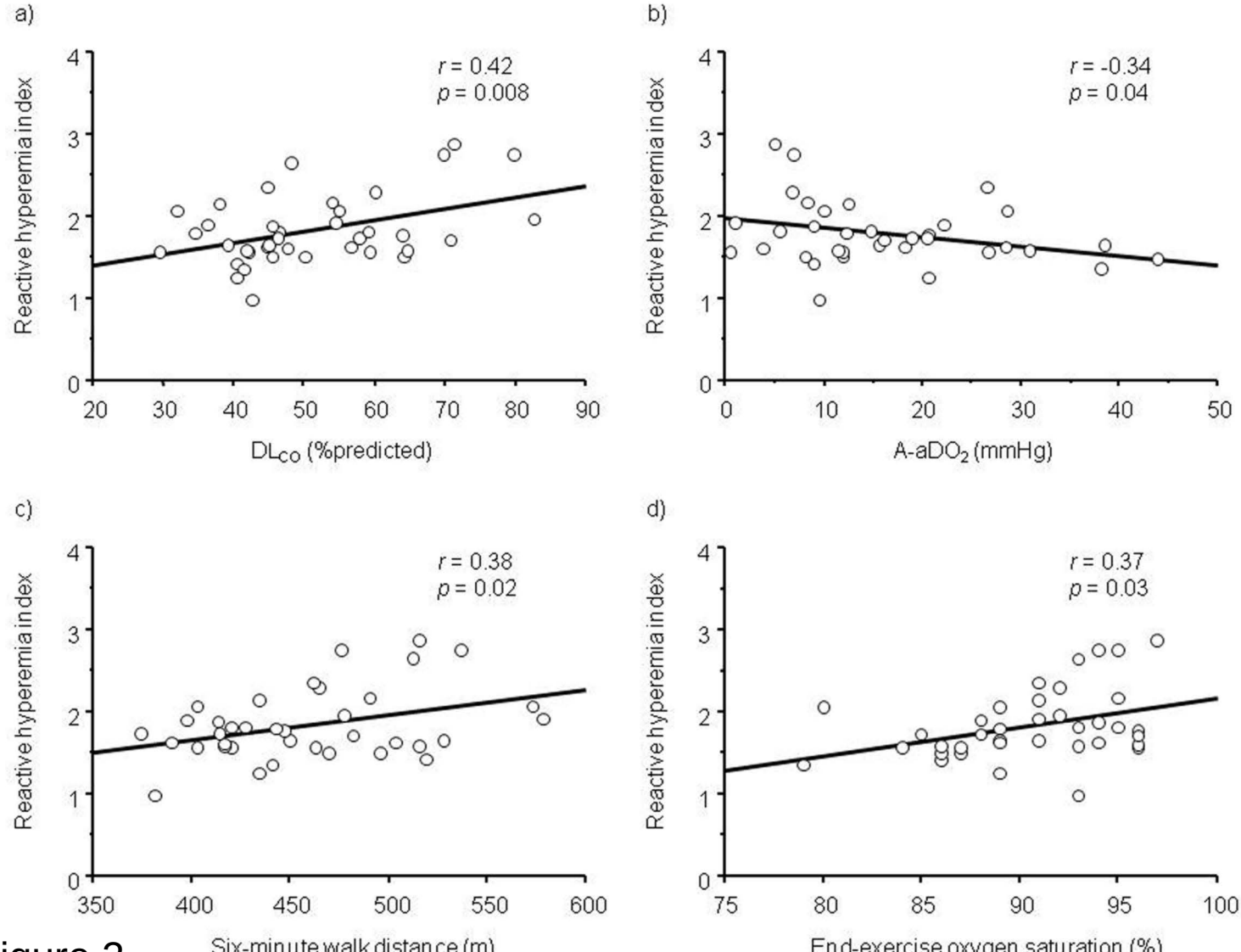

a)
r = 0.42
p = 0.008
Reactive hyperemia index
DLCO (%predicted)
b)
r = -0.34
p = 0.04
Reactive hyperemia index
A-aDO2 (mmHg)
c)
r = 0.38
p = 0.02
Reactive hyperemia index
Six-minute walk distance (m)
d)
r = 0.37
p = 0.03
Reactive hyperemia index
End-exercise oxygen saturation (%)

Figure 2.

**Table 1. Characteristics of patients and controls**

| | Patients (n=39) | Controls (n=30) | *p* value |
|---|---|---|---|
| Sex, male/female | 24/15 | 20/10 | 0.80 |
| Age, years | 65.0±8.5 | 62.7±7.6 | 0.25 |
| BMI, $kg/m^2$ | 24.2±3.4 | 23.2±3.2 | 0.23 |
| Smoking history, current/ex/never | 7/22/10 | 5/11/14 | 0.17 |
| Smoking, pack years | 29.4±27.3 | 17.5±24.3 | 0.07 |
| Systolic blood pressure, mmHg | 123.7±12.2 | 126.5±13.2 | 0.36 |
| Diastolic blood pressure, mmHg | 73.5±9.8 | 77.5±11.0 | 0.12 |
| Heart rate, bpm | 61.2±9.4 | 58.4±5.9 | 0.16 |
| ODI | 3.8±4.0 | 5.1±2.6 | 0.14 |
| Diabetic patients | 6 | 2 | 0.45 |
| Antihypertensive treatment | 9 | 10 | 0.42 |
| Statins use | 6 | 2 | 0.45 |

Data are presented as number or mean±SD.

BMI, body mass index; ODI, oxygen desaturation index.

**Table 2. Pulmonary function, arterial blood gas data and laboratory cardiovascular risk factors**

| | Patients (n=39) | Controls (n=30) | *p* value |
|---|---|---|---|
| *Pulmonary function* | | | |
| FVC, % predicted | 94.9±18.5 | 115.0±18.0 | <0.001 |
| $DL_{CO}$, % predicted | 51.2±13.0 | 87.3±13.2 | <0.001 |
| *Arterial blood gas data* | | | |
| $PaCO_2$, mmHg | 41.0±3.3 | 41.5±3.5 | 0.62 |
| $PaO_2$, mmHg | 83.5±10.7 | 83.4±11.0 | 0.98 |
| A-$aDO_2$, mmHg | 15.2±11.7 | 14.8±12.1 | 0.88 |
| *Laboratory cardiovascular risk factors* | | | |
| Total cholesterol, mg/dl | 208.4±34.2 | 207.0±32.6 | 0.86 |
| HDL, mg/dl | 47.1±11.1 | 57.6±16.3 | 0.002 |
| LDL, mg/dl | 133.7±30.6 | 116.7±20.8 | 0.01 |
| Triglycerides, mg/dl | 127.8±47.9 | 131.9±92.1 | 0.81 |
| HbA1c, % | 5.7±0.8 | 5.5±0.5 | 0.15 |
| Fasting glucose, mg/dl | 105.8±24.9 | 97.1±20.6 | 0.13 |

Data are presented as mean±SD.

FVC, forced vital capacity; $DL_{CO}$, diffusing capacity for carbon monoxide; $PaCO_2$, arterial partial pressure of carbon dioxide; $PaO_2$, arterial partial pressure of oxygen; A-$aDO_2$, alveolar-arterial oxygen pressure difference; HDL, high density lipoprotein; LDL, low density lipoprotein.

**Table 3. Correlation coefficients of reactive hyperemia index with clinical characteristics and laboratory cardiovascular risk factors**

| | All subjects (n=69) | | Patients (n=39) | |
|---|---|---|---|---|
| | *r* value | *p* value | *r* value | *p* value |
| *Patient characteristics* | | | | |
| Age, years | -0.07 | 0.58 | 0.12 | 0.48 |
| BMI, $kg/m^2$ | -0.24 | 0.045 | -0.22 | 0.19 |
| Smoking, pack years | -0.21 | 0.09 | -0.03 | 0.87 |
| ODI | 0.01 | 0.91 | -0.08 | 0.64 |
| *Laboratory cardiovascular risk factors* | | | | |
| Total cholesterol, mg/dl | 0.18 | 0.13 | 0.31 | 0.06 |
| HDL, mg/dl | 0.18 | 0.13 | 0.21 | 0.21 |
| LDL, mg/dl | 0.09 | 0.48 | 0.26 | 0.11 |
| Triglycerides, mg/dl | 0.03 | 0.81 | -0.18 | 0.26 |
| HbA1c, % | -0.12 | 0.34 | -0.05 | 0.77 |
| Fasting glucose, mg/dl | -0.05 | 0.71 | 0.06 | 0.73 |

BMI, body mass index; ODI, oxygen desaturation index; HDL, high density lipoprotein; LDL, low density lipoprotein.

**Supplementary Table 1. Characteristics of IPF-confirmed patients and others**

| | IPF-confirmed (n=17) | Others (n=22) | *p* value |
|---|---|---|---|
| Sex, male/female | 14/3 | 10/12 | 0.02 |
| Age, years | 66.2±5.2 | 64.0±10.4 | 0.44 |
| BMI, $kg/m^2$ | 23.5±2.8 | 24.8±3.8 | 0.23 |
| Smoking history, current/ex/never | 4/12/1 | 3/10/9 | 0.045 |
| Smoking, pack years | 38.4±17.5 | 22.4±31.6 | 0.07 |
| FVC, % predicted | 96.0±15.9 | 94.1±20.5 | 0.76 |
| $DL_{CO}$, % predicted | 51.0±15.1 | 51.4±11.4 | 0.93 |
| $PaCO_2$, mmHg | 41.2±2.5 | 40.9±3.9 | 0.75 |
| $PaO_2$, mmHg | 86.3±8.8 | 81.1±11.8 | 0.14 |
| A-$aDO_2$, mmHg | 12.2±9.7 | 17.8±12.9 | 0.15 |
| Six-minute walk distance, m | 466.8±42.1 | 452.9±60.0 | 0.43 |
| End-exercise oxygen saturation, % | 91.7±3.8 | 89.5±4.8 | 0.14 |
| HRCT ground-glass score | 1.3±0.4 | 1.9±0.9 | 0.02 |
| HRCT fibrosis score | 1.6±0.4 | 1.1±0.4 | <0.001 |

Data are presented as number or mean±SD.

IPF, idiopathic pulmonary fibrosis; BMI, body mass index; FVC, forced vital capacity; $DL_{CO}$, diffusing capacity for carbon monoxide; $PaCO_2$, arterial partial pressure of carbon dioxide; $PaO_2$, arterial partial pressure of oxygen; A-$aDO_2$, alveolar-arterial oxygen pressure difference; HRCT, high-resolution computed tomography.

**Supplementary Table 2. Correlation coefficients of reactive hyperemia index with disease-related factors in IPF-confirmed patients**

| | IPF-confirmed (n=17) | |
|---|---|---|
| | *r* value | *p* value |
| *Pulmonary function* | | |
| FVC, % predicted | 0.58 | 0.02 |
| $DL_{CO}$, % predicted | 0.64 | 0.006 |
| *Arterial blood gas data* | | |
| $PaCO_2$, mmHg | 0.41 | 0.10 |
| $PaO_2$, mmHg | 0.40 | 0.12 |
| A-$aDO_2$, mmHg | -0.50 | 0.04 |
| *Six-minute walk test* | | |
| Six-minute walk distance, m | 0.36 | 0.16 |
| End-exercise oxygen saturation, % | 0.53 | 0.03 |
| *HRCT scores* | | |
| HRCT ground-glass score | -0.37 | 0.14 |
| HRCT fibrosis score | -0.54 | 0.03 |

IPF, idiopathic pulmonary fibrosis; FVC, forced vital capacity; $DL_{CO}$, diffusing capacity for carbon monoxide; $PaCO_2$, arterial partial pressure of carbon dioxide; $PaO_2$, arterial partial pressure of oxygen; A-$aDO_2$, alveolar-arterial oxygen pressure difference; HRCT, high-resolution computed tomography.