PULMONARY GAS EXCHANGE RESPONSE TO EXERCISE- AND MANNITOL- INDUCED BRONCHOCONSTRICTION IN MILD ASTHMA

(MS # JAP_00108-2008.R2)

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Supported by the Fondo de Investigaciones Sanitarias (FIS) (PI05208), the CibeRes (CB06/06), the Sociedad Española de Neumología y Cirugía Torácica (SEPAR) (SEPAR-2004), and a grant-in-aid by Esteve, Barcelona, Spain; HM was a Predoctoral Research Fellow from the European Respiratory Society (ERS/SEPAR LTRF #182).

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Running Head: EiB, Mannitol and Pulmonary Gas Exchange
ABSTRACT (n = 248 words)

Both exercise (EIB) and mannitol challenges were performed in asthmatic patients to assess and compare their pulmonary gas exchange responses for an equivalent degree of bronchoconstriction. In 11 subjects with EIB (27±4[SD]yrs; FEV\(_1\), 86±8% predicted) ventilation-perfusion (V\(_A/Q\)) distributions (using multiple inert gas elimination technique) were measured 5, 15 and 45 min after cycling exercise (FEV\(_1\) fall, 35±12%) and after mannitol (33±10%), one week apart. Five min after EIB, minute ventilation (V\(_E\), by 123±60%), cardiac output (Q\(_T\), by 48±29%) and oxygen uptake (VO\(_2\), by 54±25%) increased whereas PaO\(_2\) (by 14±11mmHg) decreased due to moderate V\(_A/Q\) imbalance, assessed by increases in dispersions of pulmonary blood flow (Log SDQ, by 0.53±0.16) and alveolar ventilation (Log SDV, by 0.28±0.15) (dimensionless) (p<0.01 each). In contrast, for an equivalent degree of bronchoconstriction and minor increases in V\(_E\), Q\(_T\) and VO\(_2\), mannitol decreased PaO\(_2\) more intensely (by 24±9 mmHg) despite fewer disturbances in Log SDQ (by 0.27±0.12). Notwithstanding, mannitol-induced increase in Log SDV at 5 min (by 0.35±0.15) was similar to that observed during EIB, as was the slow recovery in Log SDV and high V\(_A/Q\) ratio areas, at variance with the faster recovery of Log SDQ and low V\(_A/Q\) ratio areas. In asthmatics, EIB provokes more V\(_A/Q\) imbalance but less hypoxemia than mannitol, primarily due to post-exercise increases in V\(_E\) and Q\(_T\) benefiting PaO\(_2\). V\(_A/Q\) inequalities during both challenges most likely reflect uneven airway narrowing and blood flow redistribution generating distinctive V\(_A/Q\) patterns, including the development of areas with low and high V\(_A/Q\) ratios.

Key words: Bronchial Provocation – Exercise – Mannitol – Multiple Inert Gas Elimination Technique – Pulmonary Gas Exchange
INTRODUCTION

Exercise-induced bronchoconstriction (EIB), often an indication that asthma is not properly controlled (3), is triggered by dehydration and an increased osmolarity of the airway surface as a consequence of evaporative water loss in conditioning the inspired air (1). The airways of asthmatics with EIB are also responsive to hyperosmolar aerosols (4; 9; 18; 30) and both exercise and inhalation of mannitol provoke release of the same inflammatory mediators (5; 7). They are both used to identify bronchial hyperresponsiveness in individuals suspected of having asthma (6; 29). The possible advantage of comparing mannitol to exercise and other osmotic stimuli is that the sensitivity to mannitol correlates very well with the reactivity to exercise (9). It also correlates with hypertonic saline response (PD_{20}) (6) and its ease of administration, without any known serious adverse effect, now facilitates its use as a surrogate for EIB in laboratories. Further EIB has a high prevalence in athletes and the effects on pulmonary gas exchange are of particular interest to those performing at the elite level.

Previous studies on EIB pathophysiology showed a distinctive ventilation-perfusion (V_{A}/Q) response (13; 35) compared to all direct and indirect pharmacological challenge tests in asthma (11; 19; 20; 27). V_{A}/Q imbalance (35) during EIB has previously been demonstrated in a limited group of adults and there is one study in children revealing regions of high V_{A}/Q ratio alone, possibly related to increased alveolar pressures thereby reducing blood flow in these areas (13). Our aim was to study the pulmonary gas exchange response to EIB in mild asthmatics and compare this with the response to mannitol-induced bronchoconstriction in the same subjects. We proposed that the interaction between intrapulmonary (i.e., V_{A}/Q mismatch) and extra-pulmonary (i.e., minute ventilation [V_{E}], cardiac output [Q_{T}] and oxygen uptake [VO_{2}]) factors governing
arterial blood gases after these two stimuli would be different. Thus, EIB would disturb $V_A/Q$ balance while causing less hypoxemia at the time of greatest bronchoconstriction compared with mannitol due to the optimizing effects on $\text{PaO}_2$ caused predominantly by parallel increases in $V_E$ and $Q_T$ after EIB (27). By contrast, for an equivalent degree of bronchoconstriction, mannitol would provoke more arterial hypoxemia than EIB in the presence of similar $V_A/Q$ imbalance due to the less influential effects of increased $V_E$, $Q_T$, and $\text{VO}_2$ on $\text{PaO}_2$. 
METHODS

Subjects: Eleven non-smoking subjects with EIB (27±4 yrs; FEV₁, 86±8%; 8 females) were recruited. For inclusion, subjects were required to have symptoms of asthma in the last year, an FEV₁ ≥ 70% predicted and ≥ 1.5 L and a decrease in FEV₁ of ≥ 20% from baseline after a standardized EIB challenge breathing dry air. The time of maximum fall in FEV₁ on the screening EIB challenge was recorded for use in the subsequent exercise tests. Subjects with an exacerbation of asthma and/or a respiratory infection within the preceding 6 weeks were excluded, as were those treated with oral or inhaled glucocorticosteroids, leukotriene-receptor antagonists or chromones within the preceding 3 months. Maintenance therapy, antihistamines (1 patient), long-acting β₂-agonists (1 patient) or rescue short-acting β₂-agonists (11 subjects), was withheld for at least 72, 48 and 12 h, respectively. For each visit, subjects were asked to refrain from heavy exercise and consumption of caffeine/tea-containing beverages/foods for at least 12 h before arrival in the laboratory. The study was approved by the Ethics Review Board at Hospital Clínic (Protocol # 2312-2004) and all subjects gave written informed consent.

Study Design. A two-period sequential study design was used. All subjects visited the laboratory on 3 separate occasions. At visit 1 (screening) visit, clinical evaluation, spirometry, and EIB challenge (23) were carried out. Subjects fulfilling the inclusion criteria were scheduled for two additional visits for invasive measurements before and after challenge with exercise (visit 2) and inhaled mannitol (visit 3) challenges, one week apart. The sequence of the challenges was intentional in order to match the severity of bronchoconstriction for exercise and mannitol. At both visits 2 and 3, a radial artery catheter and a central (superior cava vein) catheter placed percutaneously were inserted. Measurements including determinations of V_A/Q distributions by the multiple inert gas elimination technique (MIGET) (26) were performed before (baseline) and
after each challenge at 5, 15 and 45 min. After each inert gas sample set, spirometry and respiratory system resistance (Rrs) and respiratory arterial blood gases, but not MIGET measurements, were also assessed at 60, 90 and 120 min. All measurements were performed with the subject seated in an armchair breathing room air. A three-lead electrocardiogram, heart rate (HR), and arterial oxygen saturation by pulse oximetry (HP M1166A, Hewlett-Packard, Bollinger, Germany) were recorded continuously throughout the study (HP 7830A Monitor and HP 7754B Recorder, Hewlett-Packard, Waltham, MA) for reasons of safety. Inhaled salbutamol was administered as rescue medication to only one subject at the end of each study due to a persistent fall in FEV₁ (more than 10%). All study days were completed by all the subjects without adverse events.

**Exercise Challenge.** Dry-air exercise challenge was performed according to standard recommendations (23). Subjects performed cycling exercise on an electro-magnetically braked cycle ergometer (CardiO₂ cycle; Medical Graphics Corporation, St Paul, MN) while breathing dry air (temperature 22 ºC, relative humidity <10%, obtained from a compressed air supply contained in a Douglas bag) using a mouthpiece and a low resistance two-way valve (series 2730; Hans Rudolph, Kansas City, MO). A protocol of rapid increase in the work rate to a \( V_E \) target, calculated as 18 times the predicted FEV₁, within 3-4 min followed by a constant workload sustained for 4 to 6 min was performed. Recordings of breath-by-breath VO₂, carbon dioxide production (VCO₂), \( V_E \), respiratory exchange ratio (RER), and work rate were made and online calculations performed. After cycling, two acceptable FEV₁ measurements were obtained at each time point and the highest value was used for the analysis. FEV₁ measurements were performed whilst the subject was seated on the cycle ergometer at 1 and 3 min and then whilst seated on an armchair at 5, 7, 10, 15 and 20 min after exercise. The severity of EIB was expressed as the
maximum percentage decrease in FEV$_1$ after exercise expressed as a percentage of the baseline value.

**Mannitol Challenge.** Mannitol challenge was performed according to the standardized protocol described by Brannan et al. (6). Dry powder mannitol (Aridol™) was supplied in kit form (Pharmaxis Ltd., NSW, Australia). The single capsule dry powder device (RS-01 Plastiape, Italy) was used for delivering the mannitol by inhalation. The challenge started with inhaling from an empty capsule and then inhaling progressively increasing doses (5, 10, 20, 40, 80, and 160 mg) of mannitol. One min after each dose, FEV$_1$ was measured. The dose of mannitol was increased until it had induced a fall in FEV$_1$ similar (within ±5%) to that documented after EIB. If the FEV$_1$ fell by 20% on any one dose then that same dose of mannitol was repeated, if necessary, but no more inhalations were given if FEV$_1$ fell by 40% (irrespective of maximal FEV$_1$ fall after EIB). The subjects were asked to inhale from the device from near to functional residual capacity to near to total lung capacity and to hold their breath for 5 s. Subjects were encouraged to keep a nose clip on for 10 s after inhalation and then exhale through their mouth to minimize deposition of the particles in the nasopharynx. Two acceptable FEV$_1$ measurements were obtained and the highest value was used for the analysis. Pre-challenge baseline FEV$_1$ was used to calculate the maximum % fall in FEV$_1$ after mannitol. In addition the dose of mannitol that induced a fall of 15% below baseline (PD$_{15}$) was determined as a measure of sensitivity.

**Measurements.** At baseline and at 5, 15 and 45 min after challenge, subjects breathed quietly through a mouthpiece connected to a non-re-breathing two-way valve with a low dead space (series 1410, Hans-Rudolph, Kansas City, Mo) while recordings of ventilatory and hemodynamic variables were made. After achieving adequate steady-state conditions in ventilatory and hemodynamic variables, duplicate samples of arterial blood and mixed expiratory inert and
respiratory gases were collected. Then, duplicate measurements were made of FEV$_1$, inspiratory capacity (IC), and Rrs (forced oscillation technique at 5 Hz; Department of Biophysics, Universitat de Barcelona, Spain); paired measurements of IC were available in 5 subjects only. Arterial PO$_2$, PCO$_2$, pH and hemoglobin concentration were analyzed using standard electrodes (Ciba Corning, 800 series, Medfield, MA) and values were corrected for body temperature. An increase of 0.6 °C from baseline in body temperature has been assumed for corrections at 5, 15 and 45 min after EIB based on the findings of others (17). Both VO$_2$ and VCO$_2$ were calculated from mixed expired oxygen and carbon dioxide concentrations, measured by zirconia and infrared cell sensors respectively (MedGraphics, Cardiorespiratory Diagnostic Systems, St Paul, MN). Likewise, V$_E$ and respiratory rate (f) were measured using a calibrated Wright Respirameter (MK8, BOC-Medical, Essex, UK). The alveolar-arterial oxygen partial pressure difference (AaPO$_2$) was calculated according to the alveolar gas equation using the measured RER. MIGET was used to estimate the distribution of V$_A$/Q ratios without sampling mixed venous inert gases (26). Using this method, Q$_T$ needs to be measured directly. The dye dilution technique (DC-410; Waters Instruments Inc, Rochester, MN) using a bolus of 5 mg of indocyanine green injected through the central vein catheter was used to measure Q$_T$ (28).

Mixed venous inert gas concentrations were computed from mass balance equations (26). Using the measured solubilities for the six gases from each subject, and their concentrations in arterial blood and expired breath, inert gas gradient indexes (i.e., retentions [R] minus excretions [E*] corrected for acetone) were plotted against the solubility for each gas to obtain retention-solubility curves (14; 26). In addition, the dispersions of pulmonary blood flow (Log SDQ) and of alveolar ventilation (Log SDV) (normal values ≤0.60 to 0.65) (10) and an overall index of V$_A$/Q heterogeneity (DISP R-E*) (the root of the mean square difference among measured retentions
and excrections of the inert gases (except acetone) corrected for the dead space (normal value ≤ 3.0) (14)) were also calculated (all dimensionless). Intrapulmonary shunt and regions of low \( V_A/Q \) ratio were defined as the fraction of blood flow perfusing lung units with \( V_A/Q \) ratios < 0.005 and ≥ 0.005 but < 0.1, respectively. Dead space and high \( V_A/Q \) regions were defined as the fraction of alveolar ventilation to lung units with \( V_A/Q \) ratios > 100 and < 100 but > 10, respectively. The duplicate samples of each set of measurements were treated separately, the final data being the average of variables determined from both \( V_A/Q \) distributions at each time point. The residual sum of squares (RSS), the best descriptor of the quality of MIGET data, was within the expected limits (< 5.0) (26) both on the EIB day (3.74; 95% CI, 3.09 to 4.39) and on the mannitol day (3.98; 95% CI, 3.28 to 4.69).

Likewise, we manipulated the mathematical model used in the MIGET as previously reported (34) to dissociate the relative contributions of the different extrapulmonary factors (\( V_E \), \( Q_T \) and \( V_O_2 \)) that may have influenced the actual \( PaO_2 \) during EIB and compared the \( PaO_2 \) expected to result from the measured \( V_A/Q \) inequality during mannitol challenge with the \( PaO_2 \) expected for particular combinations from the EIB changes in the three extrapulmonary factors (34). To achieve this outcome, we recalculated the R - E differences from 5 min data collected during EIB first with \( V_E \) constrained at levels observed 5 min after mannitol and the same individual analyses were performed for \( Q_T \) and \( V_O_2 \). The fourth model constrained \( V_E \), \( Q_T \) and \( V_O_2 \) altogether to values obtained 5 min after mannitol.

**Statistical Analysis.** All data are expressed as mean±SD (unless otherwise stated) or 95% confidence interval (95% CI). The provoking dose of mannitol causing a 15% fall in \( FEV_1 \) (PD\(_{15}\)) was derived by linear interpolation from the cumulative dose on log-transformed data and the geometric mean was calculated for the group. Differences at baseline, before exercise
and/manitol challenges, were assessed using paired t-test. The effects of each challenge on functional and gas exchange variables were assessed by a repeated measures analysis of variance (ANOVA) followed by Bonferroni’s t test post hoc to determine statistical differences. Comparisons between the effects of exercise and mannitol challenges were assessed by two-way repeated measures ANOVA. Whenever a significant interaction between challenges over time was observed, differences at each time point were analyzed with a post-hoc paired t-test. Correlations among variables were established by calculating Pearson's correlation coefficients. Statistical analysis was performed with specialized computer software (SPSS 12.0, Inc, Chicago, IL, USA) and significance was set at p<0.05 values in all instances.
RESULTS

Baseline Findings. At screening, FEV\textsubscript{1} was within normal limits (86±8% predicted) in all but two subjects (73% and 74% each) and all subjects had bronchial hyperresponsiveness to exercise (FEV\textsubscript{1} fall, by 39±18%; range, 20-73%), without differences before challenges (Tables 1 & 2). On both challenge days, FEV\textsubscript{1} (EIB, 3.2±1.7 L; mannitol, 3.1±1.7 L) and IC (100±11% predicted each) were normal (24; 25) and not different from the baseline screening visit but Rrs was slightly increased on both EIB and mannitol visits (4.0±1.6 and 4.1±1.9 cmH\textsubscript{2}O/L/s, respectively) (22). Arterial blood gases were normal in all subjects, while V\textsubscript{A}/Q distributions were normal (i.e., narrowly unimodal) in eight and slightly broader than normal in three. In spite that inert gas gradients (R-E*) for the five gases for both EIB and mannitol (SF\textsubscript{6}, 0.003±0.001 and 0.003±0.002; ethane, 0.02±0.01 and 0.02±0.01; cyclopropane, 0.05±0.02 and 0.05±0.02; halothane, 0.05±0.03 and 0.04±0.02; and ether, 0.02±0.01 and 0.02±0.01, respectively) were not different, DISP R-E* was however slightly elevated on the EIB day. Intrapulmonary shunt and regions with both low and high V\textsubscript{A}/Q ratio were negligible or absent, while Log SDQ was normal, Log SDV was slightly elevated in three patients (11) and dead space was slightly reduced.

Response to EIB. Subjects performed exercise for 8.1±0.6 min (total time) at a constant workload (105±34 W) and over the last 5.0±0.4 min there was an average V\textsubscript{E} of 60±20 L·min\textsuperscript{-1} and heart rate (HR) of 168±8 min\textsuperscript{-1}. This exercise provoked a 37±16% fall in FEV\textsubscript{1} (p<0.01) at 3.6±1.6 min after ceasing exercise (maximal fall). Five min after the maximal response, the fall in FEV\textsubscript{1} remained almost unchanged (Table 1) while Rrs increased (by 121±71%)(p<0.01 each). Mean IC decreased at 5 and 15 min (Table 1). Likewise, V\textsubscript{E} (by 123±60%), Q\textsubscript{T} (by 48±29%), VO\textsubscript{2} (by 54±25%) and HR increased at 5 and 15 min (p<0.01 each) and respiratory frequency (f)
(p<0.05) only at 5 min. At 5 min, all but one patient (who had a milder fall of 16% in FEV1 compared to the rest and, consequently, less V_A/Q disturbances) exhibited moderate pulmonary gas exchange abnormalities. This was characterized by decreased PaO2 and PaCO2 and increased AaPO2 (p<0.01 each) (Table 2) together with deterioration of the main V_A/Q descriptors (i.e., increases in Log SDQ, Log SDV, and DISP R-E*) and increases in SF6, ethane, cyclopropane, halothane, and ether (0.009±0.005; 0.07±0.02; 0.16±0.04; 0.17±0.04; and 0.09±0.02 respectively) (p<0.01 each). At 5 min, five subjects developed broadly unimodal V_A/Q patterns (both distributions were widened) and six a bimodal alveolar ventilation profile (Table 3). At 15 min most of the changes in V_A/Q descriptors remained similarly altered, including R-E* values, Log SDQ and Log SDV (Figures 1 and 3).

All indexes of airflow obstruction (FEV1 and Rrs) and PaO2, AaPO2, Log SDQ and DISP R-E* tended to progressively improve but still remained slightly abnormal at 45 min (p<0.01 each), whereas Log SDV remained persistently increased as did SF6, ethane, cyclopropane, halothane, and ether (0.005±0.002; 0.04±0.01; 0.10±0.03; 0.10±0.03; and, 0.05±0.02, respectively) (p<0.01 each) (Figures 1 and 3). Mean FEV1, PaO2 and AaPO2 returned to baseline (±5%) at 90 min whereas Rrs recovery continued out to 120 min (Tables 1 & 2). IC remained normal between 45 and 120 min. At 5 min, FEV1 (in L) was correlated with PaO2 (r=0.67), AaPO2 (r= −0.66) and QT (r=0.74) (p<0.05 each), but only PaO2 correlated with the % fall in FEV1 (r= −0.74, p<0.01).

**Response to Mannitol.** Subjects were highly sensitive to mannitol as demonstrated by a dose of mannitol to provoke a 15% fall in FEV1, PD15 of 18.7 mg (GM) (95% CI, 8.9 to 39.3). Mean cumulative dose of mannitol administered was 113±77 mg and this dose provoked a 40±8% fall in FEV1 at matching (with EIB study) time point (p<0.01). Five min after, FEV1 was decreased
(Table 1) along with increases in Rrs (by 109±53%) (p<0.01 each) and decreases in IC (Table 1) (p<0.05). We observed mild (at 5 min only) increases in QT (by 16±13%) and VO2 (by 17±16%) (p<0.01 each) with discrete changes in VE (by 11±33%). At 5 min, PaO2 moderately decreased, while AaPO2, Log SDQ, Log SDV and DISP R-E* increased (p<0.01 each) (Table 2), as did SF6, ethane, cyclopropane, halothane, and ether (0.009±0.005; 0.07±0.02; 0.16±0.04; 0.17±0.04; and, 0.09±0.02, respectively) (p<0.01 each) (Figure 3). At five min, five subjects developed broadly unimodal VA/Q patterns alike during EIB and six a bimodal alveolar ventilation profile (five of these subjects also showed it during EIB) (Table 3). At 15 and 45 min, FEV1, Rrs, IC, PaO2, AaPO2 and one of the main descriptors of VA/Q abnormalities (Log SDQ) tended to improve but all remained slightly altered at 45 min (p<0.01 each), as were SF6, ethane, cyclopropane, halothane and ether (0.004±0.002; 0.03±0.02; 0.09±0.04; 0.09±0.04; and, 0.05±0.03; respectively) (p<0.01 each) (Figure 3). By contrast, increased Log SDV remained persistently elevated at 15 and 45 min (Table 2, Figure 1); at 90 min, all measurements had returned to baseline (±5%). No correlations were shown between gas exchange and spirometric indices.

**Comparison between EIB and Mannitol.** The reactivity to exercise (maximal FEV1 fall at the screening visit) was related to the sensitivity (expressed as PD15) to mannitol (r=−0.86; p=0.001). Maximal changes in FEV1, Rrs and IC were not different. As expected, at 5 min, VE, QT and VO2 were higher after EIB than after mannitol (Table 1, Figure 2), whereas at 45 min all these values were comparable to baseline for both challenges. At 5 min, EIB was associated with a higher PaO2 (p<0.05), Log SDQ (p<0.01) and DISP R-E* (p<0.05) and a lower PaCO2 (p<0.001), without differences in AaPO2, compared with mannitol. By contrast, Log SDV was similarly altered during EIB and mannitol (Table 2, Figure 2). No correlation was shown between pulmonary perfusion and alveolar ventilation dispersions. As for inert gas gradients (R-E*)
(Figure 3), EIB showed significantly higher levels for halothane and ether (0.17±0.04 and 0.09±0.02) at 5 min compared to mannitol challenge (0.12±0.05 and 0.06±0.02) (p<0.05 and p<0.001), respectively. At 45 min, Log SDV and its surrogate DISP R-E* remained similarly abnormal after each challenge (Table 2), as did the five inert gases (SF₆, ethane, cyclopropane, halothane, and ether) (Figure 3). FEV₁, Rrs, PaO₂ and AaPO₂ remained abnormal at 60 min.

Based on the modeling approach (Figure 4), an increase of 105% in Vₑ caused an increase in PaO₂, 5 min after EIB of the order of 29.2 mmHg, whereas increasing Qₜ by 32%, favored an increase in PaO₂ of 3.7 mmHg and increasing VO₂ by 37% reduced PaO₂ by 18 mmHg. When all factors were combined together, the result was a gain in the modeled PaO₂ of 16.9 mmHg.

Accordingly, the difference between the predicted PaO₂ according to MIGET at 5 min during EIB (17.8 mmHg) and the net change in PaO₂ estimated using this modeling was just of the order of 0.9 mmHg (changing Vₑ, Qₜ and VO₂ to mannitol levels). This finding indicates therefore that altogether these three factors played a key role in modulating the PaO₂ differences between the two challenges, although the increases in both Vₑ and Qₜ were overall more influential.
DISCUSSION

This is the first study to compare pulmonary gas exchange responses to two stimuli used clinically to measure bronchial hyperresponsiveness (BHR). For EIB the response was characterized by more low V_A/Q regions (as assessed by increased Log SDQ) but less hypoxemia than for mannitol for an equivalent level of bronchoconstriction but similar amounts of high V_A/Q areas as assessed by increases in R-E* indexes for intermediate and high soluble gases and also by increased Log SDV. This suggests that uneven airway narrowing appears to be more potent during EIB. Second, compared to the progressive normalization of intermediate soluble inert gas gradient indexes (including Log SDQ), gases with greater solubility and Log SDV remained abnormal after both broncho-provoking stimuli thus possibly alluding to residual pulmonary vasoconstriction.

Before discussing these findings, two methodological limitations of the study should be acknowledged. First, general experimental limitations of MIGET have been discussed at length in previous papers (12; 26; 32). With MIGET many experimental challenge situations have been investigated repeatedly (11; 20; 35) such that MIGET should be a more than reasonable approach to assess the pulmonary gas exchange status during both challenges. One established facet of the MIGET is that there is an association between the Log SD indexes and the RSS. If a given, initially error-free set of retentions is perturbed with progressively increasing amounts of random error and the data submitted to the inert computer gas analysis, the recovered values of Log SD indexes will be smaller the greater the errors, and the RSS will become obviously larger (12). The RSS is considered to be the best descriptor of the quality of MIGET and our results exhibited low values, thus reflecting very good quality data. From a pathophysiologic viewpoint, all V_A/Q data were internally consistent and fitted quite well with the overall functional time course exhibited by our participants. Second, we did not measure actual temperatures during exercise. Instead, we
corrected arterial blood gas data at the standard body temperature to the expected increases in PaO₂ and decreases in AaPO₂ according to the achieved metabolic demands during exercise (i.e., VO₂) (17). We consider that these temperature corrections were sufficiently reliable to modify the arterial blood gases to their actual values.

**Gas Exchange Responses to EIB and Mannitol**

The study was primarily designed to compare the pulmonary gas exchange responses to EIB and mannitol as both are used as bronchoprovocation tests. We used mannitol therefore as a comparator for its clinical usefulness in diagnosing EIB and its correlation with EIB reactivity. As expected, maximum bronchoconstriction occurred within a few minutes after the cessation of exercise when Vₑ, Qₑ and VO₂ were still considerably increased above baseline. The bronchoconstrictive effects of EIB on pulmonary gas exchange were characterized by moderate Vₐ/Q disturbances (i.e., increased inert gas gradients for halothane, ether and acetone and increases in Log SDQ and Log SDV). As shown by the modeling, the higher Vₑ and Qₑ after exercise almost certainly improved alveolar and mixed venous PO₂, respectively, likely contributing to a less marked hypoxemia than might be expected (16; 33) although the simultaneous increased VO₂ exerted significant counterbalance and contributed to the underlying levels of hypoxemia. From a gas exchange viewpoint, an increased Log SDQ even in the absence of a bimodal pattern, reflecting the presence of regions of low Vₐ/Q ratio (26), is consistent with the development of widespread, patchy, uneven airway narrowing along with pulmonary blood flow redistribution modulated by the simultaneous increased Qₑ. The precise mechanisms responsible for the observed development of persistently elevated Log SDV, hence reflecting the presence of regions of high Vₐ/Q ratio (26), along with the finding of a bimodal (high) Vₐ/Q pattern in half our patient group, remain unclear. One likely mechanism could be hypoxic
pulmonary vasoconstriction of poorly ventilated alveolar units; an alternative possible mechanism could have been hyperventilation in the less abnormal lung sections to compensate for the effects of regional gas trapping, as shown with the positron emission tomography approach (15; 31). At 5 min, IC decreased suggesting gas trapping, a finding akin to increased Log SDQ previously observed in both adults and children (13; 35). However, the contention of pulmonary vasoconstriction, which may cause the abnormal Log SDV residually shown at 45 min when IC had already returned to baseline, cannot be overlooked. Urinary PGD$_2$ and cystLTs levels, known to be active pulmonary vasoconstrictors (2; 11), are increased in asthmatics after EIB (21) and mannitol (8). This sustained unique increased Log SDV during mannitol and EIB challenges indicates that this effect may be related to osmotic challenges.

Interestingly, in terms of increased Log SDQ, the bronchoconstriction provoked by mannitol resulted in less $V_A/Q$ imbalance but more hypoxemia than EIB in the face of discrete increases in $Q_1$ and $\text{VO}_2$. However, the increased AaPO$_2$ was similar 5 and 15 min after both stimuli. This similarity is possible and consistent with our modeling because the marked hyperventilation during EIB increased alveolar PO$_2$, an effect not present during the bronchoconstriction provoked by mannitol.

**Comparison with other Bronchial Stimuli**

Both EIB- and mannitol-induced $V_A/Q$ inequalities were, in terms of increased Log SDQ and hypoxemia, similar to those occurring following other direct and indirect provocative agents, such as methacholine (20), histamine, LTD$_4$ (11), PAF, adenosine 5'-monophosphate (AMP) (20) and allergens (19) (mean Log SDQ range, 0.71-0.85) at comparable degrees of bronchoprovocation. Notwithstanding, at variance with all the abovementioned agents, there was more increased Log
SDQ along with an equivalent increased Log SDV [mean Log SDV range of previous studies (11; 20), 0.75-0.88] but a distinct V_A/Q recovery pattern. From a gas exchange viewpoint, these findings suggest that EIB and mannitol have features that are unique and distinct from the wide spectrum of other provoking agents.

In this study the mannitol was administered in doses sufficient to simulate the bronchoconstriction provoked by exercise, a protocol also used in studies to investigating the inflammatory mediators associated with mannitol-induced bronchoconstriction (7; 8). The present study demonstrates that when the bronchoconstriction provoked by mannitol is equivalent to moderate to severe EIB there is a similar degree of pulmonary gas exchange disturbances. It is of note, however, that the two challenges did exhibit slightly different responses in terms of arterial hypoxemia, showing that there are inherent pathophysiologic differences between the two challenges.

Mannitol has recently received regulatory approval as a bronchial provocation test to identify BHR and the required target fall in FEV_1 below the baseline value required for a positive test result is 15%, a value usually associated with a mean maximum fall in FEV_1 to 21% over the next 5 min (6). This is a much lower degree of bronchoconstriction than we used to simulate the moderate to severe EIB in our subjects, and has not been associated with a significant arterial deoxygenation as measured by pulse oximetry (6).

**Conclusions**

This study demonstrates that, for an equivalent degree of bronchoconstriction induced by both provoking stimuli, V_A/Q imbalance during EIB is associated with less arterial hypoxemia than during mannitol-induced bronchoconstriction. This is due to the influential roles of increased V_E and Q_T governing PaO_2. These V_A/Q inequalities resulted from predominant widespread uneven
airway narrowing and pulmonary blood flow redistribution are likely to be related to the release of inflammatory mediators. Although both stimuli are used for bronchial provocation testing the response to mannitol is normally limited to the level of 15 % fall in FEV$_1$ using a dose response protocol, an approach that is not associated with either severe bronchoconstriction or arterial hypoxemia. However, if mannitol is used to simulate moderate to severe EIB, one might expect similar disturbances in pulmonary gas exchange as can occur with exercise.

**Acknowledgements.** The authors wish to thank J.L Valera, RN for his technical assistance, C. Picado, MD and C. Vennera, MD for their support in recruiting subjects and to J.D. Brannan, PhD for his contribution in the definition of the experimental design.

**Disclosures**

PAM, FPG, HM, JR and JAB have no conflicts of interest to declare.

IHY has been an investigator on the industry supported trial of Aridol (Phase III 2004 – 2005, AUS 78,000). IHY has not received personal remuneration from the sponsor for his participation and has had no personal financial relationship with any commercial entity related to mannitol.

SDA has received an educational grant from Pharmaxis via her hospital; SDA owns stocks in excess of 30,000 Euros in Pharmaxis that she purchased herself; she does not hold any stock options. She acts as a consultant to Pharmaxis in her capacity as an employee of Sydney South West Area Service who owns the intellectual property rights relating to the application for mannitol.

RRR has participated as a lecturer and speaker in scientific meetings under the sponsorship of Almirall and Chiesi; serves on advisory boards for Chiesi and Novartis; and has received laboratory research support from Esteve.
Reference List


13. **Freyschuss U, Hedlin G and Hedenstierna G.** Ventilation-perfusion relationships during


26. **Roca J and Wagner PD.** Contribution of multiple inert gas elimination technique to


LEGENDS TO FIGURES

Figure 1: Individual time courses of FEV\textsubscript{1} (% change from baseline), PaO\textsubscript{2} (in mm Hg), dispersion of pulmonary blood flow (Log SDQ) and of alveolar ventilation (Log SDV) (dimensionless) values after EIB (left column, closed circles) and mannitol (right column, open circles) challenges. Measurements correspond to baseline (BL), 5 min post-maximal fall and 15, 45, 60, 90 and 120 min after each challenge (bars denote mean values). Note that V\textsubscript{A}/Q indices were only measured at BL and then, at 5, 15 and 45 min after challenge (See Results, for explanation).

Figure 2: Box plots showing absolute differences at 5 min from baseline in PaO\textsubscript{2}, Log SDQ, Log SDV, DISP R-E*, V\textsubscript{E}, Q\textsubscript{T} and VO\textsubscript{2} after exercise (EIB) and mannitol (MAN) challenges. The box includes observations from the 25\textsuperscript{th} to the 75\textsuperscript{th} percentile. The horizontal line within the box denotes median values. Upper and lower lines outside the box represent the 5\textsuperscript{th} and 95\textsuperscript{th} percentiles. (*) p<0.05, (†) p<0.01 for comparison with EIB.

Figure 3: Retention minus corrected excretion (R-E*) values versus log inert gas solubilities (mL/100mL/mmHg) for 5 inert gases during EIB (top) and MAN (bottom). Values shown are mean±SEM at baseline (closed circles), 5 min (open circles), 15min (closed squares) and 45min (open squares) after challenge. Significance of the interaction between the effects of EIB over time is shown as (*) p<0.05, (†) p<0.01, (‡) p<0.001 for comparison with mannitol. Note the greater values for halothane and ether during EIB indicating greater development of areas of high V\textsubscript{A}/Q ratio.

Figure 4: Pictorial analysis dissociating extrapulmonary factors in improving or worsening PaO\textsubscript{2} during EIB, 5 min after maximal bronchoconstriction. Values shown are mean±SEM. Abscissa values correspond to changes in estimated PaO\textsubscript{2} (in mmHg) mathematically manipulated using MIGET models from V\textsubscript{A}/Q distributions obtained during EIB after changing individual (V\textsubscript{E}, VO\textsubscript{2} and Q\textsubscript{T}) factors and all three extrapulmonary factors together, to values obtained during mannitol challenge (at 5 min). Ordinate values denote the differences between EIB and mannitol challenges in each extrapulmonary factor. Ventilation had the greatest increase with a very large change in favor of PaO\textsubscript{2} during EIB.
Table 1: Effects of exercise (EIB) and mannitol (MAN) on airflow parameters, cardiac output, minute ventilation and oxygen consumption.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>5 min</th>
<th>15 min</th>
<th>45 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>p value*</th>
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<tr>
<td><strong>FEV₁, % fall</strong></td>
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<tr>
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<td>35±12 †</td>
<td>25±10†</td>
<td>8±10†</td>
<td>6±8†</td>
<td>4±7</td>
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<tr>
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<td>26±11†</td>
<td>11±6†</td>
<td>8±7†</td>
<td>4±6</td>
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<td>20±14 †</td>
<td>13±15</td>
<td>1±8</td>
<td>-</td>
<td>-</td>
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<tr>
<td><strong>V̇E, L·min⁻¹</strong></td>
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<td>&lt;0.01</td>
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<tr>
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<td>13.8±4.3†§</td>
<td>8.5±2.2†§</td>
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<td><strong>Q̇t, L·min⁻¹</strong></td>
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<td><strong>VO₂, mL·min⁻¹</strong></td>
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<td>267±75†</td>
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<td>252±58†</td>
<td>221±33</td>
<td>227±32</td>
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</table>

Values are mean±SD. Definitions of abbreviations: FEV₁: forced expiratory volume in one second; IC: Inspiratory capacity; V̇E: minute ventilation; Q̇t: cardiac output; VO₂: oxygen consumption. Dash represents no measurement performed at time point.

(*) Significance of the interaction between the effects of challenges over time (repeated measures ANOVA).
†(†) p<0.01, (‡) p<0.05 for comparison with baseline.
§(§) p<0.01, (¶) p<0.05 for comparison with mannitol.
#: (n, 6 subjects) (-) Denotes measurement not performed
NS: Not statistically significant
Table 2. Effects of exercise (EIB) and mannitol (MAN) on arterial blood gases and ventilation-perfusion distributions.

<table>
<thead>
<tr>
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<th>Baseline</th>
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<th>15 min</th>
<th>45 min</th>
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<th>90 min</th>
<th>120 min</th>
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<td>91± 9</td>
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<td>71 ± 10 †</td>
<td>72± 11 †</td>
<td>87 ± 10 ‡</td>
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<tr>
<td>PaCO₂, mmHg</td>
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<td>29 ± 11 †</td>
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<td>27 ± 9 †</td>
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<tr>
<td>EIB</td>
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<td>MAN</td>
<td>0.44±0.10</td>
<td>0.71 ± 0.16†</td>
<td>0.69 ± 0.19†</td>
<td>0.58 ± 0.16 †</td>
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<td>EIB</td>
<td>0.51±0.14</td>
<td>0.79 ± 0.09†</td>
<td>0.85 ± 0.13†</td>
<td>0.80 ± 0.20 †</td>
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<tr>
<td>MAN</td>
<td>0.47±0.14</td>
<td>0.82 ± 0.13†</td>
<td>0.86 ± 0.22†</td>
<td>0.73 ± 0.23 †</td>
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<td>DISP R-E*</td>
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<td>EIB</td>
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<tr>
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<td>2.90±1.44</td>
<td>7.81 ± 2.87†‡</td>
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<td>5.69 ± 2.59 †</td>
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<td>Dead Space, %Vₑ</td>
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</table>

Values are mean±SD. Definitions of abbreviations: PaO₂: arterial oxygen tension; PaCO₂: arterial carbon dioxide tension; AaPO₂: alveolar-arterial PO₂ difference; Log SDQ: dispersion of pulmonary blood flow distribution; Log SDV: dispersion of alveolar ventilation distribution; DISP R-E*: dispersion of retention minus discretion inert gases corrected for dead space (Log SDQ, Log SDV and DISP R-E* are dimensionless). Dash represents no measurement performed at time point. For other abbreviations see Table 1.

(*) Significance of the interaction between the effects of challenges over time (repeated measures ANOVA).

(†) p<0.01, (‡) p<0.05 for comparison with baseline.

(§) p<0.01, (¶) p<0.05 for comparison with mannitol.
Table 3: Individual Vₐ/Q patterns of the pulmonary blood flow and/or alveolar ventilation distributions for exercise (EIB) and mannitol (MAN) challenges at 5, 15 and 45 min post-challenge

<table>
<thead>
<tr>
<th>Subject #</th>
<th>EIB 5 min</th>
<th>EIB 15 min</th>
<th>EIB 45 min</th>
<th>MAN 5 min</th>
<th>MAN 15 min</th>
<th>MAN 45 min</th>
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</tbody>
</table>

Definitions of Abbreviations: H: bimodal ventilation distribution (high Vₐ/Q pattern); U: broadly unimodal pattern in either one or both of the distributions; N: narrowly unimodal pattern of both distributions. Interestingly, 5 of the 6 subjects showing a high Vₐ/Q pattern during EIB also experienced this profile after mannitol. All subjects showed abnormal Vₐ/Q patterns with no subject showing a bimodal blood flow distribution (low Vₐ/Q pattern).
Figure 3

**EIB**

![Graph showing solubility and retention-excretion for EIB with different substances and time points.]

**MAN**

![Graph showing solubility and retention-excretion for MAN with different substances and time points.]

Key:
- Baseline
- 5 min
- 15 Min
- 45 min