Modulation of Airway Reactivity and Peak Flow Variability in Asthmatics Receiving the Oral Contraceptive Pill

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Female sex-steroid hormones may play an important influence in asthma. The aim of this study was to compare airway reactivity to adenosine monophosphate (AMP) in female asthmatics with natural menstrual cycles and those taking the oral combined contraceptive pill (OCP). Eighteen asthmatic subjects were evaluated. Nine subjects, mean (SEM) age, 24 (6) years, FEV_1 93% (10) predicted, with natural cycles (group 1) were compared with nine subjects, age 24 (6) years, FEV_1 93% (9) predicted taking the OCP (group 2). Group 1 subjects were evaluated at the follicular (visit 1) and luteal (visit 2) phases; group 2 subjects were evaluated during the week off OCP (visit 1) and at the end of the OCP cycle (visit 2). At each visit, serum progesterone and estradiol were measured. Airway reactivity to AMP was evaluated and expressed as PC_{20} (FEV_1; mg/ml). Morning and evening peak expiratory flow rates (PEFR) were monitored throughout the study. In group 1, there was a significant increase in serum progesterone (nmol/l) and estradiol (pmol/l). (Visit 1 vs. 2): 2.5 vs. 13.5 (95% CI 2.1 to 19.9; $p = 0.02$) and 152.3 vs. 358.1 (95% CI 113.0 to 298.5; $p < 0.001$), respectively. In group 2, however, there was no increase between visit 1 vs. 2 in hormones: 0.9 vs. 1.0 and 75.7 vs. 21.8 for progesterone and estradiol, respectively. There was a significant increase in airway reactivity in group 1 during the luteal phase. Geometric mean PC_{20} (mg/ml) was 18.8 and 4.7 at visit 1 and 2, respectively: a 4.0-fold difference (95% CI 1.25 to 13.03; $p = 0.03$) amounting to two doubling doses. In contrast, there was no change in PC_{20} in group 2. Geometric mean PC_{20} was 23.5 and 21.4: a 1.06-fold difference (95% CI 0.41 to 2.78; $p = 0.83$). In group 1, morning and evening PEFR (l/min) were significantly different at both visits: at visit 1 (A.M. PEFR vs. P.M. PEFR) 403 vs. 430 (95% CI 5 to 50; $p < 0.001$) and visit 2, 415 vs. 439 (95% CI 1 to 46; $p < 0.001$). In group 2, there was no significant difference in diurnal PEFR variability at both visits; 411 vs. 417 at visit 1 and 413 vs. 427 at visit 2. In conclusion, asthmatic patients receiving the OCP had attenuated cyclical change in airway reactivity as well as reduced diurnal PEFR variability, which was associated with suppression of the normal luteal phase rise in sex-hormones. Tan KS, McFarlane LC, Lipworth BJ. Modulation of airway reactivity and peak flow variability in asthmatics receiving the oral contraceptive pill.

AM J RESPIR CRIT CARE MED 1997;155:1273–1277.

Female sex-steroid hormones may play an important role in asthma. In particular, exacerbations of asthma have been recognized to occur during the premenstrual period, when there are large fluxes in levels of circulating sex-steroid hormones (1–4). These have also been associated with a fall in peak expiratory flow rates (PEFR) during this period, even in those who have been asymptomatic (4). It has also been reported that in a few cases of severe premenstrual asthma, intramuscular progesterone eliminated the premenstrual fall in PEFR and allowed better control of asthma (5).

With such compelling evidence for hormonal influence on airways, we have investigated whether changes in airway responsiveness occur during the menstrual cycle, and we have chosen to evaluate a group of female asthmatics with natural menstrual cycles and a group of female asthmatics who are taking the oral combined contraceptive pill (OCP). Previous studies, however, have not demonstrated any change in airway reactivity during the menstrual cycle using methacholine (4, 6) or histamine (7) challenges, which are direct bronchoconstrictor agents. In this respect, we have chosen to investigate airway reactivity using adenosine 5'-monophosphate (AMP), an indirect bronchoconstrictor, which acts by stimulating the release of mediators from mast cells (8).

METHODS

Patients

Eighteen female subjects with stable asthma were evaluated in this study. In group 1, there were nine subjects, mean (SEM) age 24 (six) years; FEV_1 in litres and % predicted was 2.93 (0.46) 1 and (10) 93% predicted, with natural menstrual cycles. In group 2, there were nine subjects, age and severity matched, who were taking the OCP, age 24 (six) years with FEV_1 2.98 (0.38) 1 and (nine) 93% predicted. A full physical examination, biochemical and hematological parameters were normal prior to inclusion. All gave written informed consent before being evaluated in

(Received in original form August 19, 1996 and in revised form December 10, 1996)

This work was supported by the National Asthma Campaign (UK).

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this study, which was approved by the Tayside Medical Ethics Committee. All had asthma according to the criteria of the American Thoracic Society (9), and all were non-smokers. In the group with natural cycles, seven subjects were receiving inhaled corticosteroid (either beclomethasone or budesonide), median dose 600 μg/d (range 200–2,000 μg/d). One subject was taking inhaled salmeterol 50 μg bid, and one was taking sustained-release theophylline. In the group on the OCP, six subjects were receiving inhaled corticosteroid, median dose 400 μg/d (range 200–1,000 μg/day). One subject was also taking inhaled salmeterol 50 μg bid, and one was taking sustained-release theophylline. All had been inhaling short-acting β_2-agonists as required in doses of < 400 μg/d. All medications were at stable dose for at least 2 mo prior to the study and were taken at constant dosage throughout the study. None had received oral corticosteriods for at least 3 mo, and none had had a recent exacerbation of asthma in the past month. All had stable asthma with no subjective history of premenstrual asthma.

In the group on the OCP, six subjects were taking monophasic and three subjects were taking triphasic preparations. Of the monophasic preparations, five were on ethinyloestradiol 30 μg/d and one was on 35 μg/d of ethinyloestradiol. There were different preparations and doses of progestogens.

Protocol

All subjects were evaluated at two visits in the laboratory. Group 1 subjects were evaluated during the follicular (visit 1) and luteal (visit 2) phases of the menstrual cycle. The follicular phase was defined as Day 1–4 and luteal phase as day 21–24, with Day 1 being the first day of the menses. Group 2 subjects were evaluated during the week while off the OCP (visit 1, during the week of menstruation) and at the end of the 21-day course of OCP (visit 2). At each visit, subjects attended the laboratory at 9:00 A.M., having withheld short-acting β_2-agonists for 8 h, long-acting β_2-agonists for 48 h, and theophylline preparations for 48 h. After a 15-minute period of rest, 10 ml of blood was withdrawn from a peripheral vein for serum estradiol and progesterone. Then, airway reactivity to adenosine 5'-monophosphate (AMP; Sigma, Poole, UK) was determined using a challenge protocol as described below. This was expressed as PC_{20}, the provocative concentration that causes a 20% fall in FEV_1.

During the study, subjects were asked to keep a diary of morning and evening peak expiratory flow readings using a Wright's peak flow meter (Airmed, London, UK) by recording the best of three consecutive readings. They were also asked to give a daily symptom score for wheeze (0–3) and for nocturnal symptoms (0–3), and the number of rescue puffs of β_2-agonists needed.

Measurements

Female sex-steroid hormones. Samples for serum estradiol and progesterone were stored at −20° C and analyzed at the end of the study. Serum estradiol (Sorin Biomedica, Saluggia, Italy) and progesterone (Incstar Ltd., Wokingham, UK) were measured by radioimmunoassay. The intra-assay coefficients of variation for analytical imprecision were 2.9% and 3.1% for estradiol and progesterone, respectively.

Airway reactivity. Fresh solutions of AMP, in a range of concentrations from 0.04 mg/ml to 200 mg/ml was made up in normal saline on each study day. A nebicheck nebulizer controller (PK Morgan Ltd., Rainham, Kent, UK) was used with a system 22 Acorn nebulizer (Medicaid Ltd., Pagham, West Sussex, UK) with a driving pressure of 20 psi (138KPa). The nebulizer was activated for 1.2 sec from the initiation of respiration. A mouthpiece was used with the nebulizer and the nose clipped during the procedure. The mouthpiece was placed between the teeth of the subject who exhaled to slightly below functional residual capacity and then inhaled slowly over 1 to 2 sec toward total lung capacity, where the breath was held for 3 sec before taking the next breath.

Pulmonary function was assessed by measurement of FEV_1 according to American Thoracic Society criteria (10) with a Vitalograph Compact spirometer with a pneumotachograph head and pressure transducer, and on-line computer-assisted determination of FEV_1 (Vitalograph Ltd., Buckinhamshire, UK). Forced expiratory maneuvers were performed from total lung capacity to residual volume. The best FEV_1 value was taken from three consistent measurements and used as baseline. A coefficient of variation of less than 3% was considered as acceptable. Subjects then inhaled five breaths of a normal saline control solution followed by sequential doubling concentrations of AMP given at 3 min-intervals. FEV_1 was measured 1 minute after administrating saline and each concentration of AMP. The test was terminated when a 20% fall in FEV_1 from the post-saline value was attained. A log-dose response curve was constructed and PC_{20} was calculated by linear interpolation.

Statistical Analysis

PC_{20} was log-transformed for analysis, as it is not normally distributed. For all variables, comparisons within groups for both visits were made by analysis of variance (ANOVA) and where appropriate followed by Bonferroni multiple range testing. Comparisons between groups was by unpaired Student's *t*-test. Data were analyzed with the Statgraphics statistical software package (STSC Software Publishing Group, Rockville, MD). A probability value of $p < 0.05$ (two-tailed) was considered as being of significance and 95% confidence intervals (95% CI) for mean differences were calculated where significant. The rescue requirements and symptom score were analyzed nonparametrically using Kruskal-Wallis analysis of ranks.

RESULTS

Baseline Airway Caliber

Baseline FEV_1 for both groups did not differ between the two visits. For group 1, (visit 1 vs. 2) FEV_1 was 2.74 vs. 2.73 ($p = 0.90$), and for group 2, FEV_1 was 2.85 vs. 2.87 ($p = 0.77$). Likewise, there was no significant difference between the groups at visit 1: 2.74 vs. 2.85 ($p = 0.67$) and at visit 2: 2.73 ($p = 0.53$).

Female Sex-Steroid Hormones

In group 1, who had natural cycles, there was a significant increase in sex hormones (Figure 1), at visit 2; for progesterone (mmol/l), visit 1 vs. 2: 2.5 vs. 13.5 (95% CI 2.1 to 19.9; $p < 0.02$) and for estradiol (pmol/l): 152.3 vs. 358.1 (95% CI 113.0 to 298.5; $p < 0.001$). However, in group 2, who were on the OCP, there was no increase between visit 1 and visit 2 in female hormones; for progesterone (visit 1 vs. 2), 0.9 vs. 1.0 and for estradiol 75.7 vs. 21.8.

Airway Reactivity

In group 1, there was a significant increase in airway reactivity during the luteal phase (visit 2) of the menstrual cycle (Figure 2). The geometric mean PC_{20} was 18.8 mg/ml at visit 1 and 4.7 mg/ml at visit 2. This was a 4.0-fold difference (95% CI 1.25 to 13.03; $p < 0.03$), which amounted to two doubling doses. One subject had shown a dramatic increase in airway reactivity from the follicular phase (log PC_{20} 1.398) to the luteal phase (log PC_{20} −0.903). When this "outlier" is removed (i.e., $n = 8$), and analysis performed with the "trimmed" geometric mean, there is still a significant difference between the follicular (visit 1) and luteal (visit 2) phases of the cycle: 18.2 mg/ml at visit 1 and 7.4 mg/ml at visit 2. This is a 2.5-fold difference (95% CI 1.68 to 3.64; $p < 0.001$) amounting to 1.3 doubling doses. In contrast, there was no change in airway reactivity in group 2 between the two visits: 23.5 mg/ml at visit 1 and 21.4 mg/ml at visit 2; a 1.06-fold difference (95% CI 0.41 to 2.78; $p = 0.83$) (Figure 2). There was no difference in geometric mean PC_{20} between the two groups at visit 1: 18.8 vs. 23.5; a 1.25-fold difference (95% CI 0.3 to 5.1; $p = 0.74$). There was a trend toward a difference between the two groups at visit 2: 4.7 vs. 21.4, a 4.57-fold difference (95% CI 0.93 to 22.4; $p = 0.06$).

Peak Expiratory Flow Readings

In group 1, with natural cycles, there was a significant difference between morning and evening PEFR (l/min): at visit 1 (morning PEFR vs. evening PEFR), 403 vs. 430 (95% CI 5 to 50; $p < 0.001$) and for visit 2, 415 vs. 439 (95% CI 1 to 46; $p < 0.001$). In group 2, on the OCP, there was no significant difference in morning

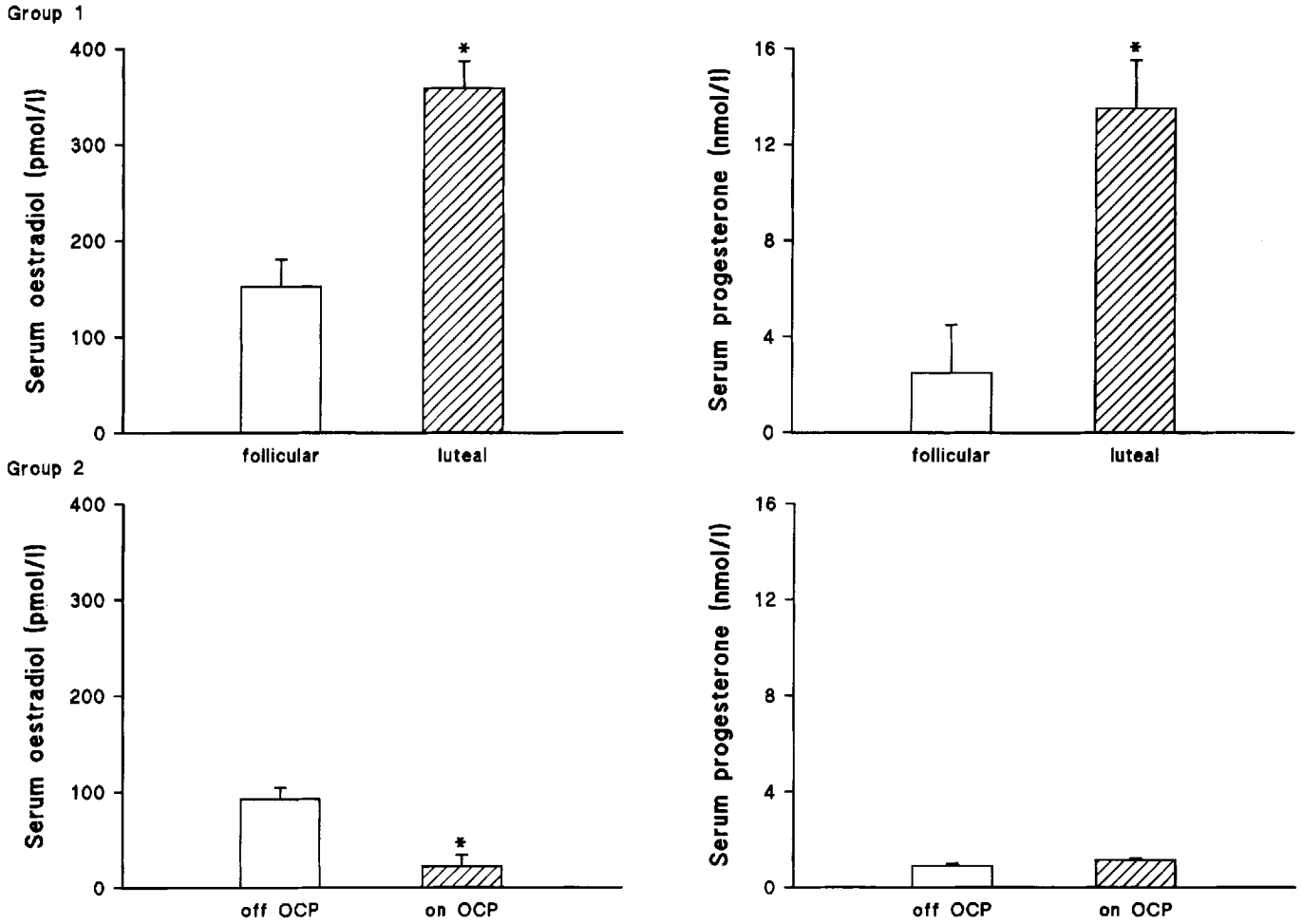

Figure 1. Female sex-steroid hormone levels at both visits in group 1 (with natural cycles) and group 2 (on the OCP). *$p < 0.05$ visit 1 vs. 2.

and evening PEFR at both visits; at visit 1, 411 vs. 417 and at visit 2, 413 vs. 427 (Figure 3).

In both groups, there were no significant differences in symptom score and rescue requirements between visit 1 and 2.

DISCUSSION

This study has demonstrated that airway reactivity to AMP shows cyclical change in female asthmatics during the menstrual cycle, with greater reactivity at the luteal phase. This was accompanied by a significant and appropriate increase in female sex-steroid hormones at this phase of the menstrual cycle. In contrast, endogenous female sex-steroid hormones were suppressed and showed no increase during visit 2 in the group of asthmatics who were taking the oral contraceptive, as would be expected. Airway reactivity in this latter group did not show any variation during the cycle, in contrast to those with natural cycles. It is also interesting to note that this cyclical change in airway reactivity occured in the group of asthmatics with natural cycles even though they were on a higher median dose of inhaled corticosteroid. Our patients had mild disease and so it may not be possible to extrapolate the present results to more severe asthmatics and particularly those with symptomatic premenstrual deterioration. It is interesting to note that in a recent study by Skobeloff and coworkers (11), almost half of all female admissions for acute asthma occurred during the perimenstrual phase.

Previous studies (4, 6, 7) using histamine or methacholine, which act directly on airway smooth muscle have not shown any change in airway reactivity during the menstrual cycle. In the studies by Pauli (4) ($n = 11$) and Weinmann (7) ($n = 9$), the subjects studied had natural cycles and none were receiving the oral contraceptive. Juniper and colleagues (6) studied 10 subjects with natural cycles and seven subjects receiving the oral contraceptive. The observation that 'direct' bronchoconstrictors like histamine and methacholine show no cyclical change together with the results in this present study would suggest that the mechanism of hormonal influence on airway reactivity is on mediator release. Since AMP mediates bronchoconstriction via mediator release from mast cells (8), it is possible that the normal postovulatory rise in sex-hormones may sensitise mast cells, and so, lower the threshold for mediator release. This in turn suggests that in the luteal phase during the premenstrual period, deterioration in asthma control may be due to augmented inflammatory mediator release. Thus, suppression of endogenous hormone levels by the contraceptive pill may offer an explanation for the attenuation of airway reactivity seen in this group of subjects.

When evaluating changes in airway reactivity it is important to consider the intrinsic variability of this measurement. This is such that PC_{20} values can vary by up to 2-fold, i.e., ± one doubling dose—even in stable asthmatics (12). In our study, we have shown that, in those with natural cycles, airway reactivity to AMP increased by four fold during the luteal phase, equiva-

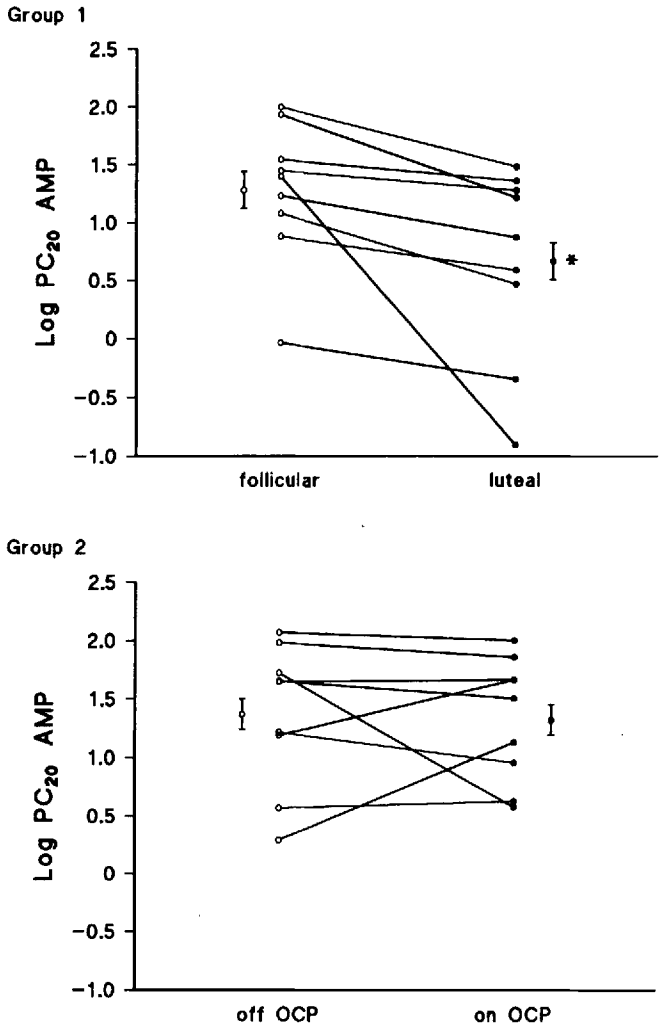

Figure 2. Airway reactivity to AMP during the menstrual cycle in group 1 (with natural cycles) and group 2 (on the OCP). *p < 0.05 visit 1 vs. 2.

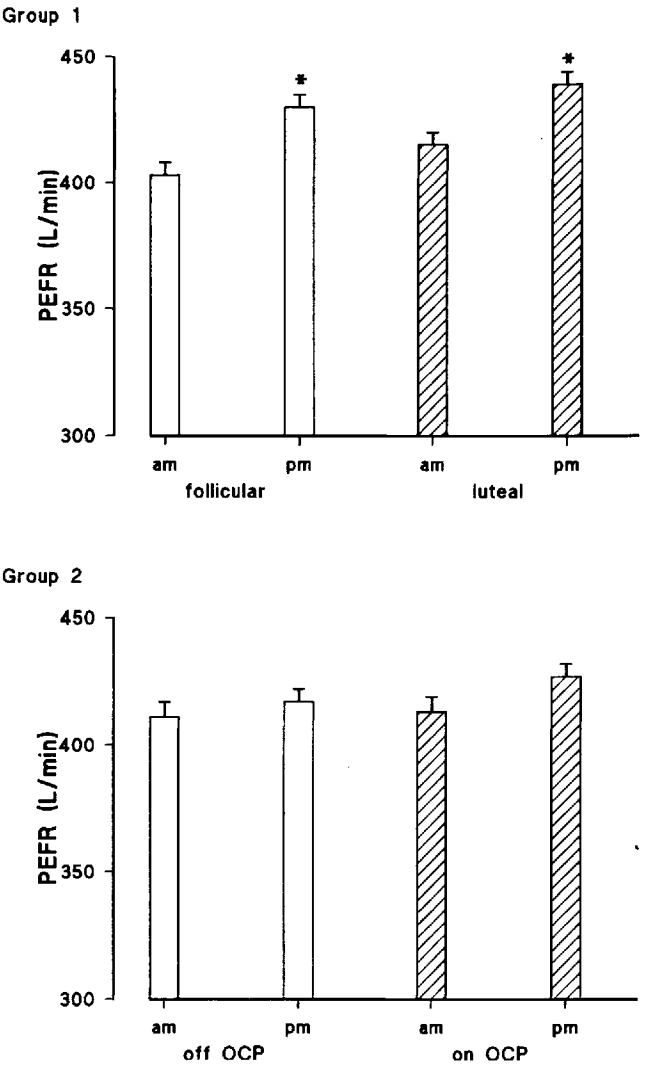

Figure 3. Morning and evening dormicillary PEFR during the menstrual cycle in group 1 (with natural cycles) and group 2 (on the OCP). *p < 0.05 morning PEFR vs. evening PEFR.

lent to two doubling doses of AMP. Thus, as well as being statistically significant, this increase is also likely to have clinical significance as it exceeds the limit of biological variability. It is also important to note that baseline airway calibre as assessed by FEV_1 was not significantly different between the two visits for the two groups. This is relevant to the evaluation of PC_{20} as airway geometry has an important influence on assessing airway responsiveness to a bronchoconstrictor agent (13).

The group receiving the contraceptive pill were also found to have attenuated diurnal PEFR variability compared with those with natural cycles. Clearly, it is important to know that the two groups were similarly matched for age and disease severity. In this respect, there were no significant differences in FEV_1 and PC_{20} at visit 1. This makes it likely that the difference in PC_{20} and PEFR variability between the two groups was due to the effect of the contraceptive pill rather than any other confounding variables due to their asthma. Ideally, we would like to have evaluated the group of asthmatics while on and off the oral contraceptive. However, this was not possible as the subjects concerned were reluctant to consent to this because of the increased risk of pregnancy while off the OCP. Likewise the Tayside Committee for Medical Research Ethics felt that this was ethically unacceptable.

Cyclical menstrual changes are not confined to the airways but are also evident in skin-prick test reactions to histamine and allergen, with greater weal-and-flame reactions during the early luteal phase (14). There are other possible mechanisms by which sex-steroids may influence the airways. In rabbit lung, estrogen and progesterone have been shown to modify β-adrenergic receptor density with estrogen increasing and progesterone decreasing the number of sites (15). Prostaglandins are known to be modulators of airway tone (16) and monthly cyclical variations in circulating $PGF_{2\alpha}$ metabolite have been reported (17). However, treatment with meclofenamate, a potent prostaglandin synthase inhibitor, did not prevent exacerbations of premenstrual asthma (18).

What then is the clinical relevance of our findings? It is possible that exogenous female sex-steroid hormones may be used therapeutically in females with unstable asthma or those with premenstrual asthma, not controlled by conventional therapy, by smoothing out cyclical changes in airway reactivity. However, the effects of exogenous female sex-steroid hormones are not entirely clear. In a recent questionnaire-based prospective cohort study reported by Troisi and coworkers (19), there was an increased risk of developing adult-onset asthma in those women who had

used hormone-replacement therapy or oral contraceptives, and this risk was related to duration of use and dose of estrogen. Nevertheless, the modulatory effect of exogenous female sex-steroids may be beneficial rather than deleterious in those with established asthma, by attenuating the cyclical changes in airway responsiveness. Clearly, further studies are needed to identify which hormone is responsible for these airway changes, perhaps by administering the hormones individually by aerosol to the lung and assessing changes in airway responsiveness.

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