

Pulmonary gas exchange responses to histamine and methacholine challenges in mild asthma

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ABSTRACT: Histamine (HIST) produces greater changes in bronchial and pulmonary vasculature, and so may produce more gas exchange abnormalities, than methacholine (MTH) after inhalational challenge. The goals of this study were to compare the effects of HIST and MTH challenge on pulmonary gas exchange in patients with mild asthma at an equivalent degree of bronchoconstriction.

Eleven patients were studied (mean±SEM age, 22±1 yr; forced expiratory volume in one second (FEV₁), 91±5% pred) using a randomized, double-blind cross-over design. Respiratory system resistance (*R_{rs}*), arterial blood gases, and ventilation-perfusion distributions were measured before and after HIST/MTH challenges when cumulative doses caused a 30% fall in FEV₁.

Compared with baseline, HIST and MTH provoked similar moderate to severe increases in *R_{rs}* (*p*<0.005 each), and mild to moderate decreases in arterial oxygen tension (*P_aO₂*) due to ventilation-perfusion abnormalities (dispersion of pulmonary blood flow -log SDQ-, 0.40±0.03–0.71±0.08 and 0.47±0.04–0.89±0.06; normal values <0.60–0.65), respectively, similar to those shown in mild to moderate acute asthma, without differences between them.

For the same degree of airflow obstruction, both histamine and methacholine bronchoprovocations induce, in patients with mild asthma, very similar disturbances in ventilation-perfusion distribution and respiratory system resistance, suggesting similar mechanisms of airway narrowing.

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The strong evidence for inflammation in the airways of patients with asthma underpins the pathophysiology of the disease. Histamine (HIST) is a well known inflammatory mediator invoked in the pathogenesis of bronchial asthma. The release of this biogenic amine has been shown to be implicated in the development of several asthmatic symptoms including bronchial smooth muscle contraction, increased mucous secretion and bronchial vasodilatation through a nitric oxide (NO) synthesis-dependent mechanism [1, 2], and pulmonary vasoconstriction [3].

The multiple inert gas elimination technique (MIGET) has shown that, in adult patients with mild asthma, methacholine (MTH) challenge produces mild to moderate arterial hypoxaemia caused by ventilation-perfusion (*V'/Q'*) heterogeneity, namely, broadened unimodal blood flow and ventilation distributions

related to maldistribution of ventilation, without changes in shunt and dead space [4, 5]. By contrast, HIST challenge produces, in childhood asthma, moderate hypoxaemia due to the development of low *V'/Q'* areas, together with a bimodal pattern in the ventilation distribution [6]. Although no information is available on *V'/Q'* response to HIST challenge in adult asthmatics, it is generally postulated, but never clearly proven, that bronchoprovocation with HIST enhances more hypoxaemia than with MTH.

There is evidence for dissociation between expiratory airflow and gas exchange disturbances in asthma [7]. This is consistent with the view that spirometry predominantly reflects airway narrowing in both larger and middle-sized airways, more closely related to bronchoconstriction, while changes in gas exchange reflect changes in more peripheral airways where

inflammation is prominent. Patients with chronic asthma have an inflammatory cellular response involving both central and distal airways [8].

The MIGET was used to study detailed pulmonary gas exchange after HIST and MTH challenges producing an equivalent degree of bronchoconstriction. It was postulated that HIST could be more deleterious to pulmonary gas exchange than MTH given its more potent vascular effects, hence typically inducing more V'/Q' disturbances, namely, areas with low V'/Q' units and bimodal blood flow patterns commensurate with the findings seen in acute severe asthma [6]. This is the first time in which the responses of alveolar ventilation to pulmonary perfusion matching to both inhalational HIST and MTH challenges are investigated comparatively while inducing the same level of airway narrowing.

Methods

Study population

Eleven nonsmoking patients (eight females) with mild asthma (22 ± 1 yr; forced expiratory volume in one second (FEV₁), 3.4 ± 0.1 L ($91 \pm 5\%$ pred); FEV₁/forced vital capacity (FVC), $79 \pm 3\%$; and provocative dose causing a 20% fall in FEV₁ (PD₂₀), 0.6 ± 0.1 μ mol) were recruited either from the outpatient Dept of the Hospital Clínic of Barcelona or through local advertisements for the study, which was approved by the Ethical Research Committee. Patients received financial remuneration for their participation. All subjects gave informed written consent after the purpose, risks and potential benefits of the study were explained to them. The inclusion criteria were: >18 yrs of age and <45 yrs; no respiratory infection or exacerbation of asthma within the preceding 6 weeks; FEV₁ $\geq 70\%$ pred and ≥ 1.5 L after withholding adrenergic agents for 12 h and inhaled steroids for 24 h, and positive methacholine (PD₂₀ <1.9 μ mol) bronchial challenge on their first visit; no previous treatment with oral steroids; and absence of any systemic or cardiopulmonary disease other than asthma. All subjects were atopic, as judged by the presence of a positive response to skin prick tests (SPT) to one or more common aeroallergens. Maintenance therapy included aerosol short-acting (n=6) and long-acting (n=4) selective beta-adrenergics and/or inhaled glucocorticosteroids (n=6).

Measurements

On the day of the study, forced spirometry (Datospir-2000; Sibel-Med, Barcelona, Spain) according to the American Thoracic Society (ATS) recommendations [9] and total respiratory system resistance (R_{rs}), measured by the forced oscillation technique [10], were performed in each patient. Predicted equations for forced spirometry were those of ROCA *et al.* [11] and reference values for R_{rs} were those of PESLIN *et al.* [10].

Blood samples were collected anaerobically through a catheter inserted into the radial artery. Arterial oxygen tension P_{a,O_2} , carbon dioxide tension (P_{a,CO_2})

and pH were analysed in duplicate using standard electrodes and haemoglobin concentration was measured by a Co-oximeter (Ciba Corning 860 System, Ciba Corning Diagnostics Corporation, Meadfield, MA, USA). Oxygen uptake ($V'O_2$) and CO₂ production ($V'CO_2$) were calculated from mixed expired O₂ and CO₂ concentrations (CPX/D, MCG Medical Graphics Corporation, St. Paul, MN, USA). Both minute ventilation ($V'E$) and respiratory frequency (f_R) were measured using a calibrated Wright spirometer (Respirometer MK8, BOC-Medical, Essex, UK). The alveolar-arterial oxygen tension gradient (P_{A-a,O_2}) was calculated according to the alveolar gas equation using the measured respiratory exchange ratio (RER).

MIGET was used to estimate the distributions of ventilation-perfusion (V'/Q') ratios without sampling mixed venous inert gases [12]. In brief, an intravenous infusion, was started and maintained of six inert gases dissolved in saline for 45 min before measurements while the infusion rate was 5 mL·min⁻¹. Duplicate samples of arterial and mixed expired gases were processed separately for six inert gas concentration measurements by gas chromatography. From the measured concentrations, retention and excretion values for each of the six inert gases were calculated, and retention-solubility and excretion-solubility curves were constructed. The distribution of ventilation and blood flow was estimated from these curves. With this approach, cardiac output (Q') needs to be directly measured by dye dilution technique (DC-410; Waters Instruments Inc., Rochester, MN, USA) using 5 mg bolus of indocyanine green injected through a catheter placed percutaneously in an arm vein, while mixed venous inert gas concentrations are computed from mass balance equations. The duplicate samples of each set of measurements were treated separately, the final data resulting in the average of variables calculated from both sets at each point in time.

A three-lead electrocardiogram, heart rate (HR) and systemic arterial pressure (P_s) and arterial oxygen saturation (S_{a,O_2}) through a pulse oximeter (HP M1166A, Hewlett-Packard, Boblingen, Germany) were continuously recorded throughout the whole study (HP 7830A Monitor and HP 7754B Recorder, Hewlett-Packard, Waltham, MA, USA).

Study design

A randomized, double-blind, two-period, cross-over design was used. After completing the first visit to check all inclusion and exclusion criteria, patients were challenged on two other days, one week apart, with HIST diphosphate or MTH chloride (Sigma-Aldrich Química SA, Madrid, Spain) by a nebulizer attached to a breath-activated dosimeter (Mefar, Brescia, Italy). The nebulizer delivered particles with a mass median aerodynamic diameter of 3.5–4.0 mm at an output of 9 mL·breath⁻¹. The dosimeter was set to nebulize for 0.8 s with a pause time of 6.0 s at a pressure of 0.176 kPa. During the challenges the patients breathed room air and were seated in an armchair. All asthma medication was withheld for 48 h before arrival to the laboratory and subjects were asked to refrain from

caffeine-containing beverages/foods for at least 12 h before testing. Maintenance of steady-state conditions was demonstrated by stability ($\pm 5\%$) of both ventilatory and haemodynamic variables, and by the close agreement between duplicate measurements of mixed expired and arterial O_2 and CO_2 (within $\pm 5\%$). These conditions were met by all patients throughout all the period of study and the residual sum of squares (RSS), a descriptor of the closeness of the fit of inert gas data, was 7.0 ± 1.4 for the HIST challenge study and 7.7 ± 2.5 for the MTH challenge study (RSS < 10.6 in 72% of the samples). The patient was then challenged with HIST/MTH following the recommended standardized procedures [13]. In each patient, increasing HIST/MTH concentrations (0.195–12.5 mg each) were used causing $\geq 30\%$ fall in FEV₁; at this time, R_{rs} was also measured. Delivery of HIST/MTH was always performed by the same observers. The challenge was terminated when the 30% fall in FEV₁ was achieved, or upon patient request because of respiratory discomfort or shortness of breath. Immediately after completing the fall in FEV₁, a set of duplicate measurements was performed. All sets of measurements consisted of the following steps in sequence: FEV₁, R_{rs} and ventilatory recordings; inert and respiratory gas sampling; and, finally, haemodynamic recording. After the completion of this set of measurements, FEV₁ was measured again (15 min after the nadir of the challenge) to estimate the degree of recovery of airflow obstruction. No patient needed rescue medication with short-acting bronchodilators during or immediately after the end of the study and all the studies were clinically well tolerated by all the patients who always completed the study without complaints or major side effects. Two puffs (200 μ g) of inhaled salbutamol were routinely administered after full completion of the study. In two patients, inert gas data were not available due to chromatographic limitations.

Statistical analysis

Results are expressed as mean \pm SEM. The effects of HIST and MTH challenges on the different end-point variables were assessed by a two-way repeated analysis of variance (ANOVA). When the F value of the ANOVA was significant, *post hoc* comparisons were performed using paired t-test. Correlation's among variables were assessed with the Spearman's rank test. All analyses were performed with SPSS version 6.1.3. (SPSS Inc, Chicago, IL, USA). Statistical significance was set at $p < 0.05$ in all instances.

Results

Baseline conditions

Eleven patients were studied with mild asthma without clinical or functional differences between HIST and MTH study days. V'/Q' relationships were mostly within the normal range [14] (table 1). Distributions of pulmonary blood flow (log SDQ) and of alveolar ventilation (log SDV) were narrowly unimodal in all but one and two patients respectively, in whom they were broadly unimodal; shunt (percentage of blood flow to units with V'/Q' ratio < 0.005) and areas with low (< 0.1 , excluding shunt) or high (10–100) V'/Q' ratios were conspicuously negligible.

Histamine and methacholine responses

After HIST and MTH challenges (cumulative doses, 0.38 ± 0.13 mg and 0.22 ± 0.05 mg), FEV₁ decreased (by $37 \pm 1\%$ and $38 \pm 2\%$) and both R_{rs} (by 83% and 78%) and $V'E$ increased, respectively. fR , HR, P_s and Q' remained unchanged; $V'O_2$ increased after MTH only (figs. 1 and 2).

Table 1. – Effects of histamine/methacholine challenges

	Histamine		Methacholine	
	Before	After	Before	After
FEV ₁ L	3.2 \pm 0.1	2.0 \pm 0.1***	3.1 \pm 0.1	2.0 \pm 0.1***
FEV ₁ % pred	88 \pm 5	56 \pm 3***	90 \pm 5	55 \pm 3***
R_{rs} cmH ₂ O \cdot L ⁻¹ \cdot s	4.1 \pm 0.5	7.5 \pm 1.2 [#]	4.1 \pm 0.6	7.3 \pm 1.2 [#]
$V'E$ L \cdot min ⁻¹	5.2 \pm 0.3	6.0 \pm 0.4*	5.3 \pm 0.4	6.1 \pm 0.3**
fR min ⁻¹	11 \pm 1	13 \pm 1	12 \pm 1	12 \pm 1
P_{a,O_2} mmHg	98 \pm 1	73 \pm 4***	95 \pm 1	68 \pm 3***
P_{a,CO_2} mmHg	36 \pm 1	35 \pm 1	37 \pm 1	35 \pm 1
P_{A-a,O_2} mmHg	6 \pm 1	31 \pm 4***	8 \pm 2	36 \pm 3***
$V'O_2$ mL \cdot min ⁻¹	216 \pm 7	223 \pm 9	207 \pm 6	229 \pm 11*
log SDQ	0.40 \pm 0.03	0.71 \pm 0.08 [#]	0.47 \pm 0.04	0.89 \pm 0.06***
log SDV	0.50 \pm 0.07	0.84 \pm 0.04 [#]	0.56 \pm 0.08	0.87 \pm 0.04 [#]
DISP R-E*	2.6 \pm 0.5	7.9 \pm 1.0***	3.6 \pm 0.7	10.4 \pm 1.1***
HR min ⁻¹	75 \pm 4	78 \pm 3	73 \pm 3	77 \pm 2
P_s mmHg	85 \pm 3	84 \pm 2	79 \pm 2	82 \pm 3
Q' L \cdot min ⁻¹	5.8 \pm 0.3	6.2 \pm 0.2	5.6 \pm 0.3	5.7 \pm 0.3

Data presented as mean \pm SEM. FEV₁: forced expiratory volume in one second; R_{rs} : resistance of respiratory system; $V'E$: minute ventilation; fR : respiratory frequency; P_{a,O_2} : arterial oxygen tension; P_{a,CO_2} : arterial carbon dioxide tension; P_{A-a,O_2} : alveolar-arterial oxygen tension; $V'O_2$: oxygen uptake; log SDQ: dispersion of pulmonary blood flow; log SDV: dispersion of alveolar ventilation; DISP R-E*: dispersion of retention minus excretion of inert gases corrected for dead space; HR: heart rate; P_s : systemic arterial pressure; Q' : cardiac output. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; #: $p < 0.005$.

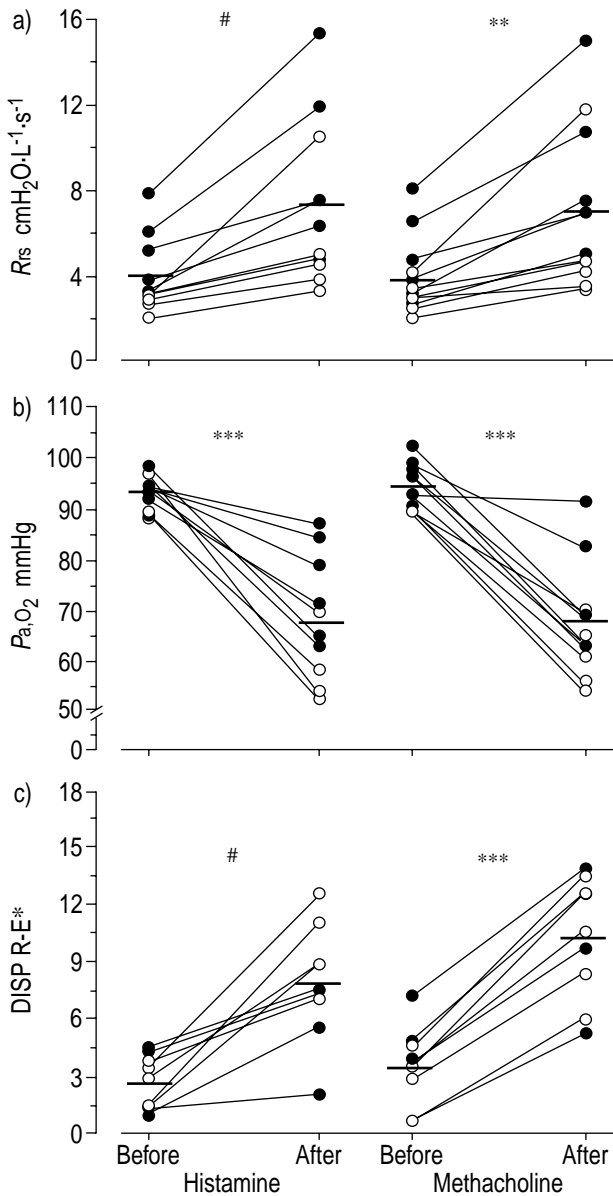


Fig. 1. – Individual and mean (thick bars) values of a) respiratory system resistance; b) arterial oxygen tension; and c) ventilation/perfusion mismatch, expressed in their own units (DISP R-E*; dimensionless), before and after histamine or methacholine challenges. ● : patients using inhaled glucocorticoids; #: $p < 0.005$; **: $p < 0.01$; ***: $p < 0.001$.

Following HIST and MTH challenges, P_{a,O_2} fell (by 26% and 28%) and P_{A-a,O_2} increased (ranges, 4.8–45.2 and 15.4–38.5 mmHg); both P_{a,CO_2} and pH (7.41 ± 0.01 and 7.40 ± 0.01), respectively, did not vary. Similarly, basal V'/Q' relationships mildly to moderately worsened after HIST and MTH challenges, as shown by increases of both log SDQ and log SDV (HIST ranges, 0.33–1.05 and 0.59–1.06; and, MTH, 0.62–1.15 and 0.70–0.98, respectively; normal values, < 0.60 – 0.65 [14]), and/or of an overall index of V'/Q' heterogeneity (DISP R-E*; root mean square difference among measured retentions (R) and excretions (E) of the inert gases (except acetone) corrected for the dead space; normal

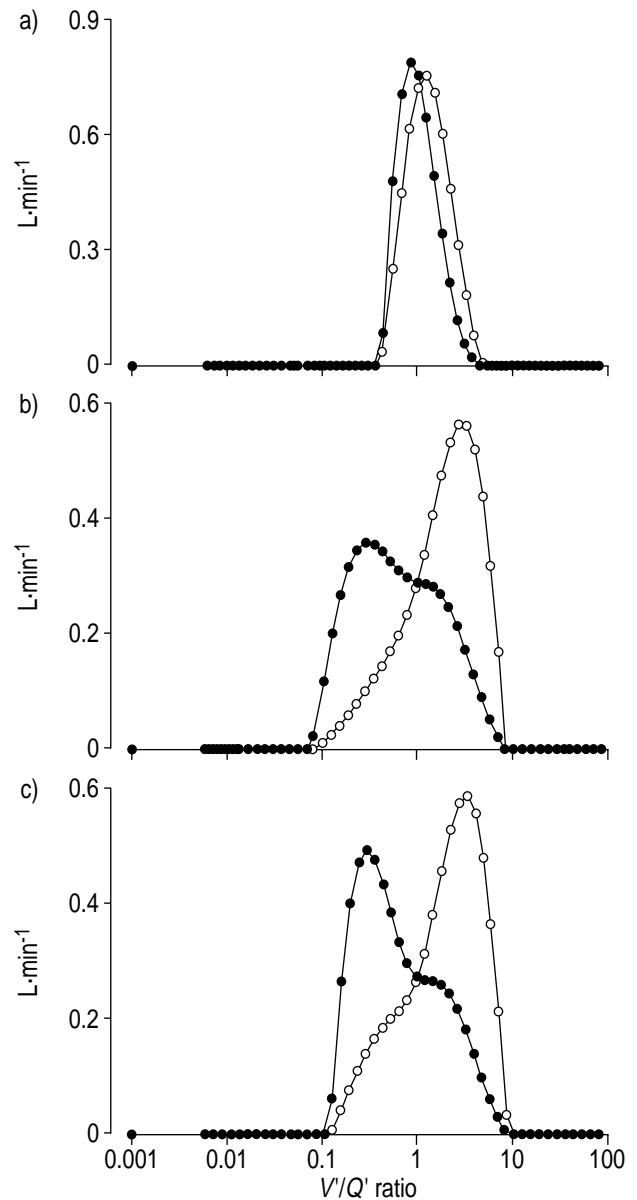


Fig. 2. – Representative ventilation/perfusion (V'/Q') ratio distributions in a patient, measured at a) baseline (arterial oxygen tension (P_{a,O_2})=94 mmHg; dispersion of retention minus excretion of inert gases corrected for dead space (DISP R-E*)=3.4; and forced expiratory volume in one second (FEV₁)=71% pred) and at the respective nadirs of b) histamine (P_{a,O_2} =58 mmHg; DISP R-E*=12.6; and FEV₁=49% pred) and c) methacholine (P_{a,O_2} =62 mmHg; DISP R-E*=13.6; and FEV₁=46% pred) challenges. Both challenges revealed similar mild to moderate V'/Q' defects, as shown by increases in the dispersion of pulmonary blood flow (log SDQ) (●) and that of alveolar ventilation (log SDV) (○), and similar broadly unimodal V'/Q' distributions reflected in marked increases of an overall index of V'/Q' heterogeneity (DISP-RE*).

values < 3.0). Distributions of pulmonary blood flow and alveolar ventilation modestly broadened in all patients. The mean V'/Q' ratio of the perfusion distribution (Q) showed a trend to decrease after HIST (from 0.70 ± 0.04 – 0.62 ± 0.03) or decreased after MTH (from 0.75 ± 0.05 – 0.62 ± 0.04) ($p < 0.05$). Similarly, the mean V'/Q' ratio of the ventilation distribution (V)

increased after both challenges (HIST, 0.86 ± 0.07 – 1.19 ± 0.10 ; and, MTH, 1.01 ± 0.08 – 1.44 ± 0.08 ; $p < 0.0001$ each). By contrast, there were no changes in intrapulmonary shunt, areas with low V'/Q' units or dead space (percentage of ventilation to units with V'/Q' ratio > 100). Mean FEV₁ after the completion of all measurements was still decreased (HIST, by $18 \pm 3\%$; and, MTH, by $19 \pm 3\%$, $p < 0.001$ each, respectively). All in all, there were no differences in the changes seen with HIST compared to MTH.

The difference in log SDV after HIST challenge as compared to baseline was correlated with the baseline log SDV ($r = -0.75$, $p < 0.02$). Likewise, the differences in P_{a,O_2} and P_{A-a,O_2} following HIST compared with baseline were correlated with those shown in log SDQ ($r = -0.79$, $p < 0.01$; and 0.68 , $p < 0.04$, respectively), and those in P_{a,O_2} as compared to those observed in DISPR-E* ($r = -0.68$, $p < 0.04$). The difference in FEV₁ following MTH as compared to baseline was correlated with the baseline FEV₁ value ($r = -0.84$, $p < 0.001$). No correlations were shown in any of the variables between pre- to post-HIST and MTH challenges.

A subanalysis was performed in the subset of the six patients using inhaled glucocorticoids to ensure that there was no effect or a lack of an effect with regard to any of the parameters investigated. Differences in pre- to post-HIST and MTH challenges between patients with and without inhaled steroids were not significant (fig. 1).

Discussion

The major finding in the present study was that HIST provoked, in patients with mild asthma, similar V'/Q' defects than MTH while undergoing an equivalent degree of bronchoconstriction. After both challenges, patients showed mild to moderate arterial hypoxaemia and/or increased P_{A-a,O_2} , due to V'/Q' mismatching. This was essentially characterized by broadly unimodal blood flow and ventilation distributions, while shunt and dead space remained unaltered; areas with low and high V'/Q' units were never observed. Both post-challenge V'/Q' findings were akin to those shown in adult patients during mild to moderate acute asthma [15], or after MTH [4], exercise [16] or allergen challenges [17], but at variance with those shown in children after HIST [6]. HIST challenge in childhood asthma uniquely developed a bimodal V'/Q' distribution centred around a V'/Q' ratio of 10, without changes in dead space, along with mild to moderate increases in the log SDQ [6]. These V'/Q' defects, never observed in adult asthmatics after different bronchial challenges [4–6, 16, 17], may be related to both gas trapping and lung hyperinflation whose pathogenic mechanism remains elusive. In contrast with this hypothesis, however, HIST challenge did not induce areas with low V'/Q' areas nor bimodal blood flow distributions, as shown in more life-threatening forms of acute asthma [18] or during acute experimental canine bronchoconstriction induced by nebulized MTH [19, 20], HIST, or *ascaris suum* extract [20], or more V'/Q' disturbances than those observed after MTH.

There were no correlations between spirometric or lung mechanic changes and gas exchange findings during both inhalational challenges. There was a good agreement, however, between respiratory and inert gas exchange indices after HIST. The latter correlations were seen because the principal extrapulmonary factors modulating arterial oxygenation, such as minute ventilation, cardiac output, and oxygen uptake, additional to V'/Q' inequalities, remained unaltered (or changed slightly) after HIST. However, since this study comprised a small number of subjects, a type 2 error may be too large to be confident that there were no major differences in variables after challenges.

The similarities between the degree of pulmonary gas exchange disturbances shown after HIST to those observed after MTH may indicate that the mechanisms of bronchoconstriction in both of these challenges are similarly heterogeneous in their anatomical basis, being mediated in both central and peripheral airways. Furthermore, areas with low V'/Q' ratio did not change after both challenges, suggesting a relatively well preserved active hypoxic pulmonary vasoconstriction, a very efficient collateral ventilation, and/or that airway occlusion was never complete [15, 18]. Although it is generally held that gas exchange impairment in asthma is mainly induced by small airways [7], experimental studies have shown that the V'/Q' imbalance becomes much more disrupted with larger airways narrowing [15]. In a previous study [4], the increases of airway resistance after MTH correlated with those of Log SDV, indicating that maldistribution of ventilation due to widespread airway narrowing was the most likely mechanism of MTH-induced V'/Q' inequalities. In another work in patients with adult asthma [5], in contrast to airway conductance changes, there was no difference in the V'/Q' responses whether the deposition of MTH was in central or peripheral airways.

It could be argued that part of the HIST challenge-induced V'/Q' disturbances may be caused by airway plasma leakage due to its potent bronchial vascular effects. MTH may also have airway vasoactive properties, potentially related to a NO-mediated vascular response [21] and aerosolized MTH may result in substantial increases in bronchial blood flow in sheep [22]. Although HIST may have more intense bronchial vascular effects than MTH, HIST-induced reinforcement of hypoxic pulmonary vasoconstriction [3] could facilitate a better matching between ventilation and perfusion, thereby minimizing its more deleterious effect on V'/Q' balance. Histamine in response to hypoxia is released from mast cells clustered primarily around the pulmonary vasculature resulting in vasoconstriction [3]. There were no differences between patients with and without regular inhaled steroid therapy. A previous anti-inflammatory treatment could favourably modulate the challenge response. Asthmatic patients using inhaled steroid therapy may have less small airway inflammation and thereby be more prone to exhibit fewer post-challenge V'/Q' defects than steroid-naïve patients at an equivalent degree of airflow obstruction [7, 18].

In summary, for the same degree of bronchoconstriction, histamine challenge does not induce more marked gas exchange disturbances than methacholine.

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