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Effects of the Menstrual Cycle on Lung Function Variables in Women with Asthma

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Abstract

Rationale: Angiogenesis is a defining pathologic feature of airway remodeling and contributes to asthma severity. Women experience changes in asthma control over the menstrual cycle, a time when vessels routinely form and regress under the control of angiogenic factors. One vital function modulated over the menstrual cycle in healthy women is gas transfer, and this has been related to angiogenesis and cyclic expansion of the pulmonary vascular bed.

Objectives: We hypothesized that changes in gas transfer and the pulmonary vascular bed occur in asthmatic women over the menstrual cycle and are associated with worsening airflow obstruction.

Methods: Twenty three women, thirteen asthmatics and ten healthy controls were evaluated over the menstrual cycle with weekly measures of spirometry, gas transfer, nitric oxide, hemoglobin, factors affecting hemoglobin binding affinity, and pro-angiogenic factors.

Measurements and Results: Airflow and lung diffusing capacity varied over the menstrual cycle with peak levels during menses that subsequently declined to nadir in early luteal phase. In contrast to healthy women, changes in lung diffusing capacity (DLCO) were associated with changes in membrane diffusing capacity and DLCO was not related to pro-angiogenic factors. DLCO was not different between the two groups, though methemoglobin and carboxyhemoglobin were higher in asthmatics than healthy women.

Conclusion: Asthmatic women experience cyclic changes in airflow as well as gas transfer and membrane diffusing capacity supportive of a hormonal effect on lung function.

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Key words: Gas transfer, angiogenesis, asthma, menstrual cycle, pro-angiogenic progenitor cell.

Introduction

The gender distribution of asthma changes at puberty, at which time there is an increase in the ratio of women to men (1-4). The female preponderance is maintained into adult life, and asthma morbidity is greater in women than men (5). Sex hormones have been suggested to play a role in mediating the gender differences in asthma, and particularly in the asthma exacerbation that many women experience around the premenstrual period (6). The menstrual cycle is divided into two phases, follicular and luteal typified by specific hormonal fluctuations and separated by ovulation at mid cycle. The follicular phase starts with the first day of menstruation and is followed by the luteal phase from ovulation till menstruation restarts. Menstrual related adverse effects on asthma have been recognized since 1931, but the causes are poorly understood (7-9). Studies investigating sex hormone/female gender effects in asthma have evaluated airflow, airway responsiveness and/or allergic-inflammatory parameters over the menstrual cycle (10-14). Diffusing capacity has not been evaluated, even though gas transfer varies in healthy women over the menstrual cycle (15, 16). Lung diffusing capacity measured by the single breath carbon monoxide diffusing capacity (DLCO) peaks at the end of the luteal phase, then rapidly drops over the time of menses to reach a nadir during the follicular phase in healthy women (16). Circulating pro-angiogenic progenitor cells, which are biomarkers of angiogenesis, change in concert with lung diffusing capacity and pulmonary capillary blood volume, suggesting cyclic changes may be due to neovascularization in the pulmonary vascular bed (16).

Pro-angiogenic progenitor cells are bone marrow-derived cells, which are identified by expression of CD34, a common cell surface marker for hematopoietic stem

cells and endothelial cells, and the co-expression of the stem cell marker CD133. They are essential for the formation of new blood vessels (17, 18). Recent study identified that circulating pro-angiogenic progenitors are increased in asthma, and related to the pathologic expansion of submucosal vessels that typifies airway remodeling (19). Increased amounts and sizes of submucosal vessels were the most striking features reported in early detailed histopathologic studies of asthmatic lungs (20) and have been repeatedly confirmed in recent reports (21-25). The expanded airway vascular bed likely contributes to the airflow limitation of asthma either through vascular tissue increasing the airway wall thickness and/or through edema formation. Pro-angiogenic progenitors as well as Th2 polarized lymphocytes and mast cells secrete vascular endothelial growth factor (VEGF), which is found at high levels in sputum and in the bronchoalveolar lavage fluid of asthmatics (19, 26, 27). As a key pro-angiogenic factor in neovascularization, VEGF contributes to the angiogenic milieu in the asthmatic lung (26, 27), and its effects are mediated in part by nitric oxide (NO), production of which is also increased in the asthmatic lung (28). Thus, asthma is characterized by a highly pro-angiogenic lung environment, i.e. high levels of pro-angiogenic progenitors, VEGF and NO.

In the context of cyclic changes in DLCO and pulmonary vascular bed in healthy women, we hypothesized that these physiologic changes may be amplified in asthmatic women and adversely be associated with airflow obstruction. To test this, asthmatic women were evaluated over the menstrual cycle with weekly measures of airflow, fraction of NO in the exhaled breath (F_ENO), and lung diffusing capacity along with its components, the pulmonary vascular capillary bed volume (Vc), membrane diffusing capacity (Dm), hemoglobin and the factors affecting hemoglobin binding affinity.

Simultaneously, parameters of angiogenesis were evaluated over the menstrual cycle, including circulating bone marrow-derived $CD34^{+}CD133^{+}$ cells, VEGF and stem cell factor (SCF). Some of the results of these studies have been previously reported in the form of abstracts and as a publication (16, 29, 30).

Materials and Methods

Thirteen asthmatic, non-smoking women (age 31± 2) with regular menstrual cycle were enrolled in the study. Four were on contraceptive pills or patch. Asthmatics were on inhaled bronchodilators as needed, seven were on inhaled corticosteroid, six were on long acting β-agonist, five were on leukotriene receptor antagonists and none were on oral corticosteroid. Two asthmatic women reported a history of second hand smoking history and three were former smokers. Ten healthy, non-smoking women were enrolled in the study as well, three were on contraceptives. They were on no other medications. Hormonal contraceptives were permitted, as previous studies have shown that changes in DLCO were detectable in menstruating women, whether or not on hormonal contraceptives (15, 16). Healthy volunteers were used as a comparative control group and some of their data have been previously published (16). All volunteers were longitudinally assessed over 4 to 5 weeks with one visit each week. The menstrual cycle was divided into weeks. Week 1 was defined by the first day of menstruation. Menstrual cycles varied from 28 to 31 days long. The phases of the cycle were verified by changes in hormonal levels, progesterone and estrogen. Asthma was verified based on a positive methacholine challenge test and/or reversible airway obstruction by documentation of change in FEV_1 or FVC by 12% and >200ml after two puffs of inhaled bronchodilator

except for 2 subjects, who were unavailable for testing (**Table 2**). Healthy subjects had no history of any lung symptoms, and all had normal spirometry. Nine of the 10 also had documentation of negative methacholine challenge and/or negative bronchodilator response. The Institutional Review Board at the Cleveland Clinic approved this study and all subjects gave written informed consent. Lung function and diffusing capacity for carbon monoxide were performed, and inspired oxygen concentrations of 21% and 42% were used to determine Dm and Vc as previously described (16) (*Detailed methods in online supplement*). Single-breath on-line measurement of fractional NO concentration in expired breath (F_ENO) was measured using the NIOX (Aerocrine, NY). The ABL 700 radiometer (Radiometer America Inc, OH) was used to measure oxygen, carbon dioxide, pH, hemoglobin (Hgb), methemoglobin (MetHgb), carboxyhemoglobin (COHgb) and to get a one-point determination of p50 using Hill's equation in venous blood collected in heparin tubes (31). Progesterone, VEGF and SCF were measured in serum using quantikine ELISA (R & D system, MN), and estrogen in serum using ELISA (Cayman chemical company, MI). The sensitivity and lower limit of detection for the variable assays were as follow: Progesterone (< 8.57 pg/ml, 15.6 pg/ml), VEGF (< 9 pg/ml, 31.2 pg/ml), SCF (< 9 pg/ml, 31.2 pg/ml) and estrogen (129 pg/ml, 19 pg/ml).

Flow cytometry evaluation of $CD34^+CD133^+$ progenitor cells

Mononuclear cells ($2X10^6$) isolated from peripheral blood were labeled with anti-human CD34-FITC (Becton Dickinson, NJ) and anti-human CD133-PE (Miltenyi Biotec, Auburn, CA) monoclonal antibodies to quantify $CD34^+CD133^+$ cells. To control for nonspecific antibody binding, isotype matched irrelevant antibodies were used.

Following incubation, cell suspensions were washed with PBS/1%BSA/0.02%sodium azide and suspended in FACS flow (Becton Dickinson, NJ). The FACScan flow cytometer (Becton Dickinson, NJ) was used to count $0.5X10^6$ events. Data was analyzed using Cell-Quest 3.3 Software (Becton Dickinson, NJ).

Statistical Analysis

Descriptive measures for quantitative variables consist of means with appropriately derived standard errors in the form 'mean ± SE'. Comparisons of asthmatic and non-asthmatic subjects were performed. For quantitative measures observed only once per patient, comparisons were performed using the Wilcoxon rank sum test. Comparisons with respect to quantitative measures observed over multiple weeks or phases were performed using linear models with parameters estimated using generalized estimating equations (GEE) to account for a correlation among observations within a subject. An exchangeable correlation structure, common to all subjects, was applied. Linear models with GEE estimation were also fit with covariate adjustments for week or phase. Within asthmatics, comparisons of weeks or phases were also carried out using linear models with GEE and exchangeable correlation structure. Linear regression was used to identify and describe relationships among pairs of quantitative variables when data was normally distributed, and Spearman correlation coefficients were used to describe relationships among pairs of quantitative variables in a manner free of the normality assumption. Pearson correlations derived through linear regression are denoted as 'R' while Spearman correlations are denoted as 'Spearman R'. Analyses were performed using R version 2.3.1 (R Foundation, Vienna, Austria) (32).

Results

Asthmatic women were similar to healthy women in terms of age, height, race, and use of contraceptives but had greater body mass, and lower $FEV_1\%$ and FEV_1/FVC and tended to have higher F_ENO over the time of measures (**Table 1**).

Airflow and F_ENO over the Menstrual Cycle

Pattern of airflow of asthmatics over the weeks of the cycle was evaluated and compared to the healthy women. None of the participants experienced an asthma exacerbation during the time of the study. Healthy women had significant decrease in both FVC% and $FEV_1\%$ from week 1 to week 3 ($p = 0.01$ and $p = 0.03$ respectively) and from week 2 to week 3 ($p = 0.01$ and $p < 0.01$ respectively), but no significant change in FEV_1/FVC (all $p > 0.1$). Similarly, FVC% and $FEV_1\%$ varied over the menstrual cycle in asthmatic women with significant decline from week 1 to week 2 ($p = 0.07$ and $p = 0.01$ respectively) and week 3 ($p=0.02$ and $p = 0.01$ respectively) (**Figure 1**). In contrast to the healthy women, FEV_1/FVC in asthmatic women decreased from week 1 to week 2 ($p = 0.08$) and week 3 ($p = 0.04$) (**Figure 1**). F_ENO did not vary over the time of the study (all $p > 0.1$). F_ENO was inversely related to airflow as measured by FEV_1/FVC ($R = -0.6$, $p<0.001$) and directly related to FVC% ($R = 0.4$, $p = 0.02$). Although the mean F_ENO of asthmatic women appears greater than healthy controls, the p value adjusted to multiple observations per subject did not achieve significance.

Gas Transfer in Asthmatic Women over the menstrual cycle

In general, asthmatics had greater single breath DLCO/Va than healthy women (**Table 1**). DLCO varied significantly over the menstrual cycle in asthmatics with the highest values on week 1 and the lowest values at week 3 ($p = 0.03$) (**Figure 2**). Parallel to DLCO, Dm changed over the menstrual cycle reaching a nadir on week 3 with a significant drop from week 1 and 2 to week 3 ($p = 0.03$ and $p = 0.01$). Overall, Dm was lower in the luteal phase than in follicular phase [Dm (ml/mmHg/min): follicular phase 92 ± 12; luteal phase 63 ± 10; $p = 0.07$]. In contrast, Vc did not vary significantly over time of weeks or from follicular to luteal phase [Vc (ml): follicular phase 49 ± 4; luteal phase 52 ± 4; $p=0.5$]. We subgrouped the asthmatics based on use of contraceptives. As a group, asthmatics on contraceptives have higher FEV_1 and FEV_1 /FVC ($p<0.01$ for both) as compared to asthmatics not on contraceptives. Regardless of contraceptive use, changes over the menstrual cycle were comparable, i.e. the highest DLCO and $CD34^+CD133^+$ cells were at week 1. On the other hand, when we divided asthmatics into 2 subgroups according to use of inhaled corticosteroid, there were no differences in lung function (all $p>0.1$), and these subgroups had similar $CD34^+CD133^+$ cells in circulation ($p>0.1$). The changes over the menstrual cycle were also comparable, i.e. the highest DLCO, FEV_1, FEV_1/FVC and $CD34^+CD133^+$ cells at week 1 in both subgroups.

Other factors that influence DLCO were also evaluated. Estimated Hgb affinity (θ) and measures of alveolar volume of gas (V_A) were unchanged over the menstrual cycle ($p = 0.8$ and 0.4 respectively). Furthermore, Hgb concentration did not change over the menstrual period (all $p >0.5$) [Hgb (gm/dl): follicular phase 13.3 ± 0.3; luteal phase 13.5 ± 0.3; $p = 0.6$]. Similarly, venous MetHgb and COHgb, which would diminish DLCO, did not change over the cycle ($p = 0.9$ and $p = 0.6$ respectively). However,

COHgb and MetHgb were significantly higher in asthmatics as compared to healthy controls (**Table 1**). Given the high affinity of carbon monoxide towards Hgb and its low plasma solubility, cardiac output effect on DLCO is typically considered trivial and was not taken into consideration as a significant determinant (33).

Angiogenic Factors in asthmatic women

Although previous study has shown increased circulating $CD34^+CD133^+$ progenitor cells in asthmatic men and women as compared to healthy individuals (19), asthmatic women in this study had circulating $CD34^+CD133^+$ progenitor cells levels similar to healthy women (%CD34+CD133+ EPC: asthma 0.06 ± 0.004, controls 0.08 ± 0.005; $p = 0.1$). Serum VEGF was lower in asthmatics as compared to controls (VEGF pg/ml: asthma 163 $\pm$ 15, controls 513 ± 47; $p < 0.01$), whereas serum SCF was higher (SCF pg/ml: asthma 1234 ± 65, controls 1024 ± 28; $p = 0.06$). Asthmatic women had changes in $CD34^+CD133^+$ progenitor cells in the circulation over the menstrual cycle ($p=0.01$) (**Figure 2**). However, VEGF and SCF did not change significantly over the menstrual cycle of asthmatic women ($p=0.2$ and $p=0.8$ respectively), and $CD34^+CD133^+$ progenitor cells were unrelated to serum VEGF or SCF (all $p > 0.3$).

The use of inhaled corticosteroids or contraceptives did not affect the changes in $CD34^+CD133^+$ progenitor cells over the menstrual cycle in asthmatics and the highest value was consistently noted at week 1.

Relation of angiogenic factors to airflow and diffusion

Previous study identified that $CD34^+CD133^+$ progenitor cells are related to changes in Vc and DLCO over the menstrual cycle of healthy women (16). In asthmatic women, the circulating numbers of $CD34^+CD133^+$ progenitor cells were unrelated to DLCO ($p = 0.5$), Vc or Dm (all $p > 0.3$). Rather, EPC were related to F_ENO ($R = 0.5$, $p < 0.01$) and inversely related to airflow (FEV_1%, $R = -0.4$, $p = 0.01$; FEV_1/FVC, $R = -0.4$, $p < 0.01$) (**Figure 3**). Serum VEGF and SCF were unrelated to circulating numbers of EPC or airflow (all $p > 0.3$).

Greater DLCO in Asthma: relationship to F_ENO, pH, pO_2, pCO_2 and Hgb

The changes in DLCO over menstrual cycle in asthma were accounted for by changes in Dm (Figure 2). Although there were no variations of pH, pO_2 or pCO_2 over the menstrual cycle [pCO_2 (mm Hg): week 1, 39.7 ± 1.0; week 2, 37.4 ± 1.3; week 3, 38.1 ± 1.3; week 4, 37.6 ± 1.8; $p = 0.4$], venous blood gases revealed differences in pH, pO_2 and pCO_2 among asthma and controls (**Table 1**). The findings showed lower pCO_2 in asthma, with resultant increases in pH. This led to predictable pH related changes in the venous blood p50; p50 was lower in asthma as compared to healthy controls (**Table 1**). F_ENO was unrelated to p50, pO_2 or pCO_2 (all $p > 0.2$).

Venous COHgb was higher in asthma than controls (**Table 1**). Similarly, MetHgb was greater in asthma than controls (**Table 1**). The greater MetHgb and COHgb may also contribute to the lower p50 of asthmatics as compared to controls. As expected, DLCO values of asthmatics were inversely related to MetHgb ($R = -0.3$, $p = 0.04$) and COHgb ($R = -0.3$, $p = 0.03$). Despite the greater MetHb and COHgb in asthma, asthmatics had

comparable DLCO and Dm to healthy women. MetHgb or COHgb levels in asthma were not related to F_ENO or airflow (all $p > 0.1$).

Relationships of DLCO to Airflow Limitation

Air trapping and thinning of the alveoli-capillary membrane might produce faster diffusion. However, better airflow was actually related to faster diffusion, i.e. FEV_1% was directly related to DLCO among asthmatics ($R = 0.5$, $p < 0.01$) (**Figure 4**). The higher levels of NO in asthma might be predicted to reduce DLCO due to its greater affinity for Hgb than CO, but higher values of NO were associated with greater DLCO ($R=0.4$, $p=0.03$), which implies that both CO and NO diffusion may be faster in asthmatics. Furthermore, the greater DLCO in asthma was not related to larger overall lungs, as the V_A was similar among the groups, and DLCO/V_A still tended to be greater in asthma than in controls (**Table 1**). Overall, it appears that peak DLCO occurs at the same time that there is the best airflow in asthmatic women, i.e. at the end of luteal phase and start of menses.

Discussion

In contrast to prior studies that did not find changes in spirometry over the menstrual cycle (10, 11, 34), airflow varied significantly in this study. Asthmatic women achieved the best airflow at the end of luteal phase through beginning of menstruation followed by a decline over the subsequent two weeks. Although there was some variation in FEV_1 in healthy women, the ratio of FEV_1/FVC did not vary among the healthy women over the cycle. Lung diffusing capacity changed in parallel to airflow in asthmatics. These findings were consistent in all asthmatic women regardless of use of contraceptives or inhaled corticosteroids. In healthy women, the changes in DLCO have been attributed to changes in the pulmonary vascular bed capacity (16). However, variation of DLCO in asthmatic women was due to changes in membrane diffusion. In further contrast to healthy women (16), circulating pro-angiogenic progenitor cells of asthmatic women were unrelated to lung diffusion. Unlike our prior report (19), the asthmatic women in this study did not have higher levels of proangiogenic progenitors than control women. Failure to show a difference between asthmatics and controls with regards to $CD34^+CD133^+$ cells could be related to the relatively mild characteristics of the asthma sample in this study, the low sample numbers, the fact that the prior study evaluated men and women, and/or the effects of inhaled corticosteroids. Our prior report, which identified higher $CD34^+CD133^+$ cells in asthma, included more severe asthmatics than in the current study, which evaluated very mild asymptomatic asthmatic women. The finding of an inverse relationship among $CD34^+CD133^+$ cells and airflow in this study supports the possibility that asthmatics with more severe airflow limitation have higher circulating levels of $CD34^+CD133^+$ cells. The relationship of $CD34^+CD133^+$ progenitor

cells to airflow obstruction in this study, and in a murine model of asthma in prior study (19), also suggests that angiogenesis may contribute to airflow obstruction, and/or inflammation, over the time of the menstrual cycle in asthmatic women. In fact, the $CD34^{+}CD133^{+}$ cells are pluripotent and can give rise to multiple lineages including mast cells and fibroblasts, both known to play a role in airway inflammation and remodeling (35).

In general, asthmatics have greater gas transfer capacity as compared to healthy individuals (36-40). Although several studies have investigated the causes for higher DLCO in asthmatics, the findings are conflicting and the physiologic mechanisms underlying the increased levels are uncertain (36, 38, 40). Stewart found that asthmatics had higher DLCO and Vc when corrected for alveolar volume in comparison to healthy controls, and concluded that the greater gas transfer was due to an increase in lung capillary blood volume (40). Collard *et al.* showed that greater DLCO in asthma was associated with an increased perfusion of the lung apices (36). On the other hand, in a study of asthmatic children with different degrees of over-inflation, Pecora *et al.* showed that DLCO was increased due to an increase in Dm, which they attributed to an increased surface area and/or thinner membrane secondary to over-inflation (38). The finding of changes in Dm over the menstrual cycle suggests that diffusion across the alveolar-capillary membrane varies in asthmatic women over the menstrual cycle, but not in healthy women. This might occur secondary to cyclic expansion of the available surface for gas exchange, i.e. gas exchange may occur more proximally than the level of the respiratory bronchioles in asthma. Alternatively, it has been proposed that children with asthma have greater growth of the lungs, which results in an increased total lung capacity

and higher DLCO (33). Although this might contribute to overall greater DLCO and Dm, asthmatic women in this study had similar levels of alveolar volume as healthy women and alveolar volume did not change over the menstrual cycle. In the context of new studies which suggest a role for membrane channels such as aquaporin in gas transfer (41, 42), the cyclic changes of gas transfer in asthma may also be due to alterations of an active transporter that modifies alveolar-capillary cell membrane permeability and/or channels. Finally, the changes of Dm over the menstrual cycle may be related to hyperinflation and/or air trapping, even though the best DLCO and Dm occurred at the times of the best airflow. In this context, limitations of this study is that measurement of lung volumes was not available to evaluate air trapping, and imaging of vascular changes were not done. More subtle measurements of small airway function and emptying, as well as the measure of ventilation and perfusion over the menstrual cycle, would have been helpful in understanding the findings. Similarly, direct assessment of the pulmonary vasculature through nuclear imaging might have provided insight into changes in DLCO, although spatial resolution of even the best imaging modalities for blood distribution in the lungs, such as single photon emission computed tomography, is only ~15 mm, which is relatively poor in comparison to other imaging modalities.

The higher level of venous COHgb in asthmatic women was similar to findings from other studies looking that investigated arterial COHgb (43, 44). The greater COHgb has been attributed to inflammation-mediated upregulation of heme oxygenases, the enzymes responsible for CO production (43, 44). Nevertheless, COHgb did not vary over the menstrual cycle, which indicates that it did not contribute to the changes in lung

functions observed in asthmatic women, and that inflammation did not vary substantially over the menstrual cycle.

Although data indicate that F_ENO serves as a surrogate marker of eosinophilic airway inflammation and airflow obstruction in asthma (45), NO has also been proposed as a method for measure of gas transfer(46). Higenbottam and coworkers originally proposed inhaled NO as a substitute for CO; NO and CO similarly diffuse and have high reactivities with hemoglobin (46). In this context, NO values are associated with DLCO over the time of the menstrual cycle in this study. The data suggest that endogenously produced NO, as measured by F_ENO, may also reflect overall gas transfer across the alveoli in asthmatics. Alternatively, the American Thoracic Society (ATS) standardized flow rate to measure exhaled NO, which was used in this study, may not be optimal for evaluating the vascular and inflammation changes occurring over the menstrual cycle. Thus, future studies might perform measures of exhaled NO at varying flow rates as previously described (47) and/or use other specific biomarkers of inflammation, such as 3-bromotyrosine for eosinophil-related oxidation and F2-isopostanes for lipid peroxidation (48, 49).

In summary, FEV_1 and gas transfer are at peak levels in all women at the end of luteal phase through the start of menstruation. In contrast to healthy women, the cyclic changes in gas transfer of asthmatic women are due to the changes in diffusion characteristics across the alveolar capillary membrane. The menstrual cycle is one of the most important biological rhythms that govern physiological processes of living beings. Here, airflow and gas transfer are shown to be among the many vital functions modulated over the menstrual cycle, but the cyclic respiratory changes occur by very different

physiologic mechanisms in asthmatic as compared to healthy women. These findings begin to provide insight into understanding features of asthma unique to women, including perhaps the much greater prevalence of difficult-to-control and severe asthma among women and the phenomenon of peri-menstrual asthma exacerbations.

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Disclosures

All authors participated in the study and have seen and approved the final version. None of the authors have any conflict of interest to declare.

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Figure Legends

Figure 1. Airflow and FE_{NO} measures over the menstrual cycle in asthmatic and healthy women. Airflow changes significantly in healthy and asthmatic women over the menstrual cycle. Closed circles represent asthmatics and open circles represent healthy controls. Asterisks represent significant changes from week 1 (all $p<0.05$).

Figure 2. Lung diffusing capacity for carbon monoxide (DLCO) and its components, the alveolar capillary membrane diffusing capacity (Dm), and lung capillary blood volume (Vc), and circulating $CD34^{+}CD133^{+}$ progenitor cells over the menstrual cycle in asthmatic and healthy women. Circulating levels of $CD34^{+}CD133^{+}$ progenitor cells also change over time in asthmatic women similar to healthy women. Closed circles represent asthmatics and open circles represent healthy controls. Asterisks represent significant changes from week 1 (all $p<0.05$).

Figure 3. Relation of $CD34^{+}CD133^{+}$ progenitor cells to airflow (%FEV_1 and FEV_1/FVC) in asthmatic women.

Figure 4. Airflow as determined by %FEV_1 is related to the lung diffusing capacity (DLCO).

Table 1. Characteristics of Study Subjects

Variable	Healthy N = 10	Asthmatics N = 13	Mean Difference ±SE (adjusted for multiple observations)	p-value
Age (years)	31 ±1	31 ±1	--	0.9
Race (Caucasian/African American/Asian)	9/1/0	12/1/0	--	1
Height (cm)	149 ±16	166 ±2	--	0.5
Weight (kg)	64 ±2	81 ±5	--	0.03
OCP use (Y/N)	3/7	4/9	--	1
Pulse (beats/min)	73 ±2	78 ±2	5 ±4	0.3
FVC %	102 ±2	100 ±2	2 ±5	0.7
FEV_1 %	106 ±2	95 ±3	11 ±6	0.07
FEV_1/FVC	83 ±1	77 ±2	6 ±3	0.08
FE_{NO} (ppb)	15 ±1	31 ±7	17 ±14	0.2
O_2 Saturation (% of Hgb)	98.7 ±0.1	98.2 ±0.3	0.5 ±0.5	0.3
Venous blood gas				
pH	7.39 ±0.005	7.42 ±0.005	0.03 ±0.008	<0.001
pO_2	36 ±2	46 ±2	11 ±5	0.02
pCO_2	44.0 ±0.8	38.1 ±0.7	6 ±1	<0.001
p50	26.7 ± 0.3	25.4 ± 0.2	1.3 ± 0.5	<0.01
COHgb (%)	0.5 ±0.04	1.4 ±0.09	1 ±0.2	<0.001
MetHgb (%)	0.5 ±0.02	1.1 ±0.06	0.6 ±0.1	<0.001
Hgb (gm/dL)	13.5 ±0.1	13.5 ±0.2	0.08 ±0.5	0.9
Va (L)	5.5 ±0.07	5.2 ±0.1	0.3 ±0.3	0.3
DLCO (ml/min/mmHg)	24.0 ±0.4	25.3 ±0.6	1.2 ±1.5	0.4
DLCO/Va (1/min/mmHg)	4.4 ±0.1	4.8 ±0.1	0.5 ±0.2	0.05
Dm (ml/min/mmHg)	62 ±6	80 ±8	19 ±13	0.1
Vc (ml)	55 ±3	50 ±3	4 ±4	0.3

Table 2. Characteristics of the Asthmatics

Study Subject	PC20 (mg/ml)	FEV_1 %	Medications used regularly*
1	0.554	123	
2	0.192	97	ICS+LABA
3	Positive BD‡	44	
4	0.109	101	ICS+LABA, Montelukast
5	--	92	ICS+LABA
6	2.5	97	Montelukast
7	10	99	ICS+LABA
8	5.572	97	Montelukast
9	3.548	85	
10	--	102	Montelukast
11	0.261	72	ICS+LABA
12	4.475	92	ICS+LABA, Montelukast
13	0.437	108	

*ICS+LABA, combination inhaled corticosteroid and long acting β-agonist; Albuterol used as needed by all subjects.

‡Positive BD, bronchodilator response = 12% and 200ml

-- Data not available

Figure 1

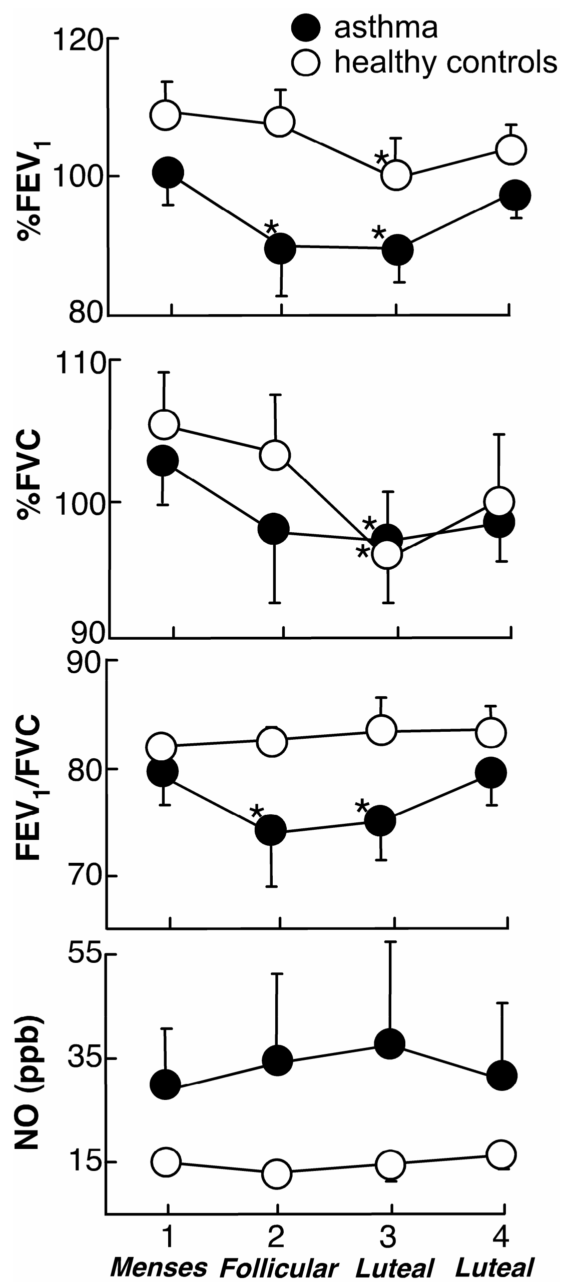


Figure 2

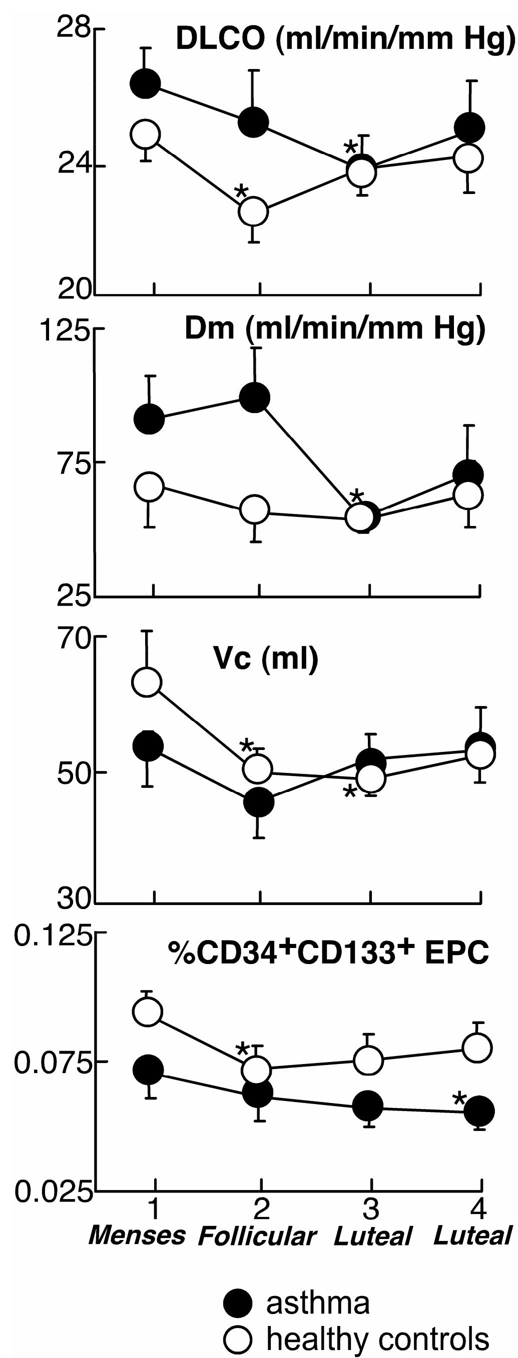


Figure 3

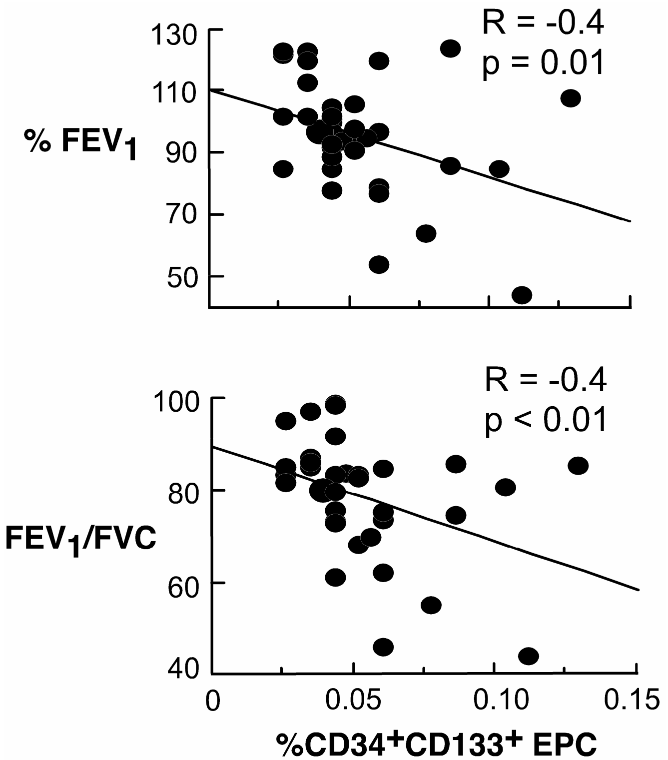
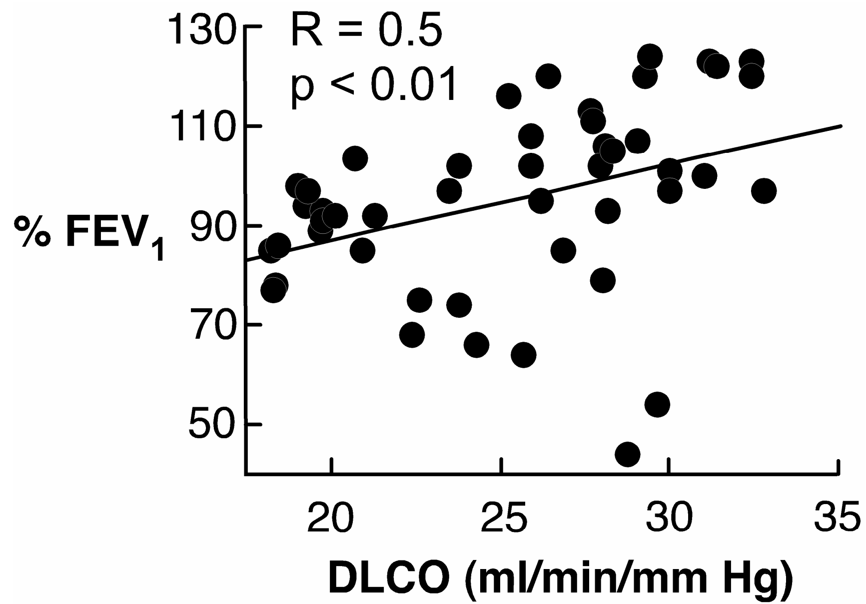


Figure 4



Online Data Supplement

Respiratory Function in Asthmatic Women over time of the Menstrual Cycle

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Herbert P. Wiedemann,Serpil C. Erzurum

Lung function and diffusing capacity for carbon monoxide

The forced expiratory volume at 1 second (FEV_1) and vital capacity (FVC) were measured using an Eagle™ spirometer (Ferraris Respiratory, Colorado). The single breath carbon monoxide diffusing capacity was performed weekly using Eagle™ equipment (Ferraris Respiratory, Colorado) with volunteers rested and in a seated position. On each visit, DLCO was measured at 2 different oxygen concentrations (21% and 42%). DLCO measurements were performed at the same time of the day on each visit to minimize the effect of diurnal variations. The single breath DLCO method was performed in duplicate, ~3 minutes apart using a washout volume of 750 ml and an alveolar volume of 750 ml. The breathhold time was ~10 seconds. DLCO was adjusted for hemoglobin (Hgb) using the Amercian Thoracic Society (ATS) guidelines. Exhaled O_2 was obtained from the alveolar gas at each measurement of the diffusing capacity. Dm and Vc were calculated from DLCO measurements at inspired O_2 concentrations 21% and 42% as described by Roughton and Forster. Theta was calculated from the formula described by Cotes (1):

$$1/\theta = [0.34 + (0.0061 \text{ x } P_cO_2)] \text{ x } (14.6/Hgb)$$

P_cO_2 represents the capillary partial pressure of oxygen and was determined to be nearly equivalent to the alveolar partial pressure of oxygen ($P_A O_2$) measured at end expiration:

$$P_cO_2 = P_A O_2 - 5$$

End expiration P_AO_2 was determined from the direct measure of end expiration oxygen concentration, and the daily barometric pressure. Since measurements of Dm and Vc are best made when Hgb is fully saturated with oxygen, DLCO was measured over a range of inspired O_2 concentrations 21%, 42%, 60% and 80% to confirm that 1/DLCO relative to

FiO_2 was linear ($R^2 = 0.97$). This validated the use of inspired oxygen concentrations of 21% and 42% to determine Dm and Vc in the study. Prior to DLCO determinations, single-breath on-line measurement of fractional NO concentration in expired breath (F_ENO) was also measured at each visit using the NIOX (Aerocrine, NY).

Reference:

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