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## Lung hyperinflation stimulates the release of inflammatory mediators in spontaneously breathing subjects

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# Lung hyperinflation stimulates the release of inflammatory mediators in spontaneously breathing subjects

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## Abstract

Lung hyperinflation up to vital capacity is used to re-expand collapsed lung areas and to improve gas exchange during general anesthesia. However, it may induce inflammation in normal lungs. The objective of this study was to evaluate the effects of a lung hyperinflation maneuver (LHM) on plasma cytokine release in 10 healthy subjects (age:  $26.1 \pm 1.2$  years, BMI:  $23.8 \pm 3.6$  kg/m<sup>2</sup>). LHM was performed applying continuous positive airway pressure (CPAP) with a face mask, increased by 3-cmH<sub>2</sub>O steps up to 20 cmH<sub>2</sub>O every 5 breaths. At CPAP 20 cmH<sub>2</sub>O, an inspiratory pressure of 20 cmH<sub>2</sub>O above CPAP was applied, reaching an airway pressure of 40 cmH<sub>2</sub>O for 10 breaths. CPAP was then decreased stepwise. Blood samples were collected before and 2 and 12 h after LHM. TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, IL-10, and IL-12 were measured by flow cytometry. Lung hyperinflation significantly increased ( $P < 0.05$ ) all measured cytokines (TNF- $\alpha$ :  $1.2 \pm 3.8$  vs  $6.4 \pm 8.6$  pg/mL; IL-1 $\beta$ :  $4.9 \pm 15.6$  vs  $22.4 \pm 28.4$  pg/mL; IL-6:  $1.4 \pm 3.3$  vs  $6.5 \pm 5.6$  pg/mL; IL-8:  $13.2 \pm 8.8$  vs  $33.4 \pm 26.4$  pg/mL; IL-10:  $3.3 \pm 3.3$  vs  $7.7 \pm 6.5$  pg/mL, and IL-12:  $3.1 \pm 7.9$  vs  $9 \pm 11.4$  pg/mL), which returned to basal levels 12 h later. A significant correlation was found between changes in pro- (IL-6) and anti-inflammatory (IL-10) cytokines ( $r = 0.89$ ,  $P = 0.004$ ). LHM-induced lung stretching was associated with an early inflammatory response in healthy spontaneously breathing subjects.

Key words: Lung hyperinflation; Ventilator-induced lung injury; Inflammation; Cytokines

## Introduction

Pulmonary collapse is a frequent complication in sedated patients with either normal or injured lungs undergoing mechanical ventilation, decreasing respiratory system compliance and worsening gas exchange (1-4).

Lung hyperinflation maneuvers (LHM) used to expand lungs up to vital capacity by means of elevated inspiratory pressures have been shown to reopen collapsed lung regions and to improve pulmonary gas exchange (5-8). Despite these short-term beneficial effects on blood oxygenation and lung mechanics, a growing body of evidence from experimental and clinical studies has shown that overinflation-induced lung stretch may induce pulmonary inflammation with the release of cytokine and promote lung tissue injury (9-13). However, few data are available about the impact of lung hyperinflation maneuvers on pulmonary inflammatory response in spontaneously breathing healthy subjects, independently of the known harmful effects of

mechanical ventilation (14,15).

We hypothesize that, even in spontaneously breathing healthy subjects, respiratory maneuvers that promote lung over-distention would induce a pulmonary-generated inflammatory response and the release of cytokines into blood. The objective of the present study was to evaluate plasma cytokine behavior after an LHM applied to healthy spontaneously breathing subjects for a short period of time.

## Material and Methods

After approval by the Institutional Ethics Committee of the Hospital das Clínicas of São Paulo University Medical School and obtaining written informed consent, 10 non-smoking spontaneously breathing healthy young adult volunteers were studied. None of the subjects had a history of lung disease, active lung or systemic infection,

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drug-induced immunosuppression or underlying disease, or was currently using anti-inflammatory medications.

LHM was performed using a non-invasive ventilator (BiPAP Vision, Respironics Inc., USA) with a 21% inspiratory oxygen fraction during spontaneous quiet breathing. The subjects were comfortably seated on a chair and a total face mask (Respironics Inc.) was tightly adjusted to avoid air leaks. Five cmH<sub>2</sub>O of continuous positive airway pressure (CPAP) was first applied, and increased by 3 cmH<sub>2</sub>O in a stepwise fashion every 5 breaths until reaching 20 cmH<sub>2</sub>O. When 20 cmH<sub>2</sub>O CPAP was reached, an additional inspiratory pressure of 20 cmH<sub>2</sub>O above CPAP was set by using the pressure bi-level ventilation mode, reaching an end-inspiratory airway peak pressure of 40 cmH<sub>2</sub>O during the successive 10 breaths. Thereafter, CPAP was decreased stepwise from 20 to 5 cmH<sub>2</sub>O every 5 breaths by 3 cmH<sub>2</sub>O steps. During the entire LHM procedure, the subjects were continuously monitored by electrocardioscopy and pulse oximetry while arterial pressure was recorded noninvasively at 1-min interval. After the end of the LHM, the subjects were carefully examined to exclude possible respiratory complications and control chest X-ray plates were obtained. Venous blood samples were collected immediately before (baseline) and 2 and 12 h after the LHM.

**Table 1.** Anthropometric data of the healthy subjects studied.

Subject	Gender	Age (years)	Weight (kg)	Height (m)	BMI (kg/m <sup>2</sup> )
1	F	25	52	1.64	19.3
2	F	26	56	1.63	21.1
3	M	26	92	1.82	27.8
4	M	27	88	1.87	25.2
5	M	27	80	1.82	24.2
6	F	26	54	1.62	20.6
7	F	24	61	1.61	23.5
8	F	27	57	1.63	21.5
9	F	28	66	1.66	24.0
10	M	25	104	1.83	31.1

BMI = body mass index.

**Table 2.** Oximetric and hemodynamic data before and after lung hyperinflation.

	Pre-LHM	Post-LHM
Pulse oximetry (%)	97 ± 1	98 ± 1*
Heart rate (bpm)	71 ± 15	70 ± 13
Mean arterial pressure (mmHg)	94 ± 13	88 ± 9

LHM = lung hyperinflation maneuver. \*P < 0.05 compared to pre-LHM (Wilcoxon paired test).

Immediately after sampling, 5-mL aliquots of venous blood containing EDTA were centrifuged at 1100 g for 3 min, and the plasma was aspirated and stored at -70°C. Simultaneous detection of multiple soluble analytes in a particle-based immunoassay was used to quantitatively measure interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12, and tumor necrosis factor (TNF)-α protein levels in serum samples by a cytometric bead array (Human Inflammation Kit, BD Biosciences, Germany).

### Statistical analysis

Statistical analysis was performed using the Sigmapstat 3.0 statistical package. Heart rate and mean arterial pressure were compared before and immediately after LHM by the paired Student *t*-test. Peripheral oxygen saturation values were tested by the Wilcoxon paired test. Blood cytokine concentrations as a function of time were analyzed by one-way analysis of variance (ANOVA) for repeated measures followed by the Student-Newman-Keuls test for *post hoc* analysis when indicated. Correlations between cytokines were studied using linear regression and Pearson product-moment correlation coefficient. Data are reported as means ± SD and the level of significance was set at P < 0.05.

## Results

Four male and 6 female healthy subjects with a mean age of 26.1 ± 1.2 years and mean body mass index of 23.8 ± 3.6 kg/m<sup>2</sup> were included in the study (Table 1). No significant changes in mean arterial pressure or heart rate were observed during or immediately after the LHM. However, we observed a significant increase in peripheral oxygen saturation after LHM (Table 2). No significant changes in respiratory pattern (tidal volume and respiratory rate) were observed.

As shown in Figure 1, LHM induced a significant increase in 2-h plasma concentration of all measured cytokines, with a return to baseline levels 12 h later. A close correlation was observed between the LHM-induced increase in inflammatory (IL-6) and anti-inflammatory (IL-10) cytokines, as shown in Figure 2. Clinical examination after the procedure did not detect harmful events. Neither pneumothorax nor other extra-alveolar air leaks were observed in control chest X-ray plates.

## Discussion

We observed that the application of an LHM (inspiratory pressure of 40 cmH<sub>2</sub>O for a short period of time) by noninvasive ventilation increased both inflammatory and anti-inflammatory cytokines in spontaneously breathing healthy subjects. To our knowledge, this is the first study showing, that under these conditions, that LHM elicits a systemic inflammatory response independently of underlying

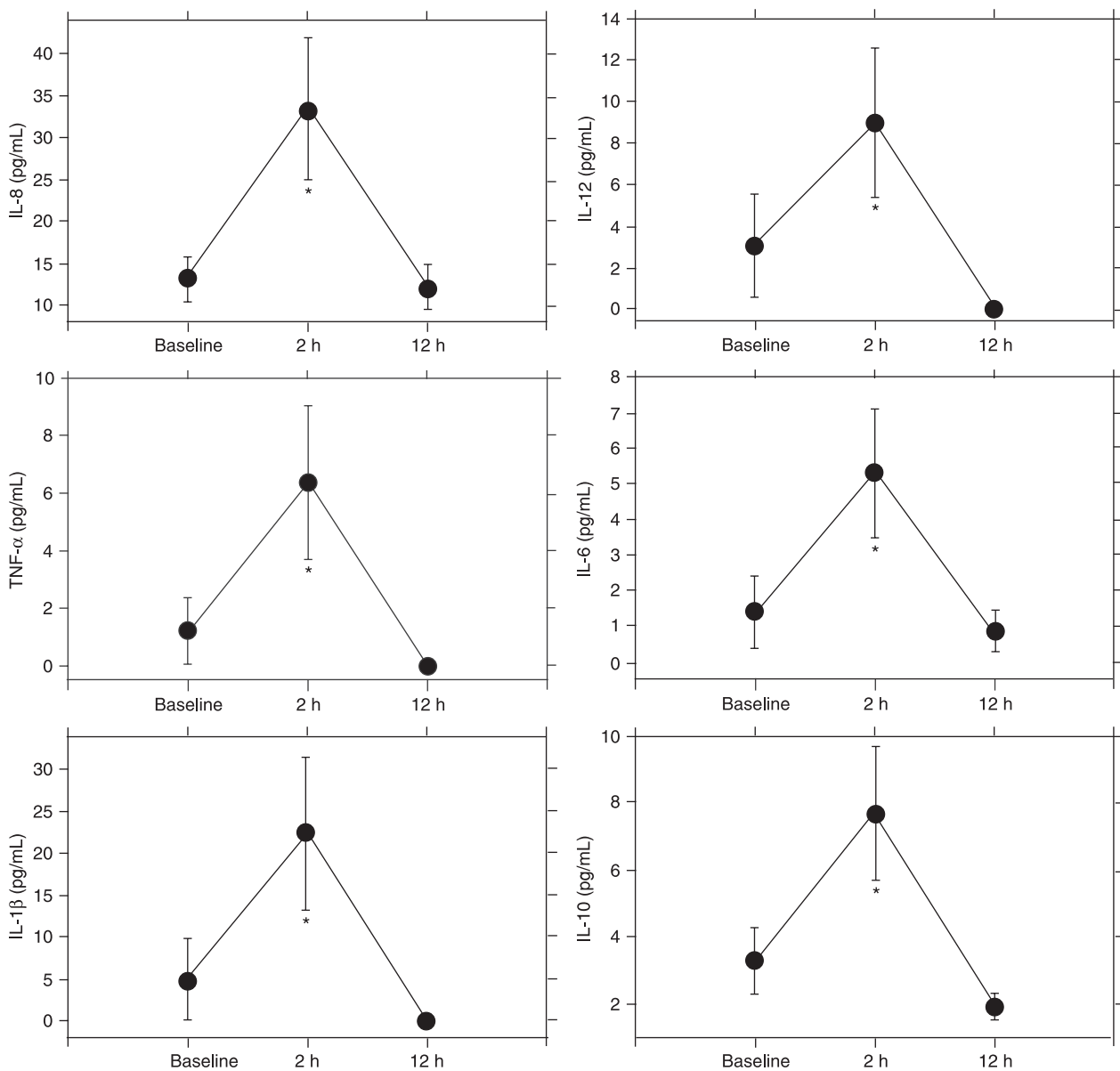
ing pulmonary disease or mechanical ventilation.

Several publications have reported beneficial effects of LHM on reopening of atelectatic lung regions, improving oxygenation and pulmonary compliance during general anesthesia and in acute respiratory failure, either in experimental or clinical settings (6,16-21). However, stretching the lung parenchyma beyond its physiological limits using high airway inspiratory pressures is associated with an over-distention-induced inflammatory response.

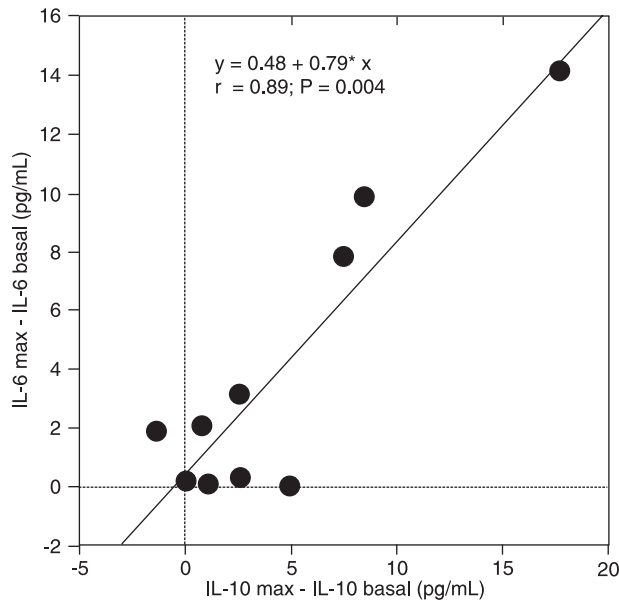
In this study, we observed that an LHM applied for a short

period of time (up to 1 to 2 min) to normal homogeneously aerated lungs induced a slight but significant increase in plasma pro- and anti-inflammatory cytokines, with a return to baseline levels within 12 h. Apart from the LHM, none of the healthy subjects participating in this study had active or chronic inflammatory conditions that could explain the release of plasma cytokines. After performing the maneuver, subjects remained in relative rest during the following 12 h in order to avoid any further inflammatory stimuli.

Lung parenchyma stretch can modify the gene ex-



**Figure 1.** Plasma cytokine (TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, IL-10, IL-12) levels. \*P < 0.05 compared to baseline value for each cytokine (one-way ANOVA for repeated measures followed by the Student-Newman-Keuls test).



**Figure 2.** Linear regression between the lung hyperinflation maneuver-induced increase in IL-6 and IL-10. Correlation tested by Pearson product-moment correlation coefficient.

pression of several inflammatory and anti-inflammatory molecules in the lung (22), which may ultimately lead to an inflammatory process. Vlahakis et al. (23) detected *in vitro* IL-8 release from alveolar epithelial type II cells stretched by 30% for up to 48 h. Wilson et al. (12) have shown that high-stretch ventilation by applying high tidal volumes induces intrapulmonary TNF- $\alpha$  and macrophage-inflammatory protein-2 (MIP-2) expression in previously healthy mice. Moriondo et al. (24) showed that mechanical ventilation could markedly damage the extracellular matrix in previously healthy rats in a tidal volume-dependent fashion. Mascheroni et al. (25) reported extensive lung injury after intense hyperventilation and increased transpulmonary pressures in spontaneously breathing previously healthy sheeps. Ranieri et al. (13) have shown that mechanical ventilation of patients with acute respiratory failure could induce a cytokine response that may be attenuated by a strategy to minimize over-distention and recruitment/de-recruitment of the lungs. More recently, Terragni et al. (26) showed that airway pressures up to 30 cmH<sub>2</sub>O might induce a marked inflammatory response in patients with acute lung injury/acute respiratory distress syndrome during invasive

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mechanical ventilation.

Evidence to the contrary has been published by Puls et al. (27), who did not find any release of inflammatory mediators after LHM using 40 cmH<sub>2</sub>O CPAP for 7 s in patients with acute respiratory failure in different stages of their diseases. Since most of their patients were recruited relatively late in the course of their disease, and did not show any increase in PaO<sub>2</sub> until 180 min, the pressure and the length of airway pressurization during LHM may not have been enough to open collapsed small airways and alveoli.

Interestingly, we found a close correlation between inflammatory and anti-inflammatory cytokines. Thus, we hypothesize that, at least in healthy subjects, the acute stretch of the lung parenchyma on the one hand stimulates the release of inflammatory cytokines, but on the other hand this effect is, at least in part, balanced by a subsequent increase in anti-inflammatory cytokines. It is possible that such a phenomenon is the physiological response to parenchymal stretch in normal conditions. Since a single LHM was applied during the study and subjects were not submitted to any stressful physiological stimuli, pro- and anti-inflammatory cytokines returned to baseline values within 12 h. However, the magnitude of increase in cytokines was not great enough to allow us to support the notion that the inflammatory response triggered by a single lung hyperinflation maneuver may damage the lungs, especially if weighed against the beneficial effects of LHM on lung collapse. On the other hand, it remains to be determined if the repeated use of such maneuvers can be detrimental in subjects with normal lungs.

Since a single LHM was performed in our study, our data cannot be generalized to other maneuvers using different airway pressures and pressurization time length. Since we applied both an increase in positive end-expiratory pressure and delta inspiratory pressure, we are unable to determine if the effects were mainly due to stress or strain or to their combination. The study was conducted on healthy subjects during spontaneous breathing in the absence of sedation or invasive mechanical ventilation and the data cannot be directly extrapolated to clinical conditions of diseased lungs that are being ventilated using elevated airway pressures.

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