

UC Davis

UC Davis Previously Published Works

Title

Enhanced response to ozone exposure during the follicular phase of the menstrual cycle.

Permalink

<https://escholarship.org/uc/item/5nb30001>

Journal

Environmental Health Perspectives, 101(3)

ISSN

0091-6765

Authors

Fox, SD

Adams, WC

Brookes, KA

et al.

Publication Date

1993-08-01

DOI

10.1289/ehp.93101242

Peer reviewed

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

Susan D. Fox,¹ William C. Adams,¹ Katherine A. Brookes,¹ and Bill L. Lasley²

¹Human Performance Laboratory, Department of Physical Education and ²Department of Reproduction, School of Veterinary Medicine, University of California, Davis, CA 95616 USA

Acute ozone (O₃) 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 E₂, F_{2a}, and thromboxane B₂ (cyclooxygenase products) in bronchoalveolar lavage fluid after exposure to O₃. In addition, prostaglandins E₂ and F_{2a} appear to stimulate pulmonary neural afferents initiating tachypnea and cough, which are responses characteristic of acute O₃ exposure (4,5). Further, Schelegle et al. (6) found that pretreatment with indomethacin, a nonsteroidal anti-inflammatory drug, significantly attenuated O₃-induced decrements in forced vital capacity (FVC) and forced expiratory volume in 1 sec (FEV₁). These findings imply that the release of prostaglandins and subsequent inflammation in the lung and airways consequent to acute O₃ 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.*, O₃)? A hypothesis not yet investigated is that exposure to O₃ 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 O₃ inhalation with respect to menstrual cycle phase and progesterone concentration. To investigate this hypothesis, healthy, young, adult females were exposed to O₃ 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, abstinence 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

Exposure to ozone (O₃), 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 O₃ inflammatory-induced pulmonary responses. In this study, we tested the hypothesis that young, adult females are more responsive to O₃ 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 O₃ 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 O₃) were observed for forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV₁), and forced expiratory flow between 25 and 75% of FVC (FEF₂₅₋₇₅; *p* < .05). More importantly, the pulmonary function flow rates, FEV₁ and FEF₂₅₋₇₅, 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 O₃ 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)

a normal hormonal profile. We randomized subjects and exposed them to either filtered air or 0.30 ppm O₃ 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

Address correspondence to W. C. Adams, Department of Physical Education, University of California, Davis, CA 95616 USA. This study was supported by the State of California Air Resources Board (Interagency Agreement A933-096) and by the Superfund Basic Research Program (P42ES04699). The authors gratefully acknowledge the technical assistance of Richard Fading, the laboratory assistance of Robin Looft, Tara McKittrick, and Kerri Winters, and Susan Shideler, who contributed help with ovarian hormone analysis and data interpretation. Sincere appreciation is extended to the subjects for their contribution of considerable time and effort.

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 _E max (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 O₂ uptake; V_Emax, maximum minute ventilation; BTPS, body temperature, pressure, saturated; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 sec.

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, FEV₁, and forced expiratory flow between 25 and 75% of FVC (FEF₂₅₋₇₅). 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 hor-

mones, 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, FEV₁, and FEF₂₅₋₇₅ 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, FEV₁, and FEF₂₅₋₇₅ and significant gas concentration and menstrual phase interaction effects for FEV₁ and FEF₂₅₋₇₅. The group mean percent changes for FEV₁ for each experimental protocol are illustrated in Figure 1. The FEV₁ 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 $\mu\text{g}/\text{mg}$ 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 $\mu\text{g}/\text{mg}$ 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 FEV₁ and FEF₂₅₋₇₅ 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

Parameter	Filtered air				0.30 O_3				Specific mean differences
	F (1)		ML (2)		F (3)		ML (4)		
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
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; FEV₁, forced expiratory volume in 1 sec; FEF₂₅₋₇₅, 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).

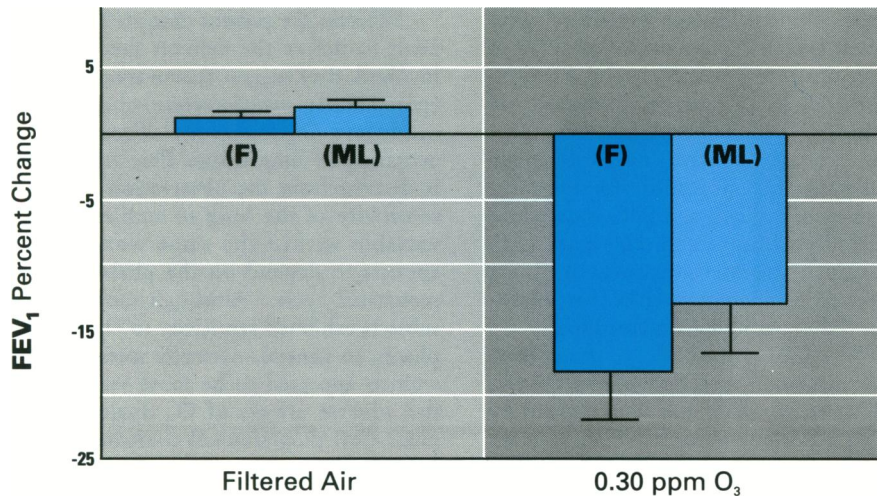


Figure 1. Group mean percent change in forced expiratory volume in 1 sec (FEV₁) on exposure to filtered air and to 0.30 ppm O₃ in the follicular (F) and mid-luteal (ML) menstrual phases. The mean difference between the two 0.30-ppm O₃ exposures was statistically significant ($p < 0.05$).

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 E₂ 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 FEV₁ and FEF_{25–75} O₃-responsiveness during the F phase could be the result of progesterone interaction with O₃-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 O₃ 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 FEV₁. Although the involvement of progesterone in modulating O₃-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.

REFERENCES

- Holtzman MJ, Cunningham JH, Sheller JR, Isigler GB, Nadel JA, Boushey HA. Effect of ozone on bronchial reactivity in atopic and nonatopic subjects. *Am Rev Respir Dis* 120:1059–1067(1979).
- Fabbri LM, Aizawa H, Alpert SE, Walters EH, O'Byrne PM, Gold BP, Nadel JA, Holtzman MJ. Airway hyperresponsiveness and changes in cell counts in bronchoalveolar lavage after ozone exposure in dogs. *Am Rev Respir Dis* 129:288–291(1984).
- Seltzer J, Bigby BG, Stulberg M, Holtzman MJ, Nadel JA, Ueki IF, Leikauf GD, Goetzl EJ, Boushey HA. O₃-induced change in bronchial reactivity to methacholine and airway inflammation in humans. *J Appl Physiol* 60:1321–1326(1986).
- Coleridge HM, Coleridge JCG, Ginzel KH, Baker DG, Banzett RB, Morrison MA. Stimulation of "irritant" receptors and afferent C-fibres in the lungs by prostaglandins. *Nature* 264:451–453(1976).
- Roberts AM, Schultz HD, Green JF, Armstrong DJ, Kaufman MP, Coleridge HM, Coleridge JCG. Reflex tracheal contraction evoked in dogs by bronchodilator prostaglandins E₂ and I₂. *J Appl Physiol* 58:1823–1831(1985).
- Schelegle ES, Adams WC, Siefkin AD. Indomethacin pretreatment reduces ozone-induced pulmonary function decrements in human subjects. *Am Rev Respir Dis* 136:1350–1354(1987).
- Bonney RC, Qizilbash ST, Franks S. Endometrial phospholipase A2 enzymes and their regulation by steroid hormones. *J Steroid Biochem* 27:1057–1064(1987).
- Kelly RW, Smith SK. Glucorticoids do not share with progesterone the potent inhibitory action on prostaglandin synthesis in human proliferative phase endometrium. *Prostaglandins* 33:919–929(1987).
- DeLucia AJ, Adams WC. Effects of O₃ inhalation during exercise on pulmonary function and blood biochemistry. *J Appl Physiol* 43:75–81(1977).
- DeMore WB, Romanovsky JC, Feldstein M, Hamming WJ, Mueller PK. Interagency comparison of iodometric methods of ozone determination. In: *Calibration in air monitoring*. ASTM Technical Publication No. 598. Philadelphia, Pennsylvania:American Society for Testing and Materials, 1976;131–143.
- Munro CJ, Stabenfeldt GH, Cragun JR, Addiego LA, Overstreet JW, Lasley BL. Relationship of serum estradiol and progesterone concentrations to the excretion profiles of their major urinary metabolites as measured by enzyme immunoassay and radioimmunoassay. *Clin Chem* 37:838–844(1991).
- Dixon WJ, ed. *BMDP statistical software*. Los Angeles:University of California Press, 1985;347–358.
- Keppel G. In: *Design and analysis: a researchers handbook*. Englewood Cliffs, New Jersey:Prentice Hall, 1991;169–170.
- Schelegle ES, Adams WC, Giri SN, Siefkin AD. Acute ozone exposure increases plasma prostaglandin F_{2a} in ozone-sensitive human subjects. *Am Rev Respir Dis* 140:211–216(1989).
- Lee L-Y, Dumont C, Djokic TD, Menzel TE, Nadel JA. Mechanism of rapid shallow breathing after ozone exposure in conscious dogs. *J Appl Physiol* 46:1108–1109(1979).
- Hazucha M, Baks D, Bromberg P. Mechanism of action of ozone on the human lung (abstract). *Am Rev Respir Dis* 133:A214(1986).
- Franz WB. Basic review: endocrinology of the normal menstrual cycle. *Primary Care* 15:607–616(1988).
- Seillan C, Ody C, Russo-Marce F, Duval D. Differential effects of sex steroids on prostaglandin secretion. *Prostaglandins* 26:3–12(1983).
- Abel MH, Baird DT. The effect of 17 β -estradiol and progesterone on prostaglandin production by human endometrium maintained in organ culture. *Endocrinology* 106:1599–1606(1980).
- Schatz F, Markiewicz, Gurdipe E. Effects of estradiol on PGF_{2a} output by cultures of human endometrium and endometrial cells. *J Steroid Biochem* 20:999–1003(1984).