Enhanced Response to Ozone Exposure during the Follicular Phase of the Menstrual Cycle

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Exposure to ozone (O_3), a toxic component of photochemical smog, results in significant airway inflammation, respiratory discomfort, and pulmonary function impairment. These effects can be reduced via pretreatment with anti-inflammatory agents. Progesterone, a gonadal steroid, is known to reduce general inflammation in the uterine endometrium. However, it is not known whether fluctuations in blood levels of progesterone, which are experienced during the normal female menstrual cycle, could alter O_3 inflammatory-induced pulmonary responses. In this study, we tested the hypothesis that young, adult females are more responsive to O_3 inhalation with respect to pulmonary function impairment during their follicular (F) menstrual phase when progesterone levels are lowest than during their mid-luteal (ML) phase when progesterone levels are highest. Nine subjects with normal ovarian function were exposed in random order for 1 hr each to filtered air and to 0.30 ppm O_3 in their F and ML menstrual phases. Ozone responsiveness was measured by percent change in pulmonary function from pre- to postexposure. Significant gas concentration effects (filtered air versus O_3) were observed for forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV_1), and forced expiratory flow between 25 and 75% of FVC (FEF_{25-75}; $p<.05$). More importantly, the pulmonary function flow rates, FEV_1 and FEF_{25-75}, showed a significant menstrual phase and gas concentration interaction effect, with larger decrements observed in the F menstrual phase when progesterone concentrations were significantly lower. We conclude that young, adult females appear to be more responsive to acute O_3 exposure during the F phase than during the ML phase of their menstrual cycles. This difference in pulmonary function response could be related to the anti-inflammatory effects of increased progesterone concentrations during the luteal phase. *Key words*: air pollution; forced expiratory flow rates; pulmonary function impairment, steroid hormones. *Environ Health Perspect* 101:242–244(1993)

Acute ozone (O_3) exposure has been shown to result in short-term airway inflammation in both animals and humans (*1,2*). This inflammatory response involves both the infiltration of neutrophils (*1–3*) and the release of cyclooxygenase products from arachidonic acid in the lung and airways (*3*). Seltzer et al. (*3*) observed significant increases in the concentrations of prostaglandins E_2, F_{2a}, and thromboxane B_2 (cyclooxygenase products) in bronchoalveolar lavage fluid after exposure to O_3. In addition, prostaglandins E_2 and F_{2a} appear to stimulate pulmonary neural afferents initiating tachypnea and cough, which are responses characteristic of acute O_3 exposure (*4,5*). Further, Schelegle et al. (*6*) found that pretreatment with indomethacin, a nonsteroidal anti-inflammatory drug, significantly attenuated O_3-induced decrements in forced vital capacity (FVC) and forced expiratory volume in 1 sec (FEV_1). These findings imply that the release of prostaglandins and subsequent inflammation in the lung and airways consequent to acute O_3 exposure are involved in routinely observed pulmonary function decrements.

Progesterone inhibits prostaglandin production in the uterine endometrium, though this inhibition fluctuates with the variant changes in progesterone concentration observed throughout the female's menstrual cycle (*7,8*). If cyclical changes in steroidal hormones alter a woman's response to uterine inflammation, could fluctuations in these hormone levels alter individual responses to a known respiratory inflammatory inducer (*viz.*, O_3)? A hypothesis not yet investigated is that exposure to O_3 during the follicular (F) menstrual cycle phase, when progesterone levels are at their lowest, might result in enhanced responses due to the absence of anti-inflammatory influences of this steroid. The purpose of this study was to determine if young, adult females respond differentially to O_3 inhalation with respect to menstrual cycle phase and progesterone concentration. To investigate this hypothesis, healthy, young, adult females were exposed to O_3 in the F and mid-luteal (ML) phases of their menstrual cycles.

Nine healthy, young, adult females, who participated in recreational aerobic exercise on a regular basis, served as subjects. We screened each woman for clinically normal pulmonary function, absence of significant allergies, abstention from taking birth control pills, and normal menstrual function [intermenstrual interval of greater than 26 days, with sustained rise of pregnanediol-3-glucuronide (PdG) greater than 3 µg/mg creatinine for more than 4 days during the luteal phase]. All subjects were nonsmokers and had not resided in an area of high air pollution within the previous 6 months.

Before initiating experimental protocols, each subject completed an orientation session in which baseline pulmonary function was obtained and specific equipment and requirements of the study were reviewed. We informed subjects of the purpose, procedures, and potential risks of participation in the study before they signed an informed consent form approved by the University of California's Human Subjects Review Committee. After completing the experimental protocols, we assessed basic anthropometry, including body composition determination via hydrostatic weighing, and maximum oxygen uptake (VO_2max). These results are presented in Table 1.

We studied subjects throughout two to four complete (though not necessarily consecutive) menstrual cycles. Early morning urine samples (3 ml) were collected throughout the study period and analyzed for estrone conjugates (E1C) and PdG (which were both indexed by creatinine concentrations of the same sample to adjust for variations in urine volume) to document a normal hormonal profile. We randomized subjects and exposed them to either filtered air or 0.30 ppm O_3 in the F and ML phases of ovulatory menstrual cycles. With day 0 representing the day of the periovulatory estrone conjugate peak, the F phase was determined to be days -6 through the first

Table 1. Group anthropometric, VO_2max, and pulmonary function data

	Age (years)	Height (cm)	Weight (kg)	Fat (% body wt)	VO_2max (ml/kg/min)	V_Emax (l/min)	FVC (l)	FEV_1 (l)
Means ± SD	26.9±5.3	165.4±4.9	64.5±8.3	26.2±4.7	45.3±4.2	111.4±17.9	4.0±0.44	3.4±0.46
Minimum	20	160.0	52.1	19.2	40.9	84.7	3.2	2.9
Maximum	34	175.3	78.8	34.1	54.7	140.6	4.7	4.4

Abbreviations: VO_2max, maximum O_2 uptake; V_Emax, maximum minute ventilation; BTPS, body temperature, pressure, saturated; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec.

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day of menses. The ML phase was similarly calculated as day +5 to +10. The exposure protocol consisted of exercise work rates such that ventilation minute volume (V_E) was about 50 l/min BTPS (body temperature, pressure, saturated). The group mean total inhaled dose of O_3 during the 0.30 ppm O_3 exposures did not differ significantly between the F (859.9 ppm·l) and ML (855.6 ppm·l) phases. We conducted all exposures in a moderate ambient environment with constant airflow provided from a floor fan. Subjects were not told whether they received O_3.

We obtained pulmonary function measurements immediately before and after each experimental protocol. At least two repeated maneuvers of forced expiration after maximal inspiration were obtained using a Collins Modular office spirometer (model 3000). An on-line data acquisition system, which interfaced the spirometer module linear potentiometer output voltage (associated with lung volume changes) and an analog-to-digital converter for reading into a Digital Equipment Corp. LSI 11/2 microcomputer, was also used. Pulmonary function on-line computer determinations included measurements of FVC, FEV_1, and forced expiratory flow between 25 and 75% of FVC (FEF_{25-75}). In addition, we monitored V_E and heart rate during each protocol.

Subjects inhaled air mixtures during experimental protocols via a blow-by obligatory mouthpiece system, described in detail previously (*9*). Inspiratory O_3 concentrations in the mixing chamber were monitored continuously by samples drawn through 0.64-cm inner diameter Teflon tubing connected to a Dasibi O_3 meter. The digital reading of O_3 concentration in ppm was compared periodically with that determined by the ultraviolet absorption photometric method (*10*).

After the orientation session, subjects initiated daily urine collection upon the first day of menses of a new cycle. Subjects placed urine samples (3 ml) in a home freezer after collection and brought them to the laboratory freezer upon the completion of a full cycle. The urinary metabolites of the two major ovarian steroid hormones, estrogen and progesterone, were assessed by enzyme immunoassay (EIA) at the Institute of Toxicologic and Environmental Health Research, University of California, Davis, to monitor ovarian function. The competitive, microtiter plate solid-phase EIA procedure for the measurement of E1Conj and PdG is described in detail by Munro and colleagues (*11*). Urinary hormone concentrations are expressed as nanogram (E1C) or microgram (PdG) per milligram creatinine.

FVC, FEV_1, and FEF_{25-75} preexposure values were subtracted from their respective post-exposure values and then divided by the preexposure values to obtain percent changes representing the treatment effect for each protocol. We analyzed all data via a two-way analysis of variance (ANOVA) with repeated measures (*12*), which tested for gas concentration effects (filtered air versus O_3 comparison), menstrual phase effects (F versus ML), and the interaction between menstrual phase and gas concentration effects. Upon obtaining a significant *F* ratio for main effects as the result of either gas concentration, menstrual phase, or their interaction, a paired-*t* post-hoc test with modified Bonferroni correction (*13*) was applied to determine which particular mean values were significantly different from others. Statistical significance was accepted at the $p < 0.05$ level.

Group mean preexposure and post-exposure data for pulmonary function parameters are presented in Table 2. Specific significant mean differences obtained by ANOVA and post hoc procedures are also included. Post hoc analysis revealed significant gas concentration effects (filtered air versus O_3) for FVC, FEV_1, and FEF_{25-75} and significant gas concentration and menstrual phase interaction effects for FEV_1 and FEF_{25-75}. The group mean percent changes for FEV_1 for each experimental protocol are illustrated in Figure 1. The FEV_1 decrement of -18.1% observed in the F phase was significantly greater than the -13.1% mean value for the ML phase. This increased effect, which was not observed even as a trend for the filtered air exposures (Fig. 1), demonstrates the interaction of O_3 and cycle phase.

Whereas the present data are not sufficient to define the cellular mechanisms involved, they suggest that ovarian steroids (particularly progesterone) may act to modulate O_3-induced inflammation occurring in lung tissue. This association is derived from the observation that the sensitivity of the lung to ambient O_3 is variable within the same woman and appears to depend on the phases of her menstrual cycle. Although most of the subjects exhibited sensitivity to O_3 in both phases, in general, normally menstruating women appeared to be more sensitive to the adverse effects of O_3 during the F phase (low progesterone) compared to the ML phase (high progesterone) of their cycle.

All menstrual cycles used in the data analysis were characterized by low, unvarying PdG concentrations in the F phase, which were 0.9 ± 0.4 and 1.2 ± 1.4 μg/mg creatine on the days of the F phase for air and O_3 exposures, respectively. In contrast, the PdG concentrations rose 1–2 days after a pronounced mid-cycle E1C peak and then declined before the next menstrual period 10–12 days later. The PdG values on the day of the ML phase filtered air and O_3 protocols (8.6 ± 5.7 and 8.5 ± 6.7 μg/mg creatine, respectively) were significantly elevated ($p<0.0001$) over F-phase levels. There were no significant differences between the treatment groups in mean cycle length, F-phase length, luteal phase length, or urinary hormone concentrations.

Acute O_3 exposure has been shown to result in the production of inflammatory mediators (prostaglandins and thromboxane) in the lungs, airways, and plasma of both animals and humans (*2,3,14*). Available data indicate that O_3-induced changes in pulmonary function are the result of prostaglandin stimulation of neural afferents in exposed airways (*15,16*). In the present study, decreases in FEV_1 and FEF_{25-75} varied significantly with respect to menstrual cycle phases, which suggests that there might be some fluctuation in the release or inhibition of prostaglandins during the F and ML phases of the menstrual cycle.

Table 2. Group pre- and post-exposure values for pulmonary function

	Filtered air				0.30 O_3				
	F (1)		ML (2)		F (3)		ML (4)		
Parameter	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Specific mean differences
FVC (l)	3.91 ± 0.40	3.93 ±0.44	4.01 ± 0.45	4.00 ± 0.43	3.90 ± 0.53	3.44 ± 0.72	3.99 ± 0.44	3.56 ± 0.58	1-3, 1-4, 2-3, 2-4
FEV_1 (l)	3.36 ± 0.41	3.40 ± 0.44	3.45 ± 0.46	3.51 ± 0.41	3.41 ± 0.50	2.82 ± 0.67	3.42 ± 0.48	2.99 ± 0.55	1-3, 1-4, 2-3, 2-4, 3-4
FEF_{25-75} (l/sec)	3.90 ± 0.93	3.97 ± 0.96	3.99 ± 1.0	4.10 ± 0.86	4.08 ± 0.93	3.14 ± 0.96	3.94 ± 1.0	3.32 ± 0.99	1-3, 1-4, 2-3, 2-4, 3-4

Abbreviations: F, follicular phase; ML, mid-luteal phase; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec; FEF_{25-75}, forced expiratory flow between 25 and 75% of FVC. Values are means ± SD. Numbers in parentheses represent protocol number. Percent change values for each protocol [(post-pre)/(pre) × 100] were used for statistical comparison to obtain specific mean differences (all significant at $p<0.05$).

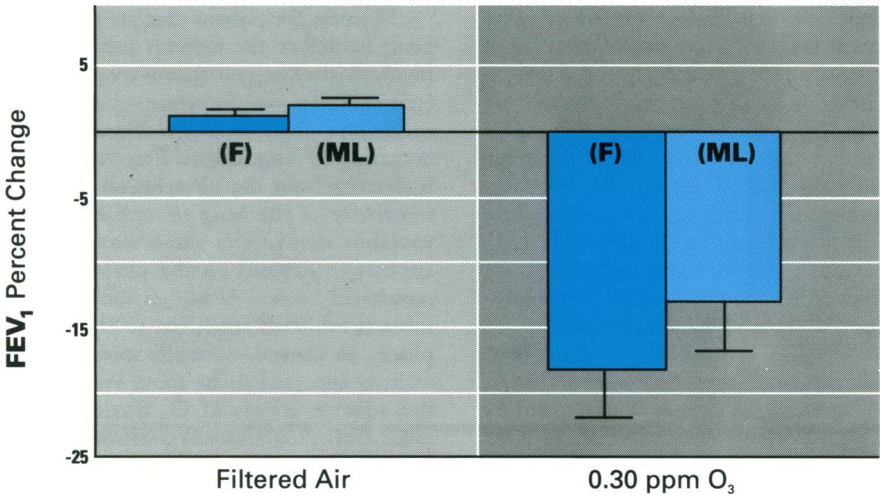

Figure. 1. Group mean percent change in forced expiratory volume in 1 sec (FEV_1) on exposure to filtered air and to 0.30 ppm O_3 in the follicular (F) and mid-luteal (ML) menstrual phases. The mean difference between the two 0.30-ppm O_3 exposures was statistically significant ($p < 0.05$).

In normally menstruating females, gonadal steroid hormones are produced at varying rates during the menstrual cycle. The F phase is characterized by baseline progesterone concentrations and increasing levels of estrogen up until ovulation. After ovulation, the luteal or secretory phase is characterized by increasing levels of progesterone. Progesterone secretion from the corpus luteum reaches a peak approximately 8 days after ovulation and is maintained for several days (*17*). Estrogen declines dramatically after ovulation and then again rises to moderate levels during the ML phase. Both progesterone and estrogen levels decrease at the end of a cycle, thus triggering menstruation (*17*).

It has been demonstrated in numerous studies that sex steroids, essentially estrogens and progesterone, exert a significant effect on prostaglandin synthesis in various tissues (*7,8,18–20*). In most cases, estradiol has been found to enhance the synthesis of prostaglandins E_2 and F_{2a}, whereas progesterone inhibits their secretion. Kelly and Smith (*8*) found that progesterone reduces prostaglandin F production by 93–96% in human proliferative-phase endometrium cultured for 2 to 3 days.

The increased FEV_1 and FEF_{25-75} O_3-responsiveness during the F phase could be the result of progesterone interaction with O_3-induced prostaglandin synthesis. Progesterone is evident in circulating serum and plasma samples in concentrations proportional to those observed in uterine tissues (*11*) and therefore can be found in respiratory blood flow. During the F phase of the menstrual cycle, when progesterone is low, there may be no inhibition of prostaglandin synthesis after O_3 exposure. In contrast, with higher concentrations of progesterone during the ML phase, there may be some inhibition of prostaglandin activity associated with a concomitant decrease in inflammation, which results in slightly smaller decrements in FEV_1. Although the involvement of progesterone in modulating O_3-induced production of prostaglandins is only suggestive, it is the only cyclic hormone that fits the profile required to have this effect (i.e., high in the ML and low in the F phases). However, additional investigation is warranted in which the role of progesterone (and other hormones) can be directly evaluated, for example, by adding progesterone to an exposed system.

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