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Real-time Lung Weight Measurement During Cellular Ex Vivo Lung Perfusion: An Early Predictor of Transplant Suitability

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Background. Increased extravascular lung water during ex vivo lung perfusion (EVLP) is associated with ischemia reperfusion injury and poor pulmonary function. A non-invasive technique for evaluating extravascular lung water during EVLP is desired to assess the transplant suitability of lungs. We investigated real-time lung weight measurements as a reliable method for assessing pulmonary functions in cellular EVLP using a porcine lung model. **Methods.** Fifteen pigs were randomly divided into 3 groups: control (no warm ischemia) or donation after circulatory death groups with 60 or 90 min of warm ischemia (n = 5, each). Real-time lung weight gain was measured by load cells positioned at the bottom of the organ chamber. **Results.** Real-time lung weight gain at 2 h was significantly correlated with lung weight gain as measured on a back table (R = 0.979, P < 0.01). Lung weight gain in non-suitable cases (n = 6) was significantly higher than in suitable cases (n = 9) at 40 min (51.6 ± 46.0 versus −8.8 ± 25.7 g; P < 0.01, cutoff = +12 g, area under the curve = 0.907). Lung weight gain at 40 min was significantly correlated with PaO_2/FiO_2, peak inspiratory pressure, shunt ratio, wet/dry ratio, and transplant suitability at 2 h (P < 0.05, each). In non-suitable cases, lung weight gain at 66% and 100% of cardiac output was significantly higher than at 33% (P < 0.05). **Conclusions.** Real-time lung weight measurement could potentially be an early predictor of pulmonary function in cellular EVLP.

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INTRODUCTION

Lung transplantation is the only established life-saving treatment for patients with end-stage respiratory diseases, although donor shortage is still a serious problem.[1] To expand the donor lung pool, ex vivo lung perfusion (EVLP) has been developed to assess pulmonary function of marginal donor lungs.[2,3] However, current evaluation methods during EVLP are imperfect, occasionally resulting in poor clinical outcomes. Thus, a reliable assessment of lung functions during EVLP is required to achieve favorable clinical outcomes in lung transplantation.

Extravascular lung water (EVLW) is the accumulation of fluid in the interstitial and alveolar space. Elevated lung weight because of EVLW during EVLP is a typical finding of ischemia reperfusion injury (IRI) and may be associated with poor lung function.[4] Recently, Okamoto et al[5] reported that heavier lungs had worse early clinical outcomes in both EVLP and straight lung transplant cases when lung weight was adjusted by body size. To date, several methods have been developed to assess EVLW during EVLP in animal models and clinical lung transplants.[6,7] Motoyama et al reported that injured lungs after warm ischemia showed significant lung weight gain at 20 to 60 min of perfusion compared with non-injured lungs in an isolated perfused rat lung model. Trebbia et al showed that transpulmonary thermodilution can be used to assess EVLW during clinical EVLP to predict postoperative outcomes. In addition, we demonstrated a non-invasive diagnostic tool to monitor EVLW using direct lung ultrasound evaluation.[8,9] Recently, Sakanoue et al[10] reported the use of real-time lung weight measurements to detect EVLW in clinical acellular EVLP. The study showed that the lung weight gain at 10 to 60 min can be a reliable predictive parameter of transplant suitability. Regarding cellular EVLP, Nilsson et al[4] reported that perfused lungs tend to be less edematous in cellular EVLP than acellular EVLP despite the high flow rate. However, there is a lack of data on real-time lung weight measurements during cellular EVLP. In this study, we hypothesized that real-time lung weight measurements may detect EVLW during cellular EVLP and differentiate the severity of IRI. We therefore investigated whether real-time lung weight measurement is a reliable method for evaluating transplant suitability by comparing lung weight gain between suitable and non-suitable cases in cellular EVLP using a porcine lung model.

MATERIALS AND METHODS

Study Design

Fifteen pigs were randomly allocated to 3 groups: a control group or donation after circulatory death (DCD) groups with 60 or 90 min of warm ischemia (n = 5, each). In the control group, EVLP was performed after 1 h of cold ischemia. In the DCD group, after 60 or 90 min of warm ischemia, lungs were stored on ice for 5 h, and then, EVLP was performed. At 2 h, transplant suitability was determined. During EVLP, real-time lung weight was continuously measured as described below. In addition, at 0 and 2 h, lung weight was measured on a back table. Lung tissue samples were collected to determine the wet/dry (W/D) ratio. The lung weight gain was calculated by subtracting the lung weight after EVLP from that before EVLP. In this study, first, we validated the proposed method by comparing back table lung weight gain with real-time lung weight gain. Second, we investigated whether real-time lung weight gain was different at any time point between suitable and non-suitable cases. Third, we investigated the correlation between real-time lung weight gain and physiological parameters or transplant suitability. This study was approved by the Institutional Animal Care and Use Committees at the National Institute of Advanced Industrial Science and Technology and Tokyo Medical and Dental University. Some cases in this study were used in other studies.[11,12]

Animal Preparation

Males (42–49 kg) crossed between Landrace and Large White pigs were used. Following injection of xylazine (2 mg/kg) and ketamine (20 mg/kg), endotracheal intubation and mechanical ventilation were started. Isoflurane of 1.5% to 3.0% was maintained. In the DCD groups, intravenous heparin (500 units/kg) was injected, and pigs were euthanized with potassium chloride injection (2.0 mEq/kg) 3 min later. Then, lungs were procured in a standard manner.[13] Briefly, 2 L of Perfadex and 10 mg/L of nitroglycerin were flushed antegrade in the pulmonary artery. In the DCD groups, after confirmation of death, pigs underwent 60 or 90 min of warm ischemia in the supine position at room temperature (21 °C.) Whole blood was collected from a dedicated blood donor pig, stored in Terumo blood bags (Terumo Co Ltd, Tokyo, Japan) at 4 °C and then washed using a cell saver (XTRA, Rivanova Japan Co, Ltd, Tokyo, Japan).

EVLP Procedure

Lungs were perfused according to the Lund protocol as summarized in Figure 1.[14,15] The EVLP circuit consisted of an organ chamber, a reservoir, a centrifugal blood pump, a membrane oxygenator, a heat exchanger, and a leukocyte filter as shown in previous reports.[11,12] The circuit was primed with 2.0 L of STEEN solution, heparin 10 000 IU, imipenem 100 mg, and 500 mL of packed red blood cells. A pulmonary artery cannula was connected to the circuit. The left atrium was kept open. At 0 to 10 min, the flow rate was 1.0 L/min with the shunt between the pulmonary artery cannula and the chamber opened. The temperature of the perfusate was incrementally increased to 37 °C. At 10 min, the flow rate was reduced to 0.2 L/min and then gradually increased in a stepwise manner, finally reaching the target flow rate (100% of estimated cardiac output: 70 mL/min/kg) at 45 min. When the upper lobe temperature was 32 °C, mechanical volume-controlled ventilation using a ventilator (ART-300, ACOMA Medical Industry Co, Ltd, Tokyo, Japan) was started with the following settings: tidal volume (TV) 4 mL/kg, respiratory rate 7 breaths/min, positive end-expiratory pressure 5 cm H_2O, and fraction of inspired oxygen (FiO_2) 0.4. When the upper lobe temperature reached 36 °C, TV increased to 6 mL/kg. At 1 and 2 h, using blood samples from the pulmonary artery and left atrium, blood gas analyses were performed at FiO_2 0.4, 1.0, and 0.21 at 10-min intervals during the evaluation phase, following the recruitment maneuver (TV 10 mL/kg, respiratory rate 10 breaths/min, positive end-expiratory pressure 5 cm H_2O, and FiO_2 0.4 for 10 min).

Criteria of Transplant Suitability

Transplant suitability was assessed according to previously published criteria by Wierup et al[16]: lungs were considered non-suitable for transplantation when the P/F ratio of the left atrium was <300 mm Hg and when there was a significant deterioration in airway parameters (peak inspiratory pressure, plateau pressure, dynamic compliance and static compliance) and vascular parameters (eg, flow <100% of estimated cardiac output). Lungs were also considered non-suitable when significant airway fluid or abnormal visual findings, such as lung edema or hematoma, were detected at the end of perfusion.[15,17]
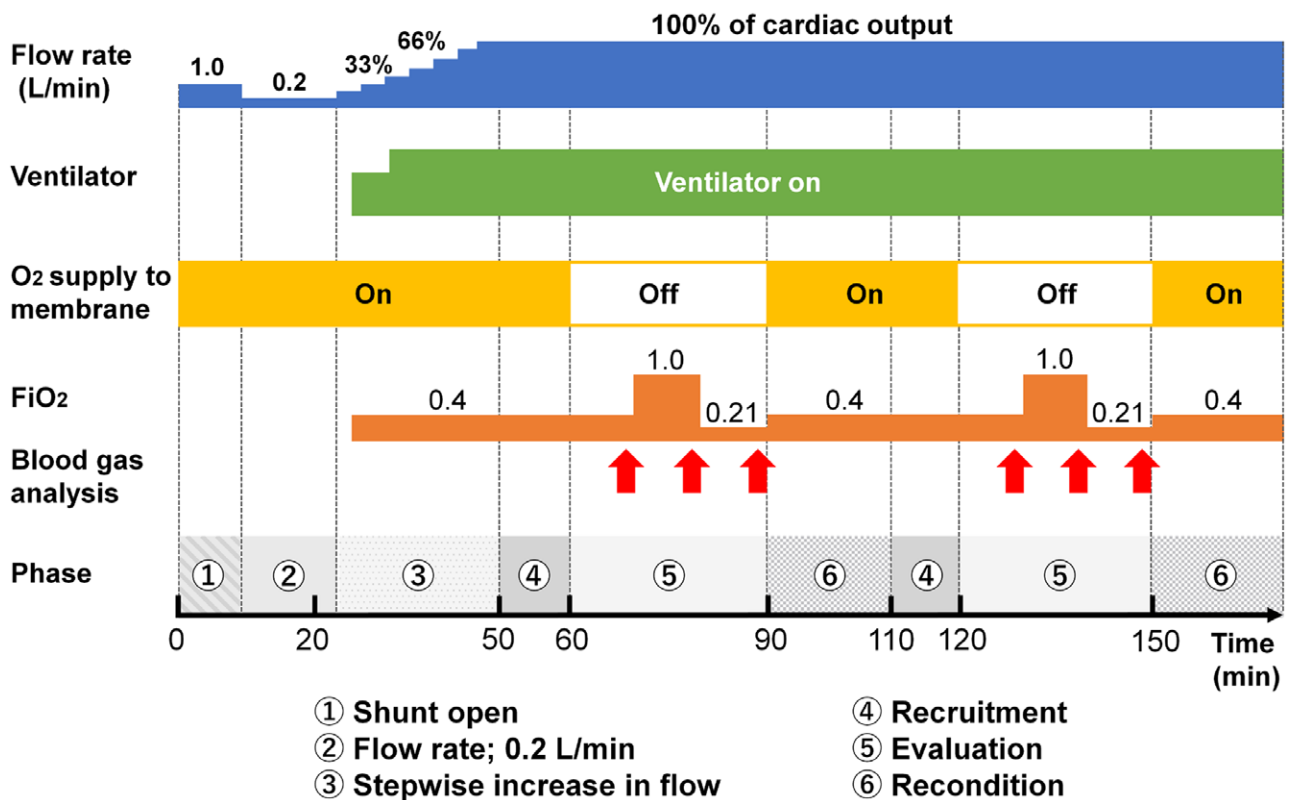
FIGURE 1. Protocol of Lund-type ex vivo lung perfusion. Perfusion started at a flow rate of 1.0 L/min for 10 min. The flow rate was reduced to 0.2 L/min. Lungs were gradually rewarmed by increasing the flow rate in a stepwise manner. When the lung temperature reached 32 °C, ventilation was started. At 60 and 120 min, O_2 supply to the membrane was stopped, and blood gas analysis (red arrow) was performed at FiO_2 0.4, 1.0, and 0.21 as an evaluation phase following the recruitment maneuver. The recondition phase was restarted after blood gas analysis at 90 and 150 min. FiO_2, fraction of inspired oxygen.

Back Table Lung Weight Measurement

Lung weight was measured without a cannula and an endotracheal tube by using an electric balance (EB-3299H, Shimadzu, Co, Ltd, Japan) after lung procurement. Heart, mediastinal fat tissue, and pericardium were detached, and intravascular fluid was removed by gravity. At 2 h, lung weight was measured after removing the cannula and the tracheal tube. Back table lung weight gain was calculated by subtracting the lung weight before EVLP from the lung weight after EVLP.

Real-time Lung Weight Measurement

Real-time lung weight was measured according to a previously reported protocol[10] by using 2 load cells (Load cell Sensor 0–5 kg, uxcell Co Ltd, Hong Kong) positioned at the bottom of the organ chamber (Figure 2A). To minimize potential artifacts related to perfusate coming out from the left atrium, the organ chamber was inclined at a 5° angle toward the outlet port to prevent perfusate from pooling in the organ chamber. A non-touch period was defined as the period when no one touched the lungs or the organ chamber, and pulmonary artery pressure, flow rate, and ventilation setting were stable. During the non-touch period, lung weight gain was continuously measured. Following the non-touch period, lung weight gain was assumed to continue at the same rate until the next non-touch period. Lung weight gain until the next non-touch period was calculated with the following equation:

$$\Delta w' = \Delta w / \Delta t \times \Delta t', \qquad (1)$$

where Δw was lung weight gain during the non-touch period, Δt. $\Delta w'$ was lung weight gain until the target period, $\Delta t'$, for 5 to 15 min (Figure 2B). Real-time lung weight gain for 2 h was calculated by adding the $\Delta w'$s together from the beginning to 2 h (Figure 2C).

Normalized Real-time Lung Weight Gain in Each EVLP Phase

Real-time lung weight gain was normalized by time and pulmonary artery pressure. The normalized lung weight gain was compared in the following different settings: 33%, 66%, and 100% of estimated cardiac output; recondition and evaluation phases; FiO_2 0.4, 1.0, and 0.21 during the evaluation phase; and recruitment and non-recruitment. The recondition phase in this analysis represents a period with a flow rate of 100% of estimated cardiac output aside from the hourly recruitment and evaluation phase.

Lung Weight Simulation Based on the Starling Equation

To investigate whether the lung weight gain data fit the Starling equation by adding reported values, the following equation was used:

$$Q_f = K_{fc} \left[(P_c - P_i) - \sigma(\pi_c - \pi_i) \right], \qquad (2)$$

where Q_f is net flow of fluid across the capillary wall, K_{fc} is the filtration coefficient, P_c is capillary hydrostatic pressure, P_i is interstitial fluid hydrostatic pressure, σ is the reflection coefficient, π_c is plasma colloid osmotic pressure,
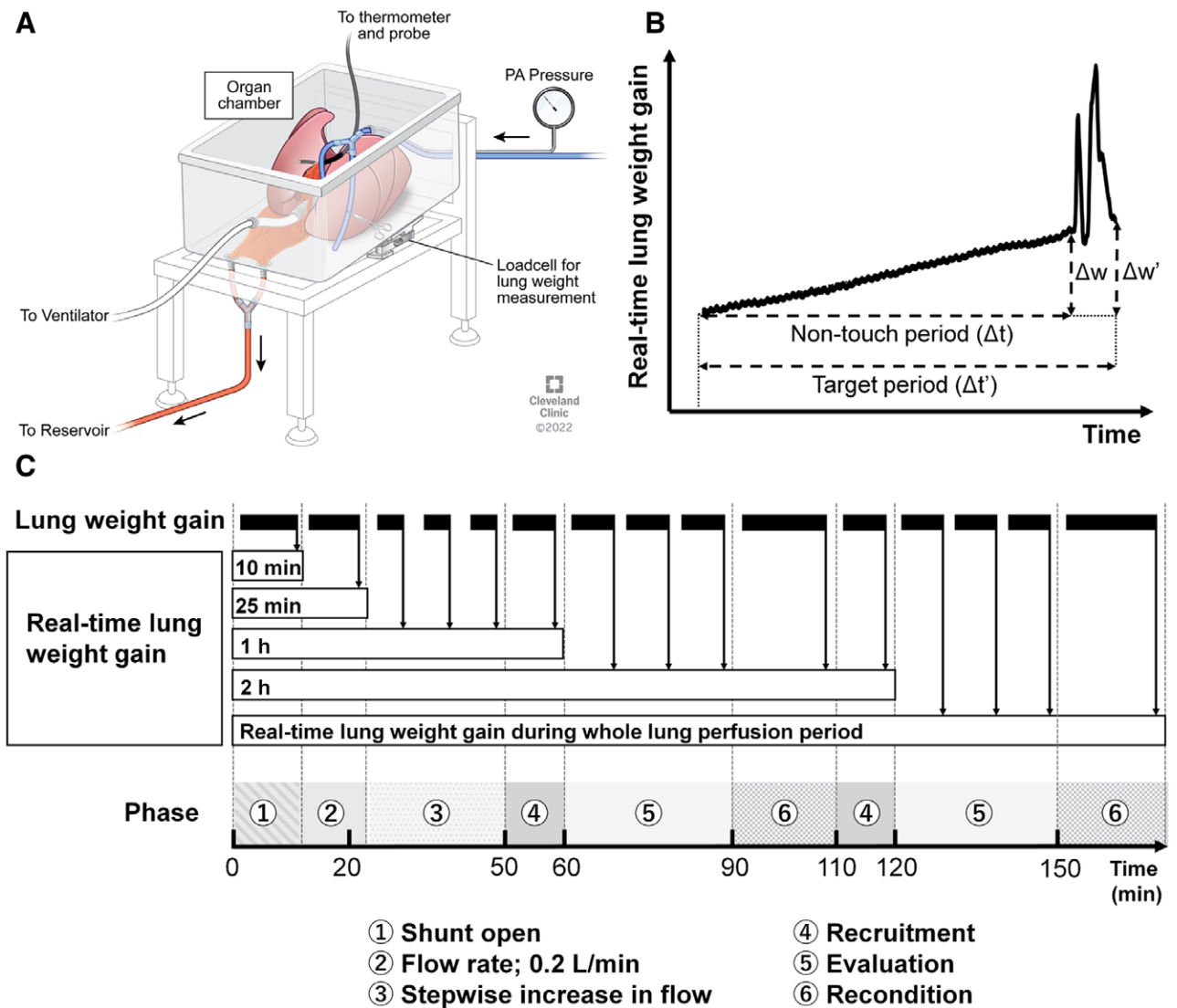
FIGURE 2. Real-time lung weight measurement. A, Developed organ chamber in an ex vivo lung perfusion circuit. Lung weight was continuously measured by using 2 load cells positioned at the bottom of the organ chamber. B, Real-time lung weight gain ($\Delta w'$) for the target period ($\Delta t'$) was calculated by real-time lung weight gain (Δw) during the non-touch period (Δt). C, Real-time lung weight gain for 2 h was calculated by adding the Δw's together from the beginning to 2 h. PA, pulmonary artery.

and π_i, is interstitial fluid colloid osmotic pressure. In the simulation, P_c was determined by measuring pulmonary artery pressure. Q_f during 2 h of EVLP was calculated by adding individual Q_f together from the beginning to 2 h. Other parameters were obtained from reported values: $\sigma = 0.65$,[18] $P_i = -10$ mm Hg,[19] $\pi_c = 32$ mm Hg,[20] and $\pi_i = 14$ mm Hg.[21] First, considering that EVLW was caused by an increase in K_{fc}, a wide range of K_{fc} was tested. Then, a single K_{fc} value was determined as when the mean squared error between the real-time lung weight gain and the simulated lung weight gain reached a minimum.

Cytokine Analysis

Perfusate samples were taken every hour and used to measure inflammatory cytokine levels including interleukin (IL)-1β, IL-6, IL-8, and IL-10 by using enzyme-linked immunoassay kits (R&D Systems, Minneapolis, MN).

Statistical Analysis

Continuous data are expressed as means and SD. In correlation coefficient analysis, Pearson's product-moment correlation coefficient was calculated. A t test was used for parametric data, and a chi-square test was used for categorical variables. A value of $P < 0.05$ was considered statistically significant. A receiver operating characteristic curve was used to select an optimal cutoff value to distinguish suitable cases from non-suitable cases. The breakpoints of the real-time lung weight gain were identified by piecewise linear regression. All statistical analyses were performed using JMP, version 16.0.0 (SAS Institute, Inc, Cary, NC).

RESULTS

EVLP Assessment

Lungs in all cases in the control group and in 4 cases in the DCD group with 60 min of warm ischemia were judged as transplantable. In contrast, lungs in the remaining 6 cases were judged as non-transplantable. Non-suitable cases were significantly associated with lower P/F ratio, higher peak inspiratory pressure, higher shunt ratio, and severe findings of pulmonary edema ($P < 0.001$; Table 1).

TABLE 1.

Physiological parameters at 2 h of ex vivo lung perfusion

Variables	Suitable cases (N = 9)	Non-suitable cases (N = 6)	P
P/F ratio, mm Hg	518 ± 80	290 ± 100	<0.001
PIP, cm H_2O	11.1 ± 0.9	24.8 ± 6.9	<0.001
PVR, dyne·s/cm^5	393 ± 84	436 ± 42	0.189
Shunt ratio, %	16.6 ± 7.6	40.9 ± 10.1	<0.001
Findings of edema, n (%)	0 (0)	6 (100)	<0.001
W/D ratio	4.8 ± 0.2	6.3 ± 0.2	<0.001

Measurement data are expressed as means and SD.
Findings of edema included severe bogginess in palpation and excessive airway fluid.
P/F ratio, P/F ratio at fraction of inspired oxygen 1.0; PIP, peak inspiratory pressure; PVR, pulmonary vascular resistance; SD, standard deviation; W/D ratio, wet/dry ratio.
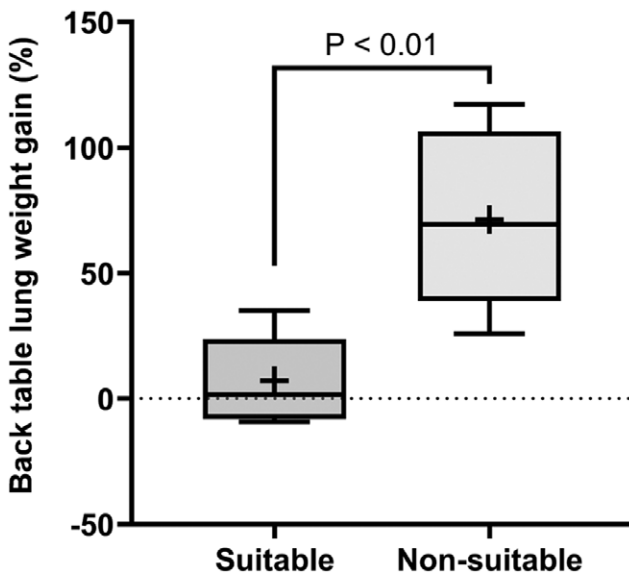

FIGURE 3. Lung weight gain on a back table at 2 h. Non-suitable cases showed significantly higher back table lung weight gain than suitable cases (71.4 ± 36.5 [range, 26 to +117] vs 7.2 ± 17.0% [range, –9 to 35], $P < 0.01$). In the box-and-whisker plots, plus marks show the mean values.

Non-suitable cases showed significantly higher back table lung weight gain than suitable cases (71.4 ± 36.5 versus 7.2 ± 17.0%; $P < 0.01$; Figure 3).

There Was a Significant Correlation Between Real-time Lung Weight Gain and Back Table Lung Weight Gain

There was a significant correlation between real-time lung weight gain at 2 h and back table lung weight gain (N = 15, $R = 0.979$, $P < 0.01$; Figure 4).

Trend of Real-time Lung Weight Gain

For real-time lung weight gain at 0 to 30 min, there was no significant difference between suitable and non-suitable cases (Figure 5A). In contrast, the real-time lung weight gain in non-suitable cases was significantly higher than in suitable cases at 40 to 170 min (40 min, 51.6 ± 46.0 versus –8.8 ± 25.7 g; 170 min, 333.9 ± 141.7 versus 31.1 ± 50.4 g; $P < 0.01$, each). Two breakpoints were identified in both suitable and non-suitable cases (Figure 5A). Breakpoints in

suitable cases were calculated as 27 and 61 min. Three regression lines were determined (0–27 min, $y = -1.27x + 0.31$, $R = 0.625$, $P < 0.01$; 27–61 min, $y = 1.93x - 82.92$, $R = 0.711$, $P < 0.01$; 61–170 min, $y = -0.12x + 43.33$, $R = 0.086$, $P = 0.418$). In non-suitable cases, breakpoints were calculated as 26 and 75 min. Three regression lines were determined (0–26 min, $y = -0.31x + 0.02$, $R = 0.223$, $P = 0.374$; 26–75 min, $y = 4.29x - 118.04$, $R = 0.794$, $P < 0.01$; 75–170 min, $y = 1.47x + 85.05$, $R = 0.346$, $P < 0.05$). Pulmonary artery pressure at 10 min in non-suitable cases was significantly higher than in suitable cases ($P < 0.05$), whereas there was no significant difference between the 2 cases at 20 to 170 min (Figure 5B). In pulmonary vascular resistance, no significant difference between the 2 cases was observed throughout EVLP (Figure 5C). Dynamic compliance in non-suitable cases was significantly lower than in suitable cases at 30 to 170 min ($P < 0.05$; Figure 5D).

Normalized Real-time Lung Weight Gain in Each EVLP Phase

In non-suitable cases, normalized real-time lung weight gain at 66% of cardiac output was significantly higher than that at 33% (33%; 0.117 ± 0.239 versus 66%; 0.831 ± 0.243 g/min/mm Hg; $P < 0.01$). In addition, normalized real-time lung weight gain at 100% was significantly higher than that at 33% (33%; 0.117 ± 0.239 versus 100%; 0.668 ± 0.225 g/min/mm Hg; $P < 0.05$; Figure 6A). In the evaluation phase, normalized real-time lung weight gain in non-suitable cases was significantly higher than in the recondition phase (0.124 ± 0.077 versus 0.096 ± 0.087 g/min/mm Hg; $P < 0.05$; Figure 6B). During the evaluation phase in non-suitable cases, normalized real-time lung weight gain at FiO$_2$ 1.0 was significantly higher than that at FiO$_2$ 0.21 (0.171 ± 0.060 versus 0.084 ± 0.087 g/min/mm Hg; $P < 0.05$; Figure 6C). In the recruitment period, normalized real-time lung weight gain was significantly higher than in the non-recruitment period for non-suitable cases (0.159 ± 0.080 versus 0.096 ± 0.087 g/min/mm Hg; $P < 0.05$; Figure 6D).

A Significant Correlation Between Real-time Lung Weight Gain at 40 min and EVLP Parameters

Real-time lung weight gain in non-suitable cases was significantly higher than in suitable cases at 40 min (51.6 ± 46.0 versus -8.8 ± 25.7 g, $P = 0.006$, cutoff = +12 g; Figure 7A). The area under the curve using receiver operating characteristic curve analysis was 0.907. There was a significant correlation between real-time lung weight gain at 40 min and the P/F ratio at FiO$_2$ 1.0 at 2 h ($R = 0.673$, $P = 0.029$; Figure 7B). A significant correlation was found between real-time lung weight gain at 40 min and peak inspiratory pressure at 2 h ($R = 0.804$, $P < 0.001$; Figure 7C). A significant correlation was also found between real-time lung weight gain at 40 min and the shunt ratio at 2 h ($R = 0.729$, $P = 0.009$; Figure 7D). There was also a significant correlation between real-time lung weight gain at 40 min and the W/D ratio ($R = 0.607$, $P = 0.014$; Figure 7E). Moreover, there was a significant correlation between real-time lung weight gain at 40 min and back table lung weight gain at 2 h ($R = 0.842$, $P < 0.001$; Figure 7F).
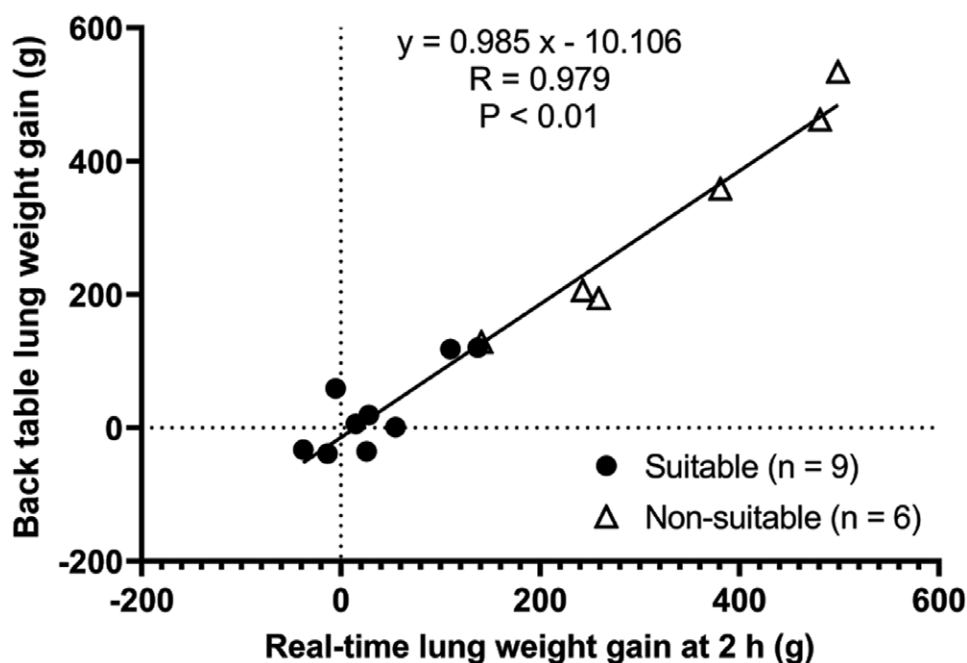
FIGURE 4. Relationship between back table lung weight gain and real-time lung weight gain at 2 h. There was a significant correlation between back table lung weight gain and real-time lung weight gain ($y = 0.985x - 10.106$, n = 15, $R = 0.979$, $P < 0.01$).

Lung Weight Simulation Based on the Starling Equation

K_{fc} values of 0.005 and 0.05 g/min/mm Hg/100 g were found to achieve a good fit in suitable and non-suitable cases, respectively (Figure 8A and B).

Cytokine Analysis

There was a significant difference in IL-1β at 2 h between suitable and non-suitable cases (37.3 ± 13.3 versus 61.9 ± 29.1 pg/mL, $P < 0.05$; Figure 9A). A significant difference in IL-6 at 2 h was found between suitable and non-suitable cases (64.3 ± 61.2 versus 191.7 ± 102.5 pg/mL, $P < 0.01$; Figure 9B). The average value of IL-8 at 2 h in non-suitable cases was higher than that in suitable cases without a statistically significant difference (80.8 ± 77.7 versus 164.6 ± 160.0 pg/mL, $P = 0.195$; Figure 9C). There was a significant difference in IL-10 at 2 h between suitable and non-suitable cases (42.8 ± 35.4 versus 145.6 ± 124.8 pg/mL, $P < 0.05$; Figure 9D).

DISCUSSION

In this study, 15 porcine lungs with different degrees of warm ischemia were perfused to simulate "real-world" varieties of donor lung injury. Lung weight was continuously measured during 2 h of cellular EVLP by using a scale under the organ chamber and with a protocol similar to that established for real-time lung weight measurement in clinical acellular EVLP.[10] First, the feasibility of real-time lung weight measurement in a porcine lung cellular EVLP was confirmed by a significant correlation between the 2 kinds of lung weight data. Second, the average lung weight gain for suitable cases on the back table was only 7%, ranging from –9% to 35%, whereas that of non-suitable cases was 74% (range, 26%–117%), with a constantly increasing trend of estimated lung weight at 26 to 170 min. Similarly, as previously reported, half of the rejected human lungs judged as suitable for transplant had negative values

of lung weight gain at the end of Lund-type EVLP.[22] Third, a significant correlation was identified between a higher estimated lung weight gain at 40 min and transplant non-suitability and typical parameters of pulmonary edema, including P/F ratio, airway parameters, and W/D ratio. The cutoff value at 40 min was +12 g, and the area under the curve was 0.907, indicating high reliability to identify non-suitable lungs before the current evaluation time at 1 or 2 h. Originally, cellular EVLP was developed to identify the likelihood of IRI at 100% cardiac output by using physiological parameters and visual findings to predict the effects of being transplanted.[2,16] Our current results suggest the possibility that real-time lung weight gain could predict transplant suitability at as early as 40 min when perfusion flow has not reached 100% cardiac output.

Historically, lung weight gain was calculated by measuring the lung weight on a back table before and after EVLP. In a previous experimental study, Motoyama et al[6] reported that the weights of injured rat lungs increased at 20 min of perfusion using an isolated rat lung perfusion model. Our results are consistent with their study in terms of the relatively early weight gain because lung weight in our study significantly increased in the non-suitable cases at 40 min. Interestingly, vascular parameters in suitable and non-suitable cases were almost identical throughout EVLP. In contrast, Sakanoue et al[10] demonstrated that injured lungs with high lung weight gain have higher pulmonary artery pressure than controls in acellular EVLP. This result is explained by excessive hydrostatic pressure according to the Starling equation.[23] Given our present data showing no significant difference in vascular parameters, the excessive EVLW was mainly caused by the other factor in the Starling equation, K_{fc}, which reflects capillary permeability. In the lung weight simulation, the increased K_{fc} value in non-suitable cases was associated with an increase in real-time lung weight gain.

In the breakpoint analysis, real-time lung weight gain was divided into 3 phases in both cases. In the first phase,
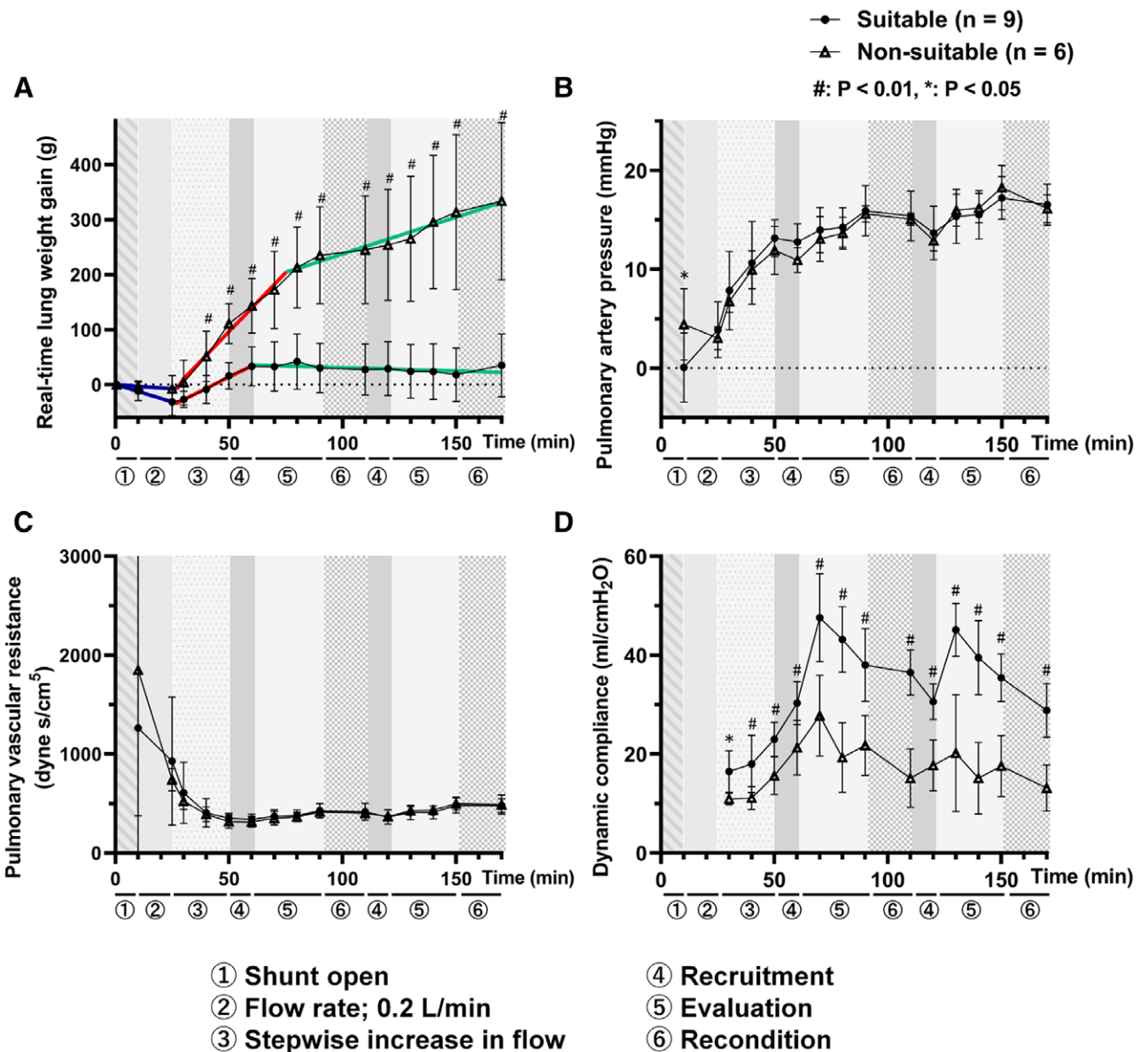
FIGURE 5. Results of real-time lung weight measurement. A, Trend of real-time lung weight gain in suitable and non-suitable cases. The real-time lung weight gain in non-suitable cases was significantly higher than in suitable cases at 40 to 170 min ($P<0.01$). Three regression lines (first phase is blue line, second phase is red line, and third phase is green line) were obtained on the basis of 2 breakpoints by piecewise linear regression. B, Trend of pulmonary artery pressure. There was no significant difference between the 2 groups at 20 to 170 min, whereas pulmonary artery pressure at 10 min in non-suitable cases was significantly higher than in suitable cases ($P<0.05$). C, Trend of pulmonary vascular resistance. No significant difference between the 2 groups was observed throughout ex vivo lung perfusion. D, Trend of dynamic compliance. Dynamic compliance in non-suitable cases was significantly lower than that in suitable cases at 30 to 170 min ($P<0.05$).

with relatively low flow, the trend decreased. Osmotic pressure of the perfusate might have contributed to this change. In the second phase, the trend increased in both cases. Especially in non-suitable cases, real-time lung weight dramatically increased. Because the flow rate increased to as high as 100% of cardiac output, perfusate was distributed throughout the whole lungs against gravity, resulting in significant fluid leakage. In the third phase, real-time lung weight gain was almost constant in suitable cases, indicating that there was no fluid leakage in healthy lungs. In contrast, real-time lung weight gain increased at a lower rate than in the second phase in non-suitable cases. This may be explained by some EVLW already existing in the interstitial area during the second phase, and

subsequently, pulmonary edema was relatively suppressed in the third phase.

Another important finding was that normalized lung weight gain in non-suitable lungs varied in different settings of flow and ventilation during EVLP. First, lung weight gain was dependent on the flow rate, with a threshold between 33% and 66% of cardiac output, indicating that a certain degree of flow allows the whole area of the lungs to be perfused against gravity. Low flow of <66% cardiac output (eg, the Toronto protocol and Organ Care System) might not cause enough fluid leakage in injured lungs, and EVLW can be underestimated. In our previous report, the lung surface temperature of injured lungs did not increase as rapidly as that of normal lungs during the
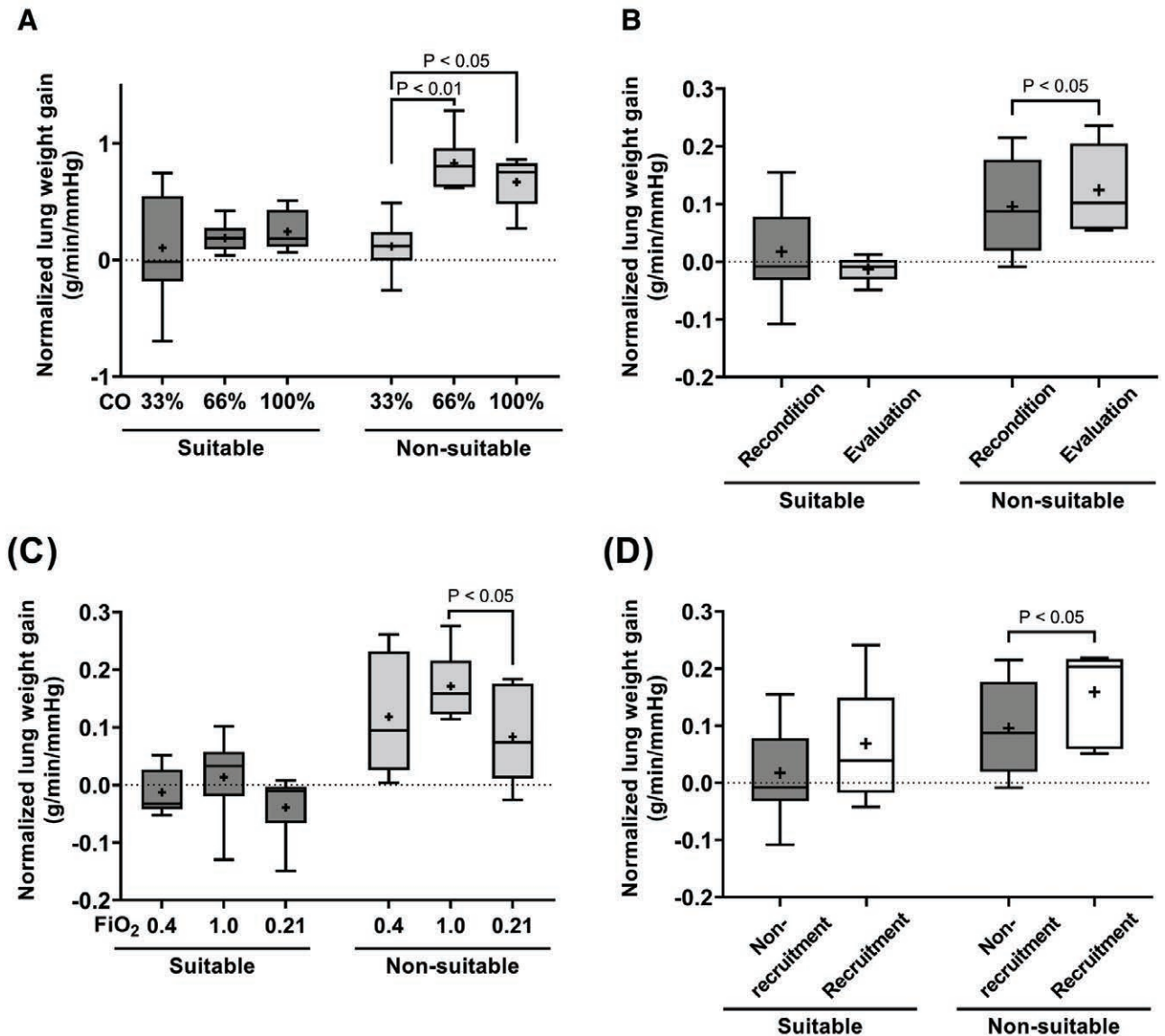
FIGURE 6. Normalized real-time lung weight gain in different settings of perfusion and ventilation. A, In non-suitable cases, normalized real-time lung weight gain at 66% and 100% of cardiac output was significantly higher than that at 33% ($P<0.01$, $P<0.05$, respectively). B, In non-suitable cases, normalized real-time lung weight gain in the evaluation phase was significantly higher than that in the recondition phase ($P<0.05$). C, During the evaluation phase in non-suitable cases, normalized real-time lung weight gain at FiO_2 1.0 was significantly higher than at FiO_2 0.21 ($P<0.05$). D, In non-suitable cases, normalized real-time lung weight gain in the recruitment period was significantly higher than in the non-recruitment period ($P<0.05$). FiO_2, fraction of inspired oxygen.

initial rewarming phase with a flow of 0.2 L/min because of poor perfusion.[12] Second, the normalized lung weight gain was greater in the evaluation phase than in the recondition phase. Moreover, normalized lung weight gain was highest at evaluation when FiO_2 was 1.0. Generally, alterations in FiO_2 influence intrapulmonary shunt and ventilation-perfusion mismatch.[24] At FiO_2 0.21, hypoxic pulmonary vasoconstriction results in the redirection of perfusion away from non-ventilated or underventilated alveolar units.[25] Insufficient perfusion in injured alveoli may lead to a misleadingly low rate of lung weight gain. In contrast, at FiO_2 1.0, the effect of ventilation-perfusion mismatch is mitigated because of the high concentration of oxygen,[24] so enough perfusion in injured alveoli may lead to a higher rate of lung weight gain. This result is consistent with our previous report showing that FiO_2 1.0 during blood gas analysis is superior to FiO_2 0.21 with

higher sensitivity in Lund-type EVLP.[17] Third, normalized lung weight gain in recruitment was higher than in non-recruitment. It is thought that the increase in TV during recruitment secondarily increases the vascular bed volume, which in turn increases the amount of fluid leakage.

Abnormal lung weight gain at 40 min can serve as a warning for assessments at 1 and 2 h, leading to more careful evaluation. In addition, lung weight data might be informative when considering therapeutic interventions during EVLP, including the use of hemoconcentration.[20] Moreover, real-time lung weight measurements enable us to identify procedural problems (eg, a kinked cannula) when sudden, abnormally high lung weight gain is observed. Most existing methods do not provide continuous measurement but rather hourly measurements with specific procedures, such as transpulmonary thermodilution, ultrasound, and chest computed tomography.[7-9,26]
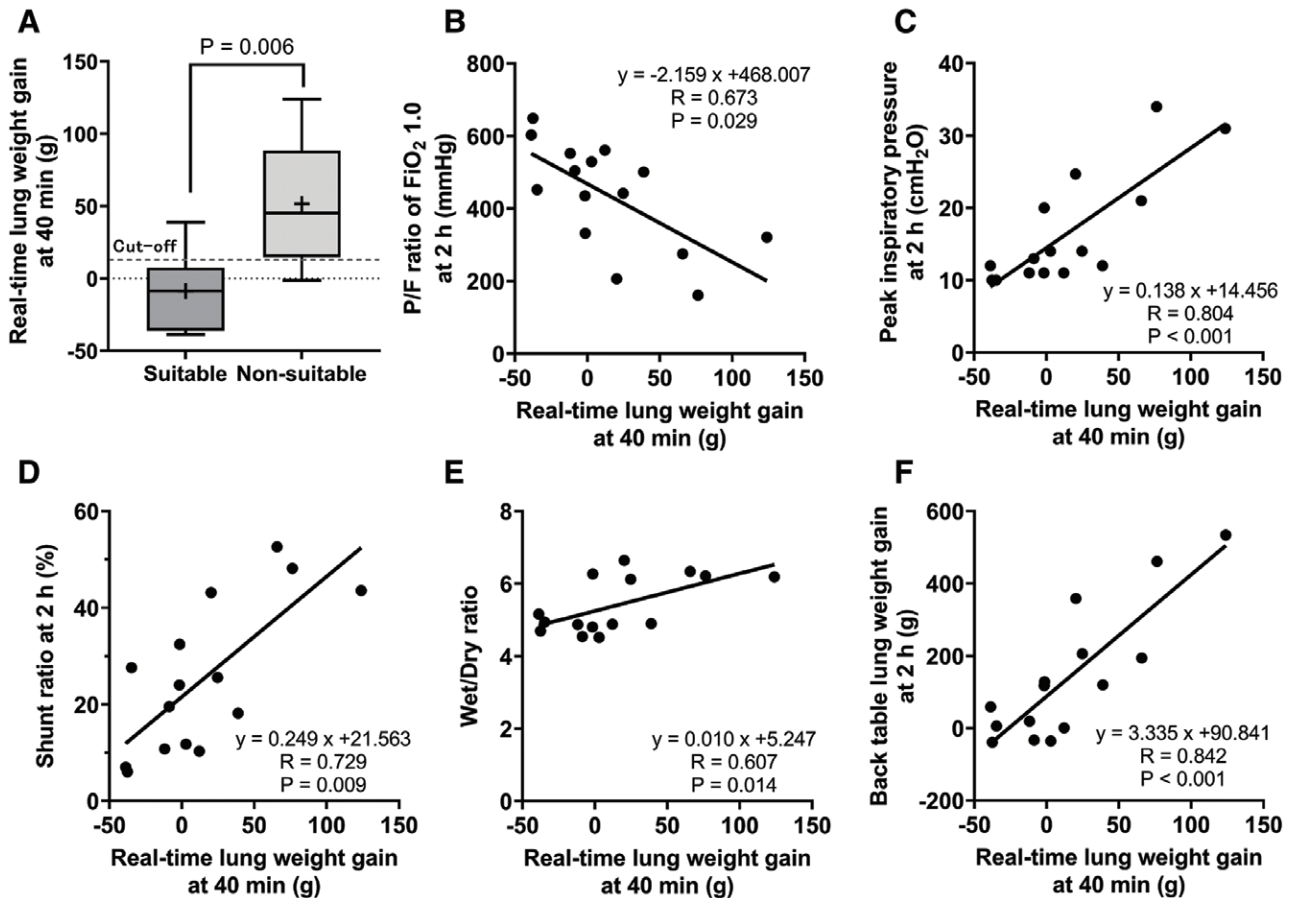
FIGURE 7. Correlation between real-time lung weight gain and ex vivo lung perfusion parameters. A, The real-time lung weight gain in non-suitable cases was significantly higher than in suitable cases at 40 min (51.6±46.0 vs −8.8±25.7 g, $P=0.006$). The cutoff was +12 g (AUC=0.907). B, There was a significant correlation between real-time lung weight gain at 40 min and P/F ratio at FiO_2 1.0 at 2 h ($R=0.673$). C, A significant correlation was found between real-time lung weight gain at 40 min and peak inspiratory pressure at 2 h ($R=0.804$). D, A significant correlation was found between real-time lung weight gain at 40 min and the shunt ratio at 2 h ($R=0.729$). E, There was a significant correlation between real-time lung weight gain at 40 min and the W/D ratio ($R=0.607$). F, There was a significant correlation between real-time lung weight gain at 40 min and back table lung weight gain at 2 h ($R=0.842$). AUC, area under the curve; FiO_2, fraction of inspired oxygen; P/F ratio, P/F ratio at FiO_2 1.0; W/D ratio, wet/dry ratio.
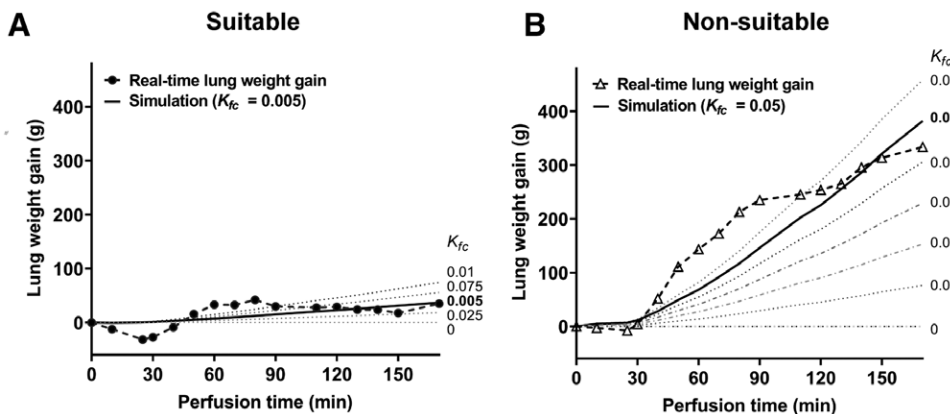

FIGURE 8. Lung weight simulation based on the Starling equation. A, K_{fc} value of 0.005 g/min/mm Hg/100 g was identified to minimize mean squared error between the real-time lung weight and the simulated lung weight gain in suitable cases. B, K_{fc} value of 0.05 g/min/mm Hg/100 g was identified to minimize mean squared error between the real-time lung weight and the simulated lung weight gain in non-suitable cases.

Therefore, we believe that real-time lung weight measurements may further improve preclinical experiments and clinical case management.

There are several limitations in this study. First, no lung transplantation was performed. However, lung function via Lund-type EVLP is reported to be well correlated with posttransplant outcomes.[27] Second, lung weight gain may be caused not only by EVLW but also by volumetric expansion of vessels, resulting in initial rapid lung weight gain 0 to 0.5 min after increasing the flow rate.[28] This is why a
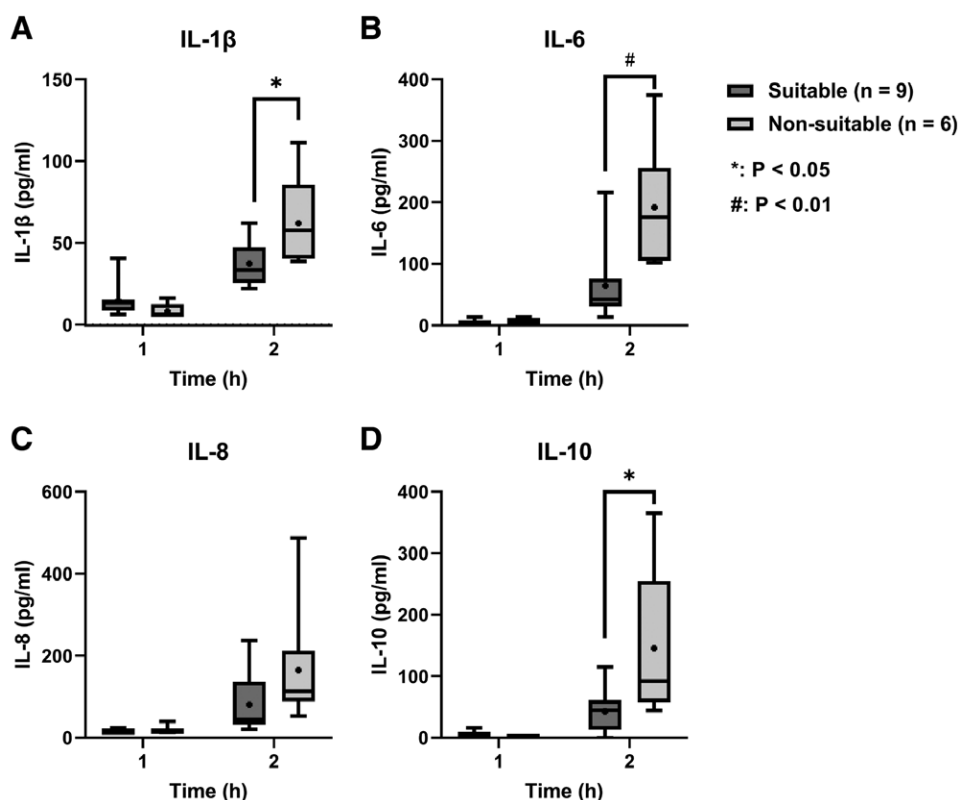
FIGURE 9. A comparison of inflammatory cytokine level between suitable and non-suitable cases. A, There was a significant difference in IL-1β at 2 h between suitable and non-suitable cases ($P < 0.05$). B, A significant difference was found in IL-6 at 2 h ($P < 0.01$). C, Although there was no significant difference in IL-8, the average value of IL-8 at 2 h in non-suitable cases was higher than that in suitable cases. D, There was a significant difference in IL-10 at 2 h between suitable and non-suitable cases ($P < 0.05$). IL, interleukin.

non-touch period was included with an interval of at least 0.5 min after the flow increase. Third, because the Lund-type EVLP was used in this study, the results might be different for other types of EVLP (Toronto protocol or Organ Care System). Fourth, in the lung weight simulation, an estimation error was observed. This is because the parameters were considered as constant to simplify the simulation. Fifth, dynamic compliance in non-suitable cases was significantly lower than in suitable cases at 30 to 170 min. This result is explained by fluid leakage into the airway. Dynamic compliance might be another early indicator of EVLW.

In conclusion, the utility of real-time lung weight measurement during cellular EVLP was investigated using a porcine lung model. Real-time lung weight gain at 40 min was significantly associated with transplant suitability and signs of pulmonary edema (P/F ratio, peak inspiratory pressure, and W/D ratio). This finding suggests that real-time lung weight gain is a potential early indicator of transplant suitability in cellular EVLP and may be of use in clinical practice.

REFERENCES

1. Chambers DC, Zuckermann A, Cherikh WS, et al; International Society for Heart and Lung Transplantation. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: 37th adult lung transplantation report—2020; focus on deceased donor characteristics. *J Heart Lung Transplant.* 2020;39:1016–1027.
2. Steen S, Liao Q, Wierup PN, et al. Transplantation of lungs from non-heart-beating donors after functional assessment ex vivo. *Ann Thorac Surg.* 2003;76:244–252.
3. Okamoto T, Niikawa H, Ayyat K, et al. Machine perfusion of lungs. *Curr Transpl Rep.* 2019;6:251–264.
4. Nilsson T, Gielis JF, Slama A, et al. Comparison of two strategies for ex vivo lung perfusion. *J Heart Lung Transplant.* 2017;8:292–298.
5. Okamoto T, Ayyat KS, Sakanoue I, et al. Clinical significance of donor lung weight at procurement and during ex vivo lung perfusion. *J Heart Lung Transplant.* 2022;41:818–828.
6. Motoyama H, Chen F, Ohsumi A, et al. Protective effect of plasmin in marginal donor lungs in an ex vivo lung perfusion model. *J Heart Lung Transplant.* 2013;32:505–510.
7. Trebbia G, Sage E, Le Guen M, et al; Foch Lung Transplant Group. Assessment of lung edema during ex-vivo lung perfusion by single transpulmonary thermodilution: a preliminary study in humans. *J Heart Lung Transplant.* 2019;38:83–91.
8. Ayyat KS, Okamoto T, Niikawa H, et al. DireCt Lung Ultrasound Evaluation (CLUE): a novel technique for monitoring extravascular lung water in donor lungs. *J Heart Lung Transplant.* 2019;38:757–766.
9. Ayyat KS, Okamoto T, Niikawa H, et al. A CLUE for better assessment of donor lungs: novel technique in clinical ex vivo lung perfusion. *J Heart Lung Transplant.* 2020;39:1220–1227.
10. Sakanoue I, Okamoto T, Ayyat KS, et al. Real-time lung weight measurement during ex vivo lung perfusion: clinical importance of early weight gain. *J Heart Lung Transplant.* 2021;40:S69–S70.
11. Sakota D, Kosaka R, Niikawa H, et al. Optical oxygen saturation imaging in cellular ex vivo lung perfusion to assess lobular pulmonary function. *Biomed Opt Express.* 2022;13:328–343.
12. Kosaka R, Sakota D, Niikawa H, et al. Lung thermography during the initial reperfusion period to assess pulmonary function in cellular ex vivo lung perfusion. *Artif Organs.* 2022;46:1522–1532.
13. Pasque MK. Standardizing thoracic organ procurement for transplantation. *J Thorac Cardiovasc Surg.* 2010;139:13–17.
14. Niikawa H, Okamoto T, Ayyat KS, et al. The protective effect of prone lung position on ischemia-reperfusion injury and lung

function in an ex vivo porcine lung model. *J Thorac Cardiovasc Surg*. 2019;157:425–433.

15. Okamoto T, Wheeler D, Liu Q, et al. Correlation between PaO_2/FiO_2 and airway and vascular parameters in the assessment of cellular ex vivo lung perfusion system. *J Heart Lung Transplant*. 2016;35:1330–1336.

16. Wierup P, Haraldsson A, Nilsson F, et al. Ex vivo evaluation of nonacceptable donor lungs. *Ann Thorac Surg*. 2006;81:460–466.

17. Okamoto T, Wheeler D, Liu Q, et al. Variability in pressure of arterial oxygen to fractional inspired oxygen concentration ratio during cellular ex vivo lung perfusion: implication for decision making. *Transplantation*. 2015;99:2504–2513.

18. Rippe B, Townsley M, Parker JC, et al. Osmotic reflection coefficient for total plasma protein in lung microvessels. *J Appl Physiol (1985)*. 1985;58:436–442.

19. Meyer BJ, Meyer A, Guyton AC. Interstitial fluid pressure. V. Negative pressure in the lungs. *Circ Res*. 1968;22:263–271.

20. Wallinder A, Hansson C, Dellgren G. Hemoconcentration in ex vivo lung perfusion: a case report of a novel technique used in clinical lung transplantation. *J Thorac Cardiovasc Surg*. 2013;145: e76–e77.

21. Hall JE. Pulmonary circulation, pulmonary edema, pleural fluid. In: Hall JE, ed, *Guyton and Hall Textbook of Medical Physiology*. 13th ed. Elsevier; 2016.

22. Okamoto T, Niikawa H, Wheeler D, et al. Significance of lung weight in cellular ex vivo lung perfusion. *J Surg Res*. 2021;260:190–199.

23. Taylor AE. Capillary fluid filtration. Starling forces and lymph flow. *Circ Res*. 1981;49:557–575.

24. Aboab J, Louis B, Jonson B, et al. Relation between $PaO2$/FiO_2 ratio and FiO_2: a mathematical description. *Intensive Care Med*. 2006;32:1494–1497.

25. Sylvester JT, Shimoda LA, Aaronson PI, et al. Hypoxic pulmonary vasoconstriction. *Physiol Rev*. 2012;92:367–520.

26. Sage E, De Wolf J, Puyo P, et al. Real-time computed tomography highlights pulmonary parenchymal evolution during ex vivo lung reconditioning. *Ann Thorac Surg*. 2017;103:e535–e537.

27. Wallinder A, Riise GC, Ricksten SE, et al. Transplantation after ex vivo lung perfusion: a midterm follow-up. *J Heart Lung Transplant*. 2016;35:1303–1310.

28. Gaar KA Jr, Taylor AE, Owens LJ, et al. Pulmonary capillary pressure and filtration coefficient in the isolated perfused lung. *Am J Physiol*. 1967;213:910–914.