CONTRACT NOS. A933-096 and A033-176
FINAL REPORT
AUGUST 1992

Studies of Young Female Responses to Acute Ozone Exposure

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

AIR RESOURCES BOARD
Research Division

STUDIES OF YOUNG ADULT FEMALE RESPONSES TO ACUTE OZONE EXPOSURE

Final Report
Contract Nos. A933-096 and A033-176

Prepared for:

California Air Resources Board
Research Division
2020 L Street
Sacramento, CA 95814

Submitted by:

Physical Education Department
University of California, Davis
Davis, CA 95616

Prepared by:

William C. Adams, Ph.D.
Principal Investigator

AUGUST 1992

ABSTRACT

The primary purposes of this research were to determine if: (1) young adult females respond with greater acute effects of ozone (O_3) than their male counterparts at a dose relative to lung size as well as at the same total dose; (2) O_3 response in females is influenced by the disparate levels of progesterone (a steroid hormone) that they experience during the various phases of their menstrual cycles; and (3) O_3 exposure has an effect on the integrity of normal menstrual cycles of healthy young adult females.

In the first study, twenty healthy young adult males and females were exposed to filtered air (FA), 0.18 parts per million (ppm) and 0.30 ppm O_3 via an obligatory mouthpiece inhalation system for 60 minutes while exercising continuously on a bicycle ergometer at work rates set to elicit an average minute ventilation of 50 l/min for female subjects and 50 and 70 l/min for male subjects. The twenty females were exposed to each gas mixture during three phases of their menstrual cycle: the early follicular (EF) (low progesterone), the mid-luteal (ML) (high progesterone) and late luteal (LL) (decreasing progesterone). In the second study, an additional eleven females were exposed in similar fashion to 0.30 ppm O_3 and to FA during the EF and ML phases of their menstrual cycles. Experimental effects were determined via pre- and postexposure standard pulmonary function and subjective symptom measurements, while values for exercise ventilation and respiratory metabolism were monitored periodically during exposures. Analysis of daily urine samples provided documentation of normal menstrual cycles as well as the concentrations of progesterone during the cycle phases.

In the first study, responses of female subjects were compared to those of male subjects following an O_3 dose that was similar (ventilation for males and females = 50 l/min), as well as one that corrected for gender differences in lung size and/ or maximal oxygen uptake (i.e., female = 50 l/min and male = 70 l/min). Although the female's responses were intermediate to those observed for the male's two exposures, no significant differences between the groups' pulmonary function and subjective symptoms of respiratory discomfort were found for either the same absolute dose or relative dose exposures to 0.18 ppm and 0.30 ppm O_3.

When exposed to O_3 during various assumed phases of their menstrual cycle, the female group did not respond differentially as reflected by the values obtained for pulmonary function parameters: forced vital capacity (FVC); forced expiratory volume in 1 s ($FEV_{1.0}$) and forced expiratory flow between 25-75 percent of forced vital capacity (FEF_{25-75}). However, merged data from the two studies utilizing a total of 13 females with

normal menstrual cycles (i.e., with respect to maintenance of intact phases at least up to the point of each exposure), revealed a nonsignificant ($P<0.12$) trend towards greater $FEV_{1.0}$ impairment during the EF phase (when progesterone levels were low) compared to the ML phase when progesterone was significantly higher. It was suggested that the pronounced difference in progesterone levels during the EF and ML phases of the menstrual cycle could be responsible for the $FEV_{1.0}$ difference via its steroidal effect (anti-inflammatory) on known O_3-induced respiratory tract inflammation, which has been shown by others to be related to pulmonary function impairment.

A significant number of females experienced menstrual cycle disruptions following exposure to 0.30 ppm O_3, as evidenced by delays in ovulation, shortened luteal phases and secondary surges in estrogen during the late luteal phase. These disturbances are most likely not attributable to changes in diet, stress or activity levels.The mechanism by which O_3 interferes with normal ovarian functioning is unknown at this time, but appears to be focused at the pituitary (rather than the ovaries), since lutenizing hormone, the hormonal signal for ovulation, also appears to be affected by O_3 exposure. Although these effects seem to be temporary (lasting 1 or 2 cycles), the reproductive viability of the females involved was clearly compromised during those cycles. It was suggested that further research be done in this area to determine the implications of this previously unreported observation.

ACKNOWLEDGEMENTS

Ms. Kathi Brookes Joye, Postgraduate Research Assistant, filled many roles that ensured successful completion of this project. She aided in recruiting subjects and assumed principal responsibility for orienting subjects regarding requirements for participation in this study. She supervised the scheduling of exposure protocols for all subjects, and was personally responsible for maintaining ongoing contact with female subjects to schedule them for exposure protocols at appropriate phases of their menstrual cycles. She trained all laboratory research assistants who conducted exposure protocols, as well as coordinating their successful completion. She conducted literature searches for data interpretation, developed efficient data collation procedures, and performed the initial data analysis. Finally, she was primarily responsible for developing the first draft of this report. Her performance in all respects was extraordinary.

Bill Lasley, Ph.D., Institute for Toxicology and Environmental Health (ITEH) was an invaluable collaborator in facilitating successful completion of this research. His contributions included financial support via Superfunds, laboratory facilities and personnel for urine analysis, and an expertise in reproductive physiology that was necessary for the development of the research designs, urine analysis interpretation, and study conclusions. Ms. Susan Shideler was primarily responsible for the supervision of all urine analysis and training of laboratory personnel. Ms. Robin Looft provided prompt analysis of collected urine samples.

Ms. Susan Fox wrote a manuscript on menstrual phase effects on O_3 response that constituted a large part of her master's degree thesis. She also was responsible for a large portion of data collection and analysis.

The expert and timely laboratory instrumentation technical support afforded by Mr. Richard Fadling was especially appreciated. Mr. Tim Duvall, California Primate Research Center, U.C. Davis, provided routine calibration of the Dasibi O_3 analyzer.

The following U.C. Davis students provided capable laboratory assistance in connection with this research: Ms. Kerri Winters, Ms. Tara McKittrick, and Ms. Candace Ireton. Administrative and clerical assistance was afforded by Ms. Sylvia Whitley. Approximately 60 individuals, almost all U.C. Davis students, served as subjects, volunteering significant time and effort.

This report is submitted in fulfillment of California Air Resources Board interagency agreement A933-096 and A033-176, project entitled "Studies of Young Adult Female Responses to Acute Ozone Exposure," by the Regents of the University of California, under the partial sponsorship of the California Air Resources Board. Work was accomplished as of 15 June 1992 .

DISCLAIMER

The statements and conclusions in this report are those of the University and not necessarily those of the State Air Resources Board. The mention of commercial products, their source or their use in connection with material reported herein, is not to be construed as actual or implied endorsement of such products.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SUMMARY AND CONCLUSIONS

The study of the young adult female's response to ozone (O_3) inhalation effects is of recent origin and relatively limited compared to that of her male counterpart. While there is suggestive evidence that the young adult female experiences greater acute effects consequent to O_3 inhalation, the appropriate procedure for metering an equivalent O_3 dose, including the effects of body size - more specifically, lung size, has not been systematically studied. In addition, there are minimal data relating physiologic mechanisms to gender differences in response to O_3 inhalation. A hormonal explanation seems plausible, considering that gonadal steroid hormones are different in females compared to males, and that gonadal steroid hormonal concentrations fluctuate during the female menstrual cycle. In addition, some of these hormones, particularly progesterone, appear to have anti-inflammatory characteristics that might be expected to reduce acute O_3 inhalation effects. Investigations that focus on the differences between male and female responses should improve our understanding of the acute effects of ambient smog alert levels of O_3 in a population that has been minimally studied, and should also enable us to determine if the female population is confronting greater health risks during acute O_3 exposure as the result of an apparent greater sensitivity.

In this study, healthy young adult male and female subjects exercised at minute ventilation rates (5 to 7 times that at rest) for 60 minutes while exposed to ambient smog alert levels of O_3 in an attempt to address three major questions: (1) Does O_3 inhalation produce a greater acute effect in young adult females at a dose relative to lung size and/or maximal oxygen uptake ($V0_{2max}$), as well as at the same total dose, compared to that experienced by her male counterpart? (2) Do disparate progesterone (a steroidal hormone) levels experienced during the early follicular (EF) and mid-luteal (ML) phases of the normal young adult female's menstrual cycle affect their response to O_3 inhalation? and (3) Does O_3 exposure affect the integrity and viability of the normal young adult female's menstrual cycle?

Subjects were exposed via an obligatory mouthpiece inhalation system to filtered air and to O_3 concentrations of 0.30 parts per million (ppm), and in some subjects, to 0.18 ppm O_3. Males were exposed at two different work rates (which elicited ventilation rates of 50 and 70 l/min), while the female's exposures were all at one work rate (50 l/min) but occurred in each of the specified menstrual cycle phases: EF, ML, and in some females, late-luteal (LL). Measurement of standard pulmonary function tests and subjective symptoms of respiratory discomfort were obtained pre- and postexposure. Exercise ventilatory and respiratory metabolic parameters were monitored periodically. The

confirmation of normal ovarian cycles, and the timing of ML, LL and EF phase protocols, were assessed by enzyme immunoassay (EIA) of daily morning urine samples.

There were no statistically significant differences between the male and female groups' responses to O_3 at the same relative dose (i.e., with V_E greater for males in direct proportion to their larger lung size and/or $V0_{2max}$). Unexpectedly, there were no statistically significant gender differences in 0_3-induced pulmonary function or subjective symptoms responses when both exercised at an equivalent V_E (50 l/min).

When female subjects in the first study were exposed to 0_3 during various phases of their menstrual cycles, they did not respond differentially in standard pulmonary function and subjective symptom parameters. Subsequent urine analysis, however, revealed that only nine of 20 subjects had actually maintained normal cycles (i.e., in terms of intact phases at least up to the point of each exposure), such that O_3-induced response comparisons could be made for distinct EF and ML phases. When four additional subjects (who also met the urine analysis confirmation of normal menstrual cycles at the time of all experimental exposures) from the second study were added to the original nine subjects, a statistically nonsignificant ($P<0.12$) trend toward enhanced pulmonary function decrements, was observed for O_3 exposures in the EF phase when progesterone concentrations were at their lowest. This trend towards an enhanced effect during the EF phase, compared to the ML phase (when progesterone reached and sustained peak levels for 5 to 7 days), suggests a potential anti-inflammatory role for progesterone during acute O_3 exposure.

An additional finding that was expanded upon in the second study, was that O_3 appears to disrupt the integrity of the normal menstrual cycle. Changes observed included: 1) delays in ovulation, with concomitant decreases in the length of the luteal phase and progesterone concentrations; 2) secondary increases in estrogen immediately following O_3 exposure that resulted in an attempted second ovulation if exposure was in the ML phase; 3) anovulation with withdrawal bleeding; and 4) stimulation of early ovulation if a sufficient surge in estrogen was evident immediately following O_3 exposure. Lutenizing hormone (LH) which, upon reaching a sufficient threshold concentration stimulates ovulation, also appeared to be altered following O_3 exposure, and resulted in short-term surges immediately postexposure, followed by depressions for approximately 3-7 days. This is suggestive of a direct effect of O_3 on pituitary functioning rather than the ovaries per se.

From our results, and in consideration of others' observations, we conclude that:

1. O_3 induced pulmonary function impairment is not significantly different between young adult males and females at a dose that is corrected relative to mean gender differences in lung size and/or maximal aerobic capacity (i.e., about 1.4 times greater for males).

However, gender difference in pulmonary function impairment when both young adult males and females are exposed to the same absolute dose, has been rarely studied and, thus far, with equivocal results.

2. Females with confirmed normal menstrual cycles (in terms of intact phases at least up to the point of each exposure), who are exposed to O_3 in the EF phase of their cycle, evidence a nonsignificantly greater pulmonary function impairment ($P<0.12$ for $FEV_{1.0}$) than that experienced during the ML phase. Greater impairment during EF may be due to uninhibited prostaglandin production during a phase in which the anti-inflammatory gonadal steroid, progesterone, is quite low (which is in contrast to the ML phase when progesterone concentrations are much higher).

3. O_3 exposure, particularly in the EF phase of the normal young adult female's menstrual cycle, can potentially disrupt the integrity of normal ovarian hormone function, including delays in ovulation, luteal phase defects, secondary rises in estrogen, early ovulation and anovulation. These effects, however, are not evident in all subjects, and do not seem to be related to the degree of pulmonary function impairment following O_3 exposure. These observations may have important implications for those charged with setting O_3 health effects standards.

RECOMMENDATIONS

1. Healthy young adult females appear to evidence greater pulmonary function response to 0.30 ppm O_3 when exposed in the EF phase of their menstrual cycle compared to that experienced from exposure in the ML phase. Further studies should be conducted to monitor menstrual cycle phase responses at lower 0_3 concentrations.

2. The slightly enhanced responsiveness to O_3 exposure during the female's EF phase seems to be associated with a low circulating progesterone concentration. Further evidence is needed, however, before any conclusive statements can be made about the possible anti-inflammatory role that progesterone may play (e.g., compare those on birth control pills to those in the ML phase of their menstrual cycles, or administer progesterone injections during the EF phase and compare to normal intact EF and ML phases).

3. Preliminary evidence from this study suggests that O_3 exposure transiently interferes with the viability and functional integrity of some trained female's menstrual cycles. Since those charged with setting air quality standards attempt to do so with a margin of safety for the most susceptible individuals, further study of those females (trained, as well as untrained) whose cycles are affected by O_3 exposure, should be completed at concentrations close to the current Federal Air Quality Standard (i.e., 0.12 ppm O_3).

4. Reexposure to high ambient levels of O_3 within 24 hours significantly enhances the pulmonary function effects observed upon initial exposure. It is possible that a consecutive day exposure to O_3 could have a greater effect on the female's menstrual cycle, as well as affecting a larger number of females, than would a single exposure.

5. Preliminary data suggest that O_3 exposure affects menstrual cycle integrity through action on lutenizing hormone (LH), the pituitary signal for ovulation. To study the mechanisms by which O_3 might induce this effect, changes in LH concentrations should be monitored in the urine of not only females but also males exposed to ambient levels of O_3.

BODY OF REPORT

Introduction

Photochemical air pollution occurs widely, particularly in the heavily populated Los Angeles basin. Inhalation of ozone (O_3), the principal constituent of photochemical air pollution, induces irritant effects on the respiratory tract that impairs pulmonary function and results in subjective symptoms of respiratory discomfort. Ozone has also been linked with human disease (Health Effects 1978).

Those charged with setting appropriate air quality standards need to consider not only the protection of the general population, but also need to include a margin of safety for groups most susceptible to the health impairing effects of O_3 exposure (Air Quality Criteria for Ozone, EPA, 1986). Populations potentially at greater risk include those with preexisting disease, as well as those within the healthy general population who may experience significantly greater effects than average (i.e., children, the elderly and, possibly, young adult females). If, as a group, young adult females of the general healthy population are more susceptible to O_3 effects due to lack of protection provided by steroidal hormones throughout 58-65% of their menstrual cycles, or due to the extreme sensitivity of the female reproductive system, then air quality standards need to reflect the greater responsiveness of this population.

The contention that young adult females of the healthy general population appear to evidence greater O_3-induced acute effects than their male counterparts has not yet been systematically investigated (Air Quality Criteria for Ozone, EPA, 1986). Further, there has been no systematic evaluation of the influence, if any, that disparate levels of progesterone (a female gonadal steroid hormone) have on the sensitivity of females to O_3 inhalation during normal menstrual cycles. If high progesterone levels provide a protective anti-inflammatory effect during the mid-luteal phases of normal female menstrual cycles, the implication is that females may be more vulnerable during all other menstrual phases of their cycles when progesterone concentrations are much lower. Therefore, they may respond with greater sensitivity to O_3 inhalation for approximately 58-65% of normal menstrual cycles.

In addition, there is a pressing need to examine the relationship between ambient O_3 exposure and the integrity of the normal female menstrual cycle. Recent data suggests that acute exposures to ambient levels of O_3 are sufficient to perturb cycles. Whether these perturbations are directly the result of O_3 exposure, the additional amount of strenuous exercise entailed in the 1-h protocols, periodic intervals of enhanced stress induced by University student academic requirements, and/ or a combination of the above, is unknown but provocative enough to examine systematically.

Theoretical Approach to the Problem. The intent of this research was to address three important aspects of acute O_3 exposure effects on humans, particularly females: 1) differential male- female responses 2) disparate female responses relative to menstrual cycle phases and 3) menstrual cycle integrity following O_3 exposure. Pulmonary function and subjective symptoms responses were used as markers. Daily morning urine samples were analyzed to assess the integrity of the female subjects' menstrual cycles as well as the timing of the menstrual phase protocols. Statements of research rationale and objectives for each of the two studies are given below.

A. Comparison of Young Adult Male and Female Responses to Ozone Inhalation Consequent to Continuous Exercise at the Same Absolute and Relative Minute Ventilations

1. Research Rationale. Until recently, laboratory investigations of human response upon exposure to O_3, utilized predominantly young adult male subjects. In early studies that included some female subjects, it was suggested that they might be more responsive to O_3 than their male counterparts when both exercised at a ventilatory rate (V_E) that was proportional to percent VO_{2max} (DeLucia et al., 1983; Lategola et al., 1980). In more recent studies, utilizing larger sample sizes, however, healthy young adult females have been found to be no more sensitive to O_3 inhalation than males, as long as their V_E was proportional to the mean gender difference in lung size (Adams et al., 1987; Horvath et al., 1986), which is essentially the same as that for VO_{2max}. These results imply that significant gender differences in pulmonary function response would occur if females exercised at the same V_E for the same duration and at the same O_3 concentration (thus, at the same total dose), but this comparison has only been made with small group sample sizes (N = 6) (Lauritzen & Adams, 1985).

In a recent study utilizing female subjects only (Messineo & Adams, 1990), the relationship between lung size and O_3 response was investigated to determine whether disparate lung sizes observed in young adult females was related to their O_3-induced responses. No significant difference was noted in O_3-induced $FEV_{1.0}$ decrement between the large-lung (FVC = 5.11 liters) and the small-lung (FVC = 3.74 liters) female groups. Therefore, it seems apparent that the gender difference in lung size is not an adequate explanation for observed differences in percent $FEV_{1.0}$ decrements for young adult males and females at the same total inhaled dose of O_3 (Lauritzen & Adams, 1985).

It is apparent that little systematic investigation has been done to determine occurrences of (and explanations for) O_3 response differences in females and males, particularly with respect to a comparison of subjects under the same exposure conditions while acting as their own controls. Further, investigation of gender

differences with respect to possible effects of the female menstrual cycle have not been addressed at all.

Females experience fluctuations in gonadal steroidal hormones during the course of each menstrual cycle, with the majority of the cycle being characterized by low steroidal hormone levels (especially when compared to male hormonal levels). It could be plausible that some female sex steroidal hormones provide a protective anti-inflammatory effect to O_3 in females during phases of the menstrual cycle in which they are evident in high concentrations (i.e., mid-luteal = high progesterone). Conversely, female responsiveness to O_3 could reflect a greater sensitivity during the phases of their cycle in which steroid hormones are at their lowest (early follicular = base-line progesterone). However, there has been no systematic investigation assessing the potential relationship between normal menstrual cycle phases and O_3 inhalation effects and its relevance to possible O_3 gender differences.

2. Statement of Research Objectives. The primary purpose of this study was to determine if the healthy young adult female's response to O_3 inhalation is greater than that evidenced by her male counterpart. This objective was accomplished by comparing the percent change in standard pulmonary function tests and the absolute change in subjective symptoms of respiratory discomfort at two exercise work rates for males to the female's one. One work rate elicited the same V_E for males as that experienced by the females, while the other permitted gender comparison at a V_E proportional to their disparate endurance capacities (i.e. VO_{2max}) and/or lung size. We also investigated whether menstrual cycle hormonal fluctuations have any effect on the female's response to O_3 by exposing each female to O_3 in various phases of the menstrual cycle: early follicular (EF), mid-luteal (ML) and late luteal (LL).

B. Effects of Acute Ozone Inhalation in Young Adult Females

1. Research Rationale. Recent data obtained in our laboratory indicates that young adult females appear to be more sensitive to O_3 inhalation during the EF phase of their menstrual cycle (when gonadal steroid hormone levels are low), compared to the ML phase when gonadal steroid hormone levels are maintained at relatively high concentrations. $FEV_{1.0}$ measurements reflected approximately a 5% greater decrement during the EF phase. However, this observation could only be considered tentative because of the small number of female subjects included in this comparison (N=9). In order to provide a more appropriate analysis of possible gonadal steroid hormone (specifically progesterone) effects on O_3 inhalation responses, additional female subjects were needed to complete EF and ML phase exposures.

Further, analysis of daily urine samples collected from twenty female subjects being exposed to O_3 protocols, revealed that a large proportion of them were experiencing one or more atypical menstrual cycles despite intensive menstrual history screening. Whether these atypical and non-functional cycles were the result of O_3 exposure, strenuous exercise, stress, diet, or other factors, is unknown. However, there is an apparent need to investigate the possible effects that O_3 exposure may have on menstrual cycle integrity, especially in view of the increasingly large number of females engaging in outdoor vocational and avocational activities that elicit substantial increases in pulmonary ventilation, resulting in increased air pollution inhalation during exposure to photochemical smog.

2. Statement of Research Objectives. The primary purpose of this study was to determine if the healthy young adult female's response to O_3 exposure is greater during the EF phase of normal menstrual cycles, compared to that for the ML phase when gonadal steroid hormones are at their highest levels. In addition, we investigated whether or not there was suggestive evidence that O_3 inhalation has a detrimental effect on menstrual cycle integrity when females are exposed to O_3 prior to ovulation (EF phase) and post-ovulation (ML phase). These objectives were accomplished by exposing 11 additional female subjects (beyond the 20 utilized in the first study) to O_3 and to filtered air (FA), both in the EF and ML phases of normal menstrual cycles occurring over 5 menstrual cycles.

During this time, female subjects collected daily early morning urine samples. Effects were assessed by comparing the percent change in standard pulmonary function tests and absolute change in subjective symptoms of respiratory discomfort during the EF and ML phases of normal menstrual cycles. In addition, the urine samples were analyzed for estrogen and progesterone metabolites to determine if changes in menstrual cycle integrity from the initial control cycle occurred in cycles containing FA and/or O_3 exposures.

Design and Methods

A. Comparison of Young Adult Male and Female Responses to Ozone Inhalation Consequent to Continuous Exercise at the Same Absolute and Relative Minute Ventilations

1. Subject Selection and Characterization. A total of 40 young adults (20 females and 20 males), aged 18-34 years, were recruited for participation in this study by in-class announcements and personal reference. Subjects were aerobically trained in order to be able to perform the most strenuous 1 h continuous exercise protocols. Further, each was screened to determine that they were nonsmokers, had clinically normal

pulmonary function and no history of asthma or significant allergies. Females also underwent an additional screening process regarding menstrual history and cycle characteristics. Determination of normal ovarian function was made following analysis of daily morning urine samples collected by each female for the duration of their participation.

Prior to 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. Subjects were informed of the purpose, procedure, and potential risks of participation in the study before signing an informed consent form approved by the campus Human Subjects Review Committee. Following completion of the experimental protocols, basic anthropometry, including body composition determination via hydrostatic weighing, and VO_{2max} were assessed. The male (N=20) and female (N=20) mean, minimum, and maximum values for basic anthropometry, VO_{2max} and baseline pulmonary function are presented in Table 1.

2. Experimental Design. Male subjects completed six protocols, which consisted of random order 1-h exposures to filtered air (FA), 0.18 ppm O_3 and 0.30 ppm O_3 while exercising continuously on a bicycle ergometer at work rates that elicited a V_E of 50 l/min or 70 l/min. Females completed nine experimental protocols including exposures to FA, 0.18 ppm O_3 and 0.30 ppm O_3 for 1 h in each of the following menstrual cycle phases: EF, ML and LL. Females exercised continuously on the bicycle ergometer at a work rate that elicited a V_E of 50 l/min. At least three days intervened between each exposure. A graphic display of the design follows:

			Gas Mixture		
Sex	V_E	Phase	FA	0.18 ppm O_3	0.30 ppm O_3
Male	50 l/min	------	X	X	X
	70 l/min	-----	X	X	X
Female	50 l/min	EF	X	X	X
	50 l/min	ML	X	X	X
	50 l/min	LL	X	X	X

Menstrual cycle phase exposures were scheduled on the basis of the following information: subject description of cycle regularity, daily morning basal temperatures and cervical mucus secretions. With day 0 defined as the day of urinary E1C peak and

representing the expected day of ovulation, the EF phase was determined to be from the first day of menses (which represented the first day of that cycle) to day -6. The ML phase was similarly calculated as day +5 to +10, with the LL phase occurring +11 to +14. Confirmation of the timing of exposure was documented via urine analysis.

The exposures were completed in an environmental chamber 3.0m wide by 2.4m high by 3.7m long, in which dry bulb temperature and relative humidity were maintained within 22-25°C and 40-60%, respectively. To facilitate convective and evaporative cooling, an appropriate airflow was directed at the subject from an industrial grade floor fan.

3. Experimental Protocol Measurements. Pulmonary function measurements were obtained immediately before and after each experimental protocol. At least two repeated maneuvers of forced expiration after maximal inspiration were obtained on 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. (DEC) LSI 11/2 microcomputer, was also utilized. Pulmonary function on-line computer determinations included measurements of FVC, $FEV_{1.0}$ and FEF_{25-75}. In addition, V_E, VO_2, f_R and heart rate (HR) were monitored during each protocol.

Subjective symptoms were collected at 10, 30 and 58 min. Each subject was asked five questions regarding specific subjective symptoms that he or she may have felt during the exposures. The subjective symptoms monitored included cough, shortness of breath, throat tickle, pain on deep inspiration, the sum of these four symptoms (total subjective symptom severity, TSSS) and an integrated rating of overall symptom severity (OSS). Subjective symptoms were rated on a severity scale of: 0, not present; 5, minimal; 10, mild; 20, moderate; 30, severe; and 40, incapacitating. Immediately after the completion of the postexposure pulmonary function tests, the subjects provided written comments on any discomforts experienced during the exposure and indicated whether or not they believed that they had received O_3.

4. O_3 Administration and Monitoring. Subjects inhaled air mixtures during experimental protocols via a blow-by obligatory mouthpiece system used in this laboratory since 1975 and described in detail previously (DeLucia and Adams, 1977). In brief, appropriate concentrations of O_3 generated by a Sander ozonizer (type II) were mixed with FA, introduced proximal to the turbulent mix, and then directed through a Teflon-coated Hans Rudolph respiratory valve to the subject.

The subject's expired air was directed through a unidirectional 5-liter stainless steel mixing and sampling chamber to an Alpha Technologies turbotachometer ventilation measurement module (VMM-2). Expired air was then routed into the distal portion of the mixing tube and, along with the volume of air mixture not inspired by the subject, passed through a Barneby-Cheney QDF multistage filter assembly and then to a laboratory ventilation exhaust outlet.

Inspiratory O_3 concentrations in the mixing chamber were monitored continuously by samples drawn through a 0.64cm 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 (DeMore et al, 1976).

5. Urine Collection and Ovarian Hormone Analysis. Following the orientation session, female subjects initiated urine collection and their random experimental protocol sequence upon the first day of their next menses. The daily urine samples (3 ml) were placed in a home freezer following collection and were brought to the laboratory freezer upon the completion of a full cycle. Basal oral temperatures and cervical mucus secretions were also monitored daily by the subjects to assist in identifying cycle phases. In addition, a daily questionnaire regarding subjective feelings of fatigue and stress were filled out by each subject for each cycle.

The urinary metabolites of the two major ovarian steroid hormones, estrogen and progesterone, were assessed in the urine samples by enzyme immunoassay (EIA) at the Institute for Toxicology and Environmental Health, University of California, Davis. The measurement of urinary estrone conjugates (E1C) and pregnanediol-3-glucuronide (PdG) to monitor ovarian function are preferable to the measurement of steroid hormones in plasma or serum in many clinical and research settings, particularly when blood samples are difficult to obtain, or when observations are required over long periods of time. The competitive, microtiter plate solid-phase EIA procedure for the measurement of E1C and PdG is described in detail by Munro and colleagues (1991). Urinary hormone concentrations are expressed as picogram (for E1C) or microgram (for PdG) per milligram creatinine (Cr). In order to compensate for variations in urine volume (due to variations in water intake), measurements of creatinine are used as the best "index" for true hormone concentrations of a given sample.

6. Statistical Analysis. Pre- and postexposure pulmonary function measurements were corrected to BTPS. FVC and $FEV_{1.0}$ preexposure values were subtracted from the postexposure values and then divided by the preexposure values to obtain percent changes representing the treatment effect for each protocol. Subjective symptom

severity scores were analyzed as the absolute change between the values reported at min 10 and those at min 58 of the exposure. E1C and PdG measurements represent the single values obtained in the morning urine collected. Cycles were categorized as typical or atypical by the following criteria: cycle length, evidence of ovulation, E1C peak concentration, luteal phase length, length and concentration of PdG plateau, and the overall configuration of the hormonal patterns.

All female data were analyzed via a two-way analysis of variance (ANOVA) with repeated measures (Dixon, 1985), which tested for gas concentration effects (FA, 0.18 and 0.30 ppm O_3 comparison) and menstrual phase effects (EF, ML and LL comparison). To evaluate gender differences following O_3 exposure, male and female data were analyzed using the two-way ANOVA with one between factor (gender) and one repeated measure which permitted comparison between gas concentrations. Upon obtaining a significant F ratio for main effects as the result of either gas concentration, gender or menstrual phase, a paired t post-hoc test with modified Bonferroni correction (Keppel, 1991) was applied to determine which particular mean values were significantly different from others. Statistical significance was accepted at the $P<0.05$ level.

B. Effects of Acute Ozone Inhalation in Young Adult Females

1. Subject Selection and Characterization. A total of 11 young adult females, aged 18-34 years, were recruited for participation in this study. The selection and characterization process was completed in the same fashion as described in the first study (see pages 17-18 of this report). Female (N=11) mean, minimum, and maximum values for basic anthropometry, VO_{2max} and baseline pulmonary function are presented in Table 1.

2. Experimental Design. Each female subject completed four experimental protocols consisting of 1-h exposures to FA and to 0.30 ppm O_3 in the EF and the ML phases of normal menstrual cycles. Each subject exercised continuously during each protocol on a cycle ergometer at a work rate that elicited a V_E of 50 l/min. Exposures occurred over a time span of 5 menstrual cycles with none in the first (control), a FA exposure in the EF phase and a 0.30 ppm O_3 exposure during the ML phase of the second cycle, none in the third (recovery), a FA exposure in the ML phase of the fourth cycle, and a 0.30 ppm O_3 exposure in the EF phase of the fifth cycle. A graphic display of the design follows:

Sex	V_E	Cycle	Phase	Gas Mixture FA	Gas Mixture 0.30 ppm O_3
Female	50 l/min	A	EF	---	---
		A	ML	---	---
		B	EF	X	---
		B	ML	---	X
		C	EF	---	---
		C	ML	---	---
		D	EF	---	---
		D	ML	X	---
		E	EF	---	X
		E	ML	---	---

All other aspects of the experimental design were identical to that described for the first study except that female subjects did not record daily oral basal temperatures for identification of cycle phase. Instead cervical mucus secretions and information about cycle regularity were used to determine when experimental protocols should be scheduled. See pages 18 and 19 of this report for more information about experimental design.

3. Experimental Protocol Measurements. Pulmonary function and subjective symptom measurements were collected in the same fashion as described for the first study (see page 19 of this report).

4. O_3 Administration and Monitoring. Subjects inhaled air mixtures during experimental protocols via a blow-by obligatory mouthpiece system used in this laboratory since 1975, and which was described briefly in the methods section for the first study (see pages 19 and 20 of this report).

5. Urine Collection and Ovarian Hormone Analysis. Urine collection and ovarian hormone analysis was identical to that described previously in the methods section for the first study (see page 20 of this report).

6. Statistical Analysis. This section is identical to the corresponding section found in the first study (see pages 20 and 21 of this report), except that the repeated measures

comparisons were made between the gas concentrations of FA and 0.30 ppm O_3 only, and the menstrual phases of EF and ML only.

Results

A. Comparison of Young Adult Male and Female Responses to Ozone Inhalation Consequent to Continuous Exercise at the Same Absolute and Relative Minute Ventilations

1. Ventilatory and Exercise Metabolism Parameters. V_E, HR and VO_2 were monitored during each experimental protocol. However, pre- and postexposure values were not analyzed since it has been repeatedly demonstrated in this laboratory that they are unaffected by O_3 exposure (Adams et al 1987, Adams and Schelegle, 1983, Brookes et al, 1988). The ventilatory parameters, f_R and tidal volume (V_T), were also monitored throughout each experimental protocol, but were not analyzed for exposure effects since changes in pre- to postexposure values reflected not only O_3 inhalation but also increases or decreases in work rate. Work rates were adjusted throughout each protocol, as necessary, to maintain ventilatory rates as close to the predetermined values of 50 l/min and 70 l/min as possible. This procedure enabled us to maintain better control of the total dose across exposure protocols.

2. Pulmonary Function and Subjective Symptom Data. Group mean pre- and postexposure pulmonary function data for the 20 females participating in the first study are presented in Table 2. Specific significant mean differences obtained by ANOVA and post-hoc procedures are also included ($P<0.05$). Final analysis revealed significant gas concentration effects (FA vs 0.18 ppm O_3 vs 0.30 ppm O_3) for FVC, $FEV_{1.0}$ and FEF_{25-75}. Since subsequent urine analysis revealed that only 9 of the 20 female subjects maintained relatively normal menstrual cycles throughout their participation in the study (i.e., intact phases at least up to the point of each exposure), any menstrual phase analysis of the entire 20 subjects is invalid with respect to exposures actually occurring within intact phases. However, each female subject completed three experimental protocols (supposedly in the EF, ML and LL phases) at each gas concentration (FA, 0.18 and 0.30 ppm O_3), with no statistically significant differences being observed between any of these three sets of exposures at any gas concentration. This is illustrated in Fig. 1, which depicts mean percent changes (and standard error bars) for $FEV_{1.0}$ for each experimental protocol for the group of 20 females. The parentheses around the EF, ML and LL indicate that the protocols did not occur within intact phases for all subjects. Similar results were obtained for OSS and TSSS, with significant mean effects occurring for gas concentration (FA vs 0.18 ppm O_3 and 0.30

ppm O_3) but not for the three sets of exposures at each gas concentration that were assumed to have occurred in specific menstrual phases (see Table 3).

Accordingly, since there were no differences in female responses for the three sets of exposures at each gas concentration that were supposed to have occurred in specific menstrual phases, the data obtained within each set (at a given gas concentration) was averaged to provide a mean value for each parameter to be used to compare male and female responses. Table 4 contains male (N = 20) and average female (N = 20) pre- and postexposure values for pulmonary function parameters at ventilatory rates of 50 and 70 l/min for males and 50 l/min for females. There were no significant mean effects at $P<0.05$ when comparing the average female values at 50 l/min to those obtained for males at 50 l/min (same absolute dose) and 70 l/min (same relative dose). Group means for percent change in $FEV_{1.0}$ and FVC for each experimental protocol are depicted in Figs. 2 and 3, respectively. 0.30 ppm O_3 decrements in FVC of 12.8% at 50 l/min and 16.7% at 70 l/min for males were not significantly different than the average female FVC decrement of 14.7% at 50 l/min.

Absolute changes for group OSS and TSSS responses and their specific significant mean differences are given in Table 5. Increases in TSSS for males at 50 and 70 l/min (40.0 and 60.6, respectively) following 0.30 ppm O_3 exposure, were not significantly different than those obtained for females at 50 l/min (47.4), thereby paralleling the results obtained for pulmonary function responses (see Fig. 4).

Upon subsequent urine analysis, there were nine young adult females that demonstrated normal ovarian function during cycles in which they received FA and 0.30 ppm O_3 in the ML and EF phase exposures. (A much smaller number of subjects maintained normal cycles for all phase exposures, including LL and 0.18 ppm O_3 protocols.) A comparison between the pulmonary function responses obtained in the EF and ML phases from the nine females that did demonstrate normal cycles for these exposures, revealed not only significant gas concentration effects (FA vs 0.30 ppm O_3) for FVC, $FEV_{1.0}$ and FEF_{25-75} but, also, borderline significant menstrual phase effects (EF vs ML) for $FEV_{1.0}$ and FEF_{25-75}. The mean $FEV_{1.0}$ decrement of 18.1% observed in the EF phase following 0.30 ppm O_3 was significantly greater than the 13.1% mean value obtained for the ML phase exposure ($P<0.05$).

Absolute changes in subjective symptom severity responses provided by these nine females demonstrated significant differences ($P<0.05$) between the O_3 and FA exposures; however, no significant differences in symptom responses were found between the EF and ML phases.

3. Urine Analysis. Analysis of daily morning urine samples from the nine females judged to have normal ovarian function throughout the study (in terms of intact phases at least up to the point of each exposure), revealed significant mean differences for both PdG (progesterone glucuronide) and E1C (estrogen conjugate) between the EF and ML phases. Typical concentrations for these phases in females with normal ovarian function would be <2.0 vs >4.0 μg/mgCr for PdG and <20 vs >35 ng/mgCr for E1C in the EF and ML phases, respectively (Munro et al, 1991). The average PdG value for both EF protocols was 1.1 (±0.9) compared to 8.6 (±6.2) μg/mgCr for both ML protocols. Group mean E1C values were 16.5 (±12.2) and 57.8 (±44.4) ng/mgCr for EF and ML exposures, respectively. This is in contrast to the other eleven female subjects whose cycles were atypical, with reported PdG values of 1.1 (±0.7) and 2.4 (±1.6) μg/mgCr and E1C values of 13.0 (±11.8) and 31.1 (±18.9) ng/mgCr for the EF and ML exposures, respectively. Thus, these eleven subjects did not experience intact ML phases.

B. Effects of Acute Ozone Inhalation in Young Adult Females

1. Pulmonary Function and Subjective Symptom Data. In this study, pulmonary function and subjective symptoms responses upon exposures to FA and to 0.30 ppm O_3, during the EF and ML phases of 13 normally menstruating young adult females, were analyzed. Nine of the females included those described in the first study (see Results A above), while four additional subjects, screened for normal menstruation in Study B from a group of eleven, were also included (thus comprising a merged data group from Study A and B of normal menstruating females, i.e., in terms of intact phases at least up to the point of each exposure). Group mean pre- and postexposure data for pulmonary function parameters are presented in Table 6. Specific significant mean differences obtained by ANOVA and post hoc procedures are also given. Post hoc analysis revealed significant ($P<0.05$) gas concentration effects (FA vs O_3) for FVC, $FEV_{1.0}$ and FEF_{25-75}, but no significant menstrual phase effects (ML vs EF) at this level. Fig. 5 illustrates the group mean percent changes in $FEV_{1.0}$ for each experimental protocol. The $FEV_{1.0}$ decrement of 16.6% observed in the EF phase following 0.30 ppm O_3 exposure was greater, at the $P<0.12$ level (but not at the $P<0.05$ level) than the 13.0% decrease obtained in the ML phase.

The absolute changes for group subjective symptom severity responses and their specific significant mean differences are given in Table 7. The total symptom severity score and the overall symptom severity mean differences for the two O_3 exposures were significantly greater than those obtained for the two FA protocols. However, no

significant differences in symptom response was found between the EF and ML phases.

2. Urine Analysis. Analysis of daily morning urine samples from the 13 normally menstruating females (merged data group from Study A and B) revealed significant mean differences for both PdG and E1C between the EF and ML phases. Typical concentrations for these phases in females with normal ovarian function would be <2.0 vs >4.0 μg/mgCr for PdG and <20 vs >35 ng/mgCr for E1C in the EF and ML phases, respectively (Munro et al, 1991). The average PdG value for both EF protocols was 0.9 (±0.9) compared to 7.4 (±5.5) μg/mgCr for both ML protocols. Group mean E1C values were 15.9 (±11.6) and 47.2 (±40.0) ng/mgCr for EF and ML exposures, respectively.

Although the four female subjects from Study B maintained normal menstrual cycles during the time of their experimental protocols (i.e., in terms of intact phases at least up to the point of each exposure), their cycles, as well as the cycles of the other seven female subjects from Study B, appeared to be affected by O_3 inhalation with respect to menstrual cycle configuration and concentrations of PdG and E1C. Four of the eleven subjects in Study B experienced atypical menstrual cycles during the first month (i.e., prior to the initiation of any exposure intervention). However, the other seven, including the four whose data were included in the EF and ML analysis, experienced **either** benign **or** detrimental changes following initiation of the experimental sequence. These included the following: 1) slight benign surges in estrogen in either the pre- or postovulatory state; 2) sufficient surges in estrogen to stimulate early ovulation when O_3 exposure was preovulatory; 3) a large surge in estrogen with a preovulatory exposure that was insufficient to stimulate early ovulation but was of enough magnitude to cause a delay in ovulation, often creating a concomitant luteal phase defect (i.e., luteal phase is too short to be functional); 4) large secondary surges in estrogen (indicative of inappropriate follicular development) just prior to menses, with postovulatory 0_3 exposures; 5) decreased estrogen and progesterone concentrations; and 6) eventual anovulation (no ovulation) with withdrawal bleeding when cycle perturbations occurred consistently.

Figs. 6, 7, 8, 9, 10 and 11 illustrate menstrual profiles of all 5 cycles from six different female subjects (color coded to protect the privacy of each individual). Exposures are marked as FA or 0.30 ppm O_3 on the individual graphs on the appropriate day they occurred. In addition, day of ovulation (OV) is indicated. Cycle length (CL), follicular phase length (FPL), luteal phase length (LPL) and the shift in PdG concentrations from the luteal to follicular phase (L/F shift) are all listed in the

upper left hand corner of each graph. Large absolute differences in these values between the control cycle (cycle A) and the experimental cycles (B, C, D and E) are indicative of delays in ovulation, early ovulation, luteal phase defects, and/or anovulation.

Subject Rose's (Fig. 6) cycle A demonstrated a normal CL (20-39 days, Keizer and Rogol, 1990), LPL (12-16 days) and L/F shift in PdG (>4 μg/mgCr). The surge in estrogen (E1C, •) occurred on day 18 indicating a mature follicle, which subsequently ovulated and became the corpus luteum. The corpus luteum secreted progesterone (PdG, ◊) which maintained high levels for at least 5 days. The concentrations of PdG were slightly lower than expected (>4 μg/mg Cr), but the L/F shift indicated a more than sufficient change in PdG from the pre- to postovulatory state. Cycle B was fairly normal despite the low E1C peak (still sufficient to allow ovulation, as evidenced by a rise in PdG). However, there was a secondary surge in estrogen immediately following a 0.30 ppm O_3 exposure in the luteal phase. Cycles C and D were normal. Following a preovulatory 0.30 ppm O_3 exposure in Cycle E, Rose experienced menstrual cycle perturbations in the form of multiple lutenizations (2 ovulations) that most likely resulted in a non-functional sequential cycle as well.

Cycles A, B and C for subject Violet (Fig. 7) showed normal CL, LPL, L/F shift and configuration. However, there was a secondary surge in E1C following a postovulatory exposure to 0.30 ppm 0_3 in cycle B in the luteal phase. In cycle D, a delay in ovulation and a concomitant luteal phase defect (LPL<12 days), which was reported to be initiated by stress from a new job that began on day 2, is evident. However, the delay in ovulation and subsequent luteal phase defect (LPL<12 days) observed in cycle E were most likely induced by a preovulatory 0.30 ppm O_3 protocol.

Subject Teal's cycles A, B, C and D (Fig. 8) were normal with respect to CL, LPL, L/F shift and configuration, despite lower than typical PdG values (>4 μg/mgCr; phase shifts indicated sufficient change in PdG). However, in cycle E, inhalation of 0.30 ppm O_3 during the EF phase appeared to have stimulated a sufficient enough surge in estrogen (E1C) to trigger premature ovulation and a short 18 day menstrual cycle.

Subject Fuschia's cycles A, B and D were normal with respect to typical menstrual cycle characteristics (Fig. 9) . In cycle B, however, following a 0.30 ppm O_3 exposure during the luteal phase, there was a small secondary surge in E1C that occurred just before menses began for cycle C. A slight luteal phase defect (LPL<12 days), which could have been influenced by the cycle B O_3 exposure, was observed during Cycle C. Surges in E1C also appeared following the ML FA exposure in cycle D and the EF 0.30 ppm O_3 exposure in cycle E.

Periwinkle's cycle profiles are shown in Fig. 10. Cycles A and B were normal, although a slight surge in E1C was noted following a postovulatory 0.30 ppm O_3 exposure in cycle B. The recovery cycle (cycle C) was atypical due to a truncated luteal phase (did not maintain a plateau of PdG $\geq$ 5 days). Etiology for this perturbation is unknown but could have been influenced by cycle B's ML O_3 exposure. The cycle C perturbation carried over into cycle D, as evidenced by a slight delay in ovulation but without a luteal phase defect. The ML FA exposure in cycle D stimulated a surge in E1C. Cycle E was greatly compromised by the 0.30 ppm O_3 exposure during the EF phase, as evidenced by a delay in ovulation and a definite luteal phase defect (LPL<12 days).

Cycle A for subject Mustard (Fig. 11) was normal with respect to CL, LPL, L/F shift and configuration. In cycle B, Mustard experienced multiple cycle perturbations in the form of preovulatory increases in PdG following the FA protocol during the EF phase, as well as a secondary rise in estrogen just prior to menses and a truncated luteal phase (early decrease in PdG) in conjunction with a 0.30 ppm O_3 postovulatory exposure. The recovery cycle (cycle C) appeared to be normal until the postovulatory phase, when a delayed rise in PdG resulted in a luteal phase defect that could have been influenced by the exposures in cycle B. Cycle D was an atypical cycle with strange surges in E1C and PdG in the preovulatory phase. Following ovulation, there were fluctuations in PdG values which made this cycle non-functional. In cycle E, there were surges in PdG and E1C following a 0.30 ppm O_3 exposure during the EF phase, but the E1C surges were not sufficient to stimulate ovulation, thus resulting in an anovulatory cycle and withdrawal bleeding following day 31.

Discussion

A. Comparison of Young Adult Male and Female Responses To Ozone Inhalation Consequent to Continuous Exercise at the Same Absolute and Relative Minute Ventilations

1. Comparison of male and female responses. Acute response to O_3 is dependent on the total dose inhaled, i.e. the product of O_3 concentration, V_E and exposure duration (Adams et al, 1981; Folinsbee et al, 1978; Silverman et al 1976). Thus, an increase in V_E effected by exercise during an exposure period increases the total inhaled dose, and results in concomitant increases in acute toxic effects of O_3 (Adams et al, 1981; Adams and Schelegle, 1983; Avol et al, 1984; DeLucia and Adams, 1977). Although quite reproducible within each individual (McDonnell, 1985), the magnitude of individual response following O_3 inhalation to a given dose is highly variable across individuals and subsets of the general population (McDonnell, 1983) . There is suggestive evidence

that young adult females are a subset of the healthy general population that appear to experience greater O_3-induced acute effects than their male counterparts at the same total dose (Lauritzen and Adams, 1985). Clarification of this observation is of practical importance, in that health professionals and regulatory agencies are concerned with the protection of population subsets that are most likely to experience distress upon acute exposure to ambient air pollution (Air Quality Criteria for Ozone, EPA, 1986).

A major observation in this investigation was that exposure of young adult females to 0.30 ppm O_3 for 1 h while engaged in heavy continuous exercise (V_E of 50 l/min) resulted in responses that were only marginally greater, but **not** significantly different (i.e., $P>0.05$) from those of young adult males exposed to the same absolute dose (V_E of 50 l/min, 1 h duration and 0.30 ppm O_3) (see Tables 4 and 5 and Figs. 2-4). When males exercised at an increased V_E (70 l/min) that corrected for gender differences in endurance capacities (i.e. VO_{2max}), their pulmonary function and subjective symptoms responses were not significantly different than those demonstrated by females despite the differences in the total inhaled dose between males ([0.30]O_3 x 1h x 70 l/min = 1260 ppm x l) and females ([0.30]O_3 x 1h x 50 l/min = 900 ppm x l) (see Tables 4 and 5 and Figs. 2-4). We define this dose, adjusted for inherent gender differences (lung size/VO_{2max}) via V_E, as the same relative dose for males and females exposed to ambient O_3.

In several recent studies, utilizing similar sample sizes as that in the present study, no significant gender differences in percent change in pulmonary function or in subjective symptom responses were observed consequent to O_3 exposure when females exercised at, or near, the same percent VO_{2max} as males (i.e., the same relative dose). Horvath et al (1986) found no significant differences in pulmonary function and subjective symptom responses between 10 young adult females and 10 young adult males of similar age, when exposed to 0.48 ppm O_3 for 2 h with light intermittent exercise (IE). The total inhaled dose was approximately 11% less for the females. Adams et al. (1987) exposed 40 young adults (20 of each sex) to 0.30 ppm O_3 for 1 h while exercising at 62% of VO_{2max}, and observed no significant difference according to gender for FVC, $FEV_{1.0}$, FEF_{25-75} or subjective symptoms. The females' mean V_E was 50 l/min, about 30% lower than that for the males (70 l/min), exactly the same ventilatory differences utilized in this study to obtain the same relative dose. Our findings are consistent in part with this earlier investigation, in that males exhibited nearly the same response ($\Delta FEV_{1.0}$ = -18.7%) as females ($\Delta FEV_{1.0}$ = -18.6%) in this study; this is similar to our previous study, in which no significant difference was observed between the male ($\Delta FEV_{1.0}$ = -23.8%) and female ($\Delta FEV_{1.0}$ = -20.3%)

decrements. However, the mean $FEV_{1.0}$ response of the male group in this study was somewhat lower than that for the male group in the earlier study (- 5%). Part of this lower $FEV_{1.0}$ response was due to the fact that four male subjects in the current investigation were extremely responsive to O_3 exposure (mean $FEV_{1.0}$ decrement -35%), and were unable to complete the 70 l/min protocol. (This probably also explains the non-significant differences between the 50 l/min and 70 l/min male exposures to 0.30 ppm O_3, since the total dose was reduced substantially in the latter due to significantly reduced exposure duration.) Four females in this study were also unable to finish their 0.30 ppm O_3, 50 l/min exposures (mean $FEV_{1.0}$ decrement -29%). Taken together, the above evidence strongly suggests that healthy young adult females are not more sensitive to O_3 inhalation at the same concentration, exposure time product than their male counterparts at a V_E that is smaller in proportion to mean gender differences in $V0_{2max}$ (i.e., at the same relative dose).

Surprisingly, despite similarities in male and female responses following exposure to the same relative dose, females in this study were not significantly more responsive than males to 0.30 ppm O_3 exposure at the same absolute dose (V_E 50 l/min for both groups). This observation is in contrast to the substantially greater responses reported by Lauritzen and Adams (1985) for females, compared to their male counterparts, at the same V_E, O_3 concentration, and duration (i.e., at the same absolute dose). The mean percent change in pulmonary function response of our females (four did not complete the entire 1h exposure) was somewhat lower ($\Delta FEV_{1.0}$ = -18.6%) than those of most other female groups studied in this laboratory at the same O_3 concentration, exposure time and V_E (Adams et al (1987), $\Delta FEV_{1.0}$ = -20.3%; Gibbons and Adams (1984), $\Delta FEV_{1.0}$ = -17.4%; Lauritzen and Adams (1985), $\Delta FEV_{1.0}$ = -24.2%; and Messineo and Adams (1990), $\Delta FEV_{1.0}$ = -22.0% for a small-lung group and -25.1% for a large-lung group). In contrast, the mean percent change in male pulmonary function response to the 0.30 ppm O_3, 50 l/min 1h exposure was greater ($\Delta FEV_{1.0}$ = -17.4%) than that observed in a group of 10 males exposed to the same dose ($\Delta FEV_{1.0}$ = -13.8%, W.C. Adams, unpublished observations) or six males exposed to a slightly greater dose ($\Delta FEV_{1.0}$ = -8.1%, Lauritzen and Adams, 1985). The greater sensitivity of our male subjects and lesser sensitivity of our female subjects (compared to that observed in our previous studies) may have substantially diminished any differences between these two groups at the same absolute dose. However, it could be contended that the reverse is true of the subjects utilized in Lauritzen and Adams's investigation (1985); i.e., sensitive females and relatively insensitive males. Thus, further research is needed on

additional male and female subjects to obtain conclusive evidence regarding whether significant gender differences at the same absolute dose actually exist.

2. Menstrual Phase Comparisons. After exposing the majority of the 20 females participating in this study to most of the nine experimental protocols (see experimental design, p. 16 of this report), urine analyses revealed that there was a very high incidence of atypical menstrual cycles in our subject population despite intensive menstrual history screening (N = 15). Six of these female subjects experienced atypical cycles prior to experimental intervention (i.e., some were anovulatory), yet they were reporting 'regular periods' every 24-30 days. Thus, they believed that their menstrual cycles were normal. The nine other subjects demonstrated atypical cycles following experimental intervention. As a result, we had a limited number of subjects whose data was valid for comparison between the three menstrual phases at the three gas concentrations. After changing the criterion to two menstrual phases at two gas concentrations (i.e., FA and 0.30 ppm O_3), we were able to increase our N to nine subjects. The nine who demonstrated normal ovarian function for these four exposures (in terms of intact phases at least up to the point of each exposure), revealed borderline significantly greater $FEV_{1.0}$ and FEF_{25-75} decrements in the EF phase compared to the ML phase. Due to the small sample size of this group and the high incidence of atypical cycles in the majority of the 20 subjects, we proposed to expose an additional eleven female subjects to FA and 0.30 ppm O_3 during the ML and EF menstrual phases. These four protocols were spaced over five menstrual cycles in order to better evaluate the effects of O_3 inhalation vs the effects of an intensive exercise protocol and other individual factors (i.e., stress). These purposes were accomplished in Study B and as a result, the discussion of menstrual phase responses is contained within the Study B Discussion (see following pages of the final report).

B. Effects of Acute Ozone Inhalation in Young Adult Females

1. Menstrual Phase Comparisons. A total of thirteen young adult females (nine from the original group of twenty subjects in Study A, and four from the second group of eleven in study B; thus comprising a merged data group), with demonstrated normal ovarian function via urine analysis (i.e., in terms of intact phases at least up to the point of each exposure), were exposed to FA and 0.30 ppm O_3 each in both the EF and ML phases of their menstrual cycles. Comparison of the EF and ML exposures did not reveal significant mean differences for pulmonary function or subjective symptoms responses at the $P<0.05$ level (see Tables 6 and 7 and Fig. 5). However, at the $P<0.12$ level, the group mean $\%\Delta$ in $FEV_{1.0}$ showed a trend towards enhanced O_3 responsiveness in the EF phase ($\Delta FEV_{1.0}$ = -16.5%) compared to the ML phase

($\Delta FEV_{1.0}$ = -13.0%). Individual percent change in $FEV_{1.0}$ responses, listed in Table 8, show that nine out of the 13 subjects exhibited greater $FEV_{1.0}$ decrements following inhalation of 0.30 ppm O_3 during their EF phase, as compared to the $FEV_{1.0}$ decreases during the ML phase.. Subjective symptoms response did not demonstrate menstrual phase effects at this level of significance.

Acute O_3 exposure has been shown to result in pulmonary inflammation, consisting of an increase in the level of inflammatory mediators including cyclooxygenase products of arachidonic acid (AA) (i.e., prostaglandins, thromboxane) and the infiltration of neutrophils into the lungs and airways of both animals and humans (Fabbri et al., 1984; Seltzer et al., 1986). The release of AA is brought about by the destruction of cell membranes by O_3-parented free radicals. Activated neutrophils are known to release reactive oxygen radicals and proteolytic enzymes, both of which are capable of producing tissue damage in addition to that created directly by O_3. Seltzer and associates (1986) observed a significant increase in neutrophils, prostaglandins E_2 and F_{2a}, and thromboxane B_2 in the bronchoalveolar lavage (BAL) fluid (3 h postexposure) of normal male subjects exposed to 0.40 ppm 0_3 for 2 h with light IE. Schelegle et al (1989) found significantly increased plasma prostaglandin F_{2a} levels at midexposure and postexposure of 80 minute, 0.35 ppm O_3 protocols with moderately heavy exercise. The observed plasma prostaglandin F_{2a} response roughly paralleled the pulmonary function, ventilatory pattern and subjective symptom responses of these young adult male subjects. Schelegle et al (1987) have also noted a 50% reduction in $FEV_{1.0}$ impairment following a 0.35 ppm O_3 exposure with pretreatment of indomethacin, an anti-inflammatory agent that inhibits prostaglandin production.

Available data indicate that O_3-induced pulmonary function decrements and ventilatory pattern changes are neurally mediated (Lee et al, 1979; Hazucha, 1986) via stimulation of lung and airway afferents by the release of prostaglandins following O_3 insult and tissue damage. While the tissue damage associated with O_3 exposure is most likely a result of O_3-parented free radicals, the pain and discomfort leading to decreased FVC and $FEV_{1.0}$ appear to be direct results of prostaglandin production. Thus, in the present study, the 3.5% non-significant difference in $FEV_{1.0}$ response (at $P<0.05$), relative to menstrual cycle phase, may be related to differential prostaglandin production.

Therefore, for females to respond differentially to O_3 exposure with respect to pulmonary function decrements, there must be some fluctuation in the release or inhibition of prostaglandins in the various phases of the menstrual cycle. In normally menstruating females, gonadal hormones are produced at varying rates during the

menstrual cycle. The follicular phase is characterized by baseline progesterone concentrations and increasing levels of estrogen until ovulation. After ovulation, the luteal 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 (5-7 days) (Franz, 1988). Estrogen declines dramatically after ovulation and then rises to moderate levels during the ML phase. Both progesterone and estrogen levels decrease at the end of a cycle, thus triggering menstruation (Franz, 1988).

Numerous studies have demonstrated that sex steroids, essentially estrogens and progesterone, exert a significant effect on prostaglandin synthesis in various tissues, including cells of the vascular wall (Seillan et al, 1983), but particularly uterine cells (Bonney et al, 1987; Kelly and Smith, 1987; Abel and Baird, 1980; Schatz et al, 1984). In most cases, estradiol (an estrogen) has been found to enhance the synthesis of prostaglandins E_2 and F_{2a}, whereas progesterone inhibits their secretion. Kelly and Smith (1987) found that progesterone reduced prostaglandin F production by 93-96% in human proliferative phase endometrium cultured for 2 to 3 days.

It is not known at which point in the synthesis of prostaglandins that progesterone exerts its inhibitory action; however, it is likely that it could potentially inhibit the release of AA through action on phospholipase A2 (PLA2), an enzyme that is responsible for the freeing of AA from cell membranes (Bonney et al .,1987). PLA2 in human endometrium shows cyclical changes in activity which are dependent on the phase of the menstrual cycle (Bonney et al, 1987): low activity in the EF phase, with increases towards mid-cycle reaching maximum levels of activity in the early luteal phase, at which point activity declines in the presence of luteal phase concentrations of progesterone. This pattern of enzyme activity is indicative of steroid hormonal influence, and suggests that estradiol stimulates and that progesterone inhibits endometrial PLA2 activity, with the latter resulting in a decreased concentration of prostaglandins and a concomitant reduction in inflammation.

The trend ($P<0.12$) of an increased $FEV_{1.0}$ O_3 responsiveness during the EF phase, which we observed in our thirteen females (merged data group of Study A and B), could be the result of gonadal hormone interaction with O_3-induced prostaglandin synthesis. Both progesterone and estradiol are evident in circulating serum and plasma samples (Munro et al, 1991) in concentrations proportional to those observed in uterine tissues. Therefore, these gonadal hormones can be found in respiratory blood flow. During the EF phase of the menstrual cycle, when progesterone is low, there may be no inhibition of PLA2 and, thus, prostaglandin synthesis following O_3 exposure. In

contrast, with higher concentrations of progesterone during the ML phase, there may be some inhibition of PLA2 activity with a concomitant decrease in prostaglandin-induced inflammation, resulting in a slightly smaller decrement in $FEV_{1.0}$. However, relatively little is known regarding progesterone's role as an anti-inflammatory agent with known inflammatory inducers such as O_3. Thus, the effects of potential cyclical prostaglandin inhibition during the female menstrual cycle on inflammatory response appears to merit further investigation.

2. O_3 Effects on Menstrual Phase Integrity. The results of this explorative study indicated that most females exposed to ambient levels of O_3 seem to experience detrimental changes in their menstrual cycles (see Figs. 6-11). These changes are not evident in all females and appear to be greatest when O_3 inhalation occurs prior to ovulation in the EF phase. Despite the recently reported conclusion that young adult females may not be more sensitive to O_3 exposure than their male counterparts with regard to pulmonary function and subjective symptoms response (see Study A results, pages 23-25 of this report), females may indeed be more vulnerable to ambient levels of O_3 as the result of their reproductive system sensitivity to O_3 exposure. Since changes, such as those observed in this study, affect the viability of menstrual cycles, there is a need to consider whether or not an appropriate margin of safety has been established to protect this large population subset.

The effects that O_3 exposure appears to induce seem to be dependent on the exposure phase and the specific individual. Many postovulatory O_3 protocols initiated a surge in E1C at the end of a cycle (see Figs. 6,7,9 and 10), a phase in which significant decreases in E1C and PdG should be noted since such decreases trigger menses and the start of a new cycle. The E1C surge, when PdG is falling, suggests that the ovary is no longer synchronous and most likely means that the corpus luteum is being compromised (since the E1C surge indicates the growth of another follicle). An intact corpus luteum is a requirement for pregnancy to occur. In addition, it is possible that the sequential cycle could exhibit carry over effects from a secondary E1C surge.

Preovulatory O_3 exposures definitely appear to have compromising effects on menstrual cycle integrity, mainly through changes in ovulation and subsequent alterations in the luteal phase, the phase that is critical for fertilization and implantation of female eggs. O_3 exposure again appears to stimulate surges in E1C. In some females, these surges are sufficient enough to trigger early ovulation (see Fig. 8) and subsequently, a very short cycle (<20 days, Keizer and Rogol, 1990). Subject Teal, who experienced such an occurrence in cycle E (Fig. 8), was still able to maintain a normal length luteal phase (12-16 days), but it was characterized by a low shift in PdG

concentrations that bordered on non-functional (>4 μg/mg Cr is acceptable). In others, the preovulatory surge in E1C following O_3 inhalation initiated the growth of a follicle but was insufficient for full maturation of that follicle for ovulation. Therefore, another follicle had to grow which resulted in delayed ovulation (see Figs. 7 and 10). Delays in ovulation are often associated with short luteal phases (luteal phase defect = LPL < 12 days), as the body attempts to compensate for the delay and maintain a relatively normal cycle length. Luteal phase defects are non-functional due to the insufficient duration provided for endometrial lining maintenance. In one subject, Rose, preovulatory O_3 exposure induced multiple ovulations resulting in a very perturbed cycle.

Evidence from our original 20 subjects in Study A (and from one subject in Study B) suggests that continued insult in some females results in a progressive deterioration of menstrual cycle integrity until the point of anovulation (see Fig. 11). There was a greater prevalence of induced anovulation in Study A's subjects (N = 4) due to as many as three 0_3 exposures occurring within any one cycle, thereby inhibiting time for recovery. This effect, as well as all other effects discussed above, appear to be transient since control urine collected several cycles beyond final O_3 exposure demonstrated patterns of resumed normality.

Following evaluation of the urine collected from the 20 subjects in Study A, it was difficult to determine if menstrual cycle changes were a function of O_3 exposure, the intensive exercise encompassed in the protocols, or other factors, because subjects received multiple exposures in most of their cycles of participation. In the second study, the four experimental protocols were spaced out over 5 cycles to provide windows of observation and time for recovery. As a result, we were able to differentiate, to a greater extent, effects that were O_3-induced and those that were protocol- (or otherwise)-induced. FA exposures appeared to have some effects on menstrual cycle characteristics in some females, such as postovulatory secondary surges in E1C (see Figs. 9 and 10) and in one individual, a preovulatory change in menstrual cycle integrity (see Fig. 11). However, the incidence of detrimental changes in menstrual cycle characteristics were far greater for O_3 exposures in comparison to FA exposures. For all the changes we noted in our eleven females' menstrual cycles, <23% of them could be attributed to FA exposures.

Numerous investigators have reported that exercise, particularly intensive training, can induce menstrual cycle alterations, including luteal phase defects, anovulation and amenorrhea (absence of menstruation) (Beitins et al, 1991; Bonen et al, 1981). Training probably accounted for the high incidence of atypical cycles (Keizer and Rogol, 1990) noted prior to our experimental intervention (36% in our N=11 female

group, compared to 7% of the general young adult female population; Vollman, 1977), especially since we selected subjects that trained regularly so that they could complete the 1 h continuous heavy exercise protocols. In trained female groups, cycle alterations are associated with decreased LH and follicle-stimulating hormone concentrations (Bonen et al, 1981; Marx et al, 1986; Rokainen, 1985). The lower than normal values of LH are the result of inhibited secretion of gonadotropin-releasing hormone (GnRH), which is the hypothalamic signal to the pituitary to release LH. Circulating LH concentrations are responsible for the ovulatory surge in estrogen once the LH pulse frequency increases significantly (Keizer and Rogol, 1990). In a normal cycle, an LH pulse frequency increase is evident at mid-cycle, with much lower pulse frequencies occurring prior to and after ovulation (Clough et al, 1992).

LH analysis was completed in a few subjects' cycles in an attempt to differentiate ovarian or pituitary mechanisms for O_3-induced effects. Following O_3 insult, there appears to be an initial surge in LH, followed by dramatic decreases for several days. The stimulatory effect of O_3 on LH could explain the surges in E1C (and sometimes PdG) following an O_3 exposure. Further, this evidence suggests that O_3 acts on ovarian function indirectly through direct pituitary action (direct ovarian action would demonstrate elevated E1C values without a concomitant increase in LH). However, more significantly, the effects of O_3 on menstrual cycle integrity appear to be mediated differentially from those induced by exercise: O_3 results in initial LH stimulation, whereas training leads to decreased LH levels. However, this difference could be due to the disparate durations: acute vs chronic insult. It does seem evident that additional research needs to be completed to determine not only the mechanisms by which O_3 affects the female menstrual cycle, but also to verify changes in cycle viability following O_3 inhalation and the associated health risks incurred with such changes (whether they be O_3-induced fertitlity problems or altered cycle-induced bone mineral density decreases).

References

1. Abel, M. H., and D. T. Baird. The effect of 17b-estradiol and progesterone on prostaglandin production by human endometrium maintained in organ culture. Endocrinol. 106:1599-1606, 1980.

2. Adams, W. C. The effect of heavy sustained exercise in combination with low levels of ozone concentration in inducing acute pulmonary function impairment in humans. Unpublished Final Report to State of California Air Resources Board, A1-158-33, Sacramento, CA., July 1984.

3. Adams, W. C. Effects of ozone exposure exposure at ambient air pollution episode levels on exercise performance. Sports Med. 4:395-424, 1987.

4. Adams, W. C., K. A. Brookes, and E. S. Schelegle. Effects of NO_2 alone and in combination with O_3 on young men and women. J. Appl. Physiol. 62:1698-1704, 1987.

5. Adams, W. C., W. M. Savin, and A. E. Christo. Detection of ozone toxicity during continuous exercise via the effective dose concept. J. Appl. Physiol. 51:415-422, 1981.

6. Adams, W. C., and E. S. Schelegle. Ozone and high ventilation effects on pulmonary function and endurance performance. J. Appl. Physiol. 55:805-812, 1983.

7. Air Quality Criteria for Ozone and Other Photochemical Oxidants. Vol. V. Washington, D. C. U. S. Environmental Protection Agency, 1986 (Publication No. EPA-600/8-84-020e F).

8. Avol, E. L., W. S. Linn, T. G. Venet, D. A. Shamoo, and J. D. Hackney. Comparative respiratory effects of ozone and ambient oxidant pollution exposure during heavy exercise. J. Air Pollut. Control Assoc. 34:804-809, 1984.

9. Beitins, I. Z., J. W. McArthur, B. A. Turnbull, G. S. Skrinar, and B. A. Bullen. Exercise induces two types of human luteal dysfunction: confirmation by urinary free progesterone. J. Clin. Endocrinol. Metab. 72:1350-1358, 1991.

10. Bonen, A., A. N. Belcastro, W. Y. Ling, and A. A. Simpson. Profiles of selected hormones during menstrual cycle of teenage athletes. J. Appl. Physiol. 50:545-551, 1981.

11. Bonney, R. C., S. T. Qizilbash, and S. Franks. Endometrial phospholipase A2 enzymes and their regulation by steroid hormones. J. Steroid Biochem. 27:1057-1064, 1987.

12. Bromberg, P. A., and M. J. Hazucha. Is adaptation in ozone protective? Am. Rev. Respir. Dis. 125:489-490, 1982.

13. Brookes, K. A., W. C. Adams, and E. S. Schelegle. 0.35 ppm O_3 exposure induces hyperresponsiveness on 24-h reexposure to 0.20 ppm O_3. J. Appl. Physiol. 66:2756-2762, 1969.

14. Clough, K., F. X. Cole, A. Seaver, A. Y. Danti, A. Kuo, and B. L. Lasley. Enzyme immunoassay method for total alpha gonadotropin in human urine samples. Fertility and Sterility 57: 1241-1246, 1992.

15. DeLucia, A. J., and W. C. Adams. Effects of O_3 inhalation during exercise on pulmonary function and blood biochemistry. J. Appl. Physiol. 43:75-81, 1977.

16. DeLucia, A. J., J. W. Whitaker, and L. R. Bryant. Effects of combined exposure to ozone and carbon monoxide in exercising humans. In: Adv. Mod. Environ. Tox., Vol. 5, The Biomedical Effects of Ozone and Related Photochemical Oxidants. Edited by S. D. Lee, M. G. Mustafa, and M. A. Mehlman. Princeton Scientific Publishers, Inc., Princeton, N. J., Chpt. 11, pp. 145-159, 1983.

17. DeMore, W. B., J. C. Romanovsky, M. Feldstein, W. J. Hamming, and P. K. Mueller. Interagency comparison of iodometric methods of ozone determination. In: Calibration in Air Monitoring. Philadelphia, PA: American Society for Testing and Materials, 1976, pp. 131-143. (ASTM Technical Publ. 598).

18. Dixon, W. J., ed. BMDP Statistical Software. Los Angeles, University of California Press, 1985; 347-358.

19. Fabbri, L. M., H. Aizawa, S. E. Alpert, E. H. Walters, P. M. O'Byrne, B. D. Gold, J. A. Nadel, and M. J. Holtzman. Airway hyperresponsiveness and changes in cell counts in bronchoalveolar lavage after ozone exposure in dogs. Am. Rev. Respir. Dis. 129:288-291, 1984.

20. Folinsbee, L. J., B. L. Drinkwater, J. F. Bedi, and S. M. Horvath. The influence of exercise on the pulmonary function changes due to exposure to low concentrations of ozone. In: Environmental Stress: Individual Human Adaptations. Edited by L. J. Folinsbee, J. A. Wagner, J. F. Borgia, B. L. Drinkwater, J. A. Gliner, and J. F. Bedi. New York: Academic Press, pp. 125-143, 1978.

21. Franz, W. B. Basic review: Endocrinology of the normal menstrual cycle. Primary Care. 15:607-616, 1988.

22. Gibbons, S. I., and W. C. Adams. Combined effects of ozone exposure and ambient heat on exercising females. J. Appl. Physiol. 57:450-456, 1984.

23. Hazucha, M. J., D. V. Bates, and P. A. Bromberg. Mechanism of action of ozone on the human lung. J. Appl. Physiol. 67:1535-1541, 1989.

24. Health Effects of Air Pollution. New York: American Lung Association, 1978, 48pp.

25. Horvath, S. M., J. F. Bedi, and D. M. Drechsler-Parks. Effects of peroxyacetyl nitrate alone and in combination with ozone in healthy young women. J. Air Pollut. Control Assoc. 36:265-270, 1986.

26. Keizer, H. A., and A. D. Rogol. Physical exercise and menstrual cycle alterations. What are the mechanisms? Sports Med. 10:218-235, 1990.

27. Kelly, R. W., and S. K. Smith. Glucocorticoids do not share with progesterone the potent inhibitory action on prostaglandin synthesis in human proliferative phase endometrium. Prostaglandins. 33:919-929, 1987.

28. Keppel, G. Design and Analysis: A Researchers Handbook. Englewood Cliffs, N. J.: Prentice Hall, 1991, pp. 169-70.

29. Lategola, M. T., C. E. Melton, and E. A. Higgins. Effects of ozone on symptoms and cardiopulmonary function in a flight attendant surrogate population. Aviat. Space Environ. Med. 51:237-246, 1980.

30. Lauritzen, S. K., and W. C. Adams. Ozone inhalation effects consequent to continuous exercise in females: Comparison to males. J. Appl. Physiol. 59:1601-1606, 1985.

31. Lee, L-Y., C. Dumont, T. D. Djokic, T. E. Menzel, and J. A. Nadel. Mechanism of rapid shallow breathing after ozone in conscious dogs. J. Appl. Physiol. 46:1108-1114, 1979.

32. McDonnell, W. F., R. S. Chapman, M. W. Leigh, G. L. Strope, and A. M. Collier. Respiratory responses of vigorously exercising children to 0.12 ppm ozone exposure. Am. Rev. Respir. Dis. 132:875-879, 1985.

33. McDonnell, W. F., D. H. Horstman, M. J. Hazucha, E. Seal, E. D. Haak, S. A. Salaam, and D. E. House. Pulmonary effects of ozone exposure during exercise: Dose-response characteristics. J. Appl. Physiol. 54:1345-1352, 1983.

34. Munro, C. J., G. H. Stabenfeldt, J. R. Cragun, L. A. Addiego, J. W. Overstreet, and B. L. Lasley. 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.

35. Rokainen, M., A. Pakarinen, and P. Kirkimen. Physical exercise-induced changes and seasonal associated differences in the pituitary-ovarian function in runners and joggers. J. Clin. Endocrinol. Metab. 60:416-422, 1985.

36. Schatz, F., L. Markiewicz, and E. Gurpide. Effects of estradiol on PGF_{2a} output by cultures of human endometrium and endometrial cells. J. Steroid Biochem. 20:999-1003,1984.

37. Schelegle, E. S., W. C. Adams, S. N. Giri, and A. D. Siefkin. Acute ozone exposure increases plasma prostaglandin F_{2a} in ozone-sensitive human subjects. Am. Rev. Respir. Dis. 140:211-216, 1989.

38. Schelegle, E. S., W. C. Adams, and A. D. Siefkin. Indomethacin pretreatment reduces ozone-induced pulmonary function decrements in human subjects. Am. Rev. Respir. Dis. 136:1350-1354, 1987.

39. Seillan, C., C. Ody, F. Russo-Marce, and D. Duval. Differential effects of sex steroids on prostaglandin secretion. Prostaglandins. 26:3-12, 1983.

40. Seltzer, J., B. G. Bigby, M. Stulbarg, M. J. Holtzman, J. A. Nadel, I. F. Ueki, G. D. Leikauf, E. J. Goetzl, and H. A. Boushey. Ozone-induced change in bronchial reactivity and airway inflammation in human subjects. J. Appl. Physiol. 60: 1321-1326, 1986.

41. Silverman, F., L. J. Folinsbee, J. Barnard, and R. J. Shephard. Pulmonary function changes in ozone-interaction of concentration and ventilation. J. Appl. Physiol. 41:859-864, 1976.

42. Vollman, R. F. The Menstrual Cycle. Philadelphia: Saunders, 1977, pp. 72-123.

Tables and Figures

Table 1. *Group subject characterization data*

	Age, yr	Ht, cm	Wt, kg	Body Fat, %wt	FVC, liters	RV, liters	$FEV_{1.0}$, liters	VO_{2max}, l/min	V_{Emax}, l/min
				Male Group N = 20					
Mean	24.6	178.6	73.4	11.1	5.57	1.61	4.35	4.36	155.8
SD	3.8	7.4	7.9	4.4	0.73	0.33	0.56	0.47	15.8
Range	(19-31)	(165.1-191.8)	(56.8-92.3)	(5.4-24.1)	(4.18-7.92)	(0.96-2.47)	(3.46-5.52)	(3.64-5.34)	(128.0-181.4)
				Female Group N = 20					
Mean	24.4	166.7	64.3	23.7	4.02	1.16	3.46	2.91	111.6
SD	4.7	4.3	8.4	6.9	0.47	0.23	0.49	0.36	15.3
Range	(20-34)	(160.0-175.3)	(51.4-78.9)	(12.2-34.4)	(3.20-4.92)	(0.79-1.59)	(2.77-4.37)	(2.06-3.46)	(84.6-140.6)
				Female Group N = 11					
Mean	25.5	169.3	66.9	25.1	4.33	1.20	3.54	2.79	115.4
SD	3.3	8.3	8.8	4.5	0.50	0.25	0.49	0.31	19.8
Range	(20-31)	(153.7-185.4)	(54.3-79.7)	(17.9-32.5)	(3.83-5.44)	(0.83-1.71)	(2.91-4.72)	(2.29-3.20)	(91.8-164.1)

FVC, forced vital capacity; RV, residual volume; $FEV_{1.0}$, forced expiratory volume in 1 s; VO_{2max}, maximum O_2 uptake; V_{Emax}, maximum minute ventilation; SD, standard deviation of the sample.

TABLE 2: *Female (N = 20) pre- and postexposure values for pulmonary function parameters*

PROTOCOL		FVC (l)		$FEV_{1.0}$ (l/s)		$FEF_{25-75\%}$ (l/s)	
		Pre	Post	Pre	Post	Pre	Post
01: FILTERED AIR	Mean	4.05	4.00	3.47	3.46	3.87	3.92
(MID-LUTEAL PHASE)	SD	0.50	0.51	0.49	0.57	0.90	1.04
02: FILTERED AIR	Mean	4.03	3.99	3.47	3.47	4.00	4.09
(LATE LUTEAL PHASE)	SD	0.50	0.53	0.53	0.58	1.03	1.09
03: FILTERED AIR	Mean	4.00	3.98	3.47	3.47	3.97	4.02
(EARLY FOLLICULAR)	SD	0.44	0.44	0.45	0.52	0.85	1.06
04: 0.18 PPM O_3	Mean	3.99	3.76	3.47	3.23	4.13	3.77
(MID-LUTEAL PHASE)	SD	0.49	0.54	0.52	0.57	1.03	1.12
05: 0.18 PPM O_3	Mean	4.05	3.86	3.48	3.26	4.02	3.70
(LATE LUTEAL PHASE)	SD	0.46	0.52	0.47	0.56	1.27	1.31
06: 0.18 PPM O_3	Mean	3.98	3.77	3.44	3.21	3.94	3.66
(EARLY FOLLICULAR)	SD	0.47	0.49	0.47	0.56	0.88	1.13
07: 0.30 PPM O_3	Mean	4.04	3.44	3.48	2.86	3.95	3.08
(MID-LUTEAL PHASE)	SD	0.50	0.58	0.52	0.65	0.95	1.11
08: 0.30 PPM O_3	Mean	4.01	3.40	3.45	2.78	3.91	2.91
(LATE LUTEAL PHASE)	SD	0.47	0.56	0.50	0.57	0.95	1.03
09: 0.30 PPM O_3	Mean	3.96	3.38	3.45	2.80	4.01	3.06
(EARLY FOLLICULAR)	SD	0.52	0.62	0.56	0.65	0.97	1.02

Significant mean differences at $p<0.05$: all filtered air vs. all 0.18 ppm O_3 protocols, all filtered air vs. all 0.30 ppm O_3 protocols and all 0.18 ppm O_3 vs all 0.30 ppm O_3 protocols for FVC, $FEV_{1.0}$ and $FEF_{25-75\%}$. There were no significant menstrual phase effects at any gas concentration for these parameters; **however, more than half of these females were not experiencing normal cycles or intact phases, thus the parentheses around the phase names.**

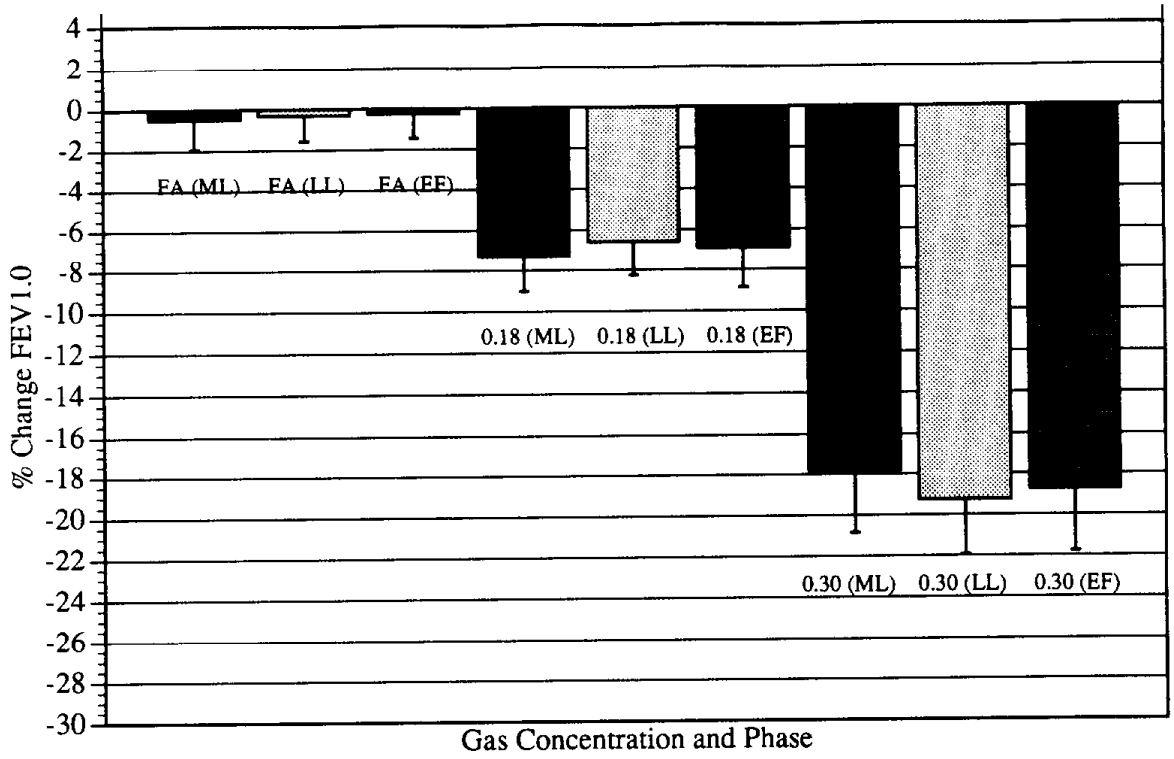

Fig. 1. Female (N = 20) mean percent change (with standard error bars) from preexposure to postexposure in FEV1.0 for filtered air (FA), 0.18 ppm O3 (0.18) and 0.30 ppm O3 (0.30) during assumed early follicular (EF), late luteal (LL) and mid-luteal (ML) phases of menstrual cycles. Not all females were experiencing normal menstrual cycles or intact phases, thus the parentheses around the phase name.

TABLE 3: *Female (N = 20) pre- and postexposure values for subjective symptom parameters*

PROTOCOL		OSS (scale 0-40)		TSSS (scale 0-120)	
		Pre	Post	Pre	Post
01: FILTERED AIR	Mean	0.8	1.3	2.6	3.7
(MID-LUTEAL PHASE)	SD	2.5	2.0	5.2	5.5
02: FILTERED AIR	Mean	1.4	1.7	4.4	5.6
(LATE LUTEAL PHASE)	SD	4.6	2.8	11.4	9.5
03: FILTERED AIR	Mean	1.7	2.4	3.6	6.5
(EARLY FOLLICULAR)	SD	3.6	3.1	8.4	8.7
04: 0.18 PPM O_3	Mean	1.0	5.4	2.7	13.7
(MID-LUTEAL PHASE)	SD	1.6	4.6	3.0	12.1
05: 0.18 PPM O_3	Mean	1.2	6.2	2.7	18.6
(LATE LUTEAL PHASE)	SD	2.2	7.5	4.8	22.8
06: 0.18 PPM O_3	Mean	1.1	3.9	2.8	12.6
(EARLY FOLLICULAR)	SD	1.9	4.9	5.5	17.0
07: 0.30 PPM O_3	Mean	0.7	16.1	2.8	48.5
(MID-LUTEAL PHASE)	SD	1.4	9.2	4.8	26.9
08: 0.30 PPM O_3	Mean	2.1	16.4	5.3	46.9
(LATE LUTEAL PHASE)	SD	3.8	9.7	8.8	31.7
09: 0.30 PPM O_3	Mean	0.6	16.0	2.3	48.2
(EARLY FOLLICULAR)	SD	1.6	9.2	4.9	28.6

Significant mean differences at $p<0.05$: all filtered air vs. all 0.18 ppm O_3 protocols (except 2-6 and 3-6), all filtered air vs. all 0.30 ppm O_3 protocols and all 0.18 ppm O_3 vs all 0.30 ppm O_3 protocols for Overall Symptom Severity (OSS) and Total Subjective Symptom Score (TSSS). There were no significant menstrual phase effects at any gas concentration for these parameters; **however, most of these females were not experiencing normal cycles or intact phases, thus the parentheses around the phase name.** Pre values were obtained at min 10 into each protocol whereas post values were collected at min 58.

TABLE 4: *Male (N = 20) and average female (N=20) pre- and postexposure values for pulmonary function parameters at ventilatory rates of 50 and 70 l/min for males and 50 l/min for females.*

PROTOCOL		FVC (l)		$FEV_{1.0}$ (l/s)		$FEF_{25-75\%}$ (l/s)	
		Pre	Post	Pre	Post	Pre	Post
01: MALE, 50 l/min	Mean	5.59	5.57	4.34	4.40	4.14	4.34
FILTERED AIR	SD	0.70	0.77	0.54	0.59	1.39	1.45
02: MALE, 70 l/min	Mean	5.58	5.61	4.38	4.49	4.21	4.45
FILTERED AIR	SD	0.78	0.74	0.58	0.59	1.40	1.45
03: FEMALE, 50 l/min	Mean	4.03	3.99	3.52	3.47	3.95	4.01
FILTERED AIR AVE	SD	0.47	0.49	0.46	0.55	0.90	1.34
04: MALE, 50 l/min	Mean	5.59	5.43	4.36	4.17	4.22	3.90
0.18 PPM O_3	SD	0.72	0.75	0.56	0.71	1.38	1.54
05: MALE, 70 l/min	Mean	5.54	5.30	4.38	4.13	4.17	3.94
0.18 PPM O_3	SD	0.73	0.80	0.58	0.73	1.35	1.51
06: FEMALE, 50 l/min	Mean	4.01	3.80	3.48	3.23	4.03	3.91
0.18 PPM O_3 AVE	SD	0.46	0.50	0.48	0.55	1.02	1.18
07: MALE, 50 l/min	Mean	5.59	4.88	4.36	3.58	4.10	3.13
0.30 PPM O_3	SD	0.76	0.97	0.56	0.79	1.42	1.38
08: MALE, 70 l/min	Mean	5.57	4.65	4.34	3.54	4.17	3.40
0.30 PPM O_3	SD	0.73	1.06	0.58	0.92	1.43	1.61
09: FEMALE, 50 l/min	Mean	4.00	3.41	3.46	2.81	3.96	3.02
0.30 PPM O_3 AVE	SD	0.49	0.55	0.52	0.59	0.94	1.02

Female values represent the mean of the values obtained for the 3 assumed menstrual cycle phases. Significant mean differences at $p<0.05$: there are no significant differences with male-female comparisons at the same absolute and relative work rate for these parameters.

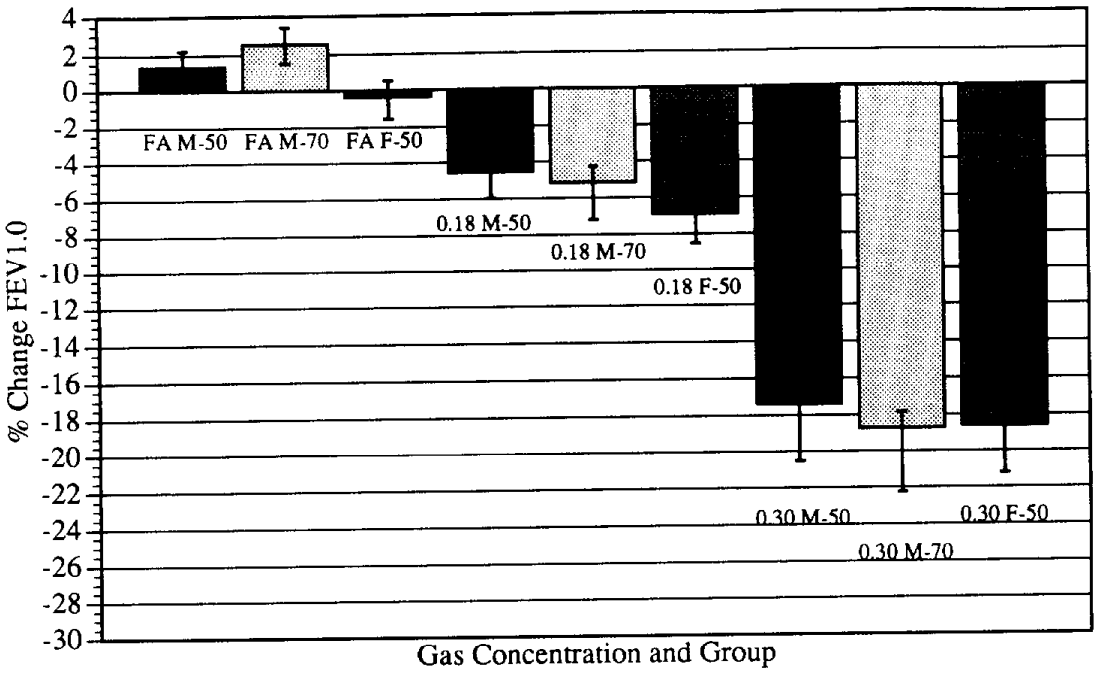

Fig. 2. Group mean percent change (with standard error bars) from preexposure to postexposure in FEV1.0 for males (N=20) at 50 l/min (M-50) and 70 l/min (M-70) and females (N=20) at 50 l/min (F-50) for filtered air (FA), 0.18 ppm O3 (0.18) and 0.30 ppm O3 (0.30).

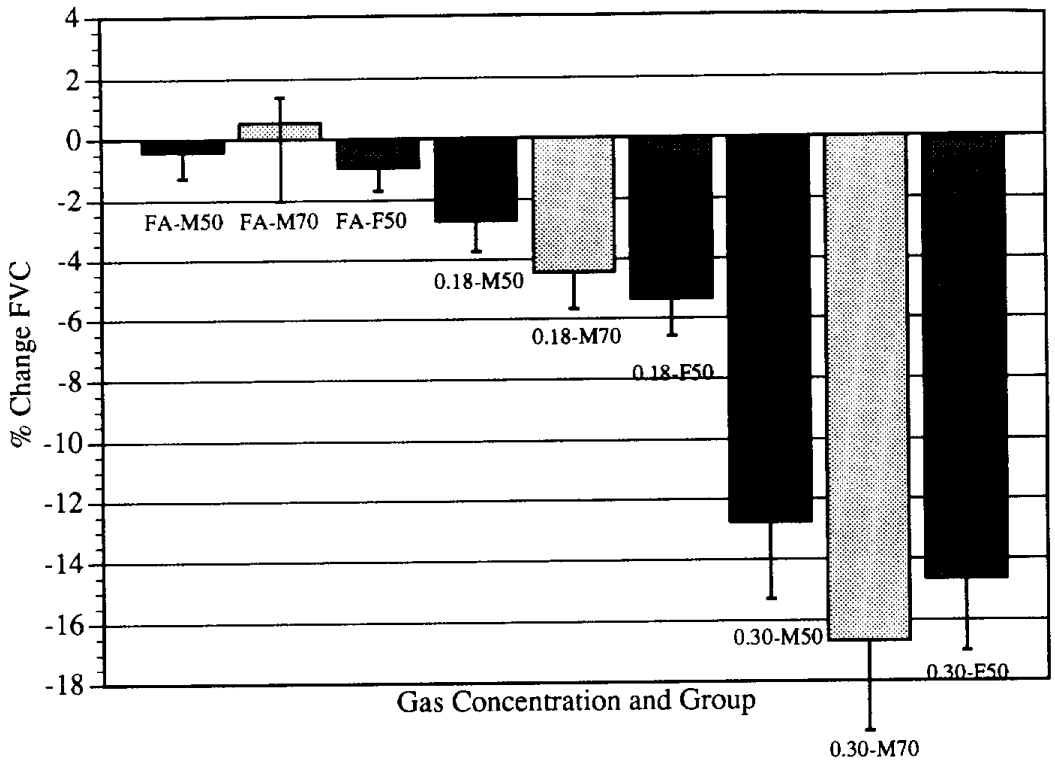

Fig. 3. **Group mean percent change (with standard error bars) from preexposure to postexposure in FVC for males (N=20) at 50 l/min (M-50) and 70 l/min(M-70) and females (N=20) at 50 l/min (F-50) for filtered air (FA), 0.18 ppm 03 (0.18) and 0.30 ppm O3 (0.30).**

TABLE 5: *Male (N = 20) and average female (N=20) pre- and postexposure values for subjective symptoms at ventilatory rates of 50 and 70 l/min for males and 50 l/min for females.*

PROTOCOL		OSS (scale 0-40)		TSSS (scale 0-120)	
		Pre	Post	Pre	Post
01: MALE, 50 l/min	Mean	0.2	0.8	0.7	1.8
FILTERED AIR	SD	0.5	2.4	1.6	4.7
02: MALE, 70 l/min	Mean	1.1	1.7	3.8	6.4
FILTERED AIR	SD	2.4	3.7	8.4	12.6
03: FEMALE, 50 l/min	Mean	1.3	1.8	3.5	5.3
FILTERED AIR AVE	SD	3.4	2.1	7.5	6.0
04: MALE, 50 l/min	Mean	0.5	2.3	1.2	8.3
0.18 PPM O_3	SD	1.3	3.0	2.7	9.5
05: MALE, 70 l/min	Mean	1.1	6.6	3.3	21.4
0.18 PPM O_3	SD	2.5	6.5	8.7	22.7
06: FEMALE, 50 l/min	Mean	1.1	5.1	2.7	14.9
0.18 PPM O_3 AVE	SD	1.5	4.4	3.8	13.7
07: MALE, 50 l/min	Mean	0.6	13.0	1.3	40.0
0.30 PPM O_3	SD	1.6	7.1	2.7	27.4
08: MALE, 70 l/min	Mean	0.9	17.8	2.0	63.4
0.30 PPM O_3	SD	1.7	9.5	3.7	39.2
09: FEMALE, 50 l/min	Mean	1.1	16.1	3.4	47.8
0.30 PPM O_3 AVE	SD	1.7	8.2	4.5	24.5

Significant mean differences at $p<0.05$: there are no significant differences with male-female comparisons at the same absolute and relative work rate for these parameters. Female values for each O_3 concentration represent the mean of values obtained during the 3 assumed menstrual cycle phases. Pre values were obtained at min 10 into each protocol whereas post values were collected at min 58.

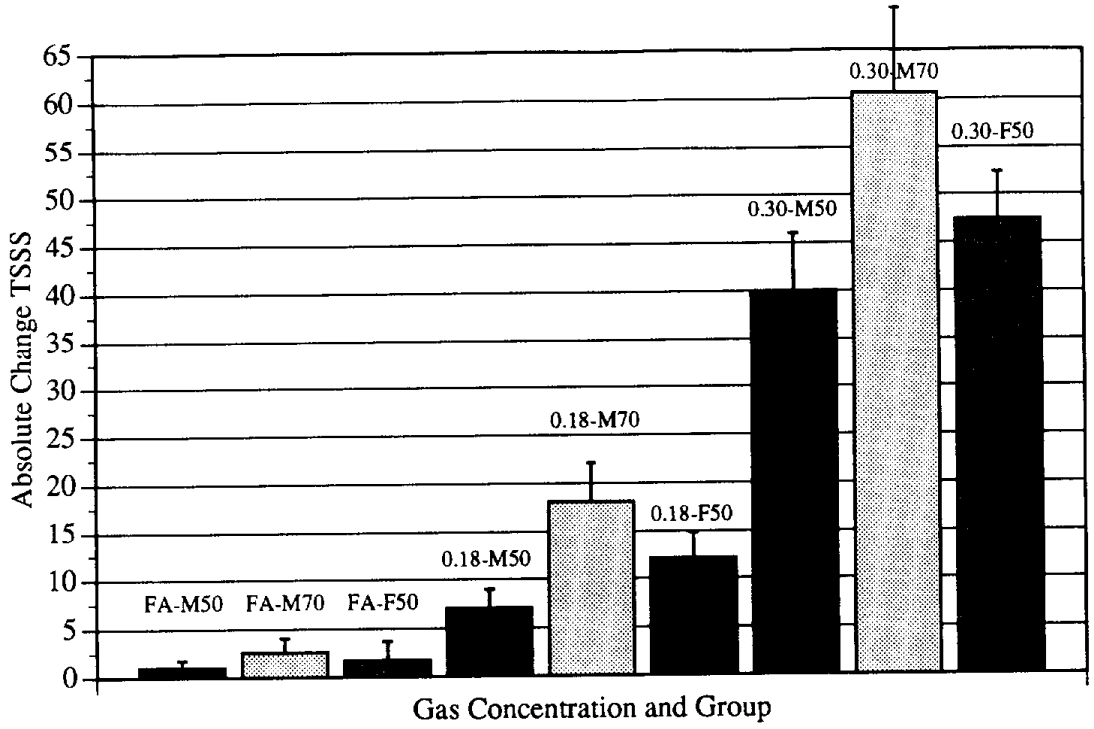

Fig. 4. **Absolute group mean change from preexposure to postexposure for TSSS for males at 50l/min, males at 70 l/min and females at 50 l/min for each gas concentration. TSSS (Total Subjective Symptom Severity) represents the summed scores from cough, pain on deep inspiration, shortness of breath and throat tickle.**

TABLE 6: *Female (N = 13) pre- and postexposure values for pulmonary function parameters during the early follicular and mid-luteal phases of normal menstrual cycles at each gas concentration.*

PROTOCOL		FVC (l)		$FEV_{1.0}$ (l/s)		$FEF_{25-75\%}$ (l/s)	
		Pre	Post	Pre	Post	Pre	Post
01: FILTERED AIR	Mean	4.14	4.10	3.47	3.51	3.79	3.87
EARLY FOLLICULAR	SD	0.56	0.51	0.51	0.48	0.98	0.91
02: FILTERED AIR	Mean	4.21	4.20	3.52	3.58	3.82	3.91
MID-LUTEAL PHASE	SD	0.55	0.54	0.53	0.45	1.12	0.92
03: 0.30 PPM O_3	Mean	4.14	3.70	3.50	2.93	4.09	3.18
EARLY FOLLICULAR	SD	0.64	0.76	0.57	0.63	1.07	0.94
04: 0.30 PPM O_3	Mean	4.22	3.84	3.50	3.05	4.04	3.33
MID-LUTEAL PHASE	SD	0.58	0.74	0.52	0.61	1.80	1.50

Significant mean differences at $p<0.05$: all filtered air vs. all 0.30 ppm O_3 protocols for FVC, $FEV_{1.0}$ and $FEF_{25-75\%}$. There were no significant menstrual phase effects at any gas concentration.

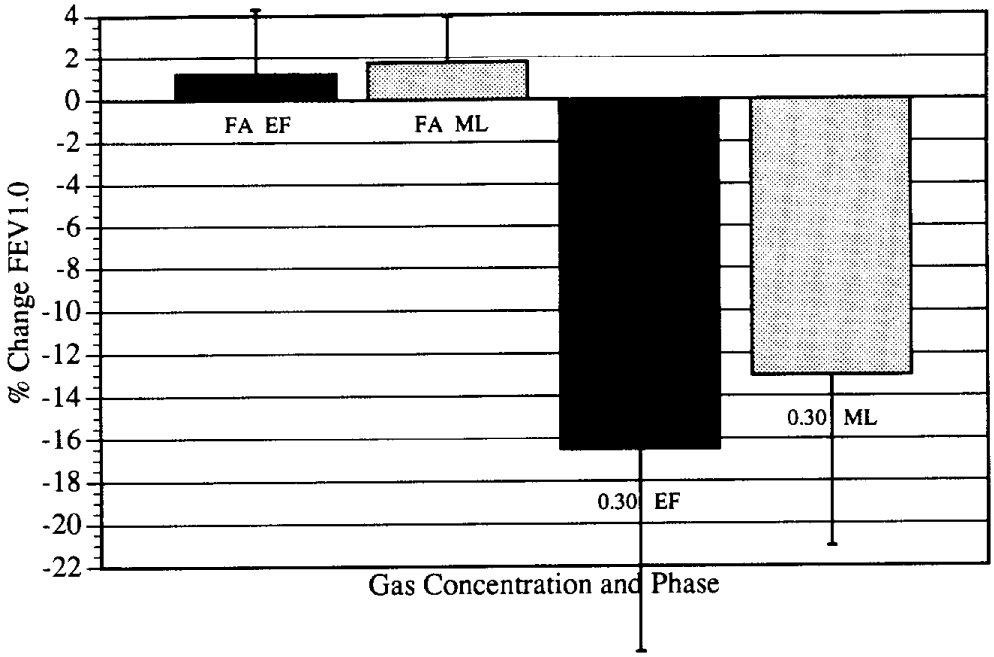

Fig. 5. Group mean percent change from preexposure to postexposure in FEV1.0 for filtered air (FA) and 0.30 ppm O3 (0.30) during the early follicular (EF) and mid-luteal (ML) phases of normal menstrual cycles.

TABLE 7: *Female (N = 13) pre- and postexposure values for subjective symptoms during the mid-luteal and early follicular phases of normal menstrual cycles at each gas concentration.*

PROTOCOL		OSS (scale 0-40)		TSSS (scale 0-120)	
		Pre	Post	Pre	Post
01: FILTERED AIR	Mean	1.2	3.0	2.3	8.2
EARLY FOLLICULAR	SD	2.2	3.7	4.8	10.8
02: FILTERED AIR	Mean	0.2	1.7	1.7	3.9
MID-LUTEAL PHASE	SD	0.6	3.1	3.1	8.2
03: 0.30 PPM O_3	Mean	0.8	14.6	3.1	38.5
EARLY FOLLICULAR	SD	1.9	9.2	6.0	22.5
04: 0.30 PPM O_3	Mean	0.7	15.4	2.1	45.2
MID-LUTEAL PHASE	SD	1.5	9.9	3.2	27.9

Significant mean differences at $p<0.05$: all filtered air vs. all 0.30 ppm O_3 protocols for Overall Symptom Severity (OSS) and Total Subjective Symptom Score (TSSS). There were no significant menstrual phase effects at any gas concentration. Pre values were obtained at min 10 of each protocol whereas post values were collected at min 58.

TABLE 8: *Individual percent change values for FEV1.0 during the early follicular and mid-luteal phases for subjects with normal menstrual cycles at each gas concentration.*

	$FEV_{1.0}$ Decrement			
SUBJECT	EF Phase FA Protocol	ML Phase FA Protocol	EF Phase 0.30 ppm O3	ML Phase 0.30 ppm O3
1	2.9	2.5	-7.9	-8.4
2	**3.0**	**1.5**	**-4.6**	**-3.9**
3	**0.2**	**1.6**	**-24.5**	**-13.8**
4	**1.6**	**-2.2**	**-17.7**	**-13.8**
5	**-0.6**	**3.1**	**-29.3**	**-22.8**
6	-0.8	2.0	-23.0	-21.3
7	**4.0**	**4.3**	**-28.6**	**-10.2**
8	**2.0**	**1.7**	**-2.2**	**-1.8**
9	**-1.7**	**1.5**	**-25.6**	**-21.8**
10	5.4	3.4	-18.8	-12.9
11	5.9	3.7	-10.6	-26.8
12	-1.2	2.9	-5.9	-4.1
13	**-4.7**	**-3.3**	**-16.6**	**-7.9**
Mean	1.2	1.8	-16.6	-13.0
SD	3.1	2.2	9.5	8.0

Bold values represent the number of subjects with a greater response in the EF phase of normal menstrual cycles as compared to the ML phase following 0.30 ppm O3 exposure when corrected for FEV1.0 response following the FA protocols in each phase.

ROSE

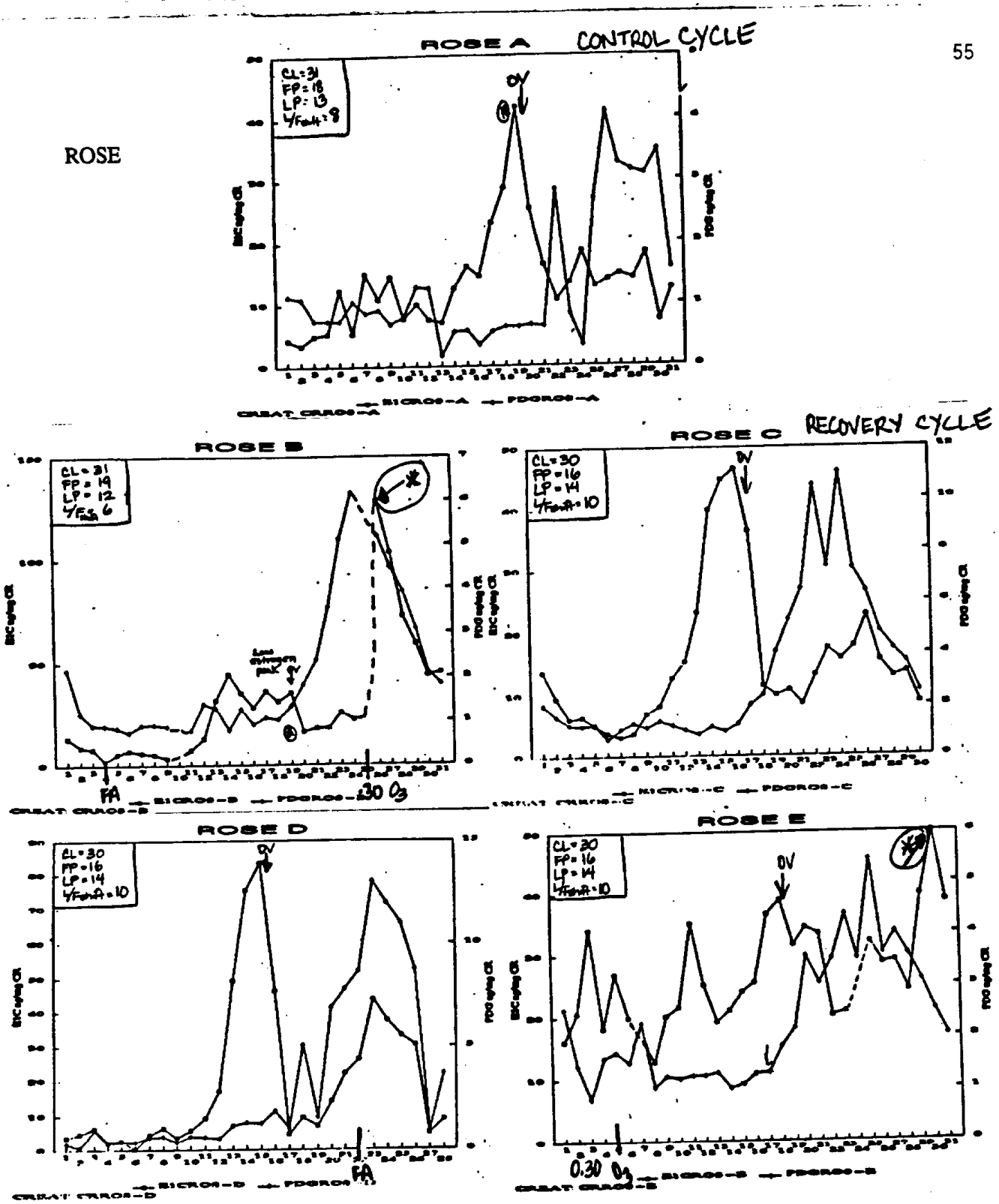

Fig. 6. Menstrual profile of all 5 cyles for subject ROSE. PdG (◊), progesterone glucuronide; E1C (•), estrogen conjugate; OV, ovulation; CL, cycle length; FP, follicular phase length; LP, luteal phase length; L/F shift, luteal to follicular phase shift in PdG concentrations; FA, day of filtered air protocol; 0.30 O_3, day of 0.30 ppm O_3 exposure. *Note E1C surges in cycle B and cycle E and the multiple leutinization in cycle E following 0.30 ppm O_3 exposure. (Dashed lines connect hormone values in periods with missing data.)

VIOLET

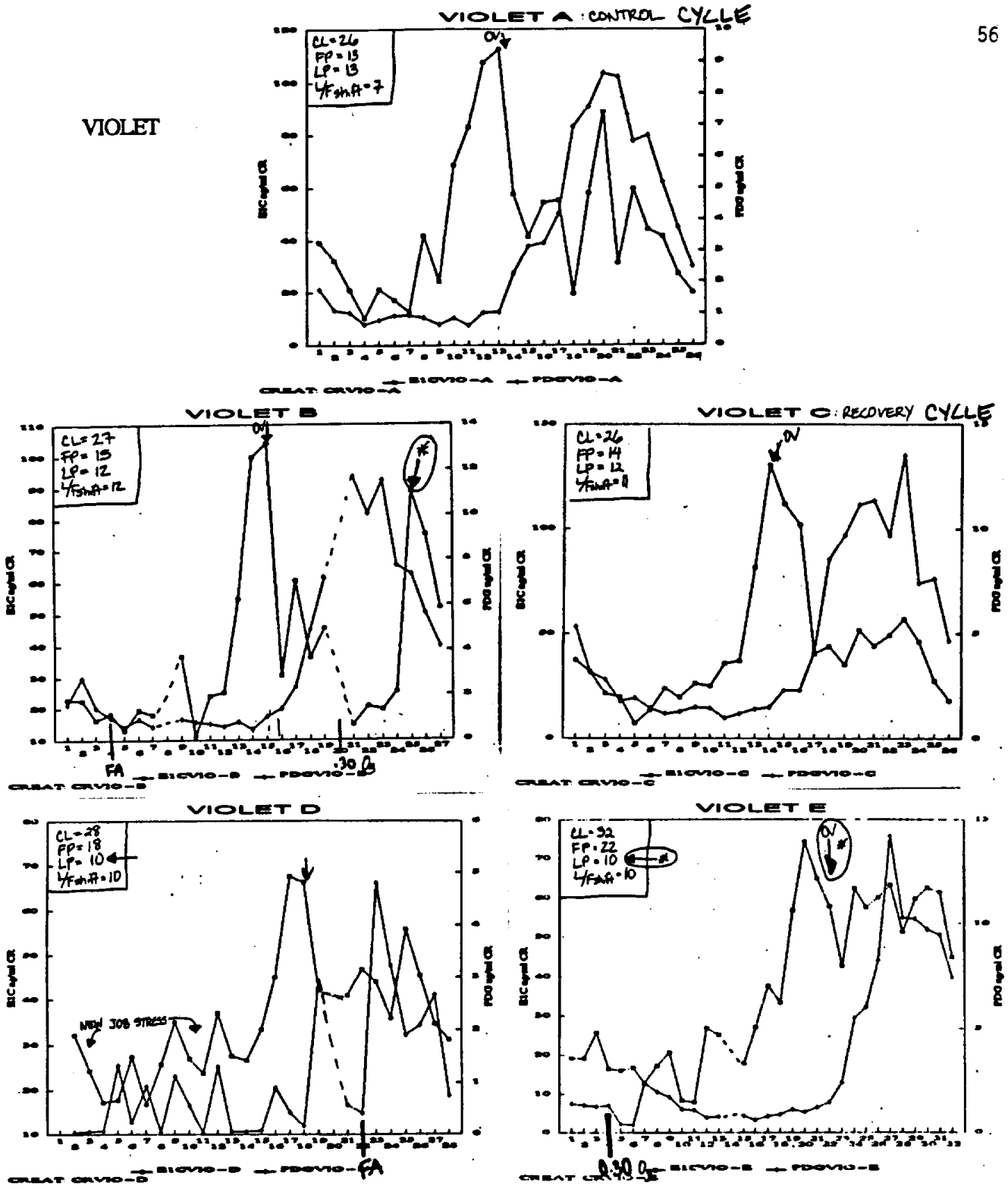

Fig. 7. Menstrual profile of all 5 cyles for subject VIOLET. PdG (◊), progesterone glucuronide; E1C (•), estrogen conjugate; OV, ovulation; CL, cycle length; FP, follicular phase length; LP, luteal phase length; L/F shift, luteal to follicular phase shift in PdG concentrations; FA, day of filtered air protocol; 0.30 O_3, day of 0.30 ppm O_3 exposure. *Note E1C surge in cycle B and the delay in ovulation and luteal phase defect in cycle E following 0.30 ppm O_3 exposure. (Dashed lines connect hormone values in periods with missing data.)

TEAL

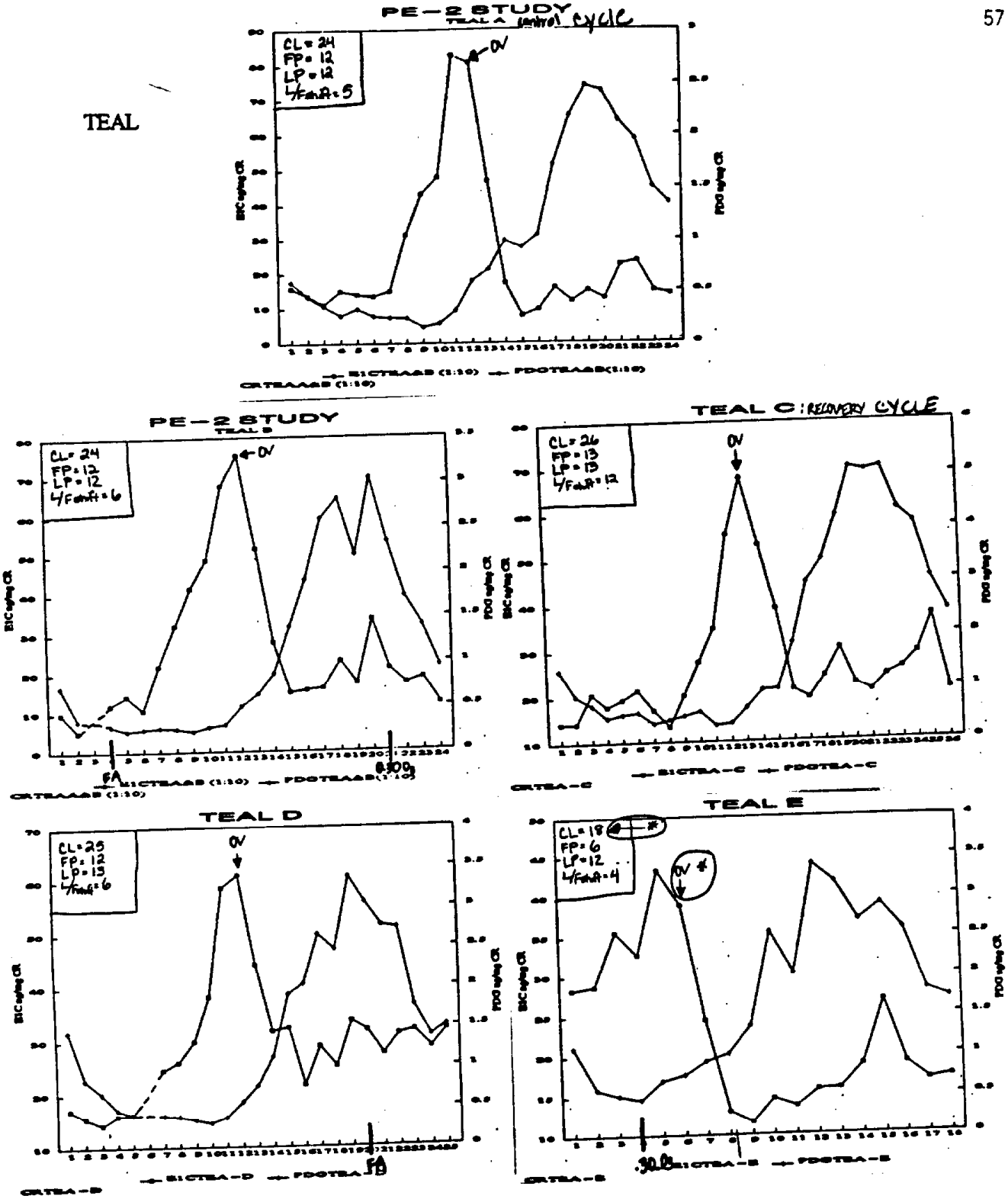

Fig. 8. Menstrual profile of all 5 cyles for subject TEAL. PdG (◊), progesterone glucuronide; E1C (•), estrogen conjugate; OV, ovulation; CL, cycle length; FP, follicular phase length; LP, luteal phase length; L/F shift, luteal to follicular phase shift in PdG concentrations; FA, day of filtered air protocol; 0.30 O_3, day of 0.30 ppm O_3 exposure. *Note the early ovulation following a preovulatory exposure to 0.30 ppm O_3 and the concomitant decrease in CL in cycle E.

FUSCHIA

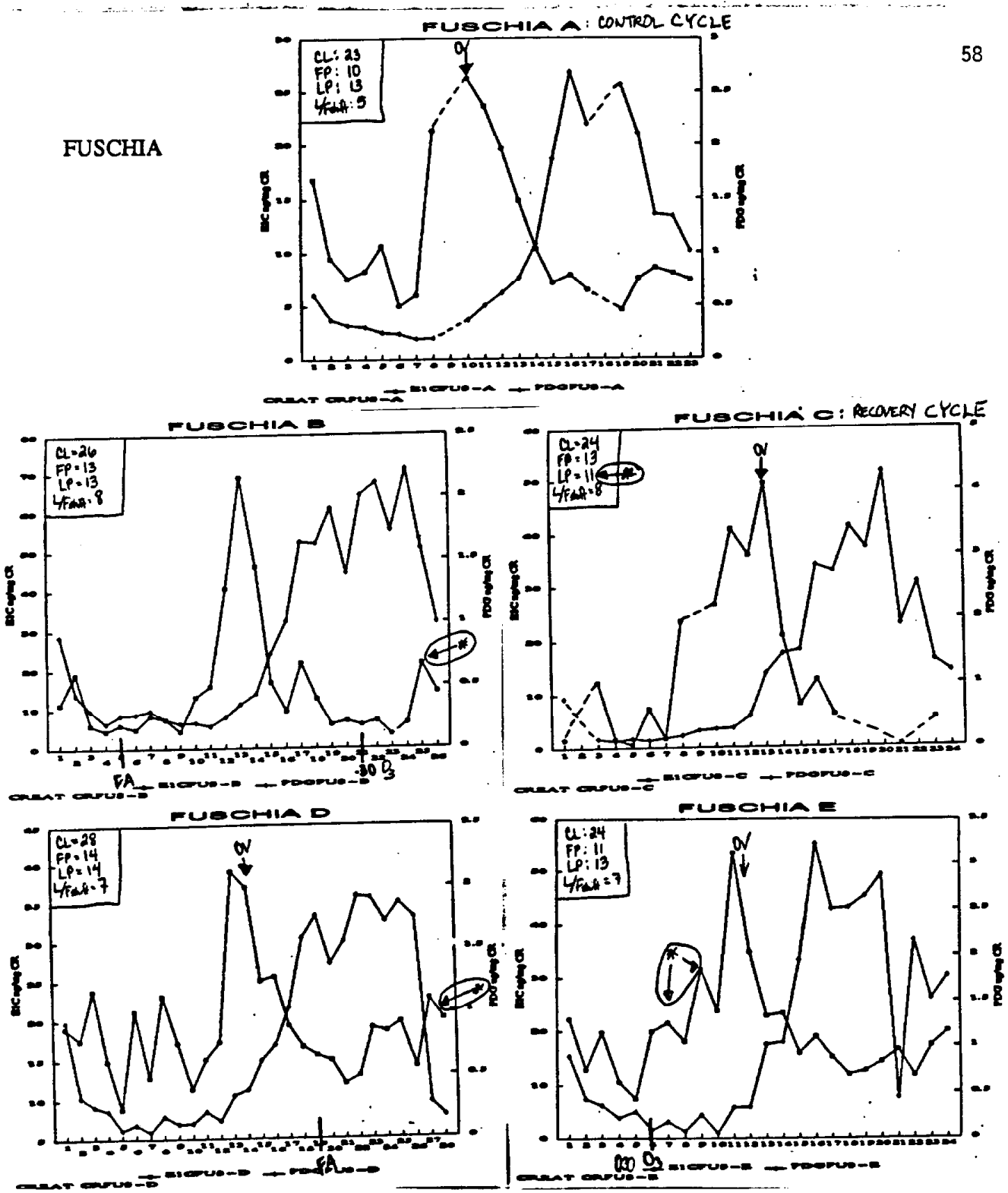

Fig. 9. Menstrual profile of all 5 cyles for subject FUSCHIA. PdG (◊), progesterone glucuronide; E1C (•), estrogen conjugate; OV, ovulation; CL, cycle length; FP, follicular phase length; LP, luteal phase length; L/F shift, luteal to follicular phase shift in PdG concentrations; FA, day of filtered air protocol; 0.30 O_3, day of 0.30 ppm O_3 exposure. *Note E1C surges following cycle B's ML 0.30 ppm O_3 exposure, cycle D's ML FA protocol and cycle E's 0.30 ppm O_3 exposure. Luteal phase defect in cycle C could be the result of cycle B's ML 0.30 ppm O_3 exposure. (Dashed lines connect hormone values in periods of missing data.)

PERIWINKLE

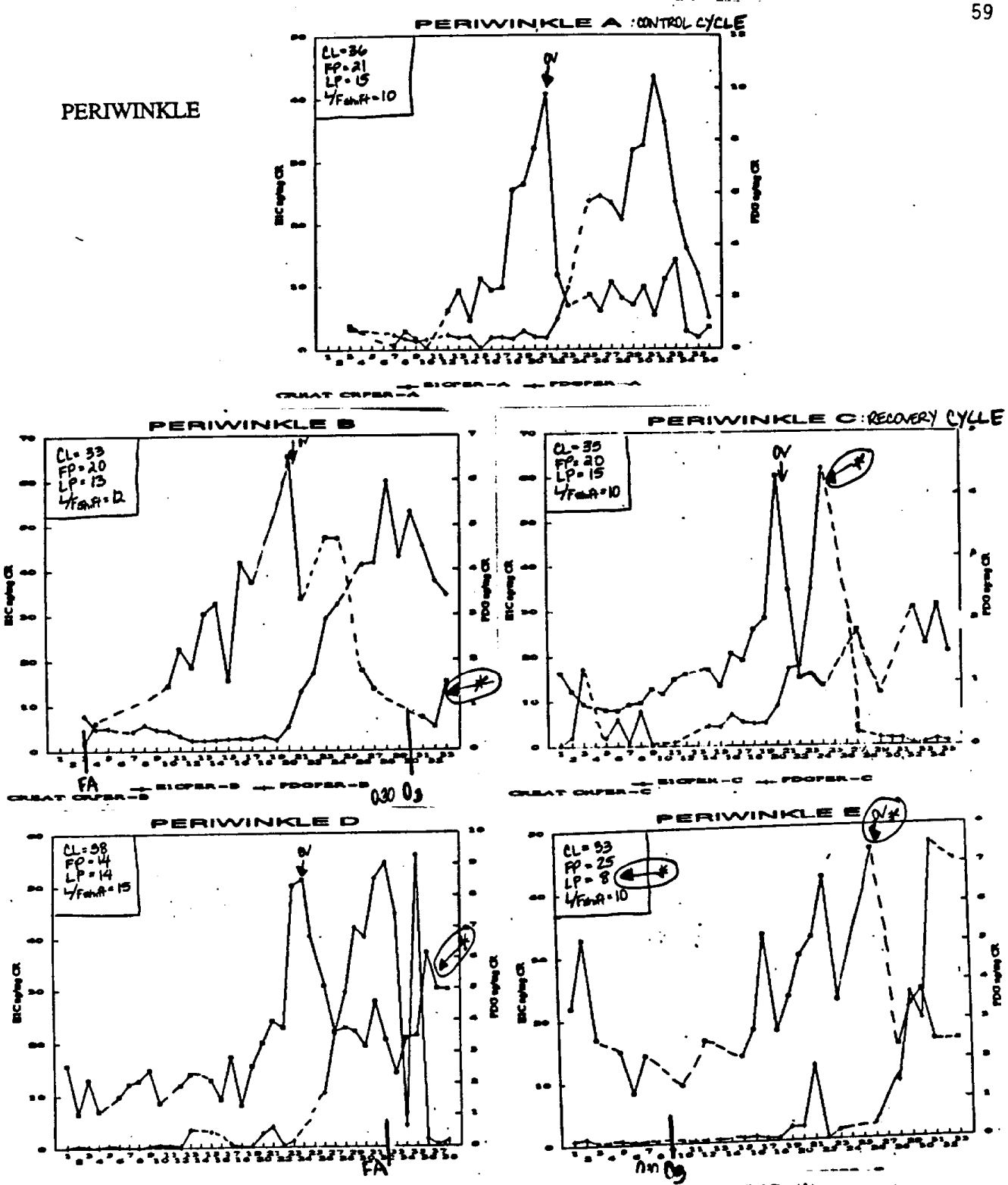

Fig. 10. Menstrual profile of all 5 cyles for subject PERIWINKLE. PdG (◊), progesterone glucuronide; E1C (•), estrogen conjugate; OV, ovulation; CL, cycle length; FP, follicular phase length; LP, luteal phase length; L/F shift, luteal to follicular phase shift in PdG concentrations; FA, day of filtered air protocol; 0.30 O_3, day of 0.30 ppm O_3 exposure. *Note E1C surges following cycle B's ML 0.30 ppm O_3 exposure. O_3 recovery cycle C was non-functional due to a inadequate maintenance of PdG. A surge in E1C following ML FA protocol was evident in cycle D. Cycle E was extremely perturbed following EF 0.30 ppm O_3 exposure, illustrated by delay in ovulation and luteal phase defect. (Dashed lines connect hormone values in periods of missing data.)

MUSTARD

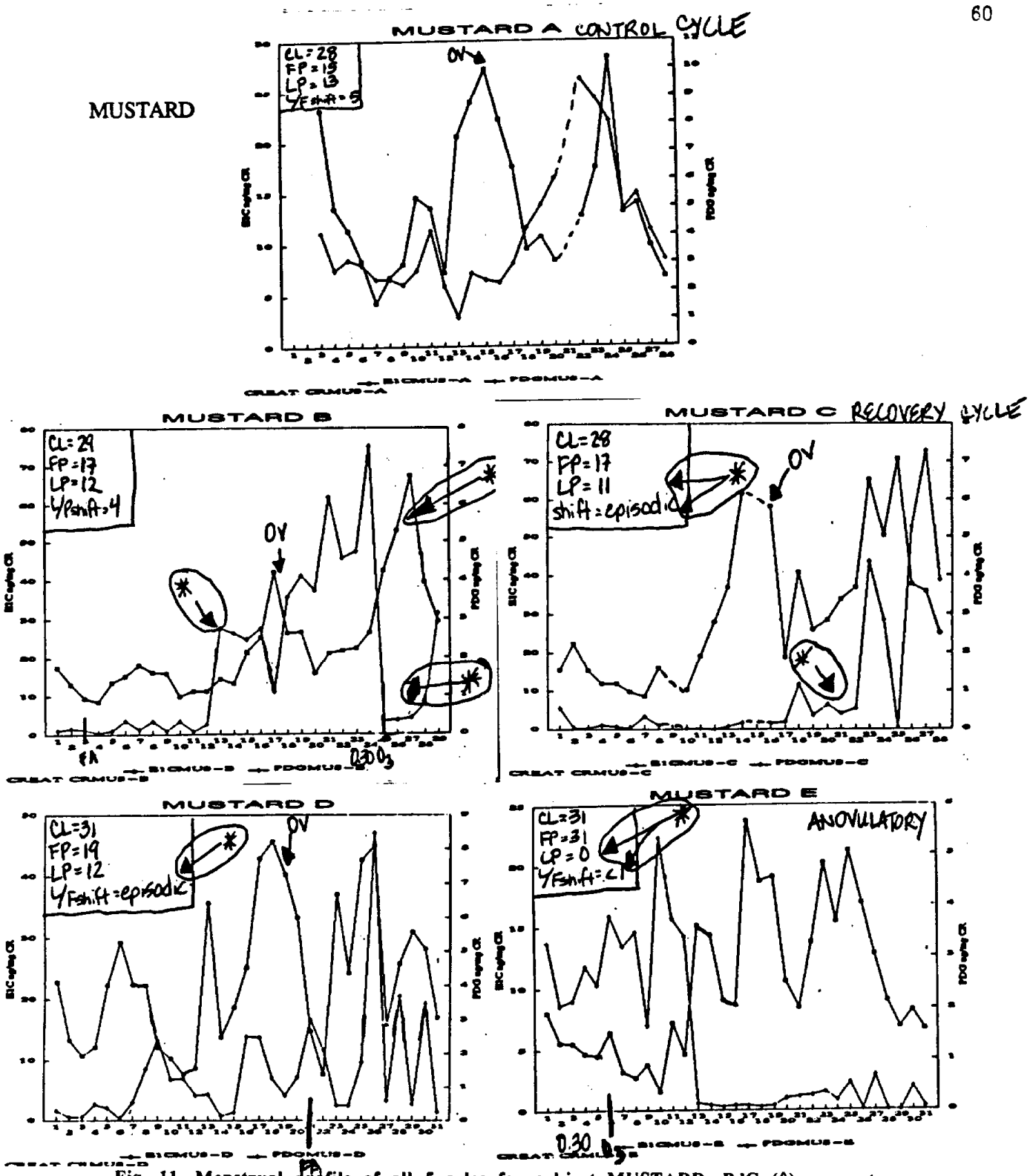

Fig. 11. Menstrual profile of all 5 cyles for subject MUSTARD. PdG (◊), progesterone glucuronide; E1C (•), estrogen conjugate; OV, ovulation; CL, cycle length; FP, follicular phase length; LP, luteal phase length; L/F shift, luteal to follicular phase shift in PdG concentrations; FA, day of filtered air protocol; 0.30 O_3, day of 0.30 ppm O_3 exposure. *Note PdG surge and E1C/PdG decrease following cycle B's EF FA and ML 0.30 ppm O_3 exposure, respectively. Cycle C and D exhibited luteal phase defects. Cycle E was anovulatory following an EF 0.30 ppm O_3 exposure. (Dashed lines connect hormone values in periods of missing data.)

Glossary of Terms and Abbreviations

AA	arachidonic acid
ACTH	corticotropin
ANOVA	analysis of variance
BAL	bronchoalveolar lavage
BTPS	body temperature, standard pressure, saturated
CL	cycle length
Cr	creatinine
EF	early follicular
EIA	enzyme immunoassay
E1C	estrone conjugates
FA	filtered air
FEF_{25-75}	forced expiratory flow between 25 and 75% of FVC
$FEV_{1.0}$	forced expiratory volume in 1 s
FPL	follicular phase length
FSH	follicle stimulating hormone
f_R	respiratory frequency
FVC	forced vital capacity
GnRH	gonadotropin releasing hormone
h	hour
HR	heart rate
IE	intermittent exercise
Kg	kilogram
L/F shift	luteal to follicular phase shift
LH	lutenizing hormone
LL	late luteal
l/min	liters per minute
LPL	luteal phase length
m	meter
min	minute
ML	mid-luteal
ml	milliliter
ng	nanogram
OSS	overall symptom severity
OV	ovulation

O_3	ozone
P	probability
PdG	pregnanediol-3-glucuronide
PLA2	phospholipase A2
ppm	parts per million
s	second
SD	standard deviation
TSSS	total subjective symptom severity
ug	microgram
V_E	minute ventilation
VO_2	oxygen uptake
VO_2max	maximal oxygen uptake
V_T	tidal volume

REPORT DOCUMENTATION PAGE

1. AGENCY USE ONLY *(Leave Blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
PB95270708	August 1992	Final Report

4. TITLE AND SUBTITLE

Studies of Young Female Responses to Acute Ozone Exposure

5. FUNDING NUMBERS

A933-096
A033-176

6. AUTHOR(S)

William C. Adams

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Physical Education Department
University of California
Davis, CA 95616

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

California Air Resources Board
Research Division
2020 L Street
Sacramento, CA 95814

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

ARB/R-95/586

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Release unlimited. Available from National Technical Information Service.
5285 Port Royal Road
Springfield, VA 22161

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

The objectives were to determine: (1) if lung function responses in young adult females were more sensitive to ozone than in males; (2) if responses to ozone were influenced by menstrual cycle phase; and (3) if ozone affected the integrity of normal menstrual cycles. Exercising female and male subjects were exposed to filtered-air, 0.18 ppm and 0.30 ppm ozone for 60 min. Average ventilation rates were 50 L min^{-1} for females, and 50 or 70 L min^{-1} for males. Responses in females were intermediate to those for males, but exposure to ozone caused significant differences in lung function and respiratory discomfort. Lung function responses in females exposed to ozone or filtered-air during three menstrual cycle phases (early follicular [EF], mid-luteal [ML] or late luteal [LL]) were not significantly different. However, in a subset exhibiting normal menstrual cycles, there was a trend toward greater impairment of forced expiratory volume responses during the EF phase, which may be related to progesterone levels. Menstrual cycle disruptions were observed following exposure to 0.30 ppm ozone (e.g., delayed ovulation, shortened luteal phase, secondary surges in estrogen). While the effects of ozone on ovarian function are unknown, effects on luteinizing hormone levels suggest a linkage to the pituitary gland.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Ozone, menstrual cycle, lung function, air pollution effects, 03	62
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited