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Chronic Elevation of Pulmonary Microvascular Pressure in Chronic Heart Failure Reduces Bi-Directional Pulmonary Fluid Flux

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Aims Chronic heart failure (CHF) leads to pulmonary vascular remodelling and thickening of the alveolocapillary barrier. We examined whether this protective effect may slow resolution of pulmonary oedema consistent with decreased bidirectional fluid flux.

Methods and results Seven weeks following left coronary artery ligation we measured both fluid flux during an acute rise in left atrial pressure ($n = 29$), and intrinsic alveolar fluid clearance ($n = 45$), in the isolated rat lung. Chronic elevation of pulmonary microvascular pressure prevented pulmonary oedema and decreased lung compliance when left atrial pressure was raised to 20 cmH₂O, and was associated with reduced expression of endothelial aquaporin 1 ($P = 0.03$). However, no other changes were found in mediators of fluid flux or cellular fluid channels. In isolated rat lungs, chronic left ventricular dysfunction (left ventricular end-diastolic pressure and infarct circumference) was also inversely related to alveolar fluid clearance ($P \leq 0.001$). The rate of pulmonary oedema reabsorption was estimated by plasma volume expansion in 8 patients with a previous clinical history of chronic heart failure and 8 without, who presented with acute pulmonary oedema. Plasma volume expansion was reduced at 24 hours in those with chronic heart failure ($P = 0.03$).

Conclusions Chronic elevation of pulmonary microvascular pressure in CHF leads to decreased intrinsic bi-directional fluid flux at the alveolar-capillary barrier. This adaptive response defends against alveolar flooding, but may delay resolution of alveolar oedema.

Keywords lung; oedema; mechanics; ion channels; aquaporins.

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Introduction

Acute left ventricular dysfunction usually leads to an acute elevation of pulmonary microvascular pressure (Pmv) followed by acute pulmonary oedema (APO). However, chronic elevations in Pmv lead to muscularisation of the pulmonary circulation, thickening of the alveolocapillary barrier, and a reduction in the capillary filtration coefficient with amelioration of the increase in lung water¹. Indeed, 6 weeks following myocardial infarction in the rat an average left ventricular end diastolic pressure (LVEDP) of 25mmHg, which would be expected to result in APO, leads only to an increase in lung dry weight². In addition to this decrease in fluid egress due to reduced capillary filtration coefficient, an increase in alveolar fluid clearance may also contribute to the dry lung when Pmv is chronically elevated³. However, gas diffusion and alveolar-membrane conductance are reduced in CHF patients⁴ consistent with a thickened alveolocapillary barrier, and reduced solute clearance from the alveolus.

Alveolar fluid is regulated by active ion transport followed by passive water movement⁵. Vectorial ion movement out of the alveolus through the epithelium occurs via apically placed amiloride sensitive cation channels (ENaC) and basolateral ouabain inhibitable Na,K-ATPase, which pump K into the cell and Na out. In addition, aquaporins (AQP), integral membrane proteins found on both epithelial and endothelial cells, function as bi-directional water channels.

As chronic elevation of Pmv leads to lung remodelling that is associated with reduced capillary filtration coefficient we hypothesised that intrinsic alveolar fluid clearance, independent of circulating factors, may be reduced. However, previous studies have suggested both downregulated⁶⁻¹⁰ and upregulated vectorial fluid flux, perhaps due to elevated circulating catecholamines^{11,12}. Therefore the aim of this study was to examine bi-directional fluid regulation, including factors affecting vectorial fluid movement, in the well established rodent

model of chronically elevated Pmv due to left ventricular dysfunction following left coronary artery ligation. As a clinical correlate we examined whether plasma refill following APO¹³ was slowed in patients with known CHF, consistent with slower fluid reabsorption from the alveolus.

Methods

The study protocols conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health, and the principles outlined in the Declaration of Helsinki, and were approved by the Flinders University Animal Welfare Committee and the Southern Adelaide Flinders Clinical Human Research Ethics Committee, respectively. Please refer to the on-line supplement for greater detail.

Cardiorespiratory Assessment

Seven weeks following recovery from left coronary artery ligation², pulmonary effects were investigated in 76 male Sprague-Dawley rats. Intrinsic to the left coronary artery ligation model¹⁴ there was a high mortality rate, (45%) and the sedentary survivors did not appear to have peripheral oedema or dyspnoea in the time leading to euthanasia. Anaesthesia was induced before catheterisation of the left ventricle via the right carotid artery for determination of systemic blood pressure, heart rate and left ventricular end diastolic (LVEDP), as well as arterial blood gas analysis (ABL-5, Radiometer, Copenhagen, Denmark). Left ventricular infarcts were graded topographically² as control (<20% LV infarct circumference, n=46); moderate (20-40%, n=18); and large (>40%, n=12). LVD (left ventricular dysfunction) refers to the combination of moderate and large infarct groups (>20% LV infarct). Animals were divided into two sequential

cohorts for measurement of lung mechanics during elevation of left atrial pressure (cohort 1), or alveolar fluid clearance followed by lavage and tissue assessment (cohort 2).

Elevated Left Atrial Pressure

In isolated perfused lungs (IPL; n=29) lung impedance, following a forced oscillation, was measured sequentially at 0, 5, 10, 15 and 20 cmH₂O left atrial pressure, referenced to the top of the lung, before determination of fluid flux.

Alveolar Fluid Clearance Measurement

In a second cohort of isolated rat lungs (n=47) alveolar fluid clearance (AFC) was measured via Evan's Blue dye concentration calculated as follows:

$$AFC = [(V_i - V_f)/V_i] \times 100$$

where V is the volume of instilled albumin solution (i) and final alveolar fluid (f), and

$$V_f = (V_i \times EB_i)/EB_f$$

where EB is the concentration of Evans blue dye in the instilled albumin solution (i) and final alveolar fluid (f).

Lung Tissue Analysis

Right lung lobes were freeze-dried for measurement of wet-to-dry weight ratio, tissue NO and for α - and β -Na,K-ATPase subunits, α -, β - and γ -ENaC, AQP1, AQP5 and nitric oxide synthase (NOS) mRNA levels by qRT-PCR (Supplementary material online, *Table S1*), before lavage of the remaining lung for assay of soluble proteins².

Immunohistochemistry was used to identify alveolar epithelial type II cells and AQP1

expression².

Clinical subjects

Sixteen consecutive patients, admitted to the Intensive Care Unit with APO, defined as sudden onset of dyspnoea and diaphoresis with tachycardia, tachypnea, hypertension, widespread pulmonary crepitations, and acute respiratory failure, in the absence of fever, were enrolled following informed consent at Flinders Medical Centre, South Australia. Patient demographics, haemoglobin and creatinine, and arterial blood gas were analyzed at ICU presentation and after 24 hours. Patients with significant renal impairment, defined as a baseline creatinine greater than 250 µM/L or receiving chronic dialysis, were excluded. The percentage changes in plasma volume (PV) were calculated using haemoglobin (Hb) before (B) and after (A) treatment¹⁵.

$$\% \text{ change in PV} = 100 \times (1 - (\text{HbA}/\text{HbB}))$$

Statistical Analyses

Statistical analyses were performed using SPSS 18.0 (PASW Inc, Chicago, IL) unless indicated. One-way analysis of variance was used to compare three groups, with between group differences tested with either Tukey's HSD or independent samples *t* test. Bivariant relationships were examined using Pearson's correlation or quadratic regression curve fit model. Repeated measures were predicted using mixed-effects linear regression (Supplementary material online, *Table S2*; Stata 11.0, Statacorp, TX). Data are expressed as mean±SD, and *P*-values ≤0.05 considered statistically different.

Results

Changes in Cardiorespiratory Variables in Response to Large Infarct

Seven weeks following left coronary artery ligation, large infarct rats had both increased LVEDP and right ventricular (RV) weight (*Table 1*) consistent with elevated pulmonary artery pressures due to left ventricular failure induced CHF. Myocardial infarcts varied from 0 to 48% of left ventricular (LV) circumference and correlated linearly with LVEDP prior to lung harvest ($R^2 = 0.772$, $P \leq 0.001$). As previously², the large infarct group with an LVEDP approximating 24mmHg resulted in an increase in dry lung weight without lung oedema (*Table 1*).

Effect of Acute Elevation in Left Atrial Pressure in Isolated Perfused Lungs from rats with Chronic Left Ventricular Dysfunction

Isolated perfused lungs of control animals (<20% LV infarct) demonstrated a deterioration in lung tissue mechanics (Gtis and Htis) from left atrial pressure elevated to 15-20 cmH₂O (*Figure 1 A&B*), and an increase in alveolar oedema following left atrial pressure elevation to 20cmH₂O (*Table 2*). However, both isolated perfused lung mechanics and lung water were unchanged in moderate and large infarct groups during incremental increase in left atrial pressure from 0 to 20cmH₂O. No change was observed in airway resistance (Raw) over the full range of left atrial pressures (*Figure 1C*), or in the volume of lymphatic drainage (lung efflux) calculated following left atrial pressure increase to 20cmH₂O (*Table 2*). Observed versus predicted outcomes by mixed models analysis is included in the online supplemental material (Supplementary material online, *Figure S1 & Table S3*).

Effect of Increasing Left Ventricular Dysfunction on Alveolar Fluid Clearance

A strong inverse quadratic relationship was demonstrated between alveolar fluid clearance and both infarct size and LVEDP (*Figure 2 A&B*). Weaker negative associations were also evident between alveolar fluid clearance and dry lung weight (*Figure 2 C*), and RV weight ($R^2 = -0.108$, $P = 0.03$; data not shown).

Effect of Left Ventricular Dysfunction on Expression of ENaC, Na,K-ATPase, AQP-1 and AQP-5 Fluid Channels

Expression of AQP1 mRNA decreased with LV dysfunction (>20% LV infarct) and correlated with alveolar fluid clearance (*Figure 3 A&B*). Immunohistochemistry also demonstrated a decrease in AQP1 protein expression in the microvasculature of lung tissue sections from animals with LV dysfunction (>20% LV infarct) (*Figure 3 C&D*).

No relationship was found between either infarct size or alveolar fluid clearance and ENaC- α , ENaC- β or ENaC- γ , Na,K-ATPase- α or Na,K-ATPase- β , or AQP5 (Supplementary material online, *Table S4*). The expression ratio of ENaC- α : β : γ was approximately 150:1:6, while the expression ratio of Na,K-ATPase- α : β was 3.7:1 and did not alter with LV dysfunction ($P > 0.05$).

Effect of Left Ventricular Dysfunction on Mediators of Pulmonary Fluid Regulation

Concentrations of soluble mediators of alveolar fluid clearance (TNF- α , TGF- β , NO) were not related to either LV dysfunction (LV infarct >20) or alveolar fluid clearance (Supplementary material online, *Table S5*). Similarly, there was no change in the mRNA expression of either inducible or constitutive nitric oxide synthase (NOS) with LV dysfunction or alveolar fluid clearance (*Table S5*).

Plasma Volume Change in Clinical APO in CHF and non-CHF patients

APO patients with a prior clinical history of CHF (n=8) and those without (non-CHF, n=8) were similar in age, gender, severity of illness and respiratory dysfunction at ICU admission (*Table 3*). CHF patients had a lower left ventricular ejection fraction. Non-CHF patients were more acidotic with higher arterial PCO₂ than CHF patients; plasma lactate was elevated equally in both groups. More CHF patients than non-CHF were taking furosemide prior to admission. Plasma volume was significantly increased 24h following ICU admission in all APO patients. However, the percent change in plasma volume was significantly lower in the CHF patients when compared with non-CHF patients.

Discussion

Chronic elevation in Pmv due to left ventricular dysfunction results in pulmonary parenchymal remodelling which protects against the development of pulmonary oedema^{1,2}. Despite this, APO in response to acute elevations of Pmv is a common clinical issue. We examined intrinsic bi-directional fluid flux in the lung following chronic elevation of Pmv due to left ventricular dysfunction and observed both increased tolerance to acute left atrial pressure elevation and impaired clearance of extraneous lung fluid. We also report a decrease in endothelial AQP1 that correlates with the reduction in alveolar fluid clearance, and an increase in dry lung weight that we have previously found to correlate with lung collagen². These data are consistent with reduced bi-directional fluid flux due to both structural and cellular remodelling. Accordingly, our clinical data suggest slower reabsorption of APO from patients with CHF compared to

patients without.

Alveolar fluid clearance is increased by dopamine, β -adrenergic stimulation, glucocorticoids, aldosterone, cytokines, thyroid hormones and pulmonary oedema fluid itself, and decreased by hypoxia, reactive oxygen species and raised left atrial pressure⁵⁻⁷. Decreased alveolar fluid clearance with raised left atrial pressure is ameliorated by extraneous increase in Na,K-ATPase abundance and, or, opening potential^{7,8}. However, our data from rats with chronic elevation of Pmv found no relationship between alveolar fluid clearance and the expression of ENaC or Na,K-ATPase subunits, the mediators of ion channel expression or activity measured. This is consistent with a previous report in which no differences in mRNA or protein expression of ENaC and Na,K-ATPase subunits were found in whole lung homogenates of control and CHF rats at 16 weeks post left coronary artery ligation¹⁶.

Catecholamine production stimulates recruitment and cell surface expression of Na,K-ATPase following an acute rise in Pmv¹². However, by measuring alveolar fluid clearance in the isolated lung we avoided the confounding effects of increased circulating catecholamines found with most stressful situations and with CHF itself¹⁷. While this may reduce the direct clinical relevance of our data, it does allow estimation of the intrinsic reabsorption of alveolar fluid. The clinical interpretation of such data would therefore need to consider whether β -blocking or β -agonist drugs were administered and the rapidly changing patterns of circulating catecholamines commonly found in this cohort.

Three alternative mediators of alveolar fluid regulation were also examined. NO and TGF- β 1 which decrease alveolar fluid clearance via inhibition of Na⁺ transport and ENaC, respectively, and TNF- α , which increases or decreases fluid clearance in a concentration and temporal relationship^{9,18-20}. While no association with tissue NO or NOS was found in the

current study, at 16 weeks following left coronary artery ligation in the rat NO synthesis by endothelial cells has been reported to be impaired, possibly restoring the rate of alveolar fluid clearance^{16,21}. TNF- α is a pro-inflammatory cytokine that plays an important role in the activation of host defence, and is associated with increased mortality in CHF patients. *In vitro* TNF- α inhibits Na⁺ transport and down regulates ENaC activity and protein expression in isolated rat alveolar epithelial cells²⁰, while *in vivo* TNF- α is associated with an increase of alveolar fluid clearance²². However, no change in alveolar TNF- α was found in our rat model at 7 weeks post-coronary artery ligation. TGF- β serves a primary role in tissue growth, repair and remodelling throughout the body, and correlates with symptom severity in CHF²³. TGF- β inhibits homeostatic fluid regulation by decreasing sodium and fluid transport in alveolar epithelium via an ERK1/2-dependant repression of α ENaC¹⁹, while increasing basolateral surface expression of Na,K-ATPase²⁴. While no change in TGF- β was discerned in bronchoalveolar lavage of animals in the current study, this may be indicative of the paracrine nature of this cytokine in the lung, or may reflect the refined alveolar fibrotic remodelling observed in this rat model².

Aquaporins are fundamental to the maintenance of fluid homeostasis. AQP1, AQP4 and AQP5 are predominant in the lung on endothelium, airway epithelium and alveolar epithelium, respectively. Expression of lung AQP appears to be regulated by inflammation via corticosteroids and cytokines. AQP1 knockout produces greater than 10-fold decrease in microvascular and alveolar-capillary water permeability resulting in decreased hydrostatic lung oedema²⁵. However, AQP1 does not affect alveolar fluid clearance in models of acute lung injury²⁶. Expression of lung endothelial AQP1 is decreased in models of viral infection, pulmonary fibrosis and, recently, consistent with our results, in CHF²⁷⁻²⁹. Differential expression

patterns of AQP1 to AQP5 in each of these models with resultant change in pulmonary oedema support an independent role for AQP1 in prevention of capillary filtration. However, as a decrease in endothelial AQP1 in the absence of concurrent changes in epithelial sodium channels and AQP5 would seem insufficient to explain the reduction in alveolar fluid clearance, we suggest that structural remodelling is likely to be the major component. Indeed, a similar paradigm is recognised both clinically and experimentally in the diabetic lung whereby protection against ARDS may be attributed to thickened endothelial and epithelial basement membranes^{30,31}, while diabetic CHF patients exhibit a more severe reduction in alveolocapillary membrane conductance than those with CHF alone³².

Alveolar fluid clearance was strongly and inversely related to both the size of the myocardial infarct and LVEDP, and more weakly inversely related to dry lung weight. Given the lack of correlation between alveolar fluid clearance and ion channels or mediators of alveolar fluid regulation, and the previously reported correlation between dry lung weight and collagen content², we suggest that basement membrane structural changes most likely explain this decrease. As deletion of AQP1 does not influence alveolar fluid clearance, perhaps due to fluid accumulation in the interstitium, this is likely to have a lesser contribution, if any.

Maron and coworkers¹⁶ reported that alveolar fluid clearance was increased with β -adrenoceptor stimulation in this model, albeit at 16 weeks post-infarct, which correlated with hyperplasia of type II cells and was greater than the increase in control lungs. We quantitated the increase in type II cell number with chronic elevation in Pmv, and found that while large infarct animals with an LVEDP of around 25 mmHg had an increase in type II cell number of about 25%, there was no evidence of hyperplasia in moderate infarct animals with an LVEDP of around 15 mmHg. This lack of a graduated increase in type II cell number suggests the decrease

in alveolar fluid clearance we report is not directly related to alveolar type II cell prevalence.

Plasma volume restoration following an episode of APO was estimated to be less in patients with CHF, consistent with reduced alveolar fluid clearance. These data derive from a previously validated methodology¹³ which is indirect in that it assumes haemoconcentration as a result of APO, and is therefore not definitive. Patients without CHF may rapidly reduce Pmv with treatment of myocardial ischemia or hypertension leading to a reversal of the hydrostatic gradient. This gradient was not measured as few patients had pulmonary artery catheters placed. However, the changes in Pmv with APO can be extremely rapid and may have normalised prior to catheter placement, despite the prolonged phase of increased extravascular lung water³³. Patients without a history of CHF were more acidaemic possibly consistent with higher acuity and higher circulating catecholamine levels which might have led to increased alveolar fluid flux. As both groups had similar impairment in oxygenation and similar elevations in plasma lactate, which we have previously found strongly correlated with plasma epinephrine and norepinephrine,³⁴ this mechanism seems a less likely explanation for the difference found in plasma volume restoration. However, we did not measure catecholamine levels in the current study.

Given the prevalence, morbidity and mortality from CHF, specifically APO, all possible interventions should be examined. The interface between the failing heart and the lung, the alveolocapillary barrier, offers a direct target for investigation and intervention in decompensated heart failure, particularly following a decade of disappointing acute cardio-renal interventions³⁵. Furthermore, the independent impact of pulmonary hypertension on mortality in CHF supports the importance of the heart lung interaction in this complex syndrome³⁶. The reduction in alveolar fluid clearance in CHF suggested by our data, and consequent prolonged period of alveolar oedema, extends our understanding of the pathophysiologic challenges in managing

decompensated heart failure patients.

This study had limitations which should be addressed in future projects. While the literature on AQP1 knockout animals supports our hypothesis that a decrease in these channels is not the primary mechanisms for decreased alveolar fluid clearance, this was not directly assessed. Studies using AQP1 agonists and antagonists should be undertaken in this model as these become available. Further, a lack of change in ion channels, AQP5 or NOS mRNA does not exclude the possibility of changes at the protein or functional level. Clinically, we estimated that plasma volume restoration following 24 hours of cardiorespiratory support subsequent to an episode of APO was less in patients with CHF, consistent with reduced alveolar fluid clearance. While this methodology was indirect and patient numbers relatively small, the finding of reduced haemodilution in CHF in the absence of differences in fluid balance is consistent with the invasive animal data. This result should be confirmed in a clinical study utilising a direct method that examines alveolar fluid clearance and the effect of confounders.

Our data support the hypothesis of a decrease in intrinsic bi-directional fluid flux at the alveolar capillary barrier where an elevation in Pmv is long standing, which may both protect against the development of pulmonary oedema, and contribute to its slow resolution once fluid has breached the alveolus. Given the clinical relevance of plasma refill rate³⁵ and extravascular lung water in the management of heart failure, our observations may have direct clinical relevance and warrant further investigation.

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Conflict of interest: none declared

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LEGENDS:

Figure 1 Seven weeks following left coronary artery ligation lung impedance mechanics (mean \pm SD) of isolated perfused lungs from rats with moderate (20-40% left ventricular (LV) infarct circumference, n=7) or large (>40% LV infarct circumference, n=9) infarcts are unchanged during sequential elevation of left atrial pressure (Pla) (0, 5, 10, 15 and 20 cmH₂O referenced to the top of the lung) analysed by linear mixed models ($P > 0.05$). However, in control lungs (0-20% LV infarct circumference, n=12) Pla elevation of 15-20 cmH₂O resulted in an increase in both A, tissue resistance (Gtis; $P \leq 0.02$) and B, tissue elastance (Htis; $P \leq 0.01$), while C, airways resistance (Raw) remained unchanged ($P > 0.2$).

Figure 2 Seven weeks following left coronary artery ligation there is a negative relationship between alveolar fluid clearance (AFC) measured by Evan's Blue dye concentration in isolated lungs (n=47) and, A, left ventricular (LV) infarct circumference ($R^2 = -0.931$, $P \leq 0.001$), B, left ventricular end diastolic pressure (LVEDP) ($R^2 = -0.782$, $P \leq 0.001$), and C, dry lung weight ($R^2 = -0.151$, $P = 0.009$), analysed by quadratic regression curve fit.

Figure 3 Seven weeks following left coronary artery ligation there is A, a difference in endothelial aquaporin (AQP) 1 mRNA expression measured by qRT PCR in lungs of rats with left ventricular (LV) dysfunction (>20% LV infarct circumference, n=4) and those of controls (0-20% LV infarct circumference, n=4) analysed by independent *t* test ($P = 0.006$). In addition, there is B, a positive linear relationship between alveolar fluid

clearance (AFC) measured by Evan's Blue dye concentration in isolated lungs and endothelial AQP 1 mRNA (n=9), analysed by Pearson correlation ($R^2 = 0.33$, $P = 0.03$). Immunohistochemical labelling of AQP1 protein (stained brown) from C, control and D, large infarct (>40% LV infarct) rats demonstrated an absence of AQP1 expression in the alveolar microvasculature of the large infarct rats compared to control.

Table 1 Effect of left coronary artery ligation on cardiorespiratory variables

	Control (n=45) (0-20% MI)	Moderate (n=18) (20-40% MI)	Large (n=12) (>40% MI)	P-value
Myocardial infarct (% LV)	5.5±7.0	28.9±4.8*	44.8±3.4* [†]	≤0.001
RV weight (mg/g body wt)	0.88±0.07	1.00±0.11	1.02±0.08*	0.003
LVEDP (mmHg)	8.5±2.7	16.0±4.8*	23.0±1.9* [†]	≤0.001
Heart rate (beats/min)	366±30	345±30	343±23	0.09
Lung dry wt (mg/g body wt)	0.07±0.02	0.08±0.03	0.10±0.04* [†]	0.01
Wet to dry lung weight ratio	5.0±0.5	5.0±0.3	5.4±0.6	0.39

One-way analysis of variance for change over the three groups, with. Values are mean ± SD. RV weight and wet to dry weight ratio for alveolar fluid clearance cohort only; Control n=19, Moderate n=10, Large n=3.

RV, right ventricle, LVEDP, left ventricular end diastolic pressure.

* $P \leq 0.05$ vs. controls, [†] $P \leq 0.05$ vs. moderate, Tukey HSD posthoc analysis.

Table 2 Pulmonary fluid balance in isolated perfused lungs following sequential left atrial pressure (Pla) increase from 0 to 20cmH₂O

	Control (n=7) (0-20% MI)	Moderate (n=6) (20-40% MI)	Large (n=6) (>40% MI)	<i>P</i> -value
Perfusate starting volume (ml)	84±9	77±3	81±11	0.42
Lymphatic leak (ml/gm body weight)	0.06±0.03	0.08±0.02	0.08±0.03	0.61
Tissue oedema (ml/gm body weight)	0.020±0.007	0.011±0.007	0.009±0.007*	0.04
Wet to dry lung weight ratio	16.8±5.2	8.3±2.7*	11.0±3.12*	0.004

One-way analysis of variance for change over the three groups. Values are mean ± SD.

Wet to dry weight of upper right lung lobe only.

Lymphatic leak: lung fluid leakage (lung efflux); Tissue oedema: difference between the decrease in volume of the perfusate reservoir (lung flux) and the volume collected from lung fluid leakage (lung efflux).

* $P \leq 0.05$ vs. controls, Tukey HSD posthoc analysis.

Table 3 Patient characteristics by CHF group

	Non-CHF (n=8)	CHF (n=8)	P-value
Age (years)	72 ± 18	71 ± 20	0.92
Gender, male (%)	4 (50)	4 (50)	1.00
Ejection fraction (%)	50 ± 20	29 ± 8	0.03
Furosemide, yes (%) [‡]	1 (12.5)	5 (62.5)	0.04
ACE/ARB, yes (%) [‡]	3 (37.5)	5 (62.5)	0.32
β-Blocker, yes (%) [‡]	2 (25)	4 (50)	0.30
APACHE II score	21.6 ± 8.9	24.8 ± 4.9	0.40
APACHE III score	118.0 ± 38.2	120.4 ± 40.0	0.89
Respiratory rate (breaths/min)	30 ± 13	28 ± 8	0.77
Heart rate (beats/min)	103 ± 29	109 ± 13	0.66
Mean arterial pressure (mmHg)	101 ± 18	105 ± 19	0.70
pH	7.15 ± 0.12	7.31 ± 0.09	0.03
PaCO ₂ (mmHg)	69 ± 23	42 ± 12	0.02
PaO ₂ (mmHg)	100 ± 71	84 ± 68	0.68
PaO ₂ -FiO ₂ ratio	134 ± 76	153 ± 68	0.65
Lactate (mM/L)	3.6 ± 2.2	3.6 ± 2.0	0.99
Creatinine (μM/L)	97 ± 40	141 ± 46	0.09
Fluid balance (ml)	-559 ± 1382	-244 ± 515	0.6
Δ plasma volume (%)	23.7 ± 8.2	16.0 ± 3.2	0.03
Length of stay (days)	6.1 ± 4.9	3.3 ± 2.6	0.16

Independent samples t test or Pearson Chi-square test. [‡]Prior to admission.

ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker.

Figure 1

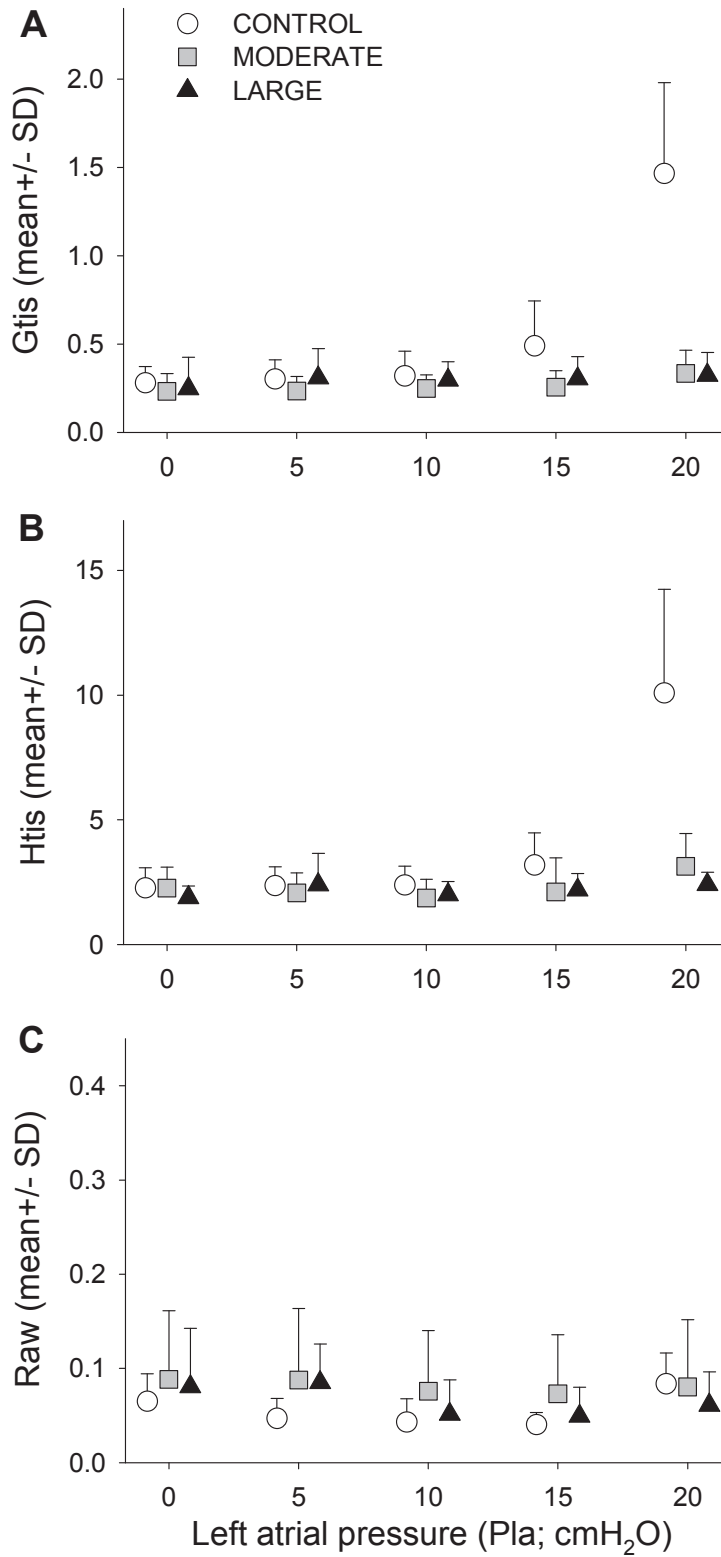


Figure 2

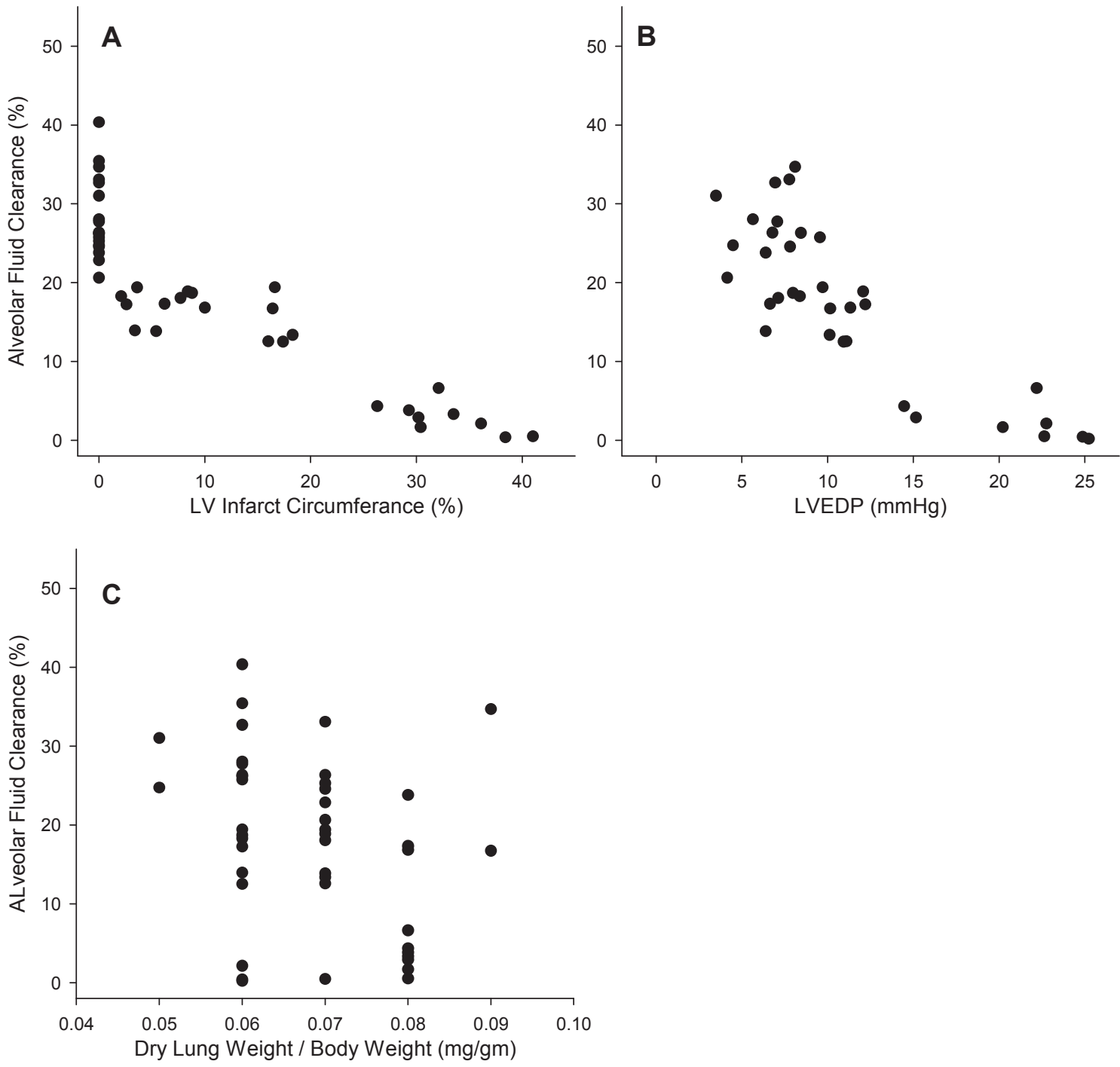


Figure 3

