RESEARCH CONTRACT FINAL REPORT TO

STATE OF CALIFORNIA AIR RESOURCES BOARD

Title of Contract: THE EFFECT OF HEAVY SUSTAINED EXERCISE IN COMBINATION WITH LOW LEVELS OF OZONE CONCENTRATION IN INDUCING ACUTE PULMONARY FUNCTION IMPAIRMENT IN HUMANS: INTERACTION OF AMBIENT HEAT AND MULTIPLE POLLUTANT EXPOSURES

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ABSTRACT

This research consisted of eight separate projects designed collectively to investigate (1) the efficacy of the ozone (O_3) effective dose concept (expressed as the product of O_3 concentration, minute ventilation ($\dot{V}_E$), and exposure duration), in predicting the acute O_3 toxicity response in young adult males; (2) the relative O_3 toxicity response of young adult females, clinically normal middle-aged males, and highly trained endurance athletes, compared to young adult males at the same effective dose; and (3) the possible interactive effects of O_3 inhalation in the presence of ambient heat and nitrogen dioxide (NO_2). A summary statement of the purpose, major observations, and their significance for each of the eight studies follows.

1. Refinement of the O_3 Effective Dose Concept. Pulmonary function impairment has been shown to be highly correlated with a second-order polynomial function of O_3 effective dose (expressed as the simple product of O_3 concentration, $\dot{V}_E$, and exposure time) in both intermittent exercise (IE) and continuous exercise (CE) exposures. However, calculated multiple regression equations, which yielded a greater weighting to O_3 concentration than to either $\dot{V}_E$ or exposure time, have been shown to provide a closer fit. To study further the physiological effects of exposure to divergent O_3 concentrations at several similar effective doses, we exposed 10 young adult males to 12 protocols varying in O_3 concentration (0 to 0.40 ppm), exercise $\dot{V}_E$ (30 and 70 $\ell \cdot min^{-1}$), and duration (30 to 100 min). The slopes of pulmonary function impairment for exposures to 0.20, 0.30, and 0.40 ppm O_3 were found to differ significantly, and to be related as an exponential function of O_3 concentration. These observations underline the need to further investigate the factors involved in O_3 toxicity response.

2. Effects of Exercise Continuity on Acute O_3 Toxicity. The use of continuous exercise (CE) during O_3 exposures, as opposed to intermittent exercise (IE) employed in other laboratories, is among several factors that can confound comparison of acute physiological responses. In this study, six aerobically trained adult male subjects were exposed by mouthpiece inhalation to 0.40 ppm O_3 during bouts of exercise that were either 1 h CE, or 2 h IE, but matched for total ventilation and effective dose. Statistical analysis revealed no significant differences in pulmonary function impairment between the 1 h CE and the 2 h IE protocols. However, alterations in exercise ventilatory pattern and subjective symptoms were more pronounced during the 1 h CE protocols at both levels of total ventilation. It was concluded that the enhanced responses with CE could not be definitively attributed to differences between the two exercise modes without assessment of filtered air (FA) control exposure responses.

3. Effects of Inspiratory Route on Physiologic Responses to O_3. Inspiratory route is among several factors that potentially confound comparison of physiologic responses to acute O_3 exposure in our obligatory oral inhalation method to that of ad-lib breathing permitted in chamber exposures. In this study, six young adult males were exposed on five occasions to 0.40 ppm O_3 while exercising continuously at one of two work loads ($\dot{V}_E$ of ~30 and 75 $\ell \cdot min^{-1}$). The V_E exposure time product was similar for all protocols. Four exposures were randomly delivered with a Hans-Rudolph respiratory valve attached to a silicone facemask, with inspiratory route effected with and without noseclip. No statistically significant differences across conditions in pulmonary function or subjective symptoms were observed. The fifth

exposure, delivered via the same respiratory valve, but without facemask, revealed significantly greater forced expiratory volume in 1 second ($FEV_{1.0}$) impairment than that observed for the respiratory valve, facemask with noseclip exposure (-20.4 and -15.9%, respectively). The latter suggests partial O_3 reactivity to the facemask and clean shaven facial surface of the subjects, but fails to negate our conclusion that inspiratory route, during moderate and heavy continuous exercise, does not affect acute physiologic responses to O_3.

4. Comparison of Aerobically Trained and Nontrained Young Adult Females' Physiologic Responses to O_3. While there are considerable data available on the response of young adult males to O_3 inhalation during exercise, there is a paucity of such data for females. In this investigation, the effects of 1 h continuous exercise at $\dot{V}_E$ of 23, 34, and 46 $\ell \cdot min^{-1}$, while exposed to FA, 0.2, 0.3, and 0.4 ppm O_3, were studied in a group of ten aerobically trained and 30 normally active, nontrained females. Both groups demonstrated significant pulmonary function impairment and a greater number of reported subjective symptoms in the O_3 exposures compared to FA. There were no statistically significant differences in the responses to O_3 inhalation observed between the trained group and their nontrained counterparts. However, the equivalent $\dot{V}_E$ imposed on both groups was elicited at absolute work loads approximately 10% less for the trained group. Thus, were the nontrained subjects to jog or ride a bicycle at the same submaximal speed as their trained counterparts in a given photochemical smog condition, they would incur a greater $\dot{V}_E$ and hence, a greater O_3 effective dose and acute toxicity response. Both groups of females incurred greater pulmonary function impairment at the O_3 effective dose imposed (~600 ppm·ℓ) than that observed for young adult males. This difference appears to be primarily associated with the mean lung size difference between the sexes, which is approximately 1½ times larger for males (i.e., a smaller lung volume to amount of O_3 inhaled for the females).

5. Comparison of Aerobically Trained and Nontrained Middle-Aged Males' Physiologic Responses to O_3. While the present South Coast Air Quality Management District (SCAQMD) advisory chart states that the elderly should stay indoors and reduce physical activity upon occurrence of a first stage alert (i.e., 0.20 ppm O_3), there are no laboratory studies of older human's response to O_3. In this investigation, we studied the effects of 1 h continuous exercise at $\dot{V}_E$ of both 34 and 46 $\ell \cdot min^{-1}$, while exposed to FA or O_3, in a group of six aerobically trained middle-aged males and 20 nontrained middle-aged males. Both groups demonstrated a trend toward pulmonary function impairment and altered exercise ventilatory pattern when exposed to O_3, but only a few differences were significantly different from FA control. Further, their pulmonary function impairment was slightly less than that incurred by young adult males at similar O_3 effective doses. There were no significant differences between the responses to O_3 inhalation of the trained and nontrained groups. It was observed, however, that the nontrained group would elicit a $\dot{V}_E$ (and thus, O_3 inhaled) approximately 30 percent greater than the trained group if they walked, jogged, or bicycled at the same speed.

6. Endurance Performance and O_3 Toxicity Responses of Competitive Athletes during Low Ambient Level Exposures. Ten highly trained endurance athletes were studied to determine the effects of exposure to low O_3 concentrations on pulmonary function and simulated competitive endurance performance. Each subject was randomly exposed to FA, and to 0.12, 0.18, and 0.24 ppm O_3

while performing a 1 h competitive simulation protocol on a bicycle ergometer. Endurance performance was evaluated by the number of subjects unable to complete rides (last 30 min at an intense work load of ~86% $\dot{V}O_{2max}$) and associated decreases in ride times. Significant decreases were observed following the 0.18 and 0.24 ppm O_3 exposures in forced vital capacity (FVC) (-7.8 and -9.9 percent, respectively), $FEV_{1.0}$ (-5.3 and -10.5 percent, respectively) and competitive simulation ride time (13.4 and 26.0 percent, respectively). All subjects completed the FA exposure, whereas one, five, and seven subjects did not complete the 0.12, 0.18 and 0.24 ppm O_3 exposures, respectively. No significant O_3 effect was observed on exercise oxygen uptake ($\dot{V}O_2$), heart rate (HR), $\dot{V}_E$, respiratory frequency (f_R) or tidal volume (V_T). The number of subjective symptoms reported increased significantly following the 0.18 and 0.24 ppm O_3 protocols. These data demonstrate decrements in pulmonary function and simulated competitive endurance performance following exposure to O_3 concentrations commonly observed in numerous urban environments during the summer months and further supports the hypothesis that endurance performance decrements following O_3 exposures are the result of physiologically induced respiratory discomfort.

7. Combined Effects of O_3 and Ambient Heat on Exercising Females. High ambient levels of O_3 during photochemical smog episodes usually occur in concert with warm to hot ambient temperatures. To study the possible interaction of O_3 and ambient heat, we exposed ten aerobically trained young adult females to FA, 0.15, and 0.30 ppm O_3 in both moderate (24°C) and hot (35°C) ambient conditions while exercising continuously at 66% of $\dot{V}O_{2max}$ for 1 h. Exposure to 0.30 ppm O_3 induced significant impairment in FVC, $FEV_{1.0}$, and other pulmonary function variables. Exercise f_R increased, while V_T and alveolar volume (V_A) decreased with 0.30 ppm O_3 exposure. Significant interactions of O_3 and ambient heat were observed for f_R and V_A, while FVC and $FEV_{1.0}$ displayed a trend toward an O_3 and temperature interaction. Although expired ventilation ($\dot{V}_E$) increased, the interactions could not be ascribed to a greater O_3 effective dose in the 35°C exposures. However, subjective discomfort increased with both O_3 and heat exposure, such that three subjects ceased exercise prematurely when O_3 and ambient heat were combined. We conclude that accentuation of subjective limitations and certain physiological alterations by ambient heat coinciding with photochemical oxidant episodes, is likely to result in more severe impairment of exercise performance, although the mechanisms remain unclear.

8. Physiologic Effects of NO_2 and NO_2 plus O_3 Consequent to Heavy, Sustained Exercise. There have been relatively few laboratory exposures of humans to NO_2 or to NO_2 plus O_3. Further, no investigation has been reported in which prolonged (>60 min), heavy exercise was utilized. We envisioned that greater exercise intensities than previously employed, in combination with exposures of 1 h, might well elicit significant pulmonary function effects at near maximum ambient levels of NO_2 (i.e., ~0.7 ppm). The purpose of the present study was two-fold. (1) To determine the effects, if any, of exposure to 0.6 ppm NO_2 on pulmonary function consequent to heavy, sustained exercise ($\dot{V}_E$ = 70 ℓ/min); and (2) to determine the combined effects of exposure to O_3 (0.3 ppm) and NO_2 (0.6 ppm) on pulmonary function under the same exercise conditions. Preliminary analysis of the complete data set available on five young adult males (a total of 10 will be studied) indicates no appreciable difference in pulmonary function, exercise ventilatory pattern or subjective symptom responses between the 1 h FA and 0.6 ppm NO_2 exposures. Those for

the 0.30 ppm O_3 exposure were substantially different from the responses observed for FA and NO_2, but essentially the same as those for 0.60 ppm NO_2 and 0.30 ppm O_3 in combination. It is suggested that a whole body plethysmograph method be developed to measure airway resistance effects more directly than can be implied from routine pulmonary function tests, in order to identify possible physiological effects incurred upon acute exposure of exercising humans to maximum ambient levels of NO_2. Furthermore, the hypothesis that NO_2 exerts its major effect in the peripheral airways points to the efficacy of developing a measurement of forced oscillatory mechanics of the lung, which may permit separation of airway resistance effects into central and peripheral components.

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CONCLUSIONS

1. In predicting the acute toxic effects of ozone inhalation upon exposure to substantially different concentrations, greater weighting must be given the ozone concentration than either exposure time or minute ventilation volume

2. There is no statistically significant difference in pulmonary function impairment when employing 1 hour continuous exercise and 2 hours intermittent exercise in ozone exposures at the same total ventilation volume and effective dose.

3. Exposure via obligatory mouthpiece inhalation to 0.40 ppm ozone during moderate and heavy exercise does not result in significantly different pulmonary function or subjective symptom responses than those observed in ad-lib (mouth and/or nose) breathing exposures.

4. Nontrained young adult females do not evidence any substantial difference in their response to ozone inhalation at the same ventilation volume and effective dose when compared to aerobically trained females.

5. Young adult females evidence greater physiologic response to ozone at the same effective dose than do their male counterparts. This difference appears to be primarily associated with the mean lung size difference between the sexes, which is approximately 1 and 1/2 times larger for the male.

6. Middle-aged males, whether aerobically trained or nontrained, incur approximately the same toxic response to ozone as do their young adult male counterparts. The possibility remains, however, that more elderly subjects are more sensitive to ozone inhalation at the same effective dose.

7. Because of the extremely high ventilation rates incurred in competitive simulation protocols, endurance athletes exposed to ozone for 1 hour at concentrations of 0.18 ppm experience pulmonary function impairment and performance decrements induced primarily by breathing discomfort.

8. Since ozone toxicity is a function of the total effective dose, those athletes engaged in competition not necessitating high ventilation volumes, will not in general, be adversely affected.

9. Trained young adult females experience impaired work performance due to accentuation of subjective symptoms and certain physiological alterations occurring when ambient heat and ozone exposure coincide, although the mechanisms remain unclear.

10. Exposure for 1 hour to high ambient levels of nitrogen dioxide while engaged in heavy continuous exercise does not induce physiologic responses comparable to those incurred in exposure to photochemical smog alert levels of ozone.

RECOMMENDATIONS

1. Further investigations of subjects exercising continuously at intensities characteristic of increasingly popular aerobic training programs should be conducted at ozone concentrations characteristic of those approaching and including the first stage smog alert level.

2. Since subjective symptoms and alterations in exercise breathing pattern affect one's desire and ability to work and appear to precede other responses to O_3 inhalation, studies should be conducted in which these parameters are carefully monitored at O_3 concentrations and effective doses that fail to elicit statistically significant pulmonary function impairment.

3. A systematic study of the apparent greater sensitivity to O_3 evidenced by young adult females compared to her male counterparts should be effected. Comparative exposures should be at the same O_3 concentration at both the same absolute effective dose and also at a lower effective dose for the female in proportion to her reduced lung size compared to the male's.

4. More apparently healthy elderly individuals should be studied to ascertain if they represent a particularly sensitive sub-population to the acute effects of O_3 inhalation. Prolonged (in excess of 1 hour), low intensity exercise, simulating the activity entailed in walking or playing 18 holes of golf, should be utilized.

5. The role of very high ventilation rates incurred by elite endurance athletes, such as marathon runners and race walkers, should be studied in combination with O_3 exposures at concentrations at or below the current Federal Air Quality Standard (i.e., $\leq$0.12 ppm).

6. Additional study of the causes of premature cessation of work performance in exercising females when exposed to O_3 and ambient heat, not seen in FA exposures to ambient heat alone or in exposures to O_3 alone, is warranted.

7. Study of the time decay of the initial O_3 exposure hypersensitivity effect upon the physiologic effects incurred on reexposure to O_3 within 1 to 5 days, should be undertaken. This has implications for those setting health effects standards, as well as those who conduct laboratory studies in which there are advantages of repeatedly exposing subjects.

ISOLATION OF OZONE CONCENTRATION AS THE PRINCIPAL FACTOR IN THE OZONE DOSE-RESPONSE RELATIONSHIP

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ABSTRACT

The effective dose, expressed as the simple product of ozone (O_3) concentration, exposure duration, and minute ventilation ($\dot{V}_E$), predicts acute toxicity effects better than O_3 concentration alone. However, there is strong evidence of the latter's greater importance, but the means to express this incongruity remains unresolved. In the present study, ten young adult males completed 12 protocols while exposed to three O_3 concentrations (0.20, 0.30, and 0.40 ppm) at two exercise intensities for varied periods (30-100 min). Second-order polynomial regression analysis of O_3 effective dose revealed statistically significant relationships with pulmonary function impairment and altered exercise ventilatory pattern. However, multiple regression analysis improved the prediction of these O_3 toxicity responses substantially, and evidenced the preeminence of O_3 concentration in all variables except respiratory frequency (f_R). Paired t test analyses of equivalent variations in the effects of $\dot{V}_E$ and exposure time (total expired volume) at the same O_3 concentration (and thus, effective dose), revealed no significant differences in pulmonary function impairment. Analysis of percent change in forced expiratory volume in 1 s ($FEV_{1.0}$), as a function of total expired volume for the three O_3 concentrations, revealed significant differences in slopes, but not in intercepts. It was shown that the best fit of predicted $FEV_{1.0}$ decrement was a function described by an exponential expression of O_3 concentration and the product of $\dot{V}_E$ and exposure time. It was suggested that although irritant receptor stimulation is dependent upon O_3 or its reaction products crossing a tissue barrier, once stimulated the dose response relationship for O_3 is similar to the curvilinear dose-response previously described for histamine.

Index terms: air pollution effects; exercise ventilatory pattern; forced expiratory flow rates; forced expiratory volume; ozone dose-response relationships; pulmonary function impairment

Ozone (O_3), a potent zootoxic component of photochemical smog, which occurs in numerous U.S. metropolitan areas at concentrations exceeding the current Federal Air Quality Standard (viz., 0.12 ppm*), was first studied in laboratory exposures of humans at rest to levels rarely if ever encountered in the ambient environment (28). Subsequently, numerous investigators have observed greater pulmonary function impairment in subjects who undertook alternate periods of 15 min of light exercise and rest, as compared to rest alone, while exposed for 2 h to 0.40-0.60 ppm O_3 (4,14,23). In similar 2 h intermittent exercise (IE) exposures, pulmonary function impairment was observed at 0.30 ppm O_3, a level that elicited no effect in resting exposures (15,16,23). Similar effects have been observed with exercise of greater intensity at O_3 concentrations between 0.18 and 0.24 ppm O_3 in 2 h IE exposures (12,20), as well as with continuous exercise (CE) in 1 h exposures (1,2,9).

The potentiating effects of exercise hyperpnea on O_3 toxicity for a given O_3 concentration and exposure time product led Silverman et al (23) to advance the effective dose concept. Subsequently, acute pulmonary function impairment has been shown to evidence a second-order polynomial function in both IE (12) and CE (1) exposures, as well as in endurance athletes consequent to CE training and varied $\dot{V}_E$ warm-up, competitive simulation protocols (2).

Both Silverman et al (23) and Folinsbee et al (12) cautioned that the effective dose, expressed as the simple product of O_3 concentration, minute ventilation ($\dot{V}_E$), and exposure duration, did not account sufficiently for the preeminent role of O_3 concentration. Similarly, we (1) observed that forced

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*All O_3 concentration referenced to buffered potassium iodide have been multiplied by a factor of 0.8 to convert to the ultraviolet photometric standard employed in the present study.

expiratory volume in 1 s ($FEV_{1.0}$) decrements at the same effective dose were substantially greater in exposures to 0.40 ppm than those at 0.20 ppm. Folinsbee and colleagues (12) proposed discarding the effective dose concept, perhaps in favor of a multiple regression approach. Indeed, the multiple R of 0.89 for $FEV_{1.0}$ supports this contention. However, it still depends on a particular data set combination of O_3 concentration, $\dot{V}_E$, and exposure time. If, though exposure time and $\dot{V}_E$ are of near similar importance, compared to O_3 concentration, this approach seems inadequate in that accounting more precisely for appropriately divergent O_3 concentrations, e.g., 0.20 and 0.40 ppm O_3, will reduce still further the variability of predicted O_3 toxicity effects.

The primary purpose of this study was to investigate further the efficacy of the O_3 effective dose concept in exposures varying widely in O_3 concentration, $\dot{V}_E$, and exposure time. Specifically, we sought to determine if the physiological effects of exposure to divergent O_3 concentrations at several similar effective doses could be best predicted by a single second-order polynomial regression, multiple regression analysis, or by a "family" of regression lines particular for each O_3 concentration employed, viz., 0.20, 0.30, and 0.40 ppm.

METHODS

Subject characterization. Ten young adult, Caucasian males, whose basic anthropometry, $\dot{V}O_{2max}$, and pulmonary function data are given in Table 1, served as subjects (Institutional Human Use Committee approval and signed individual informed consent were obtained). Each subject was engaged in an aerobic training program that was not modified significantly in intensity during the course

of the experiment. None of the subjects smoked, and all had pulmonary functions within clinically normal limits (5).

Prior to the experimental protocols, each subject completed an orientation session during which pulmonary function, basic anthropometry, including body composition via hydrostatic weighing, and $\dot{V}O_{2max}$ were measured. $\dot{V}O_{2max}$ was assessed on a Quinton electric bicycle ergometer, Model 844, using a progressive increment protocol described in detail elsewhere (1). In at least one subsequent session, to attenuate habituation effects, each subject completed 60 min of bicycle ergometer riding while breathing filtered air (FA) through the mouthpiece delivery system employed in the experimental protocols.

Experimental design. Subjects completed 12 exposures ranging from 30 to 100 min with one FA control, three at 0.20 ppm O_3, four at 0.30 ppm O_3, and four at 0.40 ppm O_3 (Table 2). Exercise intensity was set to elicit a mean $\dot{V}_E$ of approximately 30 ℓ/min or 70 ℓ/min (BTPS), according to the exposure specification listed in Table 2. The order of exposures was randomized for each subject, with a minimum of 3 days intervening between treatments. Subjects were not informed whether they were receiving O_3, and in order to prevent olfactory detection, 0.30 ppm O_3 was generated for 1-2 min prior to initiating all exposures. Following each exposure, subjects completed a subjective symptoms questionnaire, including indicating whether they had received O_3 and, if so, at what concentration.

All experimental treatments were completed in a room 3.0 x 2.4 x 3.7 m, in which dry bulb temperature and relative humidity were maintained between 21-25°C and 45-60%, respectively. A constant airflow of 2.5 m/s was directed at the subject's anterior surface via an industrial-grade floor fan to facilitate convective and evaporative cooling.

Pulmonary function measurements. A short battery of pulmonary function tests was administered before and within 10 min following the completion of each protocol. Forced vital capacity (FVC) was measured first, followed by residual volume (RV). At least two determinations of FVC were made on a Collins 10-liter Stead-Wells spirometer module (Model 3000). $FEV_{1.0}$ and the forced expiratory flow rate during the middle half of FVC (FEF_{25-75}) were calculated from the spirometric tracings. RV was determined using a modified Collins 9-liter spirometer via the O_2 rebreathing method (26), with initial and equilibrium N_2 readings taken on an Ohio 700 digital N_2 analyzer.

O_3 administration and monitoring. During all experimental exposures, specific air mixtures were inhaled by subjects through a mouthpiece system described in detail elsewhere (9). Briefly, FA blended with the appropriate O_3 concentration generated via a Sander ozonizer (Type II), was supplied to the subject at a slight positive pressure through a Teflon- coated Hans-Rudolph respiratory valve. Expired gas was directed to a Parkinson-Cowan (PC) gas meter (type CD-4), then routed into the distal portion of the mixing chamber and exhausted to the outside air along with the pumped air mixture not inspired by the subject.

A Dasibi O_3 meter was used to determine O_3 concentration by a continuous sampling of air from the inspiratory side of the Hans-Rudolph valve drawn through a 0.64 cm ID Teflon tube. The Dasibi digital reading of O_3 concentration was compared during the course of the experiment with that determined by the ultraviolet absorption photometric method (10) at the University of California, Davis, Primate Center.

Exercise measurements. Pulmonary ventilation was measured continuously on a Hewlett-Packard 7402A recorder via a potentiometer attached to a PC high

speed gas meter. Respiratory metabolism was determined via expired air volume (PC meter), and percent O_2 and CO_2 by a semiautomated sampling method incorporating a manually rotated three-way valve sampling system (27) and Applied Electrochemistry S3-A O_2 and Beckman LB-2 CO_2 gas analyzers. Heart rate (HR) was determined from the elapsed time between four consecutive R waves read from an electrocardiogram tracing taken every 10th min. Respiratory frequency (f_R) was calculated by counting the number of inspiratory plateaus occurring in one minute on the ventilation recording. Tidal volume (V_T) was calculated as a function of $\dot{V}_E$ (BTPS) divided by f_R. Respiratory metabolism and ventilatory pattern measurements were taken every 10th min.

Statistical procedures. Duplicate pulmonary function data were corrected to BTPS and averaged for pre- and postexposure measurements. The treatment effect percent change was calculated as postexposure minus preexposure, divided by the preexposure value, and multiplied by 100. Similarly, values for $\dot{V}_{O_2}$, $\dot{V}_E$, f_R, and V_T during the 10th min of exercise were subtracted from those for the 60th min, divided by the 10th min value, and multiplied by 100. Symptomatological data was reported in terms of both frequency and severity.

Data from the pulmonary function, exercise ventilatory pattern, and subjective symptom measures were analyzed using a two-way ANOVA model for uneven groups. The groups were defined as O_3 concentration and total exposure volume (the simple product of mean $\dot{V}_E$ (BTPS) and time). This model permitted examination of effects attributed to O_3 concentration alone and effects attributed to exposure volume independent of the concentration effect. Upon obtaining a significant F value from ANOVA, Bonferroni corrections were used to test for significant differences between the response variable means at differ-

ent O_3 concentrations. For all statistical analyses, the significance level was set at $P < 0.05$.

In order to facilitate comparing these data with those from other effective dose studies (1,12), both polynomial and multiple linear regression analyses of mean data were performed to determine the relationship between the components of the O_3 effective dose to the percent change in the selected response variables.

RESULTS

Table 3 contains the mean percent change and standard deviation values for pulmonary function, exercise ventilatory pattern and $\dot{V}_{O_2}$ for each exposure. Second-order polynomial regression equations, with O_3 effective dose (pmm·ℓ) as the independent variable predicting the percent changes in both pulmonary function and exercise ventilatory pattern parameters, are given in Table 4. Significant correlations between the predicted and actual responses were obtained for all parameters, indicating a dose-response relationship between the amount of change in a parameter and the inhaled O_3 dose.

The multiple linear regression analysis utilized O_3 concentration, $\dot{V}_E$, and time as predictors of percent changes in the pulmonary function and exercise ventilatory pattern parameters. For all parameters, except f_R, O_3 concentration served as the primary predictor, with $\dot{V}_E$ contributing significantly for all variables except FEF_{25-75}, and with time contributing significantly only for FVC and $FEV_{1.0}$. These regression equations, with multiple R and R^2 values, are given in Table 5. For comparative purposes, Tables 4 and 5 list the squared values for the second-order polynomial and multiple regression correlation coefficients for this study, as well as those from earlier effective dose studies (1,12).

The effects of O_3 concentration and total exposure ventilation volume (expressed as the product of $\dot{V}_E$ and exposure time), as assessed via the two-way ANOVA model on the pulmonary function and exercise ventilatory pattern parameters, revealed significant F ratios. These, and the post hoc analyses with Bonferroni correction of specific significant mean differences across O_3 concentrations for these parameters, are given in Table 6. Similarly, the ANOVA F ratios assessing the total exposure ventilation volume effects are given in Table 7. Since the experimental design did not utilize the same total exposure ventilation volume for each protocol, post hoc tests for exposure volume effects between different O_3 concentrations could not be determined. Hence, only general exposure ventilation volume effects nested within, but independent of, the O_3 concentration effects were analyzed.

Percent change values for FVC, $FEV_{1.0}$, and FEF_{25-75} for the 0.30 O_3 ppm exposure Nos. 5 and 7 (mean effective dose of 710 and 790 ppm·ℓ, respectively) and for the 0.40 ppm exposure Nos. 10 and 11 (mean effective dose of 945 and 885 ppm·ℓ, respectively), were analyzed for significant differences using paired t tests. No significant differences were observed for either the 0.30 ppm or the 0.40 ppm comparisons. Thus, for a given O_3 concentration and a given effective dose achieved through varying $\dot{V}_E$ and time combinations, there were no significant alterations in pulmonary function response.

A summary of the symptomatology response to the treatment protocols is given in Table 8. In general, the total number and severity of symptoms paralleled the effective dose, although exposures to a higher concentration at the same $\dot{V}_E$ (e.g., exposure Nos. 8 and 12 compared to No. 4) elicited an enhanced effect. This parallels the preeminent effect of O_3 concentration on pulmonary function response identified above. What is different in the

subjective symptomatology responses from that of percent change in pulmonary function is the apparent interactive effect of exercise intensity on the former. That is, in all of the exposures at similar effective doses, subjective symptom number and intensity were greater in those entailing the heavier exercise intensity. For example, subjective symptom responses were greater for exposure No. 3 than 6, and for No. 11 than 10. However, if one assumes no symptoms response in FA during prolonged exercise at low work loads, as we have observed previously (1), and subtracts the symptoms observed for the high intensity work load in FA (exposure No. 1) from those for exposures 3 and 11, they then are nearly equivalent to those for exposures 6 and 10, respectively.

DISCUSSION

Several investigators (1,12,23) have observed that pulmonary function impairment is related as a second-order polynomial function to O_3 effective dose (expressed as the simple product of O_3 concentration, $\dot{V}_E$, and exposure duration). However, Silverman et al (23) first noted that exposure to a high concentration for a short period of time produced a greater effect than did a longer exposure to a lower concentration. This divergence from the polynomial regression was later confirmed by Folinsbee et al (12) and Adams et al (1), who noted that the two exposure data points that resulted in less $FEV_{1.0}$ decrement than predicted for a given O_3 effective dose were at 0.20 ppm, while the two which showed greater decrements than predicted were for exposures to 0.40 ppm. This implies that divergent O_3 concentrations produce disparate pulmonary function impairment at a given effective dose.

Via multiple regression analysis of percent change in pulmonary function, Folinsbee and colleagues (12) substantiated the preeminent effect of O_3 con-

centration, relative to $\dot{V}_E$, in their 2 h rest and intermittent exercise (IE) exposures. Subsequently, we (1) confirmed the predominant effect of O_3, relative to $\dot{V}_E$ and exposure duration (30-80 min), in predicting percent impairment in pulmonary function parameters.

Results of polynomial regression analyses of pulmonary function impairment according to O_3 effective dose, as calculated from data obtained in the present study (Table 4), are similar to those observed in earlier studies (1,12). However, as shown in Fig. 1, there are several $FEV_{1.0}$ data points for individual protocols well away from the calculated regression line (broken line with intermittent dots). With one exception, those points notably above the line (less impairment) are for 0.20 ppm exposures, while those notably below the line are for 0.40 ppm exposures. The preeminent role of O_3 concentration, relative to $\dot{V}_E$ and exposure duration in the present study, is underlined by the multiple regression results. That is, the majority of variance in percent change for all pulmonary function parameters (except RV) and V_T, is described by O_3 concentration. The inability to predict pulmonary function impairment at low and high O_3 concentrations, using the polynomial regression model, illustrates the need to isolate the role O_3 concentration plays in acute O_3 toxicity response. To isolate the role of O_3 concentration in FVC and $FEV_{1.0}$ impairment, we regressed the percent change in these parameters against total exposure volume (i.e., the simple product of $\dot{V}_E$ and exposure time) for the 0.20, 0.30, and 0.40 ppm exposures. The intercepts and slopes for each O_3 concentration are shown in Table 9, with the separate regressions for $FEV_{1.0}$ illustrated in Figure 2a. There was no significant difference in intercepts. However, there was a significant difference between the slopes of the 0.20 and 0.40 ppm O_3 lines for both $FEV_{1.0}$ and FVC, as well as a significant difference between the 0.20 and 0.30 ppm lines for FVC.

By plotting the slope of these three regressions as a function of percent change in $FEV_{1.0}$/total exposure volume against O_3 concentration (Figure 2b), it is possible to further define the role of O_3 in pulmonary function impairment in healthy young adult males. The separate points for each concentration in Figure 2b fit an exponential relationship defined in Equation 1.

$$\% \Delta \ FEV_{1.0}/\text{Total Exposure Volume} = -0.0481[O_3]^{1.802} \qquad \text{(Eq. 1)}$$

This type of exponential relationship between percent change in $FEV_{1.0}$ per total exposure volume and O_3 concentration, supports the preeminent role of O_3 concentration identified in the multiple regression analysis. It also clarifies the inability of the effective dose polynomial regression model to accurately predict O_3 toxicity response because of the nonlinear effect of O_3 concentration. Furthermore, a similar exponential relationship was found for FVC and O_3 concentration (Equation 2).

$$\% \Delta \ FVC/\text{Total Exposure Volume} = 0.0324[O_3]^{1.52} \qquad \text{(Eq. 2)}$$

The question can be asked, is it possible to utlilize these derived exponential relationships to better predict O_3 toxicity effects? Equation 3 defines "Effective Dose Prime" (EDP) which incorporates the derived exponential coefficients.

$$EDP = a[O_3]^b \times \dot{V}_E \times T \qquad \text{(Eq. 3)}$$

Regression analysis of EDP versus percent change in $FEV_{1.0}$ shows an improvement in predictability of O_3 toxicity response, as illustrated by a higher R^2 (0.852) than for both the effective dose polynomial regression model (0.670) and that obtained by multiple regression analysis (0.821).

It is important to note, however, that the high correlation coefficients obtained in this study and previous studies (1,12,20) were obtained on mean

data points and that considerable variation exists between individual subjects (1,9,13,15,23) and that this variation increases with increasing O_3 concentration. Examples of the intersubject variability observed in this study are illustrated in Figures 3 and 4, and underlines the continuing inability to determine individual sensitivity and O_3 response within any specific population studied.

The results of the multiple regression analysis provide collective evidence of the predominant effect of O_3 concentration in the prediction of pulmonary function impairment, with $\dot{V}_E$ and exposure time contributing little of the explained variance. The paired t analyses of exposure Nos 5. and 7 (both at 0.30 ppm) and of Nos. 10 and 11 (both at 0.40 ppm) provides evidence that variation in $\dot{V}_E$ and time at a particular concentration does not significantly alter pulmonary function impairment at near equivalent effective doses. For example, in protocol No. 10, exercising at a work intensity necessitating a $\dot{V}_E$ of 31.4 $\ell \cdot min^{-1}$ for 75 min while exposed to 0.40 ppm O_3 (effective dose of 945 ppm·ℓ) elicited percent changes of -10.7, -17.6, and -21.6 for FVC, $FEV_{1.0}$, and FEF_{25-75}, respectively. The corresponding percent change values for protocol No. 11 (30 min at a $\dot{V}_E$ of 73.5 $\ell \cdot min^{-1}$ while exposed to 0.40 ppm O_3; effective dose of 885 ppm·ℓ) were -11.0, -16.6, and -23.8. The t values for these comparisons did not exceed 0.60. Figure 5 presents yet another evaluation of the effect of $\dot{V}_E$ when percent change in $FEV_{1.0}$ is plotted as a function of EDP. Although the slopes are clearly similar, the apparent difference in intercept was not statistically significant, which agrees with the analysis of Colucci (8). Again, the conclusion is that within the limitations of the O_3 concentrations, $\dot{V}_E$, and exposure times utilized in this study, $\dot{V}_E$ and time (i.e., the total ventilation volume), however combined at near

equivalent O_3 effective doses, does not contribute significantly to percent change in pulmonary function when compared to O_3 concentration. Said another way, it is the total ventilation volume, rather than $\dot{V}_E$ or exposure time, per se, that combined with O_3 concentration (preferably as an exponential function), best predicts the degree of pulmonary function impairment.

The present analysis of O_3 concentration and ventilation effects on O_3 toxicity demonstrates a complex relationship between the factors which determine O_3 dose. Furthermore, the observed subject to subject variability accentuates the complex physiological mechanisms involved in individual O_3 toxicity response. The findings of Holtzman et al (17) illustrate an initial sensitization of both bronchial smooth muscle and lung irritant receptors at O_3 doses below the threshold which elicits a significant pulmonary function impairment. Subjective symptom and ventilatory pattern changes observed in the present and previous studies (1,12,20) support the contention that increased O_3 doses result in direct stimulation of irritant receptors (7,23). Furthermore, acute O_3 exposure of cats suggests that O_3 also causes bronchial lesions, especially in peripheral airways (6,25). However, before O_3 has its effect at these two major sites, it must be taken up by the tissues. In their model, McJilton et al (21) propose that the three determining factors in O_3 uptake are: (1) O_3 solubility in the lung tissues; (2) V_T, or the surface area available for diffusion; and (3) f_R, or the rate at which O_3 is replaced at the boundary layer for diffusion. McJilton et al (21) also propose that the high O_3 affinity of organic molecules in the mucosal and submucosal layers of the airways creates a sink for O_3 diffusion. The organic molecules in the mucosal layer and the ability of the tissues to neutralize the harmful products of O_3 reaction present a barrier to O_3 stimulation of irritant

receptors in the submucosal layer of bronchi and bronchioles. Indeed, the observation of McDonnell et al (19) that O_3 sensitivity was poorly correlated ($r = 0.23$) with histamine sensitivity in a group of allergic rhinitis patients, suggests that variability in individual O_3 response is not accounted for by disparate irritant receptor sensitivity (24).

The poor correlation combined with the curvilinear dose response relationship of pulmonary function impairment to histamine (18), suggests that the major determinant of O_3 sensitivity is the ability of the mucosal and submucosal layers to act as a barrier to irritant receptor stimulation. Once stimulated, however, it is possible that a similar curvilinear dose response relationship results in the O_3 concentration effects observed in the present investigation.

At present, little is known of the factors involved between the initial input of inhaled O_3 dose and the final output of pulmonary function impairment in acute O_3 exposure in humans. This discussion points out several areas which require further research before the physiological mechanisms and health risks involved in acute O_3 exposure can be more clearly understood. These areas are: (1) the role of O_3 uptake in determining a true tissue dose; (2) the role lung tissues play in the response, including reactivity of the tissues to O_3, and the mechanisms present to neutralize its harmful effects; and (3) the relative roles of irritant receptor and bronchial smooth muscle stimulation in acute O_3 toxicity.

The polynomial regression comparison of $FEV_{1.0}$ decrement as a function of O_3 effective dose in Fig. 1 illustrates the disparity in response between the subjects in this study and subjects in previous investigations (1,11,12,22).

The issue of whether our CE obligatory mouthpiece inhalation protocols elicit greater effects than those observed in 2 h IE chamber exposures has been addressed earlier (1). In brief, the dashed line from our earlier study (1) compared to the solid line calculated by Folinsbee et al (12) are essentially similar through O_3 effective doses up to 1500 ppm-ℓ. This implies that neither exercise continuity nor the shift from nasal breathing at rest, in light IE, and recovery to primarily oral breathing at heavier work loads affects O_3 toxicity within the range studied. Thus, the difference in response of the subjects in this study compared to our earlier investigation (1) seems best attributed to two factors. First, there were a greater proportion of higher O_3 concentration exposures in this study, and perhaps more important, this group included a greater proportion of sensitive subjects. The latter point is consistent with our recent observations of $FEV_{1.0}$ responses for highly trained cyclists exposed to O_3 concentrations $\leq$0.24 ppm for 1 h (open circles) (22) compared to the notably greater response of similar caliber athletes exposed to 0.21 ppm O_3 by Folinsbee et al (11). Another factor of note is that the subjects in the present study evidenced a reduced FVC and $FEV_{1.0}$ response (-2.6 and 2.8%, respectively) in the high exercise intensity FA exposure, which we have not observed previously.

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TABLE 1. Subjects' anthropometry, pulmonary function, and maximal oxygen uptake data.

Subj. No.	Age, yr	Ht, cm	Wt, kg	Fat, % Body Wt	RV ℓ	FVC ℓ	$FEV_{1.0}$ ℓ/sec	FEF_{25-75} ℓ/sec	$\dot{V}_{O_2max}$, $\ell \cdot min^{-1}$ STPD	$\dot{V}_{Emax}$, $\ell \cdot min^{-1}$ BTPS
1	21	196	82.4	6.9	1.60	6.19	5.11	5.05	4.71	157.2
2	21	178	71.4	6.9	1.75	5.53	5.35	7.48	3.73	141.5
3	21	173	60.9	8.7	1.57	4.86	3.37	2.53	4.09	153.5
4	23	176	74.9	3.1	2.16	5.77	5.36	7.20	4.66	156.4
5	21	178	72.2	12.3	0.98	5.39	4.52	5.08	3.76	150.6
6	18	185	70.6	6.5	1.99	5.70	4.74	5.14	4.20	162.3
7	22	178	71.3	4.8	1.76	5.67	4.38	3.75	3.36	115.5
8	27	187	85.4	14.4	1.24	6.72	4.61	3.28	4.04	131.7
9	23	186	74.0	14.9	1.47	5.74	4.00	2.82	4.22	147.8
10	28	183	70.6	13.0	1.59	5.74	4.54	5.03	4.03	156.9
Mean	22.5	182	73.4	9.15	1.61	5.73	4.60	4.74	4.08	147.3
S.D.	±3	± 6.8	± 6.8	±4.2	±0.34	±0.48	±0.61	±1.69	±0.41	±14.3

RV, residual volume; FVC, forced vital capacity; $FEV_{1.0}$, expiratory volume in 1 s; FEF_{25-75}, forced expiratory flow rate during middle half of FVC; $\dot{V}_{O_2max}$, maximal oxygen uptake; $\dot{V}_{Emax}$, maximal minute ventilation.

TABLE 2. Description of experimental protocols.

Exposure No.	Air* Mixture	Exercise, $\dot{V}_E$* ℓ·min-1 BTPS	Exposure Time min	Effective Dose,* ppm·ℓ
1	FA	73.0	60	0
2	0.205	73.4	45	675
3	0.202	73.9	60	895
4	0.204	71.3	75	1090
5	0.304	31.1	75	710
6	0.301	30.6	100	920
7	0.305	74.2	35	790
8	0.304	72.7	50	1105
9	0.404	31.8	50	640
10	0.402	31.4	75	945
11	0.402	73.5	30	885
12	0.402	72.1	38	1100

*Values are means for actual observations.

TABLE 3. Summary of mean percent change in pulmonary function, exercise ventilatory pattern and $\dot{V}_{O_2}$ for the twelve exposures.

Exposure	FVC	$FEV_{1.0}$	FEF_{25-75}	$\dot{V}_E$	f_R	V_T	$\dot{V}_{O_2}$
1	-2.60 (±2.58)	-2.78 (±5.26)	-1.30 (±11.8)	+4.18 (±4.44)	+11.80 (±10.1)	-6.3 (±6.57)	+6.01 (±3.93)
2	-5.78 (±6.57)	-6.33 (±7.97)	-3.68 (±12.6)	+6.38 (±7.18)	+20.10 (±10.3)	-11.18 (±4.78)	+5.33 (±4.48)
3	-8.64 (±8.19)	-10.77 (±9.92)	-6.79 (±20.0)	+4.28 (±8.81)	+26.08 (±18.2)	-16.54 (±8.61)	+4.52 (±8.19)
4	-10.42 (±8.00)	-13.27 (±7.26)	-11.65 (±17.9)	+9.68 (±8.54)	+37.23 (±16.4)	-19.98 (±5.27)	+9.68 (±7.40)
5	-5.93 (±6.51)	-9.70 (±12.1)	-14.89 (±21.4)	-2.41 (±8.10)	+8.09 (±14.8)	-8.75 (±9.54)	+2.72 (±6.52)
6	-8.29 (±5.76)	-8.82 (±11.0)	-18.88 (±13.2)	+0.75 (±10.7)	+8.93 (±24.0)	-4.19 (±12.7)	+5.71 (±9.12)
7	-8.75 (±10.2)	-13.85 (±12.5)	-19.34 (±19.6)	+8.58 (±5.58)	+26.50 (±13.0)	-13.46 (±9.18)	+5.04 (±6.30)
8	-14.97 (±9.63)	-17.96 (±10.6)	-20.07 (±19.8)	+8.77 (±6.77)	+35.88 (±8.73)	-20.01 (±4.03)	+6.95 (±6.58)
9	-6.31 (±3.51)	-9.20 (±4.87)	-13.52 (±11.0)	+1.40 (±6.28)	+15.01 (±11.65)	-11.21 (±8.57)	+1.43 (±7.57)
10	-10.96 (±11.9)	-16.63 (±14.2)	-23.85 (±19.6)	+8.05 (±8.95)	+31.95 (±23.4)	-16.53 (±10.9)	+9.83 (±9.52)
11	-10.69 (±7.27)	-17.58 (±8.97)	-21.56 (±18.1)	-0.93 (±5.66)	+19.63 (±11.8)	-16.66 (±6.86)	+4.64 (±5.67)
12	-16.93 (±11.5)	-22.47 (±14.3)	-25.44 (±25.1)	+3.12 (±6.82)	+34.26 (±15.2)	-22.7 (±6.6)	+3.82 (±4.74)

Values are mean percent changes; values in parentheses are ± 1 standard deviation. FVC, forced vital capacity (liters); $FEV_{1.0}$, forced expiratory volume in 1.0 s (liters); FEF_{25-75} forced expiratory flow during middle half of FVC (liters·s^{-1}); $\dot{V}_E$, minute ventilation (liters·min^{-1}); f_R, respiratory frequency (breaths·min^{-1}); V_T, tidal volume (liters); $\dot{V}_{O_2}$, oxygen consumption (liters·min^{-1}).

TABLE 4. Polynomial regression analysis predicting the percent change in pulmonary function and exercise ventilatory pattern parameters from O_3 effective dose with comparison of r^2 values from earlier studies.

Parameter	Equation	r^2	S.E. of Regression Coefficients		r^2 Folinsbee et al (1978)	r^2 Adams et al (1981)
FVC	$Y=0.123x10^{-4}(x^2)+0.351x10^{-2}(x)-2.67$	0.809	$\pm 0.49x10^{-5}$	± 0.006	0.884	0.672
$FEV_{1.0}$	$Y=0.119x10^{-4}(x^2)-0.118x10^{-2}(x)-2.63$	0.670	$\pm 0.94x10^{-5}$	± 0.0115	0.922	0.681
FEF_{25-75}	$Y=0.137x10^{-5}(x^2)-0.0172(x)+0.54$	0.433	$\pm 0.184x10^{-4}$	± 0.0255	0.922	0.582
f_R	$Y=0.398x10^{-4}(x^2)-0.023(x)+12.17$	0.626	$+0.182x10^{-4}$	± 0.0223	0.722	0.712
V_T	$Y=-0.173x10^{-4}(x^2)+0.68x10^{-2}(x)-6.60$	0.603	$\pm 0.103X10^{-4}$	± 0.0125	0.504	N/A

x is effective dose in ppm·ℓ, and Y is percent change in the parameter; other abbreviations are the same as in Table 3.

TABLE 5. Multiple regression analysis predicting the percent change in pulmonary function and exercise ventilatory pattern parameters from $[O_3]$, $\dot{V}_E$, and time with comparison of R^2 values from earlier studies.

Parameter	Equation	Multiple R	Multiple R^2	S.E. of Estimate	Multiple R^2 Folinsbee et al (1978)	Multiple R^2 Adams et al (1981)
FVC	$Y=-39.47(O_3)-0.227(\dot{V}_E)-0.219(T)+23.13$	0.906	0.821	$\pm$1.96	0.746	0.653
$FEV_{1.0}$	$Y=-57.19(O_3)-0.207(\dot{V}_E)-0.130(T)+26.43$	0.924	0.853	$\pm$2.61	0.799	0.640
FEF_{25-75}	$Y=-73.81(O_3)-0.258(\dot{V}_E)-0.089(T)+19.43$	0.889	0.790	$\pm$4.65	0.655	0.604
f_R	$Y= 74.52(O_3)+0.644(\dot{V}_E)+0.350(T)-56.18$	0.757	0.573	$\pm$8.09	0.642	0.733
V_T	$Y=-44.90(O_3)-0.309(\dot{V}_E)-0.165(T)+24.10$	0.829	0.687	$\pm$3.79	NA	NA

T is time of exposure. Other abbreviations are the same as those in Table 3.

TABLE 6. F ratios and specific significant mean differences from post hoc analyses for pulmonary function and ventilatory pattern parameters: $[O_3]$ effects.

Variable	F ratio	Specific Significant Mean Differences*
FVC	7.18*	FA-0.2; FA-0.3; FA-0.4
$FEV_{1.0}$	8.46*	FA-0.2; FA-0.3; FA-0.4; 0.2-0.4; 0.3-0.4
FEF_{25-75}	9.71*	FA-0.3; FA-0.4; 0.2-0.3; 0.2-0.4
f_R	6.18*	FA-0.2; FA-0.4
V_T	9.85*	FA-0.2; FA-0.4; 0.3-0.4

*Significant at $P < 0.05$. Abbreviations are the same as those in Table 3.

TABLE 7. F ratios for pulmonary function and ventilatory pattern parameters: exposure ventilation volume effects.

Variable	F ratio
FVC	7.64*
$FEV_{1.0}$	5.21*
FEF_{25-75}	1.25
f_R	7.75*
V_T	6.63*

*Significant at $P < 0.05$. Abbreviations are the same as those in Table 3.

TABLE 8. Summary of mean symptom number and severity score (graded from 1 = just detectable to 5 = very severe) data for each experimental protocol.

Protocol No.	Mean No. of Reported Symptoms	Mean Symptom Severity Score	No. Subj. Believing They Received O_3
1	1.0	1.6	2
2	2.8	4.4	7
3	2.5	3.7	7
4	2.6	4.5	8
5	1.7	2.4	5
6	1.6	1.9	6
7	2.7	4.6	9
8	4.2	8.7	10
9	2.5	4.6	7
10	2.4	4.1	8
11	3.7	6.7	10
12	4.3	9.3	10

TABLE 9. Slopes and intercepts for the regression of percent change in FVC and $FEV_{1.0}$ against total exposure volume at three O_3 concentrations.

	0.20 ppm		0.30 ppm		0.40 ppm	
	Slope	Intercept	Slope	Intercept	Slope	Intercept
FVC	-0.003	4.505	-0.005	5.53	-0.0083	7.60
$FEV_{1.0}$	-0.003	3.11	-0.0045	0.60	-0.011	6.67

FVC, forced vital capacity; $FEV_{1.0}$, forced expiratory volume in 1 s.

FIGURE LEGENDS

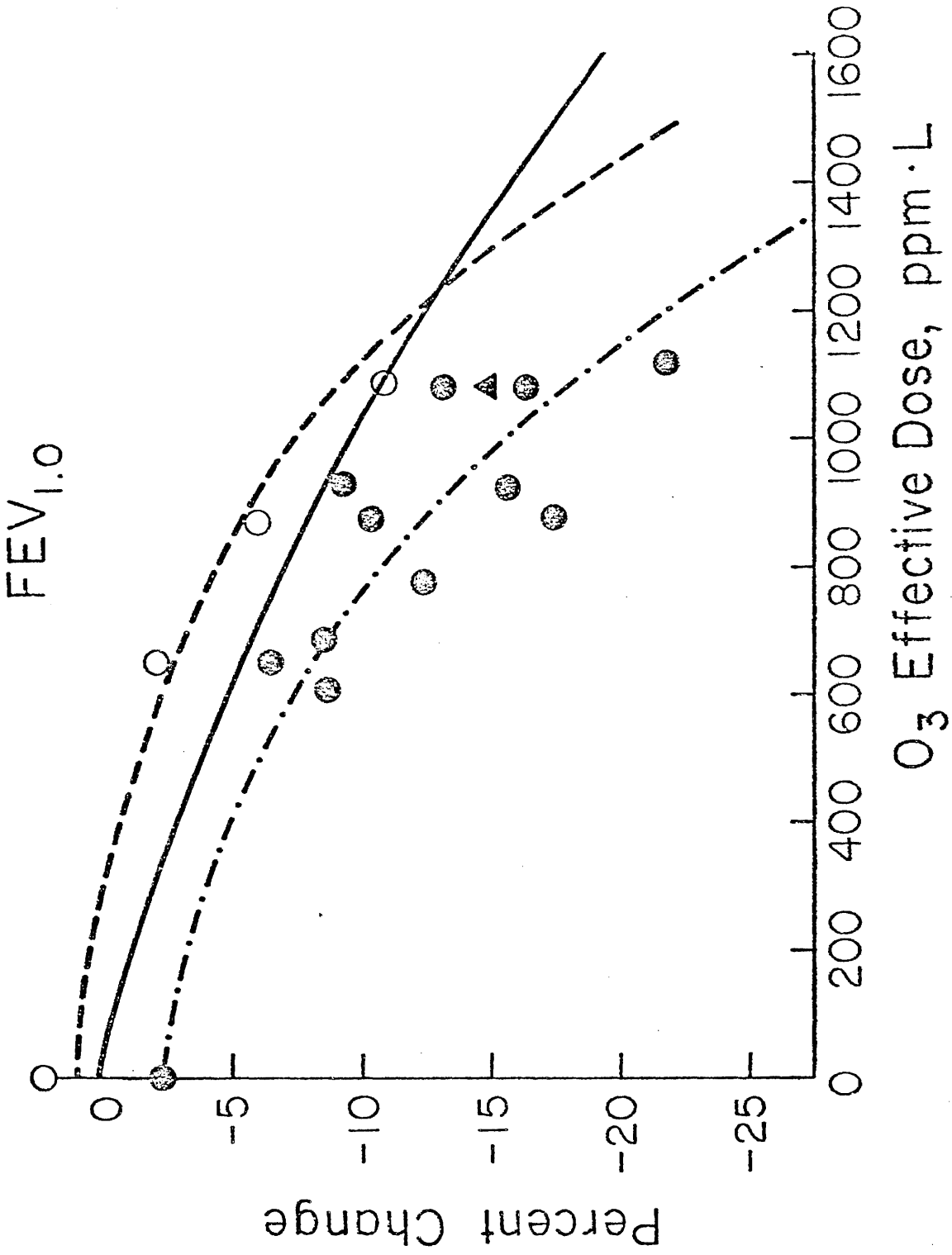
Fig. 1 Comparison of percent change in forced expiratory volume in 1 s ($FEV_{1.0}$) as a function of O_3 effective dose (ppm·ℓ) in the present study (dashed line with intermittent dots) with that in a previous study (dashed line) (1), with that observed in 2-h intermittent exercise protocols (solid line) (12), and for highly trained cyclists consequent to heavy continuous exercise (▲) (11) and competitive simulation protocols (0) (22).

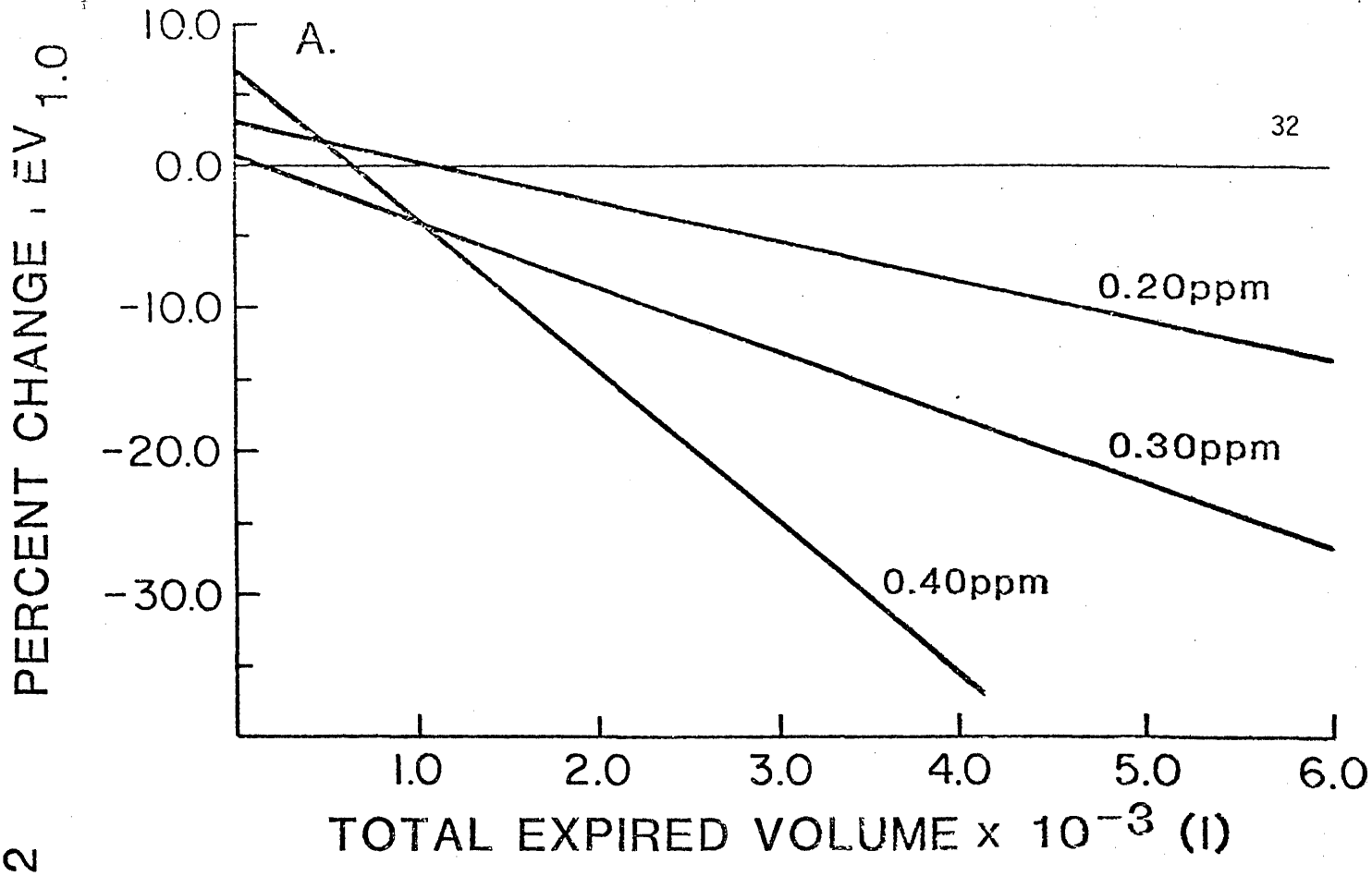
Fig. 2 (A) Comparison of percent change in forced expiratory volume in 1 s ($FEV_{1.0}$) as a function of total expired volume (ℓ x 10^{-3}) for 0.20, 0.30, and 0.40 ppm O_3 exposures. (B) Relationship between percent change in $FEV_{1.0}$ per total volume (x 10^2) as a function of O_3 concentration (ppm).

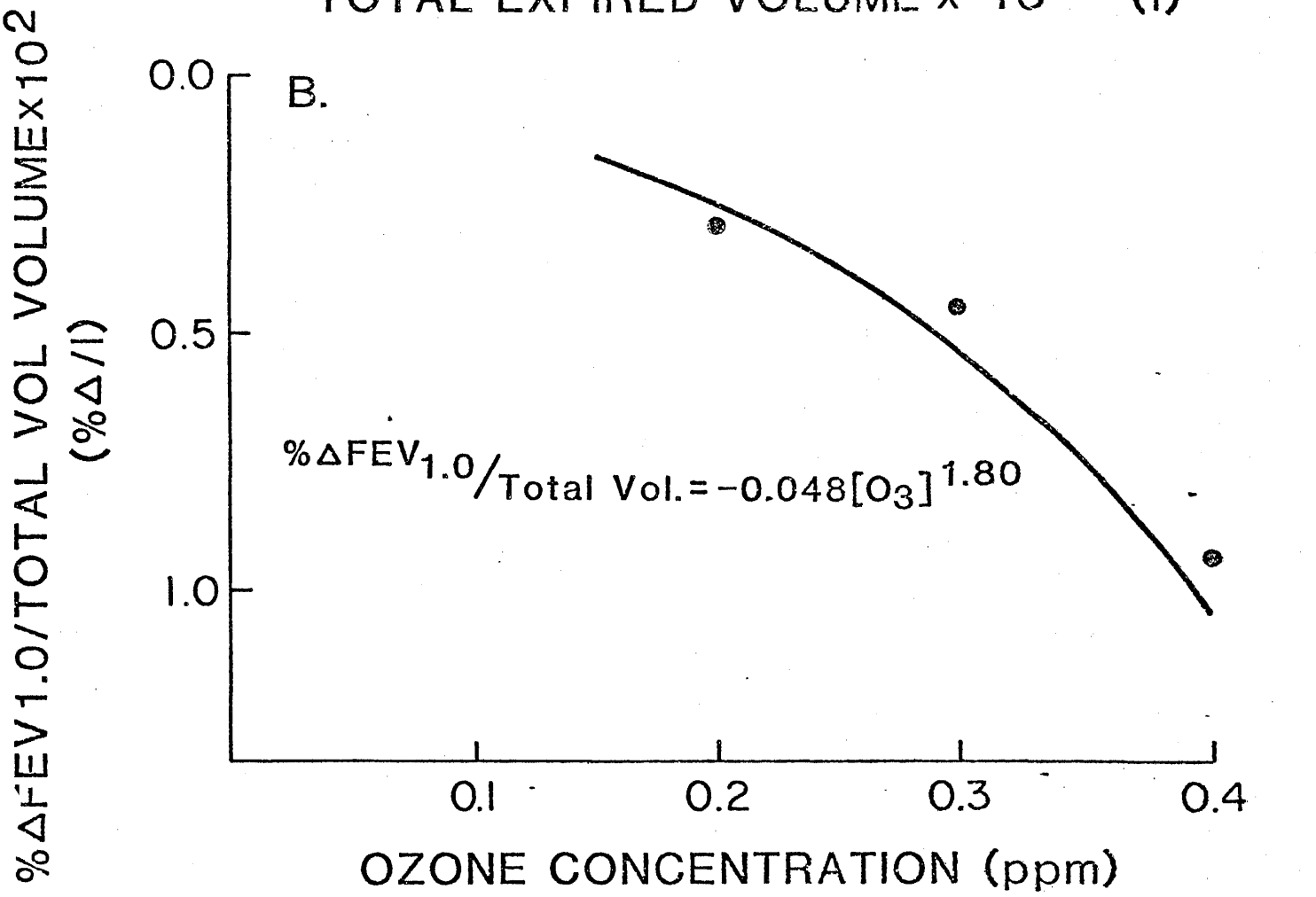
Fig. 3 Comparison of the standard deviations of the percent change in forced expiratory volume in 1 s ($FEV_{1.0}$) as a function of O_3 concentration.

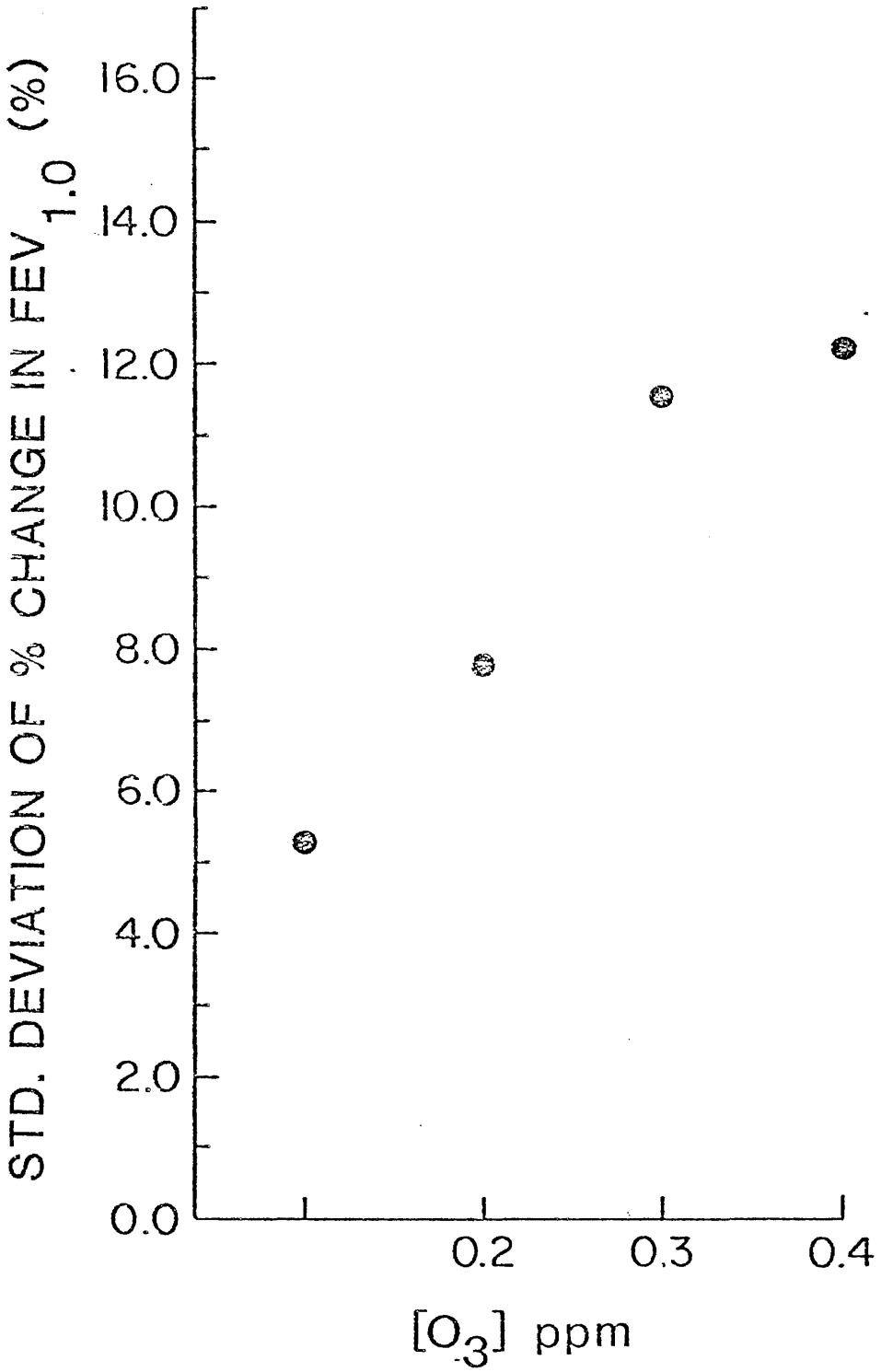
Fig. 4 Individual subject and mean regression lines for percent change in forced expiratory volume in 1 s ($FEV_{1.0}$) as a function of total expired volume (ℓ) for the 0.40 ppm O_3 exposures. Numbers on the right identify each subject's regression line.

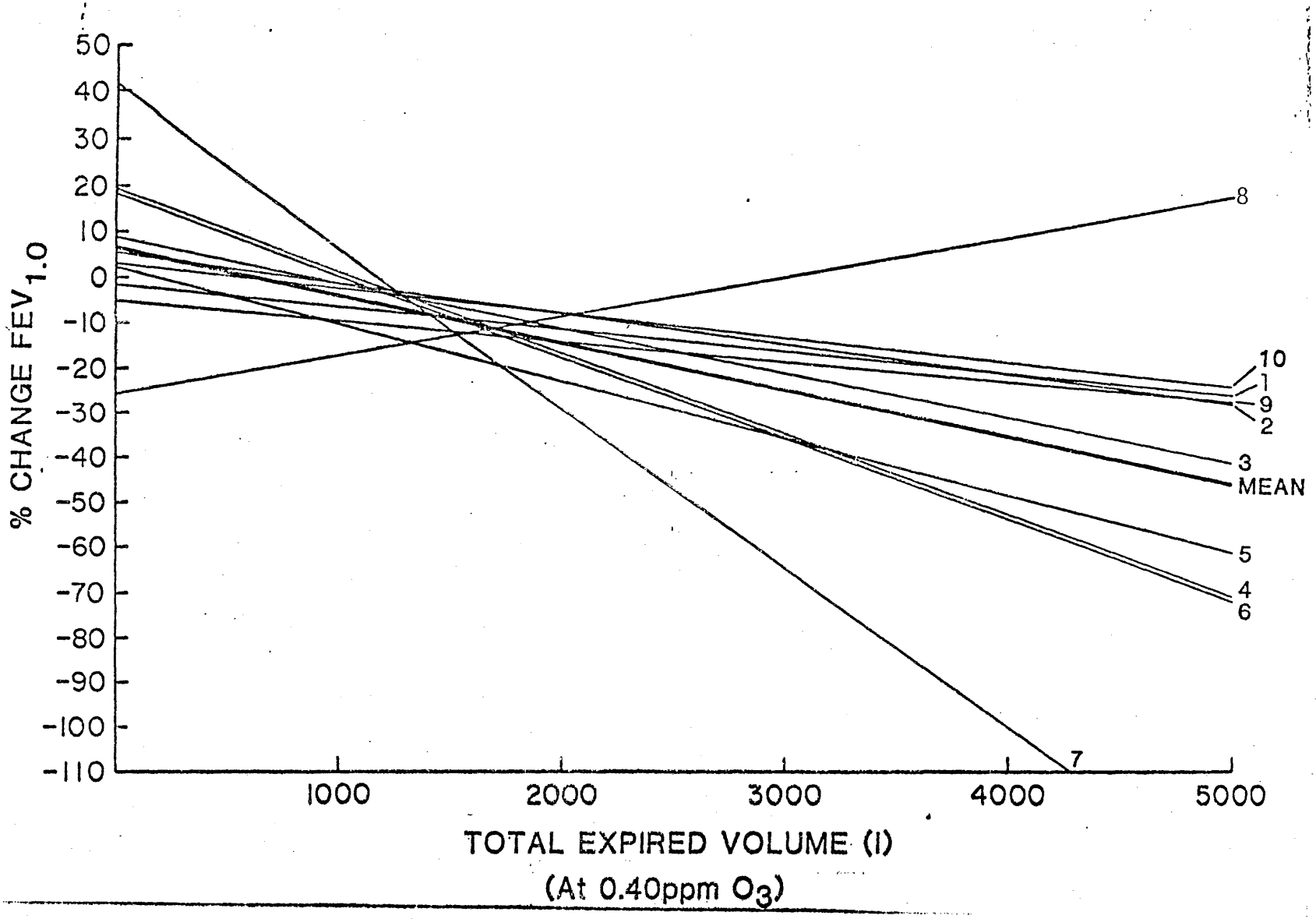
Fig. 5 Comparison of percent change in forced expiratory volume in 1 s ($FEV_{1.0}$) as a function of "effective dose prime" (EDP) for the 31 and 73 ℓ/min protocols.

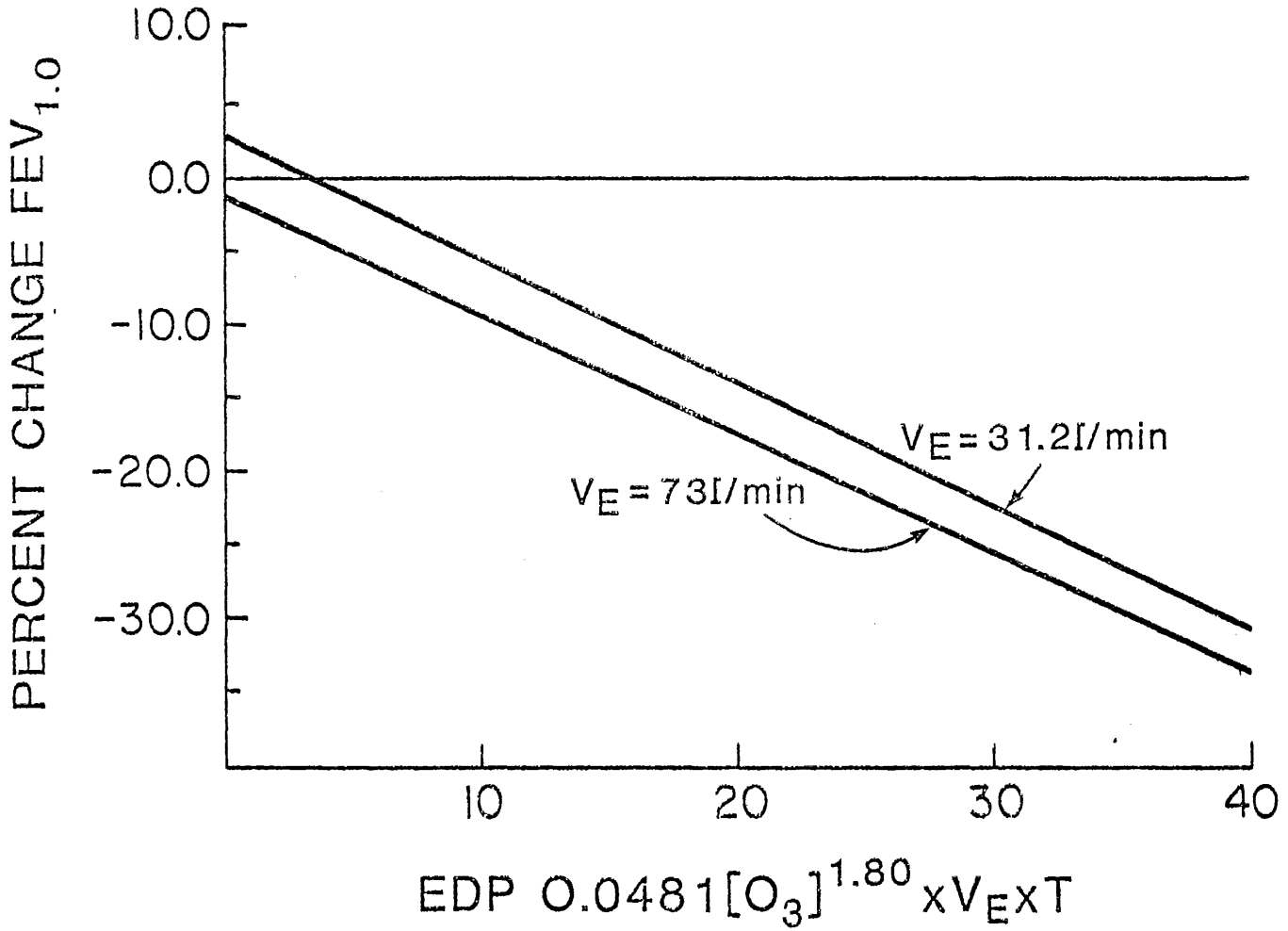

FEV1.0
0
Percent Change
0
-5
-10
-15
-20
-25
0
200
400
600
800
1000
1200
1400
1600
O3 Effective Dose, ppm·L


A.
10.0
0.0
-10.0
-20.0
-30.0
PERCENT CHANGE , $\dot{E}V_{1.0}$
0.20ppm
0.30ppm
0.40ppm
1.0
2.0
3.0
4.0
5.0
6.0
TOTAL EXPIRED VOLUME x 10^{-3} (l)


B.
0.0
0.5
1.0
%ΔFEV1.0/TOTAL VOL VOLUMEx102
(%Δ/l)
$\%\Delta FEV_{1.0}/\text{Total Vol.} = -0.048[O_3]^{1.80}$
0.1
0.2
0.3
0.4
OZONE CONCENTRATION (ppm)


STD. DEVIATION OF % CHANGE IN $FEV_{1.0}$ (%)
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0.2
0.3
0.4
$[O_3]$ ppm


50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
% CHANGE FEV1.0
1000
2000
3000
4000
5000
TOTAL EXPIRED VOLUME (l)
(At 0.40ppm O3)
8
10
1
9
2
3
MEAN
5
4
6
7


PERCENT CHANGE FEV1.0
10.0
0.0
-10.0
-20.0
-30.0
VE = 31.2l/min
VE = 73l/min
10
20
30
40
EDP 0.0481[O3]1.80 x VE x T

EFFECTS OF EXERCISE CONTINUITY AT THE SAME MEAN VENTILATION ON ACUTE OZONE TOXICITY

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ABSTRACT

Both continuous exercise (CE) and intermittent exercise (IE) laboratory exposures to ozone (O_3) have been utilized to study acute toxicity effects of this principal constituent of photochemical smog. While there is suggestive evidence that impairment in standard pulmonary function tests consequent to CE and IE exposures to O_3 at the same effective dose (i.e., the product of O_3 concentration, mean minute ventilation, and exposure duration) is similar, no direct comparison has been made. In the present study, six aerobically trained young adult male subjects were each exposed by mouthpiece inhalation to 0.4 parts per million (ppm) O_3 during bouts of exercise that were either 1 h CE, or 2 h IE, but matched for total ventilation and effective dose. These matched pairs of CE and IE protocols were performed at two levels of total ventilation (2200 liters and 3600 liters). Statistical analysis revealed no significant differences in pulmonary function impairment between the 1 h CE and the 2 h IE protocols. However, alterations in exercise ventilatory pattern were more pronounced during the 1 h CE protocols at both levels of total ventilation. While it seems likely that the greater change in exercise ventilatory pattern in CE was an artifact of "metabolic creep" from the "initial" tenth minute to the 60th minute (compared to the change in the average of the tenth and 15th minute values of the first IE bout to that of the fourth IE bout), there were also a significantly greater number of subjective symptoms reported following CE. Although it appears that O_3 toxicity at a given effective dose may be enhanced by 1 h CE protocols compared to 2 h IE exposure, despite non-significant differences in pulmonary function change between the two exercise modes, only paired filtered air exposures can elucidate this apparent anomaly. The value of subjective symptom reports, and the need to collect such data in a careful and systematic manner is emphasized.

INTRODUCTION

The degree of acute human toxicity effected by ozone (O_3), a major component of photochemical smog, varies with the level of ventilation during exposure. For example, a 2 h exposure to 0.30 ppm* O_3 had no effect on the pulmonary function of resting subjects (7,8), but resulted in pulmonary function decrements when the level of ventilation during exposure was increased via intermittent periods of light exercise (IE) (12). Similarly, in continuous exercise (CE), DeLucia & Adams (3) observed that 1 h exposure to 0.24 ppm O_3 induced significant pulmonary function impairment when subjects exercised at moderate to heavy workloads, while no effects were elicited when subjects were exposed while at rest. The role that ventilation plays in O_3 toxicity by increasing the total amount of O_3 inhaled is recognized in the effective dose concept, which relates pulmonary impairment induced by O_3 to the simple product of O_3 concentration, time of exposure, and average minute ventilation ($\dot{V}_E$) during exposure (1,5).

The pattern of ventilation induced during laboratory exposure to O_3 has varied. The two most common patterns have been 2 h exposures with light to moderate IE (5), and more recently, 1 h exposures to heavier CE (1-3). Both are valid means of increasing the mean $\dot{V}_E$ in the laboratory, and both ventilatory patterns occur in daily life: the intermittent pattern occurs in light activities such as golfing, gardening, and some forms of occupational work,

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*All O_3 concentrations referenced to buffered potassium iodide have been multiplied by a factor of 0.8 to convert to the ultraviolet photometric standard employed in the present study.

while continuous aerobic exercise has a central place in the recreational fitness programs of an increasing number of individuals, as well as the predominant training regimen of many competitive endurance athletes.

To what extent do the differences in these two commonly encountered ventilatory patterns affect the description of O_3 toxicity in the laboratory, and the subject experiences of the public? Adams et al. (1) compared the relation of effective dose of O_3 to decrement in $FEV_{1.0}$, and observed that their subjects, who performed CE for periods of 30 to 80 min, demonstrated similar impairments as that found in a group of subjects exposed to O_3 during 2 h IE protocols (5). This suggests that differences in the ventilatory pattern used to achieve a given effective dose were not of major importance. However, because this comparison was made across different groups of individuals studied in different laboratories using dissimilar exposure techniques, it cannot be regarded as conclusive.

The purpose of the present investigation, therefore, is to compare the O_3 toxicity induced by the same effective dose using two different patterns of exercise and ventilation, viz., IE of 2 h duration versus CE lasting 1 h. Comparing these two frequently employed ventilatory patterns across the same sample of subjects, using the same procedures and equipment, should provide a definitive description of any differences in O_3 toxicity.

METHODOLOGY

Subject characterization. Six Caucasian males, whose basic anthropometry and baseline pulmonary function measurements are given in Table 1, served as subjects (Approval from the Institutional Human Use Committee and written informed consent were obtained.). All subjects (non-smokers) were students or faculty familiar with exercise protocols and pulmonary function procedures.

Each subject completed an orientation session to acquaint them with the specific equipment and requirements of this study.

Fig. 1

Design. The experimental design is depicted in Figure 1. As the purpose of the investigation was to compare the effects of the same effective dose obtained via 2 h of IE or 1 h of CE, the same O_3 concentration (0.40 ppm) and total absolute ventilation (i.e., the product of $\dot{V}_E$ and time) were delivered in each of these two formats. Furthermore, the comparison of 2 h IE versus 1 h CE was made at 2 levels of absolute ventilation to determine if level of ventilation affected O_3 toxicity.

Thus, at the low level of total ventilation, cell 1 (1 h CE, $\dot{V}_E$ = 35.5 ℓ/min, total ventilation = 2130 ℓ) was contrasted with cell 2 (2 h IE, $\dot{V}_E$ ex = 25.6 ℓ/min, $\dot{V}_E$ rest = 10 ℓ/min, total ventilation = 2140 ℓ). Similarly, at the high level of total ventilation, cell 3 (1 h CE, $\dot{V}_E$ = 60.3 ℓ/min, total ventilation = 3620 ℓ) was compared to cell 4 (2 h IE, $\dot{V}_E$ ex = 51 ℓ/min, $\dot{V}_E$ rest = 10 ℓ/min, total ventilation = 3660 ℓ).

Protocols. All protocols for a given subject were performed at the same time of day. The O_3 generation and monitoring system employed has been detailed elsewhere (1,3). A battery of pulmonary function tests, including forced vital capacity (FVC), flow rates from maximal forced expiratory maneuvers, and residual volume (RV), were performed immediately before and after each O_3 exposure. CE protocols consisted of 1 h exposures while exercising on a cycle ergometer, with measurements (detailed later) made at 10 min intervals. Ventilation was recorded continuously. IE protocols consisted of 2 h of four each alternating 15 min periods of rest and exercise. During IE, ventilation was recorded continuously, and exercise measurements were made in the tenth and fifteenth minutes of each exercise bout.

Measurements. Pulmonary function tests were performed on a Collins Basic Clinical Spirometer Module, No. 3000. Data regarding subjects' symptoms were collected on a questionnaire within 12 minutes of completing any exposure. Ventilation was monitored continuously during all protocols, and total ventilation and average $\dot{V}_E$ for any exposure were calculated from this complete record. The data collected during exercise in the previously specified minutes were: oxygen consumption ($\dot{V}_{O_2}$), heart rate, breathing frequency (f_R), and tidal volume (V_T). Details of procedures used in obtaining these measurements have been described earlier (1).

Statistical procedures. The data were analyzed using an analysis of variance (ANOVA) designed to accommodate repeated measurements made on a single group of subjects (Biomedical Computer Programs, 2V). Using a repeated measures design also takes intersubject variability into account by comparing each individual to himself through the cells of the design. A "case", therefore, consisted of a single subject's four responses (e.g., the pre- to postexposure change in FVC) to the four experimental treatments. The absolute pre- to postexposure change in all pulmonary function data was analyzed in this way, as were the number of subjective symptoms reported after each exposure. The change in exercise measurements from early in the exposure to the end of the exposure was also analyzed in this manner, utilizing the change from the tenth to the last minute in CE protocols, while in IE the change between the average of the tenth and fifteenth minute values in the first exercise bout and the corresponding average from the last exercise bout was used.

RESULTS

Design validity. In order for the comparison of the effects of the two ventilatory patterns on O_3 toxicity to be valid, the total ventilation

achieved must be equivalent, i.e., the same effective dose must be delivered using either pattern. Total ventilation volume was 2118 and 2166 liters for the lower CE and IE intensities, while those for the higher intensities were 3609 and 3669 liters for CE and IE, respectively. Statistical analysis of CE and IE at the two work intensities confirmed the validity of the design, in that there was no statistical difference in total ventilation (and therefore effective dose) between the 2 h IE and the 1 h CE protocols.

Table 2

Pulmonary function. Table 2 contains a summary of the means and standard deviations of pulmonary function changes experienced at the two levels of ventilation for both the 2 h IE and 1 h CE protocols. FVC, $FEV_{1.0}$, and peak flow (PF) decreased to a greater degree at the higher level of ventilation, (i.e., greater O_3 effective dose), as expected. However, there were no significant differences in pulmonary function change between the two ventilatory patterns employed.

Fig. 2

Exercise measurements. Percent changes in f_R and V_T are depicted in Figure 2. The increase in f_R during exercise from the "initial" to the end of any exposure was significantly greater during the 1 h CE protocols than during the 2 h IE protocols ($p < .042$). A trend toward a greater drop in V_T from early to late exercise was also noted in the 1 h CE protocol ($p < .068$).

Fig. 3

Subjective symptoms. As indicated in Figure 3, subjects consistently reported a significantly greater number of symptoms after the 1 h CE exposure compared to the 2 h IE exposure ($p < .016$). The changes in severity of these symptoms were not analyzed, as it was felt that neither the questionnnaire nor the orientation session provided sufficient instructions regarding the ranking of symptom severity in a consistent manner.

Variation in total ventilation (~2150 vs ~3650 liters) in the comparison of pulmonary function, exercise, and subjective symptom measurements effects elicited by the 1 h CE and 2 h IE was examined to determine if this factor might influence the effect of exercise continuity on O_3 toxicity. No significant interactions of this type were revealed by ANOVA.

DISCUSSION

The purpose of this study was to compare the effect on O_3 toxicity of two ventilatory patterns which occur in everyday life and which have been frequently employed in laboratory investigations, viz., 2 h of IE and 1 h of CE. At equivalent total ventilation (and thus total effective dose), the results indicate that pulmonary function impairment incurred at a given effective dose was no different in the two patterns of ventilation.

There are few studies in which the effect of exercise continuity at the same total $\dot{V}_E$ (and thus, effective dose) on O_3 toxicity has been addressed. We reported in 1981 (1) that the decrements in $FEV_{1.0}$ observed in CE exposures of 30-80 min, as a function of O_3 effective dose, were similar to those observed by Folinsbee et al. (5) in IE exposures of 2 h duration. This inter-laboratory comparison, however, may be compromised by the method employed for O_3 inhalation, i.e., obligatory mouthpiece (1) and ad-lib chamber exposure (5), since previous work with dogs (13) indicates that O_3 uptake is higher when O_3 is administered orally rather than nasally. Thus, comparisons utilizing similar O_3 exposure procedures are potentially more definitive.

Recently, Horvath (9) has observed that, although not completely documented, their chamber exposure procedure appears to induce equivalent effects on pulmonary function in CE (6) and IE, provided that the products of O_3 concentration, $\dot{V}_E$, and duration of exercise (i.e., the effective dose) are taken

into account. We have also reported equivalent pulmonary function impairment upon 1 h exposure to 0.20 and 0.35 ppm O_3 when exercise continuity was varied (2). Figure 4 depicts the warm-up/competitive simulation, which elicited a mean $\dot{V}_E$ of 77.4 $\ell \cdot min^{-1}$, and the mean $\dot{V}_E$ for 1 h of CE (77.5 $\ell \cdot min^{-1}$). Even though the mean ventilatory pattern for the latter (f_R = 34.4 breaths$\cdot min^{-1}$; V_T = 2.25 ℓ) differed considerably from that for the warm-up/competitive simulation protocol (f_R = 20 breaths$\cdot min^{-1}$ and V_T = 1.1 ℓ during 7 min of rest immediately prior to 30 min of near exhaustive CE; f_R = 42 breaths$\cdot min^{-1}$ and V_T = 2.53 ℓ), there were no significant differences in pulmonary function impairment or in symptoms elicited.

Fig. 4

In the present study, however, a significantly greater number of symptoms were reported following 1 h CE than after 2 h IE protocols (Fig. 2). This observation does not appear to be simply the result of a summation of O_3 symptoms due to the slightly higher workloads used during CE. If this were the case, one would expect the symptomatic data to show a statistical interaction between level of ventilation (i.e., workload) and the type of ventilatory pattern. Such an interaction was not observed. Further, the nature of symptoms reported (e.g., pain or tightness on inspiration, cough, etc.) suggest O_3 toxicity, rather than symptoms of prolonged exercise. Tnus, there is suggestive evidence that the 1 h CE protocols elicited greated O_3 toxicity than did the 2 h IE protocols at the same effective dose, although no significant differences were observed in any pulmonary function parameter. Discrepancies between pulmonary function changes, and subjective symptoms and exercise data as indices of O_3 toxicity have been noted previously by Savin and Adams (11). In their study, no significant changes in pulmonary function occurred following graded exercise, up to a maximum work capacity, in the

presence of O_3. However, maximum $\dot{V}_E$ was reduced in a dose dependent fashion, and symptomatic responses were also present and dose related, which suggests that they may be more sensitive indicators of O_3 toxicity than standard pulmonary function tests. Such findings accent the need for careful and systematic collection of data on both the number and relative intensity of subjective symptoms in order to detect early stages of O_3 toxicity. A prerequisite to such data collection is to provide subjects with adequate instruction and explicit questionnaires to insure greater accuracy and reliability of subjective impressions.

The change in exercise breathing pattern through time (increased f_R and decreased V_T) was accentuated during CE. Although such changes sometimes accompany CE in the absence of O_3 (4), they appear dependent on thermal balance (10). In this study, however, the method of calculating ventilatory pattern changes was different. That is, the greater change in f_R seen in CE may well have been due to the effect of 50 min of continuous exercise between measurements taken in the 10th and the last (60th) min, while the average of the 10th and 15th min of the fourth IE bout at about 10 ℓ/min less than that for CE, was immediately preceded by 15 min of rest. This may have reduced thermally induced ventilatory pattern changes, and thus might not serve as a valid indicator of possible differences in the effects of CE and IE at the same total ventilation (i.e., effective dose).

While there is suggestive evidence, in terms of subjective symptomatology and exercise ventilatory response, that O_3 inhalation during 1 h CE induces greater effects than 2 h IE at the same effective dose, no pulmonary function parameters were so affected. Further, the design of the present study did not constitute a definitive test of the differential effects of CE versus IE, in

that the effects of exercise continuity, per se, were not examined in a filtered air control condition. Hence, it is recommended that a design be effected in which O_3 concentration, average $\dot{V}_E$ and exposure time are equivalent in both CE and IE protocols, and that paired filtered air exposures be employed to examine more definitively the effects of CE versus IE on O_3 toxicity at the same effective dose.

ACKNOWLEDGEMENTS

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TABLE 1. Subject's anthropometry, maximal oxygen uptake, and pulmonary function

Subj.	Age, yr	Ht, cm	Wt, kg	Fat, % body wt	$\dot{V}_{O_2}max$, $\ell \cdot min^{-1}$	RV, liters	FVC, liters	$FEV_{1.0}$, liters	FEF_{25-75}, $\ell \cdot s^{-1}$
1	50	180	73.0	17.0	4.05	1.40	4.74	3.44	2.78
2	24	195	82.5	7.2	4.65	1.79	6.62	4.61	3.70
3	26	179	71.3	7.1	4.25	1.32	6.65	4.44	3.05
4	25	179	74.0	8.0	4.37	1.13	4.61	4.02	4.47
5	25	179	73.4	6.6	4.40	1.18	4.73	3.76	3.29
6	23	189	85.6	7.5	5.43	1.56	7.13	6.20	6.78
Mean	28.8	183.5	76.6	8.9	4.52	1.40	5.75	4.41	4.01
± SD	10.4	6.9	5.9	4.0	0.48	0.25	1.17	0.98	1.48

TABLE 2. Pulmonary function response to treatments

Variable	Total Ventilation			
	Low		High	
	CE	IE	CE	IE
RV, liters	-0.15	-0.14	-0.05	-0.33
	(0.21)	(0.25)	(0.48)	(0.50)
FVC, liters	-0.72	-0.79	-1.15	-1.15
	(0.69)	(0.62)	(1.14)	(0.80)
$FEV_{1.0}$, liters	-0.91	-0.81	-1.11	-1.18
	(0.56)	(0.57)	(0.85)	(0.72)
PF, $\ell \cdot s^{-1}$	-1.26	-1.08	-1.55	-1.69
	(1.04)	(1.06)	(1.28)	(1.35)

Values represent post- minus preexposure means, while those in parenthesis are ± 1 standard deviation. CE, continous exercise; IE, intermittent exercise; RV, residual volume; FVC, forced vital capacity, $FEV_{1.0}$, forced expiratory volume in 1s; PF, peak flow.

FIGURE LEGENDS

FIG. 1 Experimental design.

FIG. 2 Percent changes in respiratory frequency and tidal volume.

FIG. 3 Number of reported subjective symptoms.

FIG. 4 Expired pulmonary ventilation response during 1 hour continuous exercise and during a 30 min competitive simulation with 30 min immediately preceding warm-up.

	Continuous Exercise (1 h)	Intermittent Exercise (2 h)
Total $\dot{V}_E$, 2150 ℓ	1 Ex $\dot{V}_E$ = 35.5 ℓ/min	2 Ex $\dot{V}_E$ = 25.6 ℓ/min
Total $\dot{V}_E$, 3650 ℓ	3 Ex $\dot{V}_E$ = 60.3 ℓ/min	4 Ex $\dot{V}_E$ = 51 ℓ/min

FIGURE 1. Experimental Design

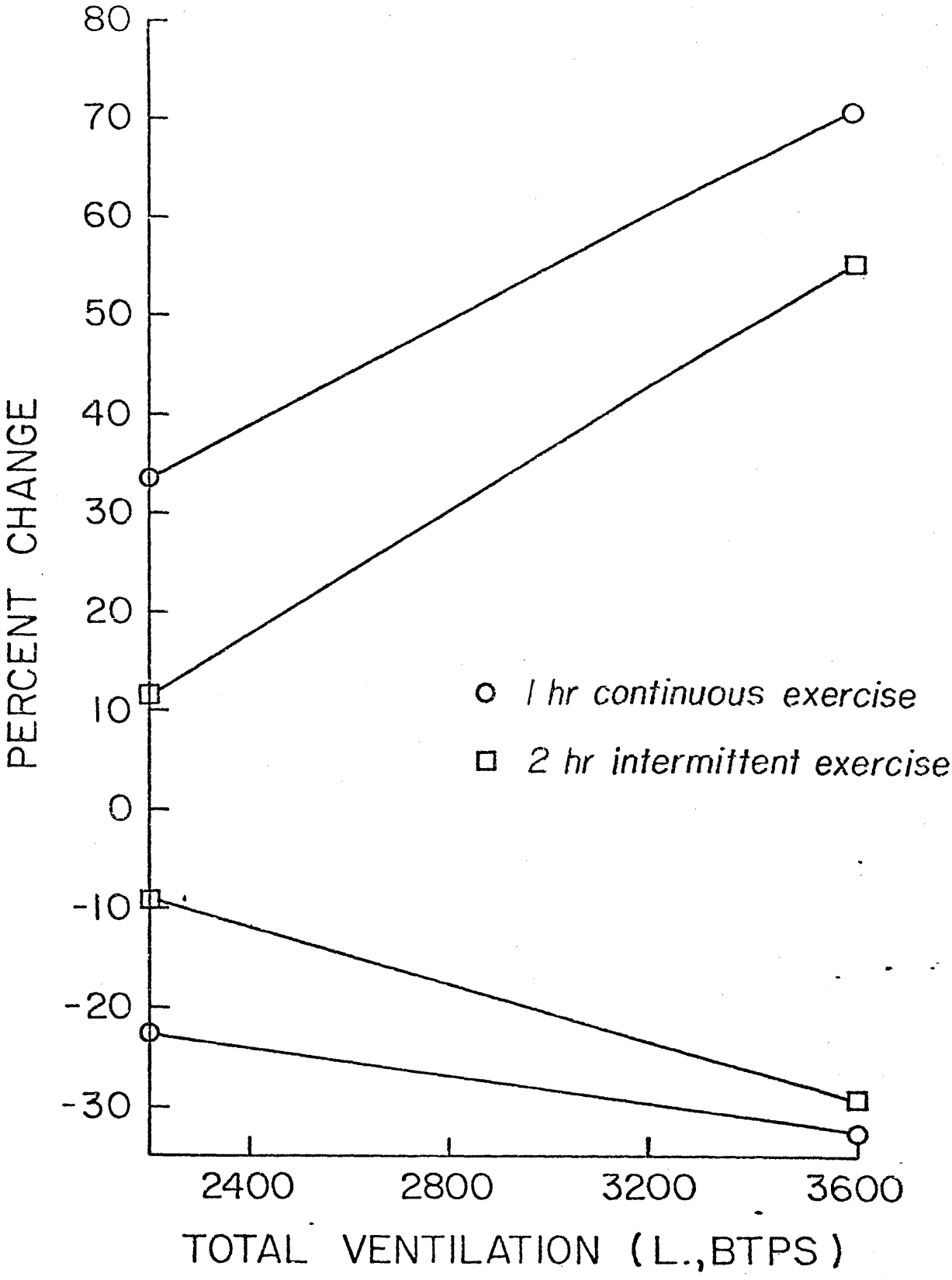


Figure 2. Percent Changes in Respiratory Frequency and Tidal Volur

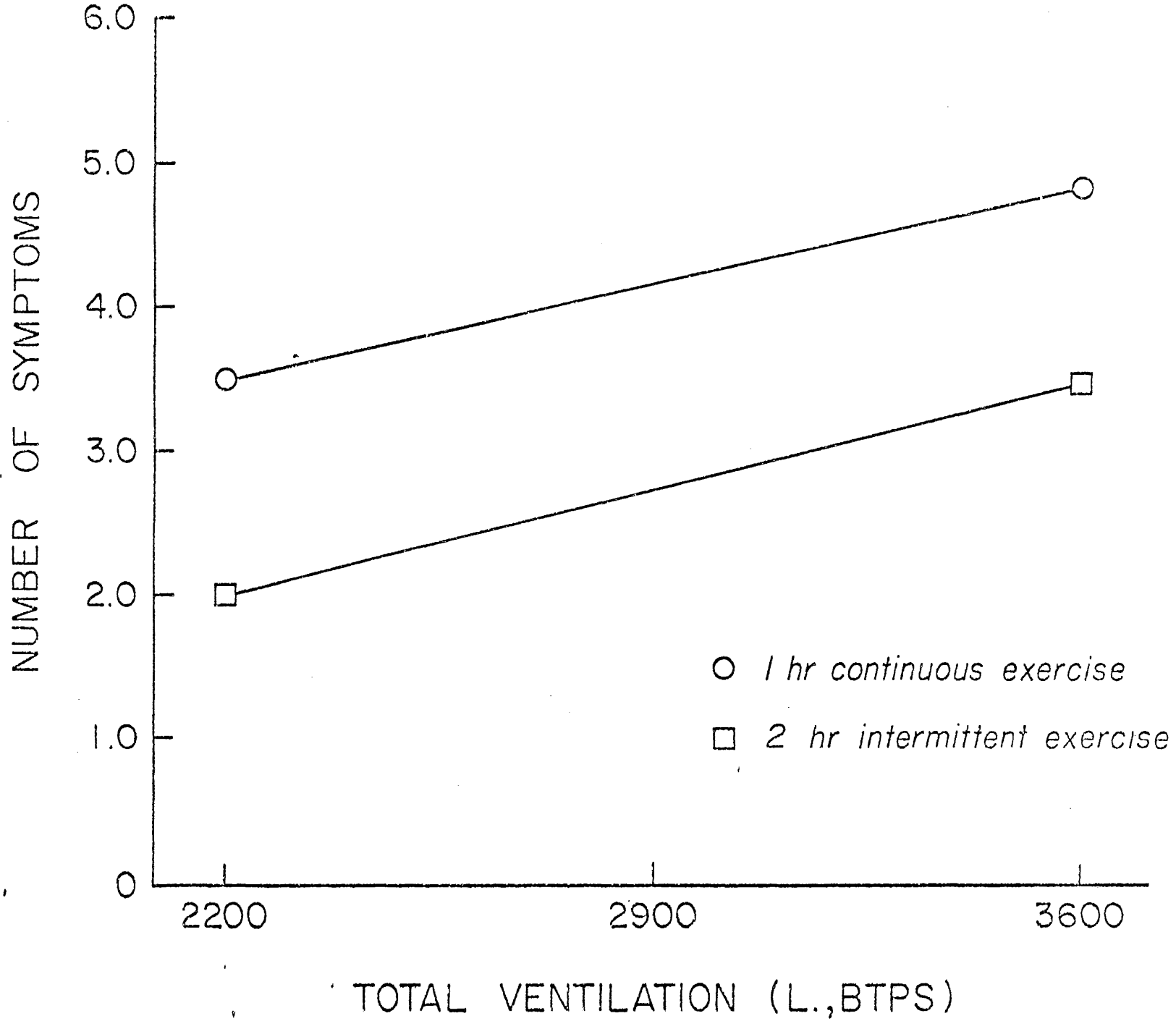
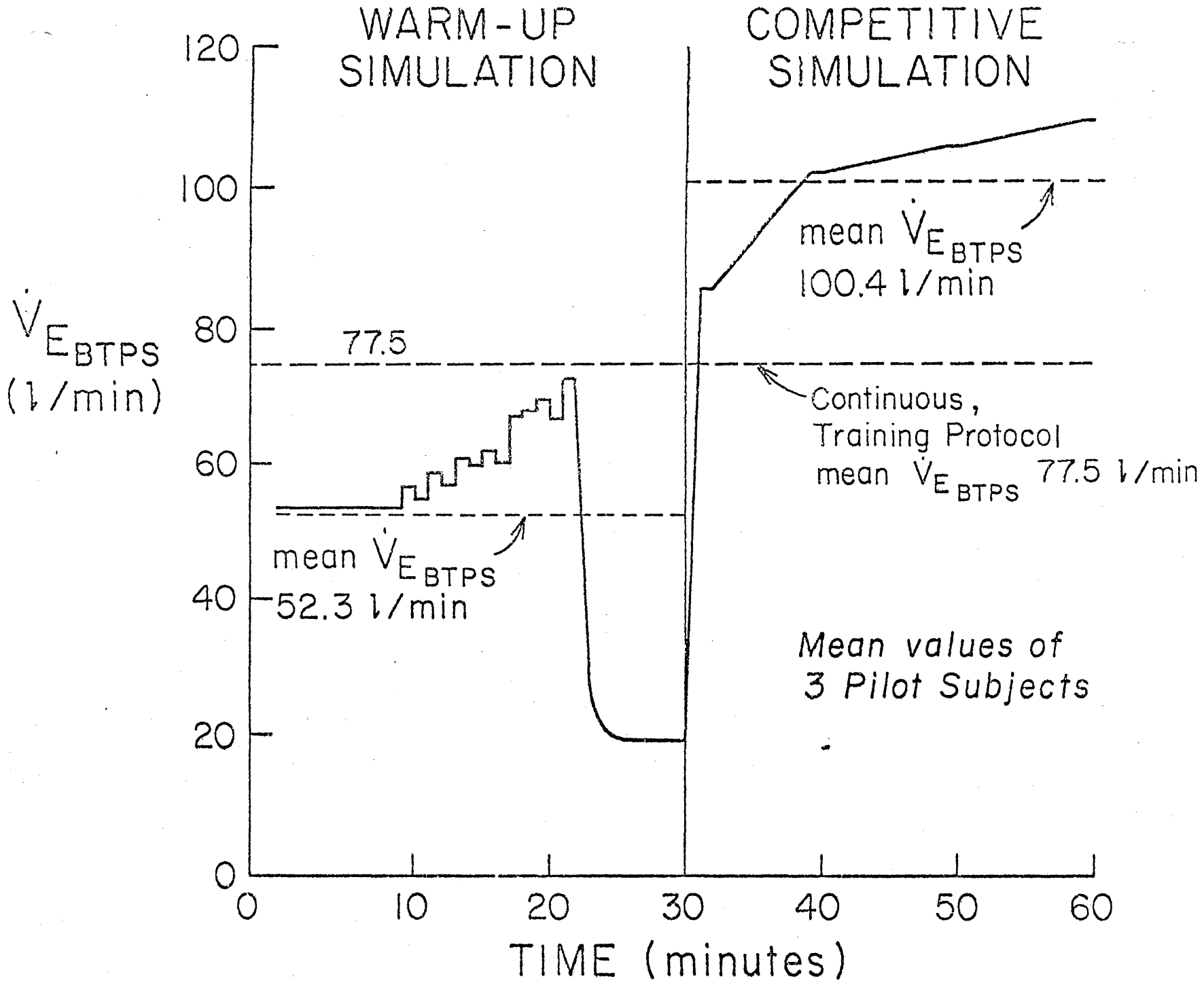


Figure 3. Mean Number of Reported Subjective Symptoms



Description of the Continuous Training and Competitive Simulation Protocols in Terms of V_E Response

EFFECTS OF INSPIRATORY ROUTE DURING CONTINUOUS EXERCISE ON PHYSIOLOGIC RESPONSES TO 0.40 PPM OZONE

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ABSTRACT

Inspiratory route is among several factors that potentially confound comparison of physiologic responses to acute ozone (O_3) exposure in our obligatory oral inhalation method to that of ad-lib breathing permitted in chamber exposures. In this study, six young adult males were exposed on five occasions to 0.40 parts per million (ppm) O_3 while exercising continuously at one of two workloads (minute ventilation, $\dot{V}_E$, of ~30 and 75 $\ell \cdot min^{-1}$). The $\dot{V}_E$, exposure time product was similar for all protocols. Four exposures were randomly delivered with a Hans-Rudolph respiratory valve (HRRV) attached to a silicone facemask, with inspiratory route effected with and without noseclip. A 2 x 2 analysis of variance revealed no statistically significant differences ($P < 0.05$) across conditions in pulmonary function or subjective symptoms. The fifth exposure, delivered via the same respiratory valve, but without facemask, revealed significantly greater forced expiratory volume in 1 second ($FEV_{1.0}$) impairment than that observed for the respiratory valve, facemask with noseclip exposure (-20.4 and -15.9%, respectively). The latter suggests partial O_3 reactivity to the facemask and clean shaven facial surface of the subjects, but fails to negate our conclusion that inspiratory route during moderate and heavy continuous exercise does not affect acute physiologic responses to O_3.

Because of the expense in building, equipping and maintaining an air pollution chamber, and our intent to investigate primarily the effects of ozone (O_3) on the physiologic response of human subjects exercising continuously at moderate to heavy work intensities, we developed an obligatory mouthpiece inhalation method (3). In this initial study we observed that 1 h of continuous exercise (CE) while exposed to 0.24 ppm O_3* induced significant pulmonary function impairment at moderate to heavy workloads, while no effects were observed consequent to resting exposures. We concluded that exercise augmented O_3 toxicity by (1) reducing the role of the upper respiratory tract in absorbing O_3 - due to greater ventilatory flow rates, predominantly via the oral inspiratory route - (2) increasing the uniformity of ventilation within the various regions of the lung, and (3) replacing reacted O_3 at a faster rate.

In 1981 (1) we showed that the decrement in forced expiratory volume in one second ($FEV_{1.0}$) consequent to 30-80 min of CE at O_3 concentrations varying from 0.20 to 0.40 ppm closely approximated that observed by Folinsbee et al. (4) in 2 h intermittent exercise (IE), chamber exposures at similar O_3 concentrations and work intensities. This comparison was made in terms of O_3 effective dose, i.e., the simple product of O_3 concentration, minute ventilation ($\dot{V}_E$), and exposure time. This suggests that although uptake of O_3 is higher in anesthesized dogs when administered orally rather than nasally (especially at low flow rates) (10), the shift from nasal breathing at rest,

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*All O_3 concentrations referenced to buffered potassium iodide have been multiplied by a factor of 0.8 to convert to the ultraviolet photometric standard employed in the present study.

in light IE, and in recovery to primarily oral breathing at heavier workloads, noted by Folinsbee et al (4) in chamber exposures, does not substantially affect O_3 toxicity in humans within the range studied.

The relatively close agreement of $FEV_{1.0}$ decrement observed in our CE, obligatory mouthpiece inhalation exposures (1) to that of Folinsbee, et al (4) in IE chamber exposures is encouraging. However, there are two important confounding variables that must be accounted for before one can accept these inter-laboratory comparisons with confidence. They are (1) the pattern of activity, CE vs IE, and (2) our use of the mouthpiece inhalation technique, while others have used environmental chamber exposures in which subjects breathed through oral and/or nasal routes, as preferred. In order to determine the effect, if any, of these two factors on O_3 toxicity, we chose to test each separately. A report on the effects of exercise continuity is presented elsewhere.

The purpose of the present investigation was to determine the effects of inspiratory route on response to O_3 during CE, as revealed by comparing obligatory mouthpiece to ad-lib inhalation. In several short (2-3 min) increments of work, most young adult males appear to switch from nasal to oronasal breathing at $\dot{V}_E$ between 35 $\ell \cdot min^{-1}$ (7) and 40 $\ell \cdot min^{-1}$ (9). Thus, we employed two disparate work intensities, necessitating $\dot{V}_E$ of approximately 30 and 75 $\ell \cdot min^{-1}$, respectively.

METHODOLOGY

Subject characterization. Six Caucasian males, whose basic characterization data, including baseline pulmonary function measurements, are given in Table 1, served as subjects (Approval from the Campus Human Subjects Review Committee and written informed consent were obtained). All subjects (non-

smokers) were regularly engaged in an aerobic training program at the time of testing, and were students familiar with exercise protocols and pulmonary function procedures. Nonetheless, each completed at least one session on the bicycle ergometer to determine $\dot{V}_E$ response to CE, and to acquaint them with the specific equipment and requirements of this study.

Experimental design. Each subject completed five exposures to 0.40 ppm O_3, as depicted in Figure 1. With O_3 effective dose maintained constant at ~900 ppm·ℓ, the exposures were delivered in randomized sequence. Exposure 5 was effected with the usual obligatory mouthpiece inhalation procedure (1). Exposures 1 and 3 were consummated via ad-lib (i.e., oral-nasal) breathing effected through the Hans Rudolph respiratory valve attached to a silicone facemask, while exposures 2 and 4 also employed the respiratory valve, facemask assembly, but permitted only oral inhalation via use of a small rubber coated wire noseclip.

Fig. 1

Protocols. The facemask was secured on the subject via spirit gum adhesive and fastening of head straps. Following placement, the subject created light positive pressure by attempted exhalation while the respiratory valve was momentarily closed at both the inspiratory and expiratory sides. The O_3 generation and monitoring system employed in this study has been described in detail earlier (3). Immediately prior to each exposure, pulmonary function was assessed via maximum forced expiratory maneuver and residual volume (RV) determinations. Following each exposure these measurements were initiated within 3 min and completed within 10 min.

Measurements. Pulmonary function measurements were obtained on a Collins 10-liter Stead-Wells spirometer assembly of the basic clinical spirometer module (Model 3000), utilizing a Collins N_2 analyzer for RV determinations.

An output voltage which was associated with volume changes was produced using a linear potentiometer attached to the spirometer bell. The analog signals produced were digitized by an A-D converter for reading into a DEC LSI 11/2 microcomputer. Within 12 min following completion of each exposure, subjects recorded any subjective symptoms (and, if present, their severity) on a questionnaire. Ventilation was monitored continuously on a Hewlett- Packard 7042A recorder via a potentiometer attached to a Parkinson Cowan (PC) high speed gasometer, Type CD4. Total expired ventilation and average $\dot{V}_E$ were calculated from this record. Other data collected during exercise every 10th minute (and in the last minute), included respiratory frequency (f_R), counted from the recorder output, tidal volume (V_T) (calculated from $\dot{V}_E/f_R$), and oxygen uptake ($\dot{V}_{O_2}$). Percent O_2 and CO_2 were obtained by a semiautomated sampling system and Applied Electrochemistry S-3A and Beckman LB-2 gas analyzers.

Statistical procedures. Duplicate pulmonary function measurements (which were periodically verified by hand tabulations from simultaneous spirometric tracings) were averaged for pre- and postexposure. The preexposure value for each parameter was subtracted from the postexposure value to obtain differences representing the treatment effect for each protocol. The pulmonary function data obtained for the four exposures utilizing the respiratory valve, facemask assembly were analyzed using an analysis of variance (ANOVA) with repeated measures made on a single group of subjects, utilizing two trial factors (ventilation and inhalation route) (Biomedical Computer Programs P2V). The 10th min value was subtracted from the last min value for each exercise ventilatory and respiratory metabolism parameter to obtain differences observed for each exposure. These differences were then analyzed statistically in the same

manner as those for pulmonary function, while the number of subjective symptoms and, if present, their severity were analyzed in a similar manner.

Statistical comparison of all data for Exposure 5 (respiratory valve without facemask) to Exposure 4 (oral inhalation with the respiratory valve, facemask assembly) only, was done via a matched pairs t test. A significance level set at $P < 0.05$ was applied in all statistical analyses.

RESULTS

Design validity. It was intended to deliver the same O_3 effective dose employing two workloads necessitating disparate $\dot{V}_E$ (30 and 75 $\ell \cdot min^{-1}$), and counterbalanced by exposure durations of 75 and 30 min, respectively. While total ventilation was marginally higher in the two longer exposures, ANOVA indicated no significant difference ($P > 0.14$).

Pulmonary function. Table 2 contains the means and standard deviations for the pulmonary function parameters for the five exposures, while Fig. 2 depicts the percent change in $FEV_{1.0}$. There were no statistically significant differences across treatments with the respiratory valve, facemask assembly (i.e. Exposures 1-4). However, FVC and $FEV_{1.0}$ for Exposure 5 (respiratory valve only) was significantly different from that observed for Exposure 4 ($P = 0.02$ and 0.03, respectively).

Exercise measurements. The means and standard deviations for the exercise ventilatory and respiratory metabolism parameters are given in Table 3. Although there was a tendency for greater changes in f_R, V_T and $\dot{V}_E$, in the high intensity 30 min exposures, ANOVA revealed no significant differences in any parameter in the four exposures with the respiratory valve, facemask assembly. Except for f_R ($P < 0.02$), t test comparison of Exposure 4 to Exposure 5

(respiratory valve without facemask) revealed no statistically significant differences.

Subjective symptoms. The mean number of subjective symptoms and their severity are given in Table 4. ANOVA revealed no significant difference in symptom number ($P > 0.17$) or severity ($P > 0.18$). t test comparison of Exposures 4 and 5 also revealed no significant differences.

Table 4

DISCUSSION

The purpose of the present study was to compare the effects of inspiratory route, as revealed by comparing obligatory oral to ad-lib (oral and/or nasal) inhaltion of 0.40 ppm O_3 during CE at the same effective dose (i.e., 900 ppm·ℓ). The latter was effected by equating total ventilation via matching $\dot{V}_E$ at two work intensities, viz., ~30 and 75 $\ell \cdot min^{-1}$, with exposure durations of 75 and 30 min, respectively. Amongst the four exposures utilizing the respiratory valve, facemask assembly, there was no significant difference in total ventilation, although that for the two low work intensity, 75 min exposures averaged 5% greater than that for the two high intensity, 30 min exposures.

ANOVA revealed no significant differences in any pulmonary function or exercise ventilatory response parameter. Further, there were no significant differences in subjective symptomatology (Table 4), although there was a tendency toward greater subjective symptom number and severity in the two high work intensity, 30 min exposures.

The latter, together with the marginally greater alteration in exercise ventilatory pattern observed in these two exposures, suggests a local work intensity factor interacting with a central O_3 inhalation factor, which is contrary to the observations by Mihevic, et al (6) in light IE exposures. Effec-

tive analysis of this factor remains to be resolved, as no filtered air (FA) control exposures were utilized in this study.

As noted earlier, the primary purpose of this study was to determine if the difference in inhalation pattern, i.e., obligatory oral vs ad-lib (nasal and/or oral) resulted in different physiologic effects at the same O_3 effective dose. To effect a large difference in the relative amount of oral and nasal inhalation in the ad-lib conditions, we employed two disparate work intensities, necessitating mean $\dot{V}_E$ of 32.7 and 77.8 $\ell \cdot min^{-1}$, respectively. It has been observed previously that most young adult males switch from nasal to oronasal breathing between 35 $\ell \cdot min^{-1}$ (7) and 40 $\ell \cdot min^{-1}$ (9). Nonetheless, Proctor (8) reports (Niinimaa and Cole, personal communication) that 56% of airflow continued nasally at this point, and that at 90 $\ell \cdot min^{-1}$, 39% of airflow was still nasal. In the present study, the greatest disparity in nasal breathing was presumed to occur between Exposure 1 (presumably mostly nasal breathing) and that for Exposures 2 and 4 (obligatory oral inhalation). Review of pulmonary function changes denoted in Table 2 reveal no apparent systematic tendency toward greater difference for the two obligatory oral inhalation protocols. Further, the close agreement in subjective symptoms number and severity, as well as exercise ventilatory pattern changes for Exposures 1 (ad-lib inhalation) and 2 (oral inhalation), strongly suggest that inspiratory route in humans exercising continuously at moderate workloads (33% of maximal oxygen uptake) does not alter O_3 toxicity.

The results of the present study indicate a significant difference in the physiological response of our subjects to Exposure 4 (obligatory oral inhalation with the respiratory valve, facemask assembly) compared to Exposure 5 (obligatory oral inhalation via the respiratory valve only). The increased

respiratory dead space of the former (200 mℓ vs 77 mℓ) resulted in expected responses at 10 min of exercise, viz., no difference in $\dot{V}_{O_2}$ and $\dot{V}_E$, but decreased V_T (~12%) and increased f_R (~10%) (2,5). Since there was no difference in total ventilation and O_3 effective dose, it is unlikely that increased respiratory dead space exacerbated the impairment in FVC and $FEV_{1.0}$ (Fig. 2) noted in Exposure 5. Rather, partial O_3 reactivity to the silicone facemask and clean shaven facial surface of the subjects seems a more likely explanation. Thus, we conclude that inspiratory route during moderate and heavy CE does not affect O_3 toxicity in humans.

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TABLE 1. Subject's anthropometry, maximal oxygen uptake, and pulmonary function.

Subject	Age (yr)	Ht (cm)	Wt (kg)	Fat (%)	FVC (ℓ)	RV (ℓ)	TLC (ℓ)	$FEV_{1.0}$ (ℓ)	FEV_1/FVC (%)	FEF_{25-75} (ℓ/sec)	$\dot{V}O_{2max}$ (ℓ/min)
1	23	172	65	15.9	5.78	1.33	7.11	4.37	75.6	3.80	3.30
2	28	171	75	12.7	5.92	1.20	7.12	4.70	79.4	4.50	3.31
3	22	180	67	12.3	4.86	1.01	5.87	4.32	88.9	5.71	3.92
4	30	178	65	9.2	5.75	1.60	7.35	4.44	77.2	3.87	4.58
5	25	184	81	19.3	6.05	1.65	7.70	4.32	71.4	3.31	3.98
6	26	177	69	8.5	5.34	1.08	6.42	4.39	82.2	4.67	3.60
Mean	25.7	177.0	70.3	13.0	5.61	1.31	6.93	4.42	79.1	4.31	3.78
± SD	3.0	4.9	6.4	4.1	0.43	0.27	0.67	0.14	6.0	0.85	0.49

Fat, percent of body weight; FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity; $FEV_{1.0}$, forced expiratory volume in 1 s; FEF_{25-75}, forced expiratory flow rate during middle half of FVC; $\dot{V}O_{2max}$, maximal oxygen uptake

TABLE 2. Pulmonary function response to treatments.

Variable	Exposure 1	Exposure 2	Exposure 3	Exposure 4	Exposure 5
RV, liter	-0.04	-0.02	-0.04	-0.09	-0.18
	(0.15)	(0.15)	(0.19)	(0.15)	(0.22)
FVC, liter	-0.60	-0.70	-0.80	-0.78	-0.98
	(0.30)	(0.52)	(0.28)	(0.24)	(0.24)
$FEV_{1.0}$, liter	-0.72	-0.77	-0.67	-0.70	-0.92
	(0.38)	(0.56)	(0.42)	(0.32)	(0.44)
FEF_{25-75}, $\ell \cdot s^{-1}$	-1.18	-1.21	-0.87	-0.97	-1.17
	(0.74)	(0.84)	(0.99)	(0.92)	(1.04)

Values represent post- minus preexposure means, while those in parentheses are + 1 standard deviation. RV, residual volume; FVC, forced vital capacity; $FEV_{1.0}$, forced expiratory volume in 1 s; FEF_{25-75}, forced expiratory flow rate during middle half of FVC.

TABLE 3. Exercise ventilatory and respiratory metabolism response to treatments.

Variable	Exposure 1	Exposure 2	Exposure 3	Exposure 4	Exposure 5
f_R, breaths·min^{-1}	6.6	8.1	8.4	9.4	12.4
	(5.4)	(7.8)	(2.5)	(3.4)	(3.5)
V_T, liters	-0.26	-0.38	-0.38	-0.50	-0.47
	(0.26)	(0.31)	(0.07)	(0.20)	(0.18)
$\dot{V}_E$, ℓ·min^{-1}	1.30	0.25	4.37	4.56	7.13
	(3.4)	(5.4)	(4.9)	(5.7)	(7.7)
$\dot{V}_{O_2}$, ℓ·min^{-1}	0.04	-0.01	-0.02	0.13	0.16
	(0.12)	(0.16)	(0.13)	(0.14)	(0.20)

Values represent last minute minus 10th min means, while those in parentheses are ± 1 standard deviation. f_R, respiratory frequency; V_T, tidal volume; $\dot{V}_E$, expired minute ventilation; $\dot{V}_{O_2}$, oxygen uptake.

TABLE 4. Subjective symptoms response to treatments.

Variable	Exposure 1	Exposure 2	Exposure 3	Exposure 4	Exposure 5
Symptom number	2.33	2.33	3.00	3.17	3.67
	(1.03)	(1.21)	(1.41)	(1.94)	(1.51)
Symptom severity	4.67	4.75	6.83	7.83	8.38
	(2.73)	(3.22)	(3.66)	(7.11)	(3.63)

Values represent the means for each exposure, while those in parentheses are $\pm$ 1 standard deviation.

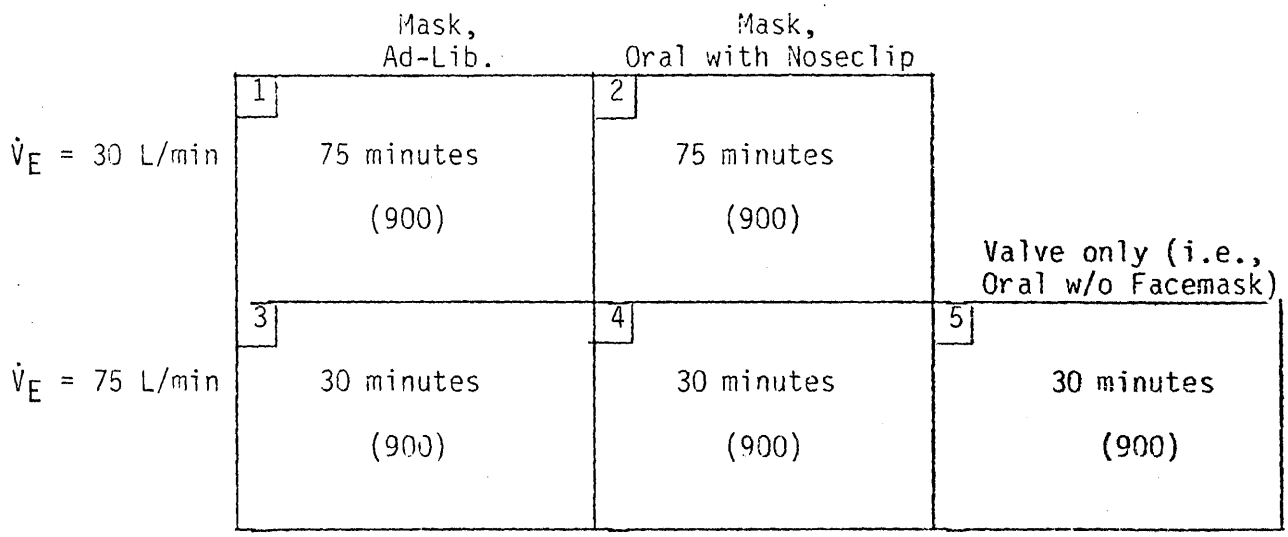

FIGURE 1. Experimental Design

	Mask, Ad-Lib.	Mask, Oral with Noseclip	Valve only (i.e., Oral w/o Facemask)
$\dot{V}_E$ = 30 L/min	1 75 minutes -16.3, ± 8.5	2 75 minutes -17.3, ± 12.6	
$\dot{V}_E$ = 75 L/min	3 30 minutes -15.2, ± 9.5	4 30 minutes -15.9, ± 7.3	5 30 minutes -20.4, ± 10.0

FIGURE 2. % $FEV_{1.0}$ Impairment as a Function of Inspiratory Route.

RESPONSES OF FEMALES TO O_3 EXPOSURE DURING CONTINUOUS EXERCISE: COMPARISON OF TRAINED AND NONTRAINED SUBJECTS

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ABSTRACT

While there are now considerable data available on the response of young adult males to ozone (O_3) inhalation during exercise, there is a paucity of such data for females. In the present study, the effects of 1 h continuous exercise at minute ventilations of 23, 34, and 46 $\ell \cdot min^{-1}$, while exposed to FA, 0.2, 0.3, and 0.4 ppm O_3, were studied in a group of ten aerobically trained and 30 normally active, nontrained females. Both groups demonstrated significant pulmonary function impairment and a greater number of reported subjective symptoms in the O_3 exposures compared to FA. There were no statistically significant differences in the responses to O_3 inhalation observed between the trained group and their nontrained counterparts. The equivalent $\dot{V}_E$ imposed on both groups was elicited at absolute workloads approximately 10% less for the trained group. Thus, were the nontrained subjects to jog or ride a bicycle at the same submaximal speed as their trained counterparts in the prevailing photochemical smog condition, they would incur a greater $\dot{V}_E$ and hence, a greater O_3 effective dose and acute toxicity response. Both groups of females incurred greater pulmonary function impairment at the O_3 effective dose imposed (~600 ppm·ℓ) than that observed for young adult males. This difference appears to be primarily associated with the mean lung size difference between the sexes, which is approximately 1½ times larger for males.

With increasing public health interest in setting appropriate health standards, identification of "no effect" levels of ozone (O_3) inhalation has become a subject of considerable importance. Exercise induced enhancement of O_3 inhalation effects, initially demonstrated in rats by Stokinger et al (16), was first observed in humans by Bates et al (4), and later by Silverman et al (15), who advanced the O_3 effective dose concept (i.e., the simple product of O_3 concentration, minute ventilation volume, and exposure duration). Folinsbee et al (9) showed that O_3 toxicity at a given concentration was enhanced at moderately heavy exercise intensities, even as low as 0.24 ppm (which did not elicit any effect following 2 hours exposure at rest). More recent studies, employing exercise intensities characteristic of individuals engaged in aerobic adult fitness training or athletic competition, have demonstrated transient pulmonary function impairment and reported subjective symptoms at O_3 concentrations ranging from 0.18 to 0.24 ppm (2,14). However, these and most other laboratory studies of O_3 inhalation have been restricted primarily to young adult males.

It is readily apparent that an increasingly large number of adult females are engaging in moderate to heavy work entailed in voluntary recreational exercise, as well as in industrial and government work applications. Hence, the enhanced ventilation volume requisite to the females' participation in these vigorous activities, and the consequent increased air pollutant inhalation

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*All O_3 concentrations referenced to buffered potassium iodide have been multiplied by a factor of 0.8 to convert to the ultraviolet photometric standard employed in this study.

incurred on exposure to photochemical smog, needs to be studied in a systematic fashion.

Most previous studies of O_3 exposure effects in humans, including the few utilizing female subjects have noted a large variation in individual response. One factor that affects one's response to O_3 during exercise is their level of aerobic fitness, as the less fit person will demonstrate a greater minute ventilation at the same absolute workload, compared to her fit counterpart (3). Since the pattern of breathing is also different at the same minute ventilation rate, there may be a dissimilar response to O_3 inhalation according to one's level of aerobic fitness. The purpose of the present study was to determine the acute O_3 toxicity effects consequent to 1 h of continuous exercise in young adult females, including comparison of the response of aerobically trained subjects to their normally active, nontrained counterparts.

METHODS

Subject selection, description, and characterization. In order to complete a more adequate sample size than that utilized in a previously reported study, i.e., N=6 (12), four aerobically trained young adult females agreed to serve as subjects. In addition, 30 normally active young adult females volunteered to participate in the study (Institutional Human Use Committee approval and individual signed informed consent were obtained). None of the subjects smoked nor had lived in a high air pollution area within three months prior to the study.

Prior to their initial exposure, each subject completed at least two sessions, during which anthropometry, including body composition via hydrostatic

weighing, base-line pulmonary function, and maximal oxygen uptake ($\dot{V}O_{2max}$) were measured (see Table 1). To decrease habituation effects, each subject also completed at least 30 min riding on a bicycle ergometer while breathing filtered air (FA) through the obligatory mouthpiece inhalation system (5).

Table 1

Expermimental design. Each of the ten aerobically trained subjects completed ten 1 h exposures while breathing FA, or 0.20, 0.30, or 0.40 ppm O_3. Exercise intensities were set individually to induce minute ventilations ($\dot{V}_E$) of approximately 23, 35, and 46 $\ell \cdot min^{-1}$. respectively, at each of the three O_3 concentrations. The FA exposure was carried out at the high exercise $\dot{V}_E$ (46 $\ell \cdot min^{-1}$) only. Except for the FA exposure, which was conducted first, the order of experimental protocols was randomized, with a minimum of three days intervening between treatments. The experimental design, including protocol numbers and the O_3 effective dose in ppm·ℓ, is depicted in Fig. 1.

Fig. 1

Following completion of this phase of the study, three protocols that elicited pulmonary function impairment in the aerobically trained females, which we anticipated could be consummated by normally active young adult females, were identified. These 1 h protocols were of similar O_3 effective doses (~600 ppm·ℓ): viz., (1) 0.2 ppm at a $\dot{V}_E$ of ~46 $\ell \cdot min^{-1}$; (2) 0.3 ppm at ~34 $\ell \cdot min^{-1}$; and (3) 0.4 ppm at ~23 $\ell \cdot min^{-1}$ (i.e., protocols 5, 7, and 6, respectively). Thirty subjects completed one of these protocols (ten in each), together with a FA exposure at the same exercise intensity as their O_3 exposure.

Subjects were not informed of the level of O_3 received. Following each exposure, subjects completed a subjective symptoms questionnaire, and indicated whether they believed they had received O_3. All exposures were completed in

a room in which ambient conditions were maintained within limits described previously (1).

O_3 administration and monitoring. Air mixtures during all exposures were generated and delivered via an obligatory mouthpiece inhalation procedure described in detail previously (5). O_3 concentration was routinely determined by samples from the inspiratory side of a Teflon coated Hans-Rudolph respiratory valve, drawn through a 0.64-cm-ID Teflon tube connected to a Dasibi O_3 meter. The Dasibi meter was calibrated on several occasions according to the ultraviolet absorption photometric method (7) at the University of California, Davis, Primate Research Center.

Pulmonary function measurements. Pulmonary function tests were administered just prior to, and completed again within 15 min following, each exposure. Residual volume (RV) was measured on a modified Collins 9-liter spirometer utilizing an O_2 rebreathing method (17), with initial and equilibrium N_2 readings obtained from an Ohio 700 N_2 analyzer. At least two forced expiratory maneuvers were performed, with forced vital capacity (FVC), forced expiratory volume at 1 second ($FEV_{1.0}$), and forced expiratory flow during the middle half of FVC (FEF_{25-75}) calculated from the spirometric tracings. The average of duplicate determinations was utilized for subsequent analyses.

Exercise measurements. To assess possible effects of O_3 inhalation on selected exercise parameters, 1 min observations were made at minutes 9-10, 19-20, 29-30, 39-40, 49-50, and 59-60. $\dot{V}_E$ was determined by use of a potentiometer mounted in a Parkinson-Cowan (PC) gas meter, type CD-4, connected to either a Hewlett-Packard 680M or 7402A strip-chart recorder. Respiratory frequency (f_R) was recorded similarly, thence counted manually, and used with

$\dot{V}_E$ corrected to BTPS to calculate tidal volume (V_T). Respiratory metabolism was determined from measurement of expired air volume and percent O_2 and CO_2 by a semiautomated sampling method incorporating a manually rotated 3-way valve sampling system (18) and Applied Electrochemistry S-3A and Beckman LB-2 gas analyzers. Heart rate (HR) was determined from the elapsed time of four consecutive R waves recorded from electrocardiogram tracings obtained on a Sanborn visocardette

Statistical procedures. Duplicate pulmonary function measurements were averaged for pre- and postexposure. Treatment effect percent change for each variable was calculated as postexposure minus preexposure, divided by the preexposure value, and multiplied by 100. Similarly, values observed for $\dot{V}_{O_2}$, HR, $\dot{V}_E$, f_R, and V_T during the 10th minute of exercise were subtracted from those obtained in the 60th min, divided by the 10th min value, and then multiplied by 100. The percent change data obtained on the four aerobically trained subjects were compared to that observed for the six subjects studied by Lauritzen (12) by t tests for independent samples. As no statistically significant differences were observed, data for the two groups were combined (i.e., N=10) for subsequent analyses.

The pulmonary function percent change data for the trained group was analyzed using an ANOVA with repeated measures design to determine if the differences observed in the three O_3 and the single FA exposures were significant. Data from protocols which were completed by both the trained and nontrained female groups were compared by t tests for independent samples using a Bonferroni correction. The FA and O_3 exposure data for the nontrained subjects was compared for each exposure group using paired t-tests. O_3 responses for the nontrained groups were calculated by subtracting the FA values from the O_3 values. The difference in O_3 groups responses were then analyzed

using an ANOVA. Upon observation of a significan F value, post hoc analysis for specific significant mean differences were done using t tests with Bonferroni correction applied for the number of comparisons made. In all analyses, the significance level was set at a $P < 0.05$.

RESULTS

The aerobically trained group's mean absolute and percent changes in pulmonary function, exercise ventilatory pattern and $\dot{V}O_2$ are given in Table 2. Statistical analyses of the 10 protocols, in terms of effective dose ppm·ℓ level, as well as the effects of $\dot{V}_E$ and O_3 concentration, for the original six subjects have been presented earlier (12). Since there were no substantial differences between the mean values observed for the four subjects of this study, similar statistical analyses seemed unwarranted. In any case, there is a near consistent increased effect (according to the O_3 effective dose ppm·ℓ product) in each of the pulmonary function parameters, as well as in f_R and V_T.

A summary of the statistical analyses of the mean percent changes of the aerobically trained group for the FA exposure (No. 1) and for the three exposures ranging from 550 to 620 ppm·ℓ (i.e., Exposure Nos. 5-7), is given in Table 3. ANOVA revealed that, except for RV, all of the pulmonary function changes were statistically significant. Post hoc analyses, revealed statistically significant differences in FVC between FA and each of the three O_3 exposures, but for $FEV_{1.0}$, only between FA and O_3 exposures 6 and 7, and for FEF_{25-75}, only between FA and exposure 7. Further, although there is a tendency toward greater effects for the 0.3 and 0.4 ppm exposures (Nos. 7 and 6, respectively), none of the changes were significantly different from those observed for the 0.2 ppm exposure (No. 5).

A summary of pulmonary function, exercise ventilatory pattern, and $\dot{V}_{O_2}$ changes for the nontrained subjects is given in Table 4. Significant results from the ANOVA and post hoc analyses of the difference in changes in pulmonary function and exercise ventilatory and metabolism variables between the three nontrained groups' FA and O_3 exposures are given in Table 5. Differences between FA and all O_3 exposures for FVC and $FEV_{1.0}$ were significant, while for FEF_{25-75}, only the 0.20 ppm group was significant. The difference between FA and O_3 exposure in f_R and V_T changes were statistically significant for the 0.3, but not for the 0.2 and 0.4 ppm groups. While there was tendency for changes in FVC, $FEV_{1.0}$ and FEF_{25-75} to be less for the 0.2 ppm group compared to those for the 0.3 and 0.4 ppm groups, only the comparison of the 0.2 and 0.3 ppm groups for FVC was statistically significant.

t test analysis of the comparison of pulmonary function changes consequent to the O_3 exposures between the aerobically trained group and the nontrained groups, revealed no significant differences. A comparison of $FEV_{1.0}$ changes as a function of O_3 effective dose, including that observed for young adult males in previous studies (1,9), that observed for ten exposures of the 10 aerobically trained females, and that observed for the three exposures completed by the nontrained females in this study, is depicted in Figure 2.

The number of subjective symptoms reported by the trained group for the FA and three O_3 exposures is shown in Table 6. The values for each of the three O_3 exposures were significantly different from that for FA, but were not significantly different from each other. The nontrained groups' subjective symptoms responses for the O_3 exposures followed a similar trend, and did not differ significantly from those for the aerobically trained group. The 0.2 ppm

nontrained group, exercising at 46 ℓ/min and 71% of $\dot{V}_{O_{2max}}$, reported significantly more subjective symptoms than did the other nontrained groups.

DISCUSSION

The purpose of this study was to examine the responses of young adult females consequent to 1 h continuous exercise while exposed to O_3 at an effective dose of ~600 ppm·ℓ, and to compare an aerobically trained group to normally active nontrained, subjects. In general, the females responded to O_3 inhalation in a similar qualitative manner as that observed previously in young adult males. That is, O_3 exposure induced significant decrements in FVC, $FEV_{1.0}$, FEF_{25-75} and in the number of reported subjective symptoms when compared to those observed upon exposure to FA. There were no statistically significant differences in the responses to O_3 inhalation observed for the aerobically trained group compared to their nontrained counterparts.

Results of this study provide additional support for the usefulness of the effective dose concept (i.e., the simple product of O_3 concentration, minute ventilation, and exposure time), in that there were no statistically significant differences for the trained mean group responses to the three variations in O_3 concentration and $\dot{V}_E$ utilized, and only one for the nontrained group. The trend toward a greater effect at a similar effective dose in the 0.3 and 0.4 ppm exposures (Tables 2 and 4) is consistent with the preeminent effect of O_3 concentration (compared to $\dot{V}_E$ and exposure time) noted by others (1,9,15).

Table 7

Table 7 summarizes the pulmonary function responses of our female subjects to those of a limited number of females studied in previous investigations. O_3 inhalation protocols utilized involved either rest (11), continuous exercise (6,8) or light intermittent exercise (10) at effective doses varying

from 525 to 700 ppm·ℓ (O_3 concentration ranged from 0.24 ppm to 0.60 ppm). While the total number of female subjects previously studied is small (N=31), the percent decrement in all studies closely approximates that observed in the present investigation. This seems even more noteworthy when one considers that the studies of Gliner et al (10) and Horvath et al (11) utilized not only different activity patterns, but also permitted ad lib chamber breathing.

As shown in Fig. 2, both the aerobically trained and nontrained females demonstrated consistently greater $FEV_{1.0}$ decrements than their male counterparts, as indicated by the dashed line for an aerobically trained group (1) and the continuous regression line calculated by Folinsbee et al (9) for nontrained, normally active males. Explanation of this enhanced response of females at the same O_3 effective dose is not entirely clear, but several factors appear to be involved. Since the males' total lung capacity (TLC) is approximately 1.5 times greater than the females', the latter may demonstrate a greater response at the same effective dose because of a higher amount of pollutant to lung volume ratio. However, comparisons of the females response to those of males when the effective dose was 1½ times greater for the males still revealed a somewhat greater sensitivity for the females (12). In these comparisons, the female had a 10% greater f_R, which would result in a more rapid replacement of reacted O_3. Further, Leith (13) has observed that alveolar surface area (SA) lung volume is a function of $BW^{3/4}/BW^{1.06}$, which if lung SA is dimensioned similarly to alveolar SA, would yield an 8% greater lung SA to volume ratio for the females (12).

While there were no statistically significant differences between the responses of the aerobically trained females and their normally active

nontrained counterparts to O_3 inhalation at the same effective dose (i.e., ~600 ppm·ℓ), there are several potential differences of considerable practical importance. The trained group had significantly less body fat than the mean for the 30 nontrained subjects (20.2 vs 24.5% of BW), although their BW was similar (58.6 kg vs 58.4 kg, respectively). Further, their FVC was approximately 10% greater, while their $\dot{V}O_{2max}$ was 20% greater.

In this study, $\dot{V}_E$ was equated for both the trained and nontrained groups in order to deliver an equivalent O_3 effective dose. However, the mean absolute workloads utilized to elicit 23, 34½ and 46 $\ell \cdot min^{-1}$ $\dot{V}_E$ for the trained group were 330, 580, and 800 $kpm \cdot min^{-1}$, respectively, while those for the nontrained groups were 270, 520, and 710 $kpm \cdot min^{-1}$. Thus, at an equivalent absolute workload, such as if the nontrained subjects were jogging or riding a bicycle outdoors at the same speed as their trained counterparts in the same photochemical smog conditions, they would incur a considerably greater $\dot{V}_E$ (~12-18%) and hence, a greater O_3 effective dose. This effect would be compounded at higher work intensities, i.e., >70% $\dot{V}O_{2max}$, due to the additional stimulus of anaerobic metabolism on $\dot{V}_E$, which would be encountered at slower speeds by the nontrained subjects. Hence, further study of nontrained young adult subjects engaged in continuous exercise for up to 1 h seems warranted.

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TABLE 1. Group anthropometry, pulmonary function, and maximal oxygen uptake data.

GROUP	Age, yr	Ht, cm	Wt, kg	Fat, % of BW	FVC ℓ	RV, ℓ	$FEV_{1.0}$ ℓ	FEF_{25-75} $\ell \cdot sec^{-1}$	$FEV_{1.0}$/FVC %	$\dot{V}_{Emax}$, min BTPS	$\dot{V}_{O_{2max}}$, min STPD	Max Test Duration, min
Aerobically Trained	23.7 (2.8)	166.4 (3.8)	58.6 (3.7)	20.2 (5.31)	4.20 (0.29)	1.16 (.32)	3.41 (.43)	3.75 (1.03)	81.1 (6.2)	107.2 (16.8)	2.68 (0.37)	15.9 (2.7)
Nontrained #1	21.3 (2.1)	163.8 (6.6)	59.6 (5.7)	24.4 (5.8)	3.95 (.41)	1.02 (.21)	2.99 (.33)	3.38 (0.61)	75.8 (7.9)	83.5 (16.9)	2.30 (0.37)	13.9 (3.1)
Nontrained #2	20.9 (1.2)	165.0 (7.2)	57.4 (8.9)	23.3 (5.5)	3.79 (.53)	1.25 (.16)	3.18 (.44)	3.61 (0.90)	83.9 (5.2)	90.0 (16.7)	2.19 (0.43)	14.2 (3.9)
Nontrained #3	20.5 (1.9)	163.9 (5.3)	58.2 (8.7)	25.7 (4.2)	3.74 (.49)	1.22 (.27)	2.89 (.42)	3.38 (.98)	77.4 (13.4)	86.9 (13.6)	2.19 (0.26)	13.2 (2.6)

Values are means, with ± 1 standard deviation values in parentheses. FVC, forced vital capacity; RV, residual volume; $FEV_{1.0}$, forced expiratory volume in 1 s; FEF_{25-75}, forced expiratory flow during middle half of FVC; $\dot{V}_{Emax}$, maximal minute ventilation; $\dot{V}_{O_{2max}}$, maximal oxygen consumption.

TABLE 2. Summary of pulmonary function, exercise ventilatory pattern and $\dot{V}_{O_2}$ changes for the ten aerobically trained subjects.

Protocol	FVC	RV	$FEV_{1.0}$	FEF_{25-75}	f_R	V_T	$\dot{V}_E$	$\dot{V}_{O_2}$
1	-.063 (-1.5)	+.077 (+6.6)	-.072 (-2.2)	+.058 (+1.7)	+2.76 (+9.7)	-.103 (-5.9)	+1.37 (+2.9)	+.068 (+4.1)
2	- .105 (-2.5)	+ .074 (+6.3)	-.086 (-2.6)	-.208 (-5.6)	+0.70 (+3.1)	-.074 (-7.9)	+0.26 (+1.0)	+.046 (+5.3)
3	-.140 (-3.3)	+.078 (+7.5)	-.177 (-5.3)	-.148 (-4.1)	+2.40 (+9.5)	-.055 (-3.4)	+0.70 (+1.9)	+.052 (+4.0)
4	-.207 (-5.0)	+.054 (+5.2)	-.386 (-11.9)	-.545 (-15.6)	+1.60 (+6.8)	-.070 (-6.8)	-0.55 (-1.9)	+.012 (+1.9)
5	-.266 (-6.5)	+.108 (+8.6)	-.332 (- 9.7)	-.520 (-13.9)	+5.70 (+19.7)	-.170 (-11.6)	+2.49 (+5.6)	+.022 (+1.2)
6	-.345 (-8.4)	+.144 (+13.2)	-.366 (-11.7)	-.648 (-17.9)	+5.04 (+21.7)	-.194 (-18.5)	-0.38 (-1.4)	-.006 (-0.1)
7	-.387 (-9.2)	+.080 (+ 7.7)	-.651 (-19.4)	-.980 (-26.1)	+4.58 (+17.7)	-.206 (-15.1)	-0.91 (-2.3)	+.010 (+1.2)
8	-.684 (-16.2)	+.163 (+14.8)	-.781 (-23.1)	-1.185 (-30.6)	+10.40 (+36.5)	-.316 (-21.5)	+2.86 (+6.5)	+.054 (+3.1)
9	-.649 (-15.5)	+.186 (+16.2)	-.710 (-21.3)	-1.146 (-31.0)	+8.86 (+33.5)	-.290 (-22.0)	+1.00 (+3.0)	+.050 (+4.6)
10	-.844 (-20.3)	+.242 (+18.0)	-.892 (-26.7)	-1.382 (-37.2)	+14.88 (+51.3)	-.396 (-26.3)	+3.61 (+8.5)	+.030 (+5.1)

Values are mean changes; values in parentheses are mean percent changes. FVC, forced vital capacity (liters); RV, residual volume (liters); $FEV_{1.0}$, forced expiratory volume in 1.0 s (liters); FEF_{25-75} forced expiratory flow during middle half of FVC (liters$\cdot s^{-1}$); f_R, respiratory frequency (breaths$\cdot min^{-1}$); V_T, tidal volume (liters); $\dot{V}_E$, minute ventilation (liters$\cdot min^{-1}$); $\dot{V}_{O_2}$, oxygen consumption (liters$\cdot$min-1).

TABLE 3. F ratios and specific significant mean differences from post hoc analysis for pulmonary functions for the trained group.

Variable	F Ratio	Specific Significant Mean Differences*
RV	1.13	NA
FVC	3.91*	1-5, 1-6, 1-7
$FEV_{1.0}$	4.45*	1-6, 1-7
FEF_{25-75}	3.13*	1-7

RV, residual volume; FVC, forced vital capacity; $FEV_{1.0}$, forced expiratory volume at 1 s; FEF_{25-75}, forced expiratory flow rate during middle half of FVC; FA, filtered air; NA, not applicable.
*Significant at $P < 0.05$.

TABLE 4. Summary of pulmonary function, exercise ventilatory pattern, and $\dot{V}_{O_2}$ changes for the nontrained subjects.

Group No.		FVC	RV	$FEV_{1.0}$	FEF_{25-75}	f_R	V_T	$\dot{V}_E$	$\dot{V}_{O_2}$
Nontrained, No. 1	FA	-.042 (-1.0)	+.090 (+ 8.8)	-.009 (-0.3)	+.138 (+4.0)	+5.25 (+17.3)	-.216 (-13.9)	+.50 (+1.1)	+.042 (+2.9)
	0.2	-.263 (-6.7)	+ .079 (+7.7)	-.287 (-9.1)	-.260 (-7.0)	+7.1 (+23.7)	-.215 (-14.1)	+2.14 (+4.7)	+.11 (+8.1)
Nontrained, No. 2	FA	-.023 (- .62)	+.025 (+2.1)	-.088 (-2.8)	-.166 (-4.4)	+1.80 (+7.6)	-.071 (-7.1)	-.088 (- .37)	+.025 (+3.8)
	0.4	-.311 (-8.2)	+.028 (+2.2)	-.551 (-17.9)	-.909 (-23.4)	+2.93 (+12.2)	-.128 (-12.7)	-0.44 (-1.9)	+.021 (+4.2)
Nontrained, No. 3	FA	-.021 (- .57)	+.049 (+4.4)	+.079 (+2.7)	- .076 (-2.2)	+1.65 (+5.3)	-.071 (-5.8)	-.138 (-.36)	+.017 (+1.7)
	0.3	-.558 (-14.9)	+.143 (+11.4)	-.602 (-20.6)	-1.046 (-29.8)	+5.25 (+17.0)	-.247 (-19.5)	-1.93 (-4.7)	-.005 (-0.4)

Values are mean changes; values in parentheses are mean percent changes. Abbreviations same as in Table 3.

TABLE 5. F ratios and specific mean differences from post hoc analysis for pulmonary function and exercise ventilatory and metabolism variables for the nontrained group.

Variable	F Ratio	Specific Significant Mean Differences*
RV	0.70	NA
FVC	26.25*	FA-5; FA-6; FA-7; 5-7
$FEV_{1.0}$	18.17*	FA-5; FA-6; FA-7
FEF_{25-75}	10.93*	FA-5
HR	0.26	NA
$\dot{V}_{O_2}$	0.40	NA
$\dot{V}_E$	0.00	NA
f_R	4.61*	FA-7
V_T	6.37*	FA-7

*Significant at $P < 0.05$

Abbreviations same as in Table 2.

TABLE 6. Total number of subjective symptoms reported by each group.

GROUP	Exposure Description			
	FA	0.2 ppm	0.3 ppm	0.4 ppm
Trained	14	25	41	29
Nontrained, No. 1	23	37	--	--
Nontrained, No. 2	9	--	--	28
Nontrained, No. 3	7	--	32	--

TABLE 7. Comparison of mean percent pulmonary function impairment of female subjects consequent to O_3 inhalation exposures.

Parameter	DeLucia et al (1983) (625 ppm·ℓ)	Dillard et al (1976) (550 ppm·ℓ)	Gliner et al (1983) (700 ppm·ℓ)	Horvath et al (1979) (525 ppm·ℓ)	Present Study (600 ppm·ℓ) Trained	Present Study (600 ppm·ℓ) Nontrained
FVC	- 9.5	- 7.7	--	-12.4	- 8.0	- 9.9
$FEV_{1.0}$	- 8.9	-10.9	-11.9	-19.6	-13.6	-15.9
FEF_{25-75}	-15.0	-14.4	--	-20.9	-19.3	-20.1

$\dot{V}_E$, $l \cdot min^{-1}$ →	23	34.5	46
$[O_3]$ FA ↓			1 0
0.20	2 276	3 414	5 552
0.30	4 414	7 621	8 828
0.40	6 552	9 828	10 1104

O_3, ppm·l = $[O_3]$ x $\dot{V}_E$ x min (60)

FIGURE 1. Experimental Design for Aerobically Trained Females

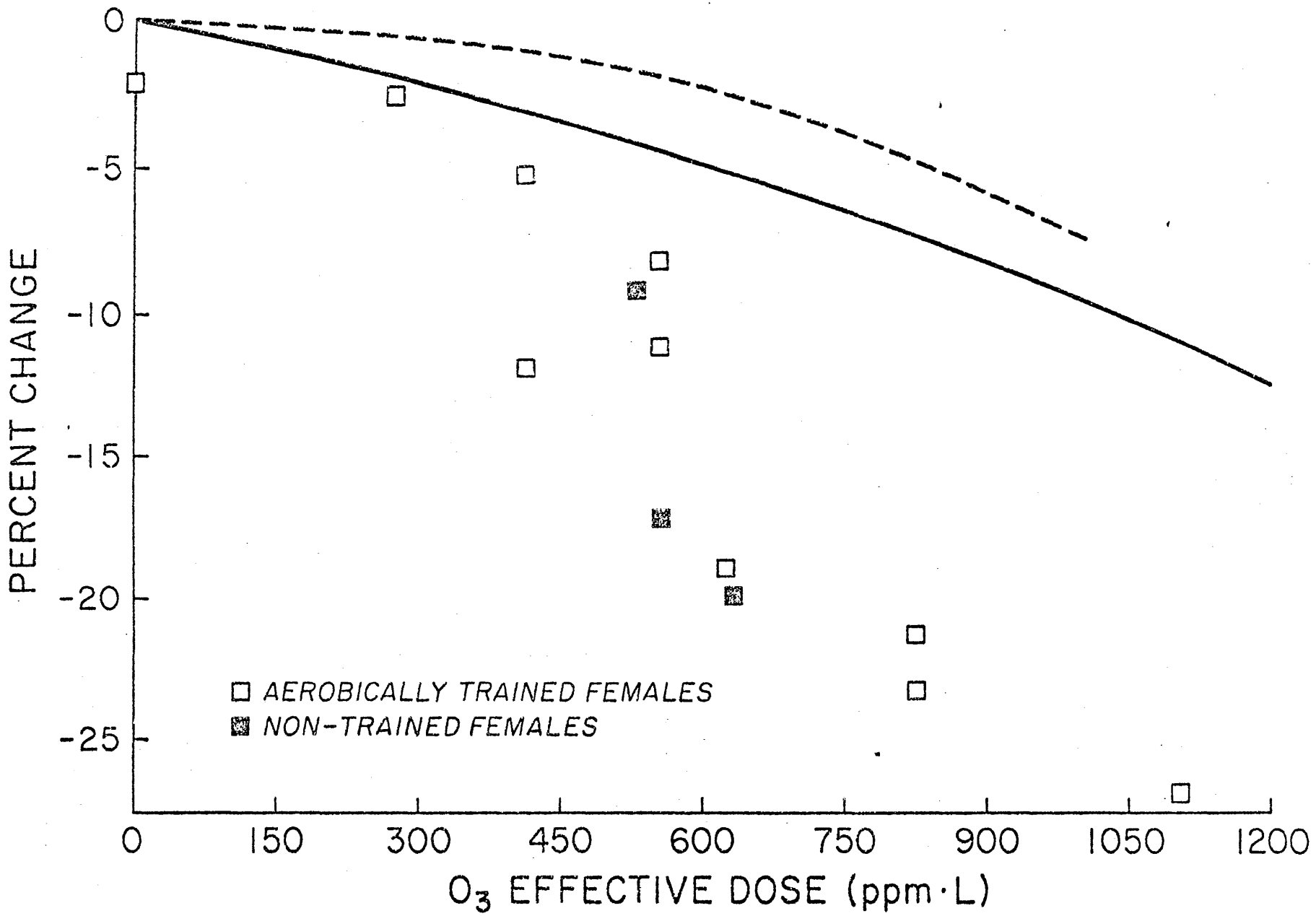

Figure 2. Comparison of Percent Change in $FEV_{1.0}$ as a Function of O_3 Effective Dose.

COMPARISON OF AEROBICALLY TRAINED AND NONTRAINED MIDDLE-AGED MALES' RESPONSES TO O_3 EXPOSURE DURING PROLONGED CONTINUOUS EXERCISE

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ABSTRACT

The present South Coast Air Quality Management District (SCAQMD) advisory chart states that the elderly should stay indoors and reduce physical activity upon occurrence of a first stage alert (i.e., 0.20 ppm ozone), yet there are no laboratory studies of the older human's response to ozone (O_3). In the present investigation, we studied the effects of 1 h continuous exercise at minute ventilations ($\dot{V}_E$) of both 34 and 46 $\ell \cdot min^{-1}$, while exposed to FA or O_3, in a group of six aerobically trained middle-aged males and 20 nontrained middle-aged males. Both groups demonstrated a trend toward pulmonary function impairment and altered exercise ventilatory pattern when exposed to O_3, but only a few differences were significantly different from filtered air (FA) control. Further, their pulmonary function impairment was slightly less than that incurred by young adult males at similar O_3 effective doses. There were no significant differences between the responses to O_3 inhalation of the trained and nontrained groups. It was observed, however, that the nontrained group would elicit a $\dot{V}_E$ (and thus, O_3 inhaled) approximately 30 percent greater than the trained group if they walked, jogged, or bicycled at the same speed.

As noted by Folinsbee et al (1978), until recently, almost all laboratory exposures to ozone (O_3) have utilized young adult male subjects, although children, females, the elderly and those with significant cardiovascular and pulmonary disease are presumed to be more susceptible to the adverse effects of photochemical air pollution episodes. The supposition that advancing age predisposes one to enhanced susceptibility to O_3 toxicity is implied in the current South Coast Air Quality Management District (SCAQMD) advisory chart, which stipulates that at the occurrence of a first stage alert (i.e., 0.20 ppm O_3), "the elderly ..... should stay indoors and reduce physical activity."

While the role of light exercise in enhancing pulmonary function impairment above that observed at rest when exposed to O_3 concentrations between 0.40-0.60 ppm (Folinsbee et al, 1975; Hazucha et al, 1973; Silverman et al, 1976) and of heavy exercise in eliciting effects at concentrations between 0.20-0.30 ppm (Adams et al, 1981; Adams & Schelegle, 1983; DeLucia & Adams, 1977; Folinsbee et al, 1978; McDonnell et al, 1983), has been well documented, preliminary study of aerobically trained middle-age ($\bar{x}$ = 48.3 yrs) males revealed no difference in their response as compared to that of similarly trained young adult males (Superko et al, 1982 ARB Final Report). The possibility remains, however, that older, less well trained subjects might prove to be more sensitive to O_3 inhalation, as the less fit will have a greater minute ventilation ($\dot{V}_E$) at the same absolute work load (Andrew et al, 1966). Thus, the total effective dose of O_3 for a given concentration, exposure time product

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*All O_3 concentrations referenced to buffered potassium iodide have been multiplied by a factor of 0.8 to convert to the ultraviolet photometric standard employed in this study.

will be increased. Further, we (Adams et al, 1972) have noted that older subjects have a higher breathing frequency (f_R) at a given $\dot{V}_E$, which would result in reacted O_3 being replaced at a faster rate.

The purpose of this investigation was to determine if pulmonary function impairment and alterations in exercise ventilatory pattern consequent to 1 h of continuous exercise while exposed to O_3 are different in a group of aerobically trained middle-age males, as compared to their nontrained counterparts.

METHODS

Subject selection, description, and characterization. Initially, six aerobically trained middle-age males were solicited to serve as subjects. Subsequently, 20 normally active (nontrained) middle-age males agreed to participate in the comparative aspect of the study (Institutional Human Use Committee approval and individual signed informed consent were obtained). The subjects were nonsmokers, although several had smoked, though not in the past 10 years. None had resided in an area where O_3 levels had exceeded 0.12 ppm in the past year.

Prior to their initial exposure, each subject completed at least two 1 h sessions in the laboratory. The first entailed acquisition of a brief medical history, resting ECG and auscultatory blood pressures, a physician supervised maximal exercise stress test (including monitoring of a 12-lead ECG and assessment of maximal oxygen uptake, $\dot{V}_{O_{2max}}$), and practice of pulmonary function tests. During the second session, anthropometry, including body composition via hydrostatic weighing, and approximately 30 min of submaximal bicycle ergometer exercise at several work loads to determine $\dot{V}_E$ response, were completed. Additional practice of pulmonary function tests was also performed.

Experimental design. Each of the six aerobically trained subjects completed seven 1 h exposures while breathing filtered air (FA), or 0.20, 0.30, or 0.40 ppm O_3. Exercise intensities were set individually to induce $\dot{V}_E$ of approximately 34 and 46 $\ell \cdot min^{-1}$, respectively, at each of the three O_3 concentrations. The FA exposure was carried out at the high exercise $\dot{V}_E$ (46 $\ell \cdot min^{-1}$) only. The order of experimental protocols was randomized, with a minimum of three days intervening between treatments. The experimental design, including protocol numbers and the O_3 effective dose in ppm·ℓ, is depicted in Fig. 1.

Following completion of this phase of the study, two protocols that elicited pulmonary function impairment in the aerobically trained males, which we anticipated could be completed by nontrained middle-age males, were identified. These 60 min protocols were of similar O_3 effective doses (~830 ppm·ℓ): (1) 0.3 ppm at a $\dot{V}_E$ of ~46 $\ell \cdot min^{-1}$; and (2) 0.4 ppm at ~34 $\ell \cdot min^{-1}$ (i.e., protocols 5 and 6, respectively). Twenty subjects completed one of these protocols (ten in each), together with a FA exposure at the same exercise intensity as in their O_3 exposure.

Subjects were not informed of the level of O_3 received. Following each exposure, subjects completed a subjective symptoms questionnaire, and indicated whether they believed they had received O_3. All exposures were completed in a room in which ambient conditions were maintained within limits described previously (Adams et al, 1981).

O_3 administration and monitoring. Air mixtures during all exposures were generated and delivered via an obligatory mouthpiece inhalation procedure described in detail previously (DeLucia & Adams, 1977). O_3 concentration was routinely determined by samples from the inspiratory side of a Teflon coated

Hans-Rudolph respiratory valve, drawn through a 0.64-cm-ID Teflon tube connected to a Dasibi O_3 meter. The Dasibi meter was calibrated on several occasions according to the ultraviolet absorption photometric method (DeMore et al, 1973) at the University of California, Davis, Primate Research Center.

Pulmonary function measurements. Pulmonary function tests were administered just prior to, and completed again within 15 min following, each exposure. For the trained subjects, residual volume (RV) was measured on a modified Collins 9-liter spirometer utilizing an O_2 rebreathing method (Wilmore, 1969), with initial and equilibrium N_2 readings obtained from an Ohio 700 N_2 analyzer. At least two forced expiratory maneuvers were performed, with forced vital capacity (FVC), forced expiratory volume in 1 s ($FEV_{1.0}$), and forced expiratory flow during the middle half of FVC (FEF_{25-75}) calculated from the spirometric tracings obtained on the 9-liter spirometer. The average of duplicate determinations was utilized for subsequent analyses.

Similar procedures were used to obtain RV, FVC, and flow rate determinations for the nontrained subjects, but on a Collins Clinical Modular Lung Analyzer, Model 3000, with a 10-liter Stead-Wells spirometer, x-y recorder, and RV modules. An output voltage which was associated with volume changes was produced using a linear potentiometer attached to the spirometer belt. The analog signals produced were digitized by an A-D converter for reading into a Digital Equipment Corporation (DEC) LSI 11/2 microcomputer.

Exercise measurements. To assess possible effects of O_3 inhalation on selected exercise parameters, 1 min observations were made at minutes 9-10, 19-20, 29-30, 39-40, 49-50, and 59-60. For the trained subjects, $\dot{V}_E$ was determined by use of a potentiometer mounted in a Parkinson-Cowan (PC) gas

meter, type CD-4, connected to either a Hewlett-Packard 680M or 7402A strip-chart recorder. Respiratory frequency (f_R) was recorded similarly, thence counted manually, and used with $\dot{V}_E$ corrected to BTPS to obtain tidal volume (V_T). Respiratory metabolism was determined from measurement of expired air volume and percent O_2 and CO_2 by a semiautomated sampling method incorporating a manually rotated 3-way valve sampling system (Wilmore and Costill, 1974) and Applied Electrochemistry S-3A and Beckman LB-2 gas analyzers. Heart rate (HR) was determined from the elapsed time of four consecutive R waves recorded from electrocardiogram tracings obtained on a Sanborn visocardette.

For the nontrained subjects, an on-line computerized data acquisition software package was developed to assess one minute average values for $\dot{V}_E$, HR, V_T, f_R, F_{EO_2} and F_{ECO_2} in expired gas, expired gas temperature, and $\dot{V}_{O_2}$ every minute. Data acquisition instruments interfaced to the DEC LSI 11/2 microcomputer, included a LB-1 CO_2 analyzer, an Applied Electrochemistry S-3A O_2 analyzer, an Alpha Technologies Turbotachometer Ventilation Module, an electrocardiograph with R-wave detector, and a temperature thermistor located in the expired gas line.

Statistical procedures. Percent change in pulmonary function parameters were calculated from the postexercise value minus the preexercise value divided by the preexercise value. Similarly, percent change for f_R, V_T, $\dot{V}_E$, $\dot{V}_{O_2}$, and HR were calculated from the value obtained in the last (60th) min. The pulmonary function percent change data for the trained group was analyzed using an ANOVA with repeated measures design to determine if the differences observed in the two O_3 and the single FA exposures were significant. Upon obtaining a significant F ratio, a paired t post hoc test (with Bonferroni correction) was applied to determine which means were significantly different from other(s).

Data from protocols which were completed by both the trained and nontrained groups were compared by t tests for independent samples using a Bonferroni correction. The FA and O_3 exposure data for the nontrained subjects were compared for each exposure group using paired t tests. Comparison of the O_3 responses for the two nontrained groups was made by first subtracting the FA values from the O_3 values, and then analyzing the differences using t tests for independent samples. Statistical significance for all comparisons was set at $P < 0.05$.

RESULTS

A summary of the three groups anthropometry, pulmonary function, and maximal oxygen uptake data is given in Table 1. The aerobically trained group was notably of lighter body weight, leaner, had higher FEF_{25-75}, and demonstrated significantly greater $\dot{V}_{Emax}$, and $\dot{V}_{O_{2max}}$.

Table 1

The group mean absolute and percent changes in pulmonary function, exercise ventilatory pattern and respiratory metabolism for the aerobically trained group are given in Table 2. In general, there was no systematic difference in the changes noted for RV, $\dot{V}_E$, $\dot{V}_{O_2}$ or HR, and none for FVC, $FEV_{1.0}$, FEF_{25-75}, f_R and V_T until the last three exposures (i.e., O_3 effective dose $\geq$ 800 ppm·ℓ). Table 3 details the statistical analyses results from comparisons of pulmonary function measurements for exposure protocols 1, 5, and 6 (Fig. 1) for the aerobically trained subjects.

Table 2

Table 3

The mean absolute and percent changes in pulmonary function and exercise ventilatory pattern parameters for the nontrained subjects is presented in Table 4. Statistical analysis, shown in Table 5, revealed that FVC, $FEV_{1.0}$, and FEF_{25-75} and RV changes for the O_3 exposures were significantly different from those observed for the FA exposures for one group, but not both. Changes

Table 4

Table 5

in $\dot{V}_E$, f_R, and V_T did not differ significantly from those observed for FA exposures. None of the differences in O_3 exposure changes between the two nontrained groups were statistically significant.

t test comparison of the pulmonary function and exercise ventilatory pattern responses to exposure Nos. 5 and 6 revealed no significant differences between the aerobically trained group and the nontrained groups. A comparison of $FEV_{1.0}$ changes as a function of O_3 effective dose (ppm·ℓ), including that observed for young adult males in previous studies (Adams et al, 1981; Folinsbee et al, 1978), that observed for each of the seven exposures completed by the aerobically trained middle-age males, and that observed for the two O_3 exposures completed by the nontrained males in this study, is depicted in Fig. 2. A comparison of the mean percent pulmonary function impairment of the middle-age male subjects for exposure Nos. 5 and 6 combined, and that experienced by other groups of young adult males (at similar effective dose) is presented in Table 6.

Fig. 2

Table 6

DISCUSSION

In the present investigation, we studied the physiological responses of middle-aged male subjects consequent to 1 h continuous exercise while exposed to FA and to O_3. The principal comparison was that between a group of aerobically trained subjects and two groups of nontrained subjects exposed to 0.3 and 0.4 ppm O_3 at exercise intensities eliciting $\dot{V}_E$ of 46 $\ell \cdot min^{-1}$ and 35 $\ell \cdot min^{-1}$ respectively (effective dose of ~830 ppm·ℓ). Both the trained and nontrained groups demonstrated a trend toward pulmonary function impairment and altered exercise ventilatory pattern when exposed to O_3, but only a few of the differences were significantly different from FA. However, in general these

middle-aged subjects responded to O_3 inhalation at approximately the same relative degree (i.e., percent change) as that observed previously in young adult males.

Since there were no statistically significant differences for either the trained or nontrained mean group responses to the two variations in O_3 concentration and $\dot{V}_E$ utilized, additional support for the effective dose concept (i.e., the simple product of O_3 concentration, $\dot{V}_E$, and exposure time) is evidenced. The slight trend toward a greater effect at a similar effective dose in the 0.4 ppm exposure (Tables 2 and 4) is consistent with the preeminent effect of O_3 concentration (compared to $\dot{V}_E$ and exposure time) noted by others (Adams, et al, 1981; Folinsbee et al, 1978; Silverman et al, 1976).

As shown in Fig. 2, both the aerobically trained and the nontrained middle-aged groups evidenced somewhat less $FEV_{1.0}$ decrements (as a function of O_3 effective dose) than did young adult males studied previously in this laboratory (dashed line) (Adams et al, 1981) and by Folinsbee et al (1978) (continuous line). This somewhat reduced response to O_3 inhalation is verified in the comparison of the middle-aged trained and nontrained groups' percent changes for FVC, $FEV_{1.0}$, and FEF_{25-75} to those of the young adult males from earlier studies (Adams et al, 1981; Folinsbee et al, 1978), given in Table 6. In these comparisons at similar effective doses, all values represent the means from exposures to both 0.3 and 0.4 ppm at near proportionally different $\dot{V}_E$. Hence, in spite of the expected age associated decline in FVC (which is only partially accounted for by an increased RV) and flow rates ($FEV_{1.0}$ and FEF_{25-75}), the changes observed for the middle-age subjects were somewhat less - rather than greater, as implied in the SCAQMD chart, which advises the elderly to refrain from physical activity and to remain indoors upon the occurrence

of a first stage alert (O_3 concentration $\geq$ 0.20 ppm). Thus, it appears that the primary factors effecting lung function deterioration with age advanced by Cotes (1975), viz., (1) deterioration in the tissues of which the lung is composed, (2) reduction in the strength of the respiratory muscles, and (3) an increase in stiffness of the thoracic cage, do not materially affect the O_3 toxicity response of clinically normal middle-age individuals, at least within the effective dose range studied. That is, middle-aged males, whether trained or nontrained, appear to incur the same - or even reduced - toxic response to O_3 as their young adult male counterparts. The possibility remains, however, that more elderly subjects (age > 60 yrs) would be more sensitive to O_3 inhalation at the same effective dose. Further, the significantly reduced FVC and flow rates seen with advancing age leave a smaller margin of reserve to meet the stress imposed by photochemical air pollution.

Although the aerobically trained and nontrained (N=20) groups did not differ significantly in their response to O_3 exposure, there were several differences of practical importance noted in the anthropometric, pulmonary function, and $\dot{V}_{O_{2max}}$ data (Table 1). The nontrained subjects (N=20) were 6½ yrs older, slightly taller, and considerably heavier (86.8 kg vs 72.7 kg). Their percent body fat was double that for the aerobically trained group, so that their lean body weights were the same (62.4 kg). There was no significant difference in FVC or RV, but the nontrained subjects had marginally lower $FEV_{1.0}$ and percent of $FEV_{1.0}$/FVC, and a substantially lower FEF_{25-75}. As expected, the greatest difference between the trained and nontrained groups was in exercise capacity, as evidenced by $\dot{V}_{O_{2max}}$. Indeed, there was a disparity in the two nontrained groups' $\dot{V}_{O_{2max}}$, with the original intention being to randomly

assign the nontrained subjects. This proved unfeasible, as of 31 individuals who agreed to participate, five failed to pass the diagnostic exercise stress test. Further, four others who passed this test, were unable or unwilling to complete 1 h of continuous exercise at the prescribed work intensity. Thus, it became necessary to assign preferentially the more fit and somewhat younger subjects to the higher work intensity group (i.e., $\dot{V}_E$ = 46 $\ell \cdot min^{-1}$).

In this study, $\dot{V}_E$ was equated for both the trained and nontrained groups in order to deliver an equivalent O_3 effective dose. However, the mean absolute work loads utilized to elicit ~34½ and 46 $\ell \cdot min^{-1}$ $\dot{V}_E$ for the trained group were 560 and 790 $kpm \cdot min^{-1}$, respectively, while those for the nontrained groups were 510 and 690 $kpm \cdot min^{-1}$. Thus, at an equivalent absolute work load in the same photochemical smog conditions, the nontrained subjects would incur a considerably greater $\dot{V}_E$ (~10-14%) and hence, a greater O_3 effective dose than their nontrained counterparts. More importantly, though, should the nontrained individuals undertake walking, jogging, or bicycling at the same speed as their trained counterparts, their $\dot{V}_E$ would be an additional 20% higher because of their greater body weight.

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TABLE 1. Group anthropometry, pulmonary function, and maximal oxygen uptake data.

	Age, yr	Ht, cm	Wt, kg	Fat, % of Wt	FVC, ℓ	RV, ℓ	$FEV_{1.0}$, ℓ	FEF_{25-75}, ℓ·sec^{-1}	$FEV_{1.0}$/FVC, %	$\dot{V}_{Emax}$, min BTPS	$\dot{V}O_{2max}$, min STPD
Aerobically Trained	48.3 (3.3)	176.5 (7.8)	72.7 (8.6)	14.2 (7.1)	5.04 (0.42)	1.89 (0.31)	3.88 (0.33)	4.34 (0.75)	77.1 (4.2)	154.5 (39.6)	3.71 (0.62)
Nontrained 1	50.3 (8.2)	182.0 (5.2)	88.3 (10.7)	25.2 (4.0)	5.62 (0.76)	1.89 (0.46)	4.18 (0.61)	3.55 (0.94)	74.5 (5.3)	131.2 (23.5)	2.97 (0.58)
Nontrained 2	59.6 (6.0)	178.0 (5.7)	85.4 (11.2)	31.0 (7.4)	4.62 (0.45)	1.83 (0.42)	3.40 (0.41)	2.60 (0.81)	73.4 (6.1)	86.3 (13.2)	2.45 (0.50)

Values are means, with ± 1 standard deviation values in parentheses. Ht, height; Wt, body weight; FVC, forced vital capacity; RV, residual volume; $FEV_{1.0}$, forced expiratory volume in 1 s; FEF_{25-75}, forced expiratory flow during middle half of FVC; $\dot{V}_{Emax}$, maximal minute ventilation; $\dot{V}O_{2max}$, maximal oxygen consumption.

TABLE 2. Summary of pulmonary function, exercise ventilatory pattern and respiratory metabolism changes for the six aerobically trained subjects.

Protocol No.	FVC	RV	$FEV_{1.0}$	FEF_{25-75}	f_R	V_T	$\dot{V}_E$	$\dot{V}_{O_2}$	HR
1	+.088 (+1.74)	-.033 (-1.74)	+.053 (+1.38)	-.111 (-2.56)	+4.4 (+22.9)	-0.45 (-19.2)	-0.33 (-0.73)	+.011 (+0.63)	+5.2 (4.66)
2	-.006 (-0.12)	+.018 (+0.99)	+.030 (+0.77)	-.283 (-6.12)	-0.8 (-4.1)	-0.08 (-4.4)	-3.07 (-8.6)	-.015 (-1.11)	+2.7 (+2.8)
3	+.018 (+0.36)	+.029 (+1.62)	-.039 (-0.98)	-.144 (-3.17)	+1.3 (+6.65)	-0.15 (-6.85)	-0.47 (-1.07)	-.002 (-0.12)	+2.5 (+2.23)
4	+.069 (+1.38)	0 (0)	-.039 (-0.99)	-.028 (-0.64)	+2.0 (+10.9)	-0.16 (-8.7)	-0.87 (-2.59)	+.019 (+1.45)	-0.8 (-0.83)
5	-.109 (-2.17)	+.137 (+6.11)	-.084 (-2.14)	+.109 (+2.59)	+5.2 (+26.7)	-0.48 (-21.4)	-0.23 (-0.53)	+.036 (+2.11)	+1.2 (+1.06)
6	-.020 (-0.39)	+.016 (+0.90)	-.258 (-6.34)	-.452 (-10.22)	+1.5 (+7.4)	-0.21 (-11.5)	-1.73 (-4.69)	-.017 (-1.32)	+2.5 (+2.70)
7	-.216 (-4.3)	+.164 (+9.4)	-.284 (-7.22)	-.576 (-13.11)	+9.6 (+46.4)	-0.63 (-29.4)	+1.4 (+3.16)	-.014 (-0.84)	-1.1 (-0.97)

Values are mean changes; values in parentheses are mean percent changes. FVC, forced vital capacity (liters); RV, residual volume (liters); $FEV_{1.0}$, forced expiratory volume in 1.0 s (liters); FEF_{25-75}, forced expiratory flow during middle half of FVC (liters·s^{-1}); f_R, respiratory frequency (breaths·min^{-1}); V_T, tidal volume (liters); $\dot{V}_E$, minute ventilation (liters·min^{-1}); $\dot{V}_{O_2}$, oxygen consumption (liters·min^{-1}); HR, heart rate (beats·min^{-1}).

TABLE 3. F ratios and specific significant mean differences from post hoc analysis for pulmonary function for the trained group.

Variable	F Ratio	Specific Significant Mean Differences*
RV	2.11	NA
FVC	1.06	NA
$FEV_{1.0}$	2.79	NA
FEF_{25-75}	8.27*	1-6, 5-6

RV, residual volume; FVC, forced vital capacity; $FEV_{1.0}$, forced expiratory volume in 1 s; FEF_{25-75}, forced expiratory flow rate during middle half of FVC; FA, filtered air; NA, not applicable.
*Significant at $P < 0.05$.

TABLE 4. Summary of pulmonary function and exercise ventilatory pattern changes for the nontrained subjects.

Group No.		FVC	$FEV_{1.0}$	FEF_{25-75}	RV	f_R	V_T	$\dot{V}_E$
Nontrained, No. 1	FA	+.135 (+2.4)	-.033 (-0.8)	+.053 (+1.5)	-.078 (-3.5)	+2.67 (+14.1)	-.074 (-3.2)	+1.91 (+4.1)
	0.3 ppm	-.180 (-3.2)	-.248 (-5.9)	-.577 (-15.6)	+.128 (+7.2)	+4.89 (+24.0)	-.389 (-16.9)	+0.62 (+1.4)
Nontrained, No. 2	FA	+.032 (+0.7)	+.039 (+1.1)	+.075 (+3.2)	-.033 (-1.8)	+2.65 (+14.4)	-.097 (-5.2)	+1.65 (+4.7)
	0.4 ppm	-.106 (-2.3)	-.131 (-3.8)	-.067 (-2.6)	+.147 (+8.3)	+1.75 (+9.4)	-.180 (-9.5)	-0.46 (-1.3)

Values are mean changes; values in parentheses are mean percent changes. Abbreviations same as in Table 2.

TABLE 5. t values for filtered air and O_3 exposure comparisons for pulmonary function and exercise ventilatory pattern changes for the nontrained groups.

Variable	Nontrained, No. 1	Nontrained, No. 2
FVC	3.51*	2.03
$FEV_{1.0}$	1.28	2.66*
FEF_{25-75}	4.25*	0.50
RV	2.14	3.66*
f_R	1.04	0.66
V_T	1.95	0.65
$\dot{V}_E$	1.13	1.58

Significant at $P < 0.05$.

Abbreviations same as in Table 2.

TABLE 6. Comparison of mean percent pulmonary function impairment of middle-aged and young adult male subjects consequent to O_3 inhalation exposures.

Parameter	Folinsbee et al (1978) (905 ppm·ℓ)	Adams et al (1981) (310 ppm·ℓ)	Present Study (830 ppm·ℓ)	
			Trained	Nontrained
FVC	- 6.4	-3.4	-1.3	-2.7
$FEV_{1.0}$	- 9.5	-6.6	-4.2	-4.9
FEF_{25-75}	-13.5	-12.7	-3.8	-8.2

$\dot{V}_E$, BTPS ℓ min^{-1} →	34	46
$[O_3]$ ppm ↓ FA		1 0
0.20	2 408	3 552
0.30	4 612	5 828
0.40	6 816	7 1104

FIGURE 1. Experimental Design for Aerobically Trained Middle-Aged Males (O_3, ppm·ℓ = $[O_3]$ x $\dot{V}_E$ x min (60)).

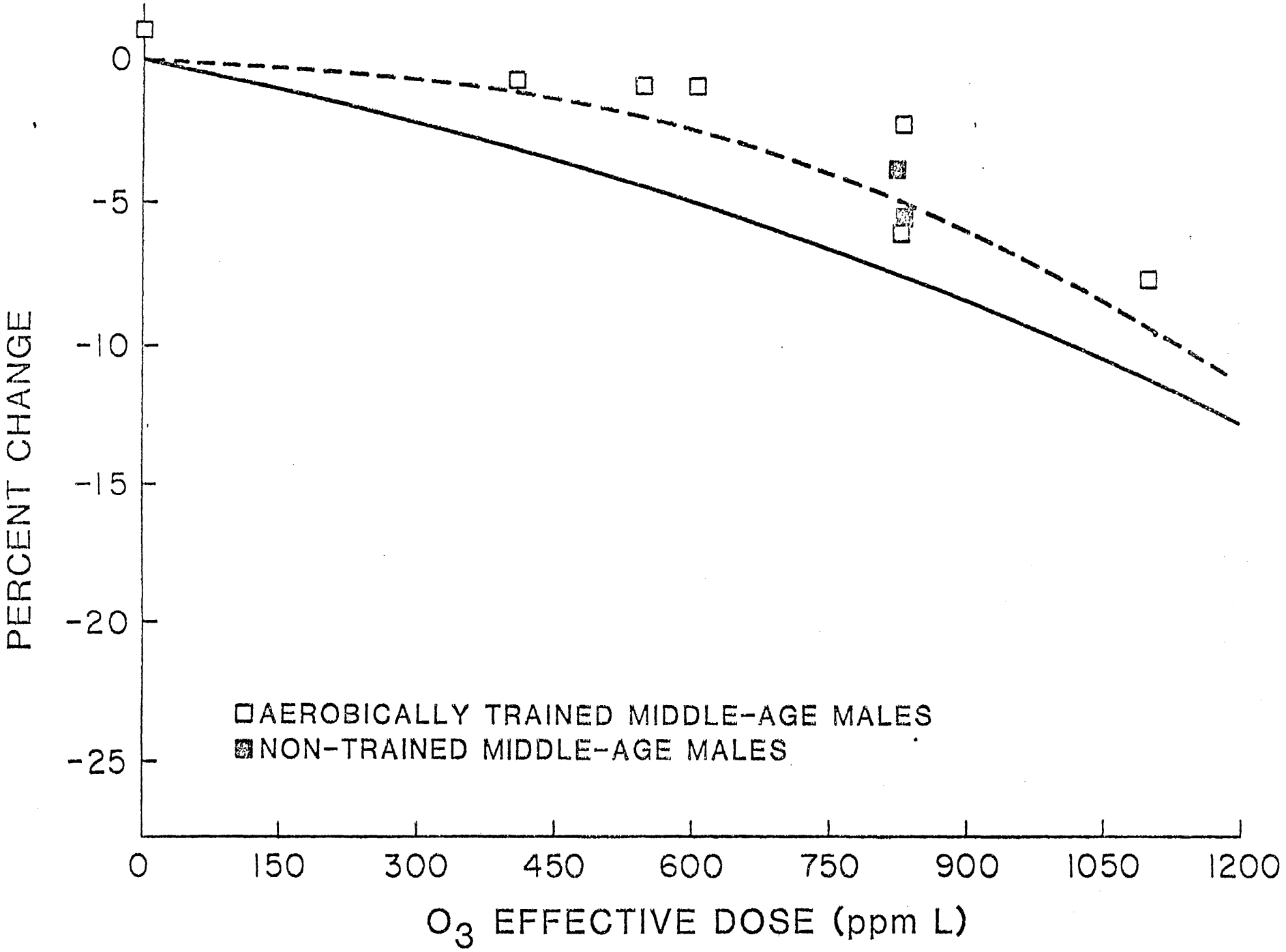

Figure 2. Comparison of Percent Change in $FEV_{1.0}$ as a Function of O3 Effective Dose.

ENDURANCE PERFORMANCE DURING LOW LEVEL OZONE EXPOSURE

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Short Running Head: Endurance performance and ozone exposure

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ABSTRACT

Ten highly trained endurance athletes were studied to determine the effects of exposure to low ozone (O_3) concentrations on pulmonary function and simulated competitive endurance performance. Each subject was randomly exposed to filtered air (FA), and to 0.12, 0.18, and 0.24 ppm O_3 while performing a 1 h competitive simulation protocol on a bicycle ergometer. Endurance performance was evaluated by the number of subjects unable to complete rides (last 30 min at an intense work load of ~86% $\dot{V}O_{2max}$) and associated decreases in ride times. Significant decreases ($P < 0.05$) were observed following the 0.18 and 0.24 ppm O_3 exposures in forced vital capacity (FVC) (-7.8 and -9.9 percent, respectively), forced expiratory volume in 1 second ($FEV_{1.0}$) (-5.8 and -10.5 percent, respectively) and competitive simulation ride time (13.4 and 26.0 percent, respectively). All subjects completed the FA exposure, whereas one, five, and seven subjects did not complete the 0.12, 0.18 and 0.24 ppm O_3 exposures, respectively. No significant O_3 effect was observed on exercise oxygen uptake ($\dot{V}O_2$), heart rate (HR), minute ventilation ($\dot{V}_E$), respiratory frequency (f_R) or tidal volume (V_T). The number of subjective symptoms reported increased significantly following the 0.18 and 0.24 ppm O_3 protocols. These data demonstrate decrements in pulmonary function and simulated competitive endurance performance following exposure to low O_3 levels commonly observed in numerous urban environments during the summer months and further supports the hypothesis that endurance performance decrements following O_3 exposure are the result of physiologically induced respiratory discomfort.

Index Terms: air; pollution; endurance performance; forced expiratory flow rate; lung volume measurements; ozone threshold.

Bates and colleagues (5) were the first to demonstrate that the acute effects of ozone (O_3), a major constituent of photochemical air pollution, were enhanced in humans when exposures entailed exercise. Subsequently, Silverman et al (17), Folinsbee et al (10), Adams et al (2), and McDonnell et al (14) have elucidated the dose-response relationship of acute O_3 effects in humans. Pulmonary function impairment, changes in exercise ventilatory pattern, and increased subjective symptoms response have been shown to be a function of O_3 concentration, minute ventilation ($\dot{V}_E$) and exposure duration, termed O_3 effective dose by Silverman et al (17).

Endurance athletes appear to evidence similar response to O_3 exposure at the same effective dose as nontrained young adult males (3). However, this capacity to maintain high work intensities necessitating $\dot{V}_E$ in excess of 80 $\ell \cdot min^{-1}$ for 1 h or longer, combined with the enhancing role of $\dot{V}_E$ on acute O_3 toxicity response, would make endurance athletes more susceptible to the toxic effects of a given O_3 concentration when training or competing in a photochemically polluted environment (3).

We have recently reported decrements in ride time in 4 of 10 endurance athletes engaged in 1 h competitive simulations when exposed to 0.35 ppm O_3 (3). Our observations suggest that decrements in ride time were due to subjective respiratory discomfort, as no significant alteration in heart rate (HR), oxygen uptake ($\dot{V}_{O_2}$), $\dot{V}_E$, alveolar ventilation ($\dot{V}_A$), or dead space volume (V_D) occurred as a result of 0.35 ppm O_3 exposure. These findings are consistent with the hypothesis advanced by Wayne et al (18) and Folinsbee et al (11) that respiratory discomfort may cause decreased maximal work performance.

Our previous study of endurance athletes (3) was not specifically designed to assess endurance performance decrements, as work loads were selected in part

to elicit a mean target $\dot{V}_E$ of ~80 $\ell \cdot min^{-1}$, instead of the highest possible work intensity the subjects could maintain during the competitive simulations. Had they been selected according to this maximal workload criteria, $\dot{V}_E$ during the competitive simulation would have been considerably higher, and would be expected to lower the O_3 concentration at which performance would be compromised.

The purpose of the present study was to determine what effects, if any, O_3 exposure at concentrations of 0.24 ppm or lower, have on highly trained endurance athletes exercising for 1 h in competitive simulation protocols.

METHODS

Subject description and base-line measurements. Ten competitive endurance athletes, whose basic anthropometry, $\dot{V}_{O_{2max}}$, and pulmonary function data are given in Table 1, served as subjects (Institutional Human Use Committee approval and signed individual informed consent were obtained). One of the subjects was a distance runner, while the others were cyclists training for competition at the time of the study. None smoked and all had pulmonary functions within normal limits.

Table 1

Each subject first completed an orientation to all testing procedures. Subsequently, base-line pulmonary function and basic anthropometry, including body composition via hydrostatic weighing, were obtained. Maximal oxygen uptake ($\dot{V}_{O_{2max}}$) was assessed via a progressive increment protocol described previously (3). Because of the very high power outputs and subject preference, pedal frequency was maintained at 80 rpm, until the heaviest work load attempted when subjects were encouraged to increase pedal frequency. Cessation occurred when pedal frequency dropped significantly below 80 rpm.

Experimental design. The subjects attempted four 1 h competitive simulation exposures at O_3 concentrations of 0, 0.12, 0.18, and 0.24 ppm. The protocol utilized was the same as described previously (3), i.e., 30 min warm-up, followed immediately by 30 min at a constant workload simulating competitive effort (Fig. 1). However, in this study a concerted effort was made to identify work loads that were just achievable in preliminary practice sessions while exposed to filtered air (FA). Initially, the work load for each subject was set to elicit 85% of his previously measured $\dot{V}O_{2max}$. If the subject was unable to complete this ride, or if the work load was too easily managed, he returned for another trial ride. This procedure was repeated until the highest work load the subject could maintain during the full 30 min was determined.

Fig. 1

The order of experimental protocols was randomized for each subject, with a minimum of 5 ($\bar{x}$ = 7.0) days intervening between treatments. Subjects were not informed whether they were receiving O_3. After each protocol, subjects completed a subjective symptoms questionnaire, indicating whether they had received O_3 and, if so, at what concentration. Also, they were asked, taking into account their subjective symptoms, if they thought they would have performed maximally in an actual competitive situation.

All experimental treatments were completed in a room, 3.0 m x 2.4 m x 3.7 m, in which dry bulb temperature and relative humidity were maintained within 23-26°C and 45-60%, respectively. To facilitate convective and evaporative cooling, a constant airflow of 2.5 m/sec was directed at the subject's anterior surface via an industrial-grade floor fan.

Pulmonary function measurements. A short battery of pulmonary function tests was administered immediately prior to each experimental protocol and repeated within 10 min following exercise. Forced vital capacity (FVC) was mea-

sured first, followed by residual volume (RV). At least two determinations each of FVC were made on a Collins 10-liter Stead-Wells Spirometer assembly of the Basic Clinical Spirometer Module, No. 3000. Forced expiratory volume in 1_s ($FEV_{1.0}$) and forced expiratory flow rate during the middle half of FVC (FEF_{25-75}) were calculated from the spirometric tracings. Residual volume (RV) was determined utilizing a modified Collins 9-liter spirometer by the O_2 rebreathing method (19), with initial and equilibrium N_2 readings taken on an Ohio 700 digital N_2 analyzer.

O_3 administration and monitoring. Specific air mixtures during all experimental protocols were inhaled by subjects through a mouthpiece system described in detail elsewhere (7). In brief, FA blended with appropriate concentrations of O_3 generated by a Sander Ozonizer, were supplied to the subject through a Teflon-coated Hans-Rudolph respiratory valve. Expired gas was directed through a 5-ℓ stainless steel mixing and sampling chamber to a Parkinson-Cowan (PC) gas meter, type CD-4, and thence routed into the distal portion of the mixing tube and, along with the pumped air mixture not inspired by the subject, exhausted to the laboratory outside air ventilation outlet.

O_3 concentration was routinely determined on the inspiratory side of the Hans-Rudolph valve, from sampled air drawn through a 0.64-cm Teflon tube connected to a Dasibi O_3 meter. The Dasibi digital reading of O_3 concentration in ppm was compared on several occasions during the course of the study to that determined by the ultraviolet absorption photometric method (8) at the University of California, Davis, Primate Research Center (no change in calibration was noted).

Exercise measurements. Pulmonary ventilation was measured continuously on a Hewlett-Packard 7402A recorder via a potentiometer attached to a PC high-

speed gas meter, Type CD4. Respiratory metabolism, observed every tenth minute, was determined via expired air volume (PC meter) and percent O_2 and CO_2 by a semiautomated sampling method incorporating a manually rotated three-way valve sampling system (20), and Applied Electrochemistry S-3A and Beckman LB-2 gas analyzers. Heart rate (HR) was determined from the elapsed time between four consecutive R waves read from an electrocardiogram tracing taken every 10th min. Respiratory frequency (f_R) was calculated by counting the number of inspiratory plateaus occurring in one minute on the ventilation recording. Tidal volume (V_T) was calculated as a function of $\dot{V}_E$ (BTPS) divided by f_R.

Statistical procedures. Duplicate pulmonary function measurements were corrected to BTPS and averaged for pre- and postexposure measurements. The postexposure value for each parameter was subtracted from the preexposure value, divided by the preexposure value, and multiplied by 100 to obtain percent changes representing the treatment effect for each protocol. Values for $\dot{V}O_2$, $\dot{V}_E$, f_R, tidal volume (V_T), and HR obtained during the last minute of exercise were subtracted from those obtained in the sixth min at the competitive simulation work load, then divided by these values and multiplied by 100 to obtain percent changes.

Data were analyzed using a one-way ANOVA with repeated measures. Post hoc comparisons were done using repeated paired t-tests with Bonferroni correction (16). Significance was set at the 0.05 level.

One subject did not complete the 0.12, 0.18, and 0.24 ppm O_3 exposures, while four others did not complete the 0.18 and 0.24 ppm O_3 exposures. Two subjects did not complete the 0.24 ppm O_3 exposure only. Data from these

exposures were included in all statistical analyses, including that for percent decrease in ride time.

RESULTS

Exercise response. Group means for the sixth min and for the final minute at the competitive simulation work load are given in Table 2. No significant changes in HR, $\dot{V}O_2$, $\dot{V}_E$, f_R, or V_T response for any of the O_3 exposures, as compared to FA, were observed. Failure of the percent change in f_R and V_T to reach statistical significance in this type of competitive simulation protocol is due, in part, to the fact that the more sensitive subjects were already "reacting" to the 0.24 ppm O_3 exposure when the "initial" 36th minute values were obtained. This contention is supported by the observation of near significant ($P < 0.06$) treatment effect for V_T.

Table 2

As shown in Table 3, all subjects completed the FA exposure protocols, whereas, one, five and seven subjects, respectively, did not complete the 30 minute competitive simulation when exposed to 0.12, 0.18 and 0.24 ppm O_3. The mean percent decrease in competitive simulation ride time for all 10 subjects was 2.5, 13.4, and 26.0, respectively, for the 0.12, 0.18, and 0.24 ppm O_3 exposures. The decreases in ride time for the 0.18 and 0.24 ppm O_3 exposures were significantly different from FA.

Table 3

Pulmonary function response. Group mean percent changes in RV, FVC, $FEV_{1.0}$, and FEF_{25-75} for the four exposures, are given in Table 4. The F ratios, together with the statistically significant individual mean comparisons by post hoc analysis, are given in Table 5. A significant O_3 concentration effect for FVC, $FEV_{1.0}$ and FEF_{25-75} was observed.

Table 4

Table 5

Subjective symptoms response. A summary of the symptomatic response to treatments is given in Table 6. The number of symptoms reported by each sub-

Table 6

ject increased significantly following the 0.18 and 0.24 ppm O_3 exposures, compared to FA. In response to the question of whether they would have been able to perform maximally in actual competition (considering their subjective symptoms), one subject indicated that he could not have performed maximally after the FA and 0.12 ppm O_3 exposures. Following the 0.18 and 0.24 ppm O_3 exposures, 5 and 7 subjects, respectively, indicated that they could not have performed maximally. All subjects who completed the rides when exposed to O_3 stated that they believed they could have performed maximally in actual competition.

DISCUSSION

In the present investigation, exposure of well-trained endurance athletes to as low as 0.18 ppm O_3, while engaged in 1 h exercise protocols simulating endurance competition, was sufficient to produce significant pulmonary function impairment. Further, these prolonged high-intensity exercise protocols induced sufficient $\dot{V}_E$, such that exposure to 0.12, 0.18 and 0.24 ppm O_3 resulted in premature cessation of exercise performance in one, five, and seven subjects, respectively.

Relation of acute O_3 toxicity to the inhaled effective dose. Several investigators (2,7,10,12,17), utilizing either intermittent exercise (IE) or continuous exercise (CE) protocols, have demonstrated that the minimum O_3 concentration required to cause significant impairment in pulmonary function is decreased as the mean $\dot{V}_E$ is increased during exposure. Results of the present study confirm recent observations by McDonnell et al (14) that exposure to O_3 effective dose $\geq$ 900 ppm·ℓ, when O_3 concentration is as low as 0.18 ppm is sufficient to elicit significant pulmonary function impairment in healthy young adult males.

In a previous study of competitive endurance athletes engaged in 1 h training and competitive simulation protocols (3), we noted that the percent decrement in $FEV_{1.0}$ as a function of the effective dose was essentially equal to that observed by Folinsbee and colleagues in IE chamber exposures (10) and by Adams et al using a CE obligatory mouthpiece inhalation method (2). This comparison is shown in Fig. 2, in which the dashed line represents the mean response calculated for ppm liter products from our laboratory (2) the solid line is the calculated relationship obtained by Folinsbee et al (10), and the squares represent values obtained in our previous study of endurance athletes (3). Also shown (as open circles) are the values from the present study for FA and the O_3 exposures at 0.12, 0.18 and 0.24 ppm. There appears to be no systematic variation observed in the present study from that represented by either line.

Fig. 2

As noted in our previous study of endurance athletes (3), the relationship between percent change in FVC as a function of O_3 effective dose is greater for endurance athletes than for nonathletes working at lower absolute work loads (2,10). The greater decrement of FVC in both studies was due to greater decrease in inspiratory volume (IV), as RV was unaffected in both. This suggests that as in our previous study (3), decreased IV plays a larger role in the $FEV_{1.0}$ decrement of endurance athletes to O_3 exposure than that noted in the response of nonathletes in earlier studies (2,10). This difference in pulmonary function response of endurance athletes suggests an increase in airway caliber and/or change in lung mechanics consequent to intense exercise.

Effect of O_3 inhalation on endurance performance. Others have observed reduced work performance consequent to O_3 inhalation (3,11,18), although causes of this decrement are not well defined. Concurrent with impaired work

performance, Folinsbee and colleagues (11) observed a decreased $\dot{V}_{O_{2max}}$ consequent to O_3 exposure, which was associated with a decrement in maximum $\dot{V}_E$ and HR. They concluded that these decrements were due to a ventilatory limitation of maximum effort, probably related to respiratory discomfort. Although in our previous investigation of endurance athletes, we did not study the effects of O_3 inhalation on work capacity, per se, 1 h CE training and competitive simulations protocols at work loads which elicited a mean $\dot{V}_E$ of 77.5 $\ell \cdot min^{-1}$ became limiting to the four most sensitive subjects when exposed to 0.35 ppm O_3. Further, while all subjects completed similar protocols when exposed to 0.20 ppm O_3, four stated that, given their postexposure symptoms, they could not have achieved actual maximal competitive performance.

In the present study, the highest work loads the subjects could maintain during the full 30 minutes of competitive simulation when exposed to FA were selected. Establishing work loads in this manner made it possible to assess endurance performance decrements upon exposure to O_3. Failure to complete the competitive simulation protocols in this study upon O_3 exposure was dependent on individual sensitivity, but was consistent. That is, one subject did not complete the 0.12, 0.18 and 0.24 ppm O_3 exposures, four did not complete the 0.18 and 0.24 ppm O_3 exposures, while two others were unable to complete only the 0.24 ppm O_3 exposure. The failure of some subjects to complete the competitive simulation protocols upon exposure to O_3, resulted in a significantly reduced group mean ride time following exposure to 0.18 and 0.24 ppm O_3. These decrements in ride time were not associated with any significant changes in HR, $\dot{V}_{O_2}$, $\dot{V}_E$, f_R, or V_T responses. Furthermore, a comparison of the five subjects who were unable to complete both 0.18 and 0.24 ppm O_3 protocols and the five remaining subjects, indicated that these two groups' cardio-

respiratory responses were similar when exposed to O_3. Therefore, as in our previous investigation of endurance athletes (3), decrements in competitive simulation ride time do not appear to be associated with any change in exercise cardiorespiratory parameters that would imply a reduced alveolar ventilation-perfusion ratio, decreased oxygen saturation in arterial blood, and/or an increased energy requirement of respiratory muscular effort, as have been suggested by Bates (4). However, significant decrement in mean competitive simulation ride time was associated with a significant increase in subjective symptoms in the present study. This supports the hypothesis of Wayne et al (18) and Folinsbee et al (11) that respiratory discomfort may cause decreased maximal work performance.

The lower number of subjective symptoms reported, combined with the greater number of subjects unable to complete the competitive simulation rides in this study compared to our earlier investigation (3), can be attributed to the associated perception of exertion with the higher work loads utilized in this study. Thus, less of an increase in the number of O_3-induced subjective symptoms is needed to result in subjects discontinuing exercise. This suggests a local work intensity factor interacting with a central O_3 inhalation factor, which is contrary to observations of Mihevic et al (15) in light IE exposures. However, the precise relationship between symptoms of respiratory discomfort and subjective perception of exertion and their effect on work performance, cannot be defined from data collected in this investigation.

Results of the present study underscore the importance of very high $\dot{V}_E$ characteristic of sustained competitive performance in lowering the minimum O_3 concentration at which significant pulmonary function impairment and increased incidence of reported subjective symptoms are observed. In our previous study (3), with mean $\dot{V}_E$ of 77.5 $\ell \cdot min^{-1}$ for 1 h exposure to 0.20 ppm, we

observed an 8.3 percent decrement in $FEV_{1.0}$, while Folinsbee et al (9) have recently reported a 14.8 percent decrement consequent to 1 h exposure of competitive cyclists to 0.21 ppm O_3, with $\dot{V}_E$ = 89 $\ell \cdot min^{-1}$. Mean $\dot{V}_E$ during the last 30 min of the competitive simulation protocol used in the present study was ~120 $\ell \cdot min^{-1}$, with that for the full 60 min (including warm-up) averaging 86.2 $\ell \cdot min^{-1}$. This produced significant decrements in FVC and $FEV_{1.0}$ in the 0.18 ppm exposure, as well as a near threefold increase in subjective symptoms and a 13.5 percent decrease in ride time. Had any of these studies of endurance athletes entailed a group of international caliber, who would be capable of sustaining $\dot{V}_E$ of >100 $\ell \cdot min^{-1}$ for periods in excess of 1 h (1,6,13), significant O_3-induced effects might be evidenced at concentrations below 0.18 ppm.

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TABLE 1. Subject's anthropometry, maximal oxygen uptake, and pulmonary function.

Subj.	Age, yr	Ht, cm	Wt, kg	Fat % BW	$\dot{V}_{O_{2max}}$ $\ell \cdot min^{-1}$	RV, liters	FVC, liters	TLC, liters	$FEV_{1.0}$ $\ell \cdot s^{-1}$	$FEV_{1.0}/FVC$ %	FEF_{25-75} $\ell \cdot s^{-1}$
1	29	178	65.0	8.5	4.50	1.49	5.93	7.42	4.63	78.1	4.17
2	19	183	71.0	13.3	4.33	1.51	5.74	7.25	4.96	86.4	5.21
3	22	188	70.0	6.5	5.03	1.08	5.59	6.67	4.71	84.3	5.11
4	21	178	68.0	5.4	4.31	1.17	5.61	6.78	4.50	80.2	4.17
5	24	194	82.5	3.6	5.15	2.06	7.12	9.18	4.73	66.4	3.24
6	23	193	81.5	9.0	4.65	1.73	6.78	8.51	4.69	69.2	3.66
7	23	183	72.4	11.5	4.51	1.46	7.00	8.46	6.28	89.7	7.50
8	25	184	70.2	7.1	4.93	1.69	7.46	9.15	5.31	71.2	4.42
9	20	188	78.3	12.1	5.33	1.12	7.08	8.20	5.59	79.0	5.47
10	22	193	80.0	10.0	4.75	1.68	7.23	8.91	5.25	72.6	3.89
Mean	23	186	73.9	8.7	4.75	1.50	6.55	8.05	5.07	77.7	4.68
± SD	3	6	6.2	3.1	0.35	0.31	0.75	0.95	0.59	7.7	1.22

$\dot{V}_{O_{2max}}$, maximal oxygen uptake; RV, residual volume; FVC, forced vital capacity; TLC, total lung capacity; $FEV_{1.0}$, expiratory volume in 1 s; FEF_{25-75}, forced expiratory flow rate during middle half of FVC.

TABLE 2. Comparison of mean exercise responses.

Variable	FA		0.12 ppm		0.18 ppm		0.24 ppm	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
HR, beats·min^{-1}	164	173	164	173	163	172	163	170
$\dot{V}_{O_2}$, ℓ·min^{-1}	3.93	4.06	3.86	4.10	3.88	3.93	3.92	4.09
$\dot{V}_E$, ℓ·min^{-1}	114	134	116	131	119	135	114	128
f_R, breaths·min^{-1}	42	52	42	51	41	53	41	54
V_T, liters	2.71	2.58	2.76	2.58	2.90	2.54	2.78	2.37

Initial values represent observations during the 36th min of the competitive simulation protocol. Final values are those observed during the last minute of the protocols. FA, filtered air; HR, heart rate; $\dot{V}_{O_2}$, oxygen uptake; $\dot{V}_E$, expired minute ventilation; f_R, respiratory frequency; V_T, tidal volume.

TABLE 3. Summary of exercise performance.

	FA	0.12 ppm	0.18 ppm	0.24 ppm
No. of subjects unable to complete protocol	0	1	5	7
Group mean, percent decrease in ride time	0	2.5	13.4	26.0
t value (vs FA)	-	0.49	2.62*	5.09*

*Significantly different from FA exposure ($P < 0.05$).

TABLE 4. Percent Pulmonary Function Response to Treatments.

Variable	FA	0.12 ppm	0.18 ppm	0.24 ppm
RV	9.26	5.49	6.95	5.56
FVC	-2.30	-2.86	-7.79	-9.88
$FEV_{1.0}$	2.36	1.79	-5.76	-10.51
FEF_{25-75}	10.71	10.02	1.47	-5.03

RV, residual volume; FVC, forced vital capacity; $FEV_{1.0}$, forced expiratory volume in 1 s; FEF_{25-75}, forced expiratory flow rate during middle-half of FVC; FA, filtered air.

TABLE 5. F ratios and specific significant mean differences from post hoc analysis for pulmonary function variables.

Variable	F Ratio	Specific Significant Mean Differences
RV	0.23	NA
FVC	5.33	FA-0.18; FA-0.24
$FEV_{1.0}$	7.25	FA-0.18; FA-0.24
FEF_{25-75}	3.46	FA-0.24

Abbreviations same as in Table 4.

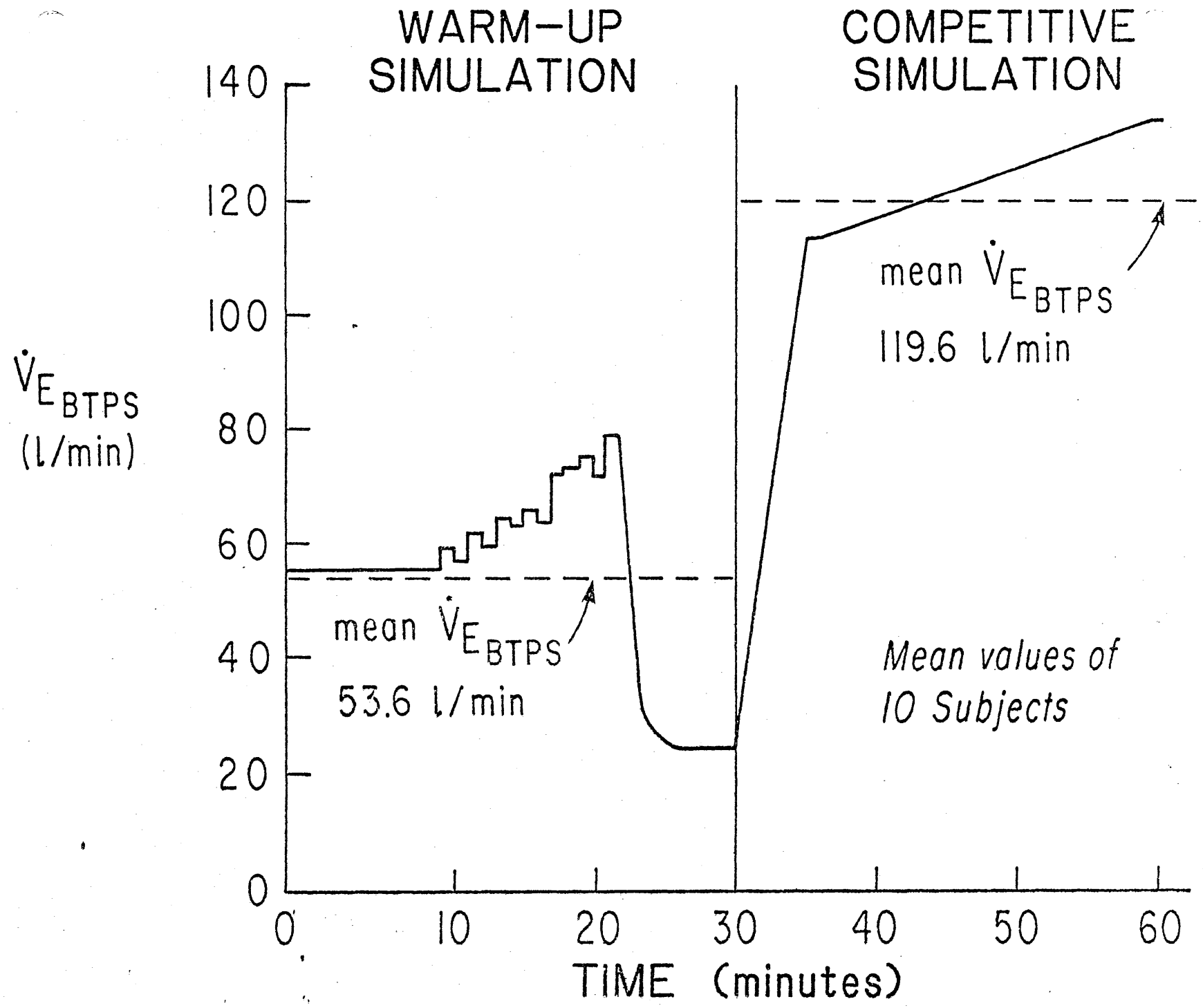
TABLE 6. Summary of total no. of subjects reporting symptoms for each exposure.

Symptoms	FA	0.12 ppm	0.18 ppm	0.24 ppm
Shortness of breath	2	1	5	8
Cough	1	5	7	7
Excess sputum	2	2	3	4
Throat tickle	2	2	4	5
Raspy throat	4	4	5	5
Wheezing	2	1	1	2
Congestion	1	4	3	4
Headache	0	1	1	3
Nausea	0	0	4	3
"Other" symptoms	0	2	5	3
Total symptoms for treatments	14	22	38	44
No. of subj. believing they received O_3	4	5	7	10
No. of subj. believing they were unable to perform maximally	1	1	5	7

FIGURE LEGENDS

Fig. 1 Description of the competitive simulation protocol in terms of expired minute ventilation ($\dot{V}_E$) response.

Fig. 2 Comparison of percent change in forced expiratory volume in 1.0 s ($FEV_{1.0}$) as a function of O_3 effective dose (ppm·ℓ) in the present study (O) with that from an earlier study of endurance athletes (▨ □)(Ref. 3), and from earlier studies of male nonathletes utilizing continuous exercise protocols (dashed line) (Ref. 2) and 2 h intermittent exercise protocols (solid line) (Ref. 10).



Description of the Competitive Simulation Exposure in Terms of $\dot{V}_E$ Response

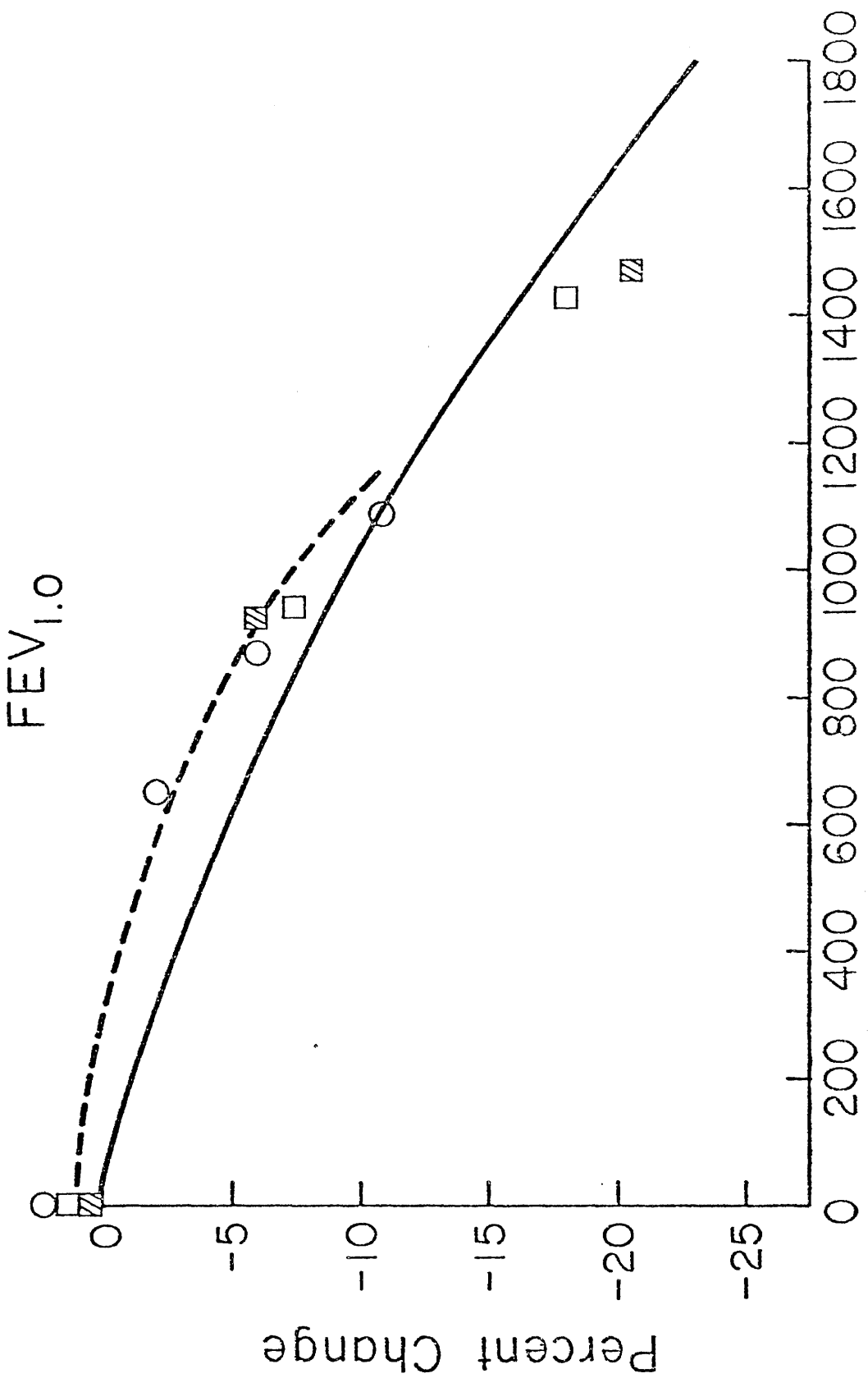


Fig. 2. Comparison of Percent Change in $FEV_{1.0}$ as a Function of O_3 Effective Dose.

COMBINED EFFECTS OF OZONE EXPOSURE AND AMBIENT HEAT ON EXERCISING FEMALES

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Short Running Head: Ozone, Heat, and Exercise

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ABSTRACT

Ten aerobically trained young adult females exercised continuously at 66% of maximum oxygen uptake for one hour while exposed orally to filtered air, 0.15, and 0.30 ppm ozone (O_3) in both moderate (24°C) and hot (35°C) ambient conditions. Exposure to 0.30 ppm O_3 induced significant impairment in forced vital capacity (FVC), forced expiratory volume in one second ($FEV_{1.0}$), and other pulmonary function variables. Exercise respiratory frequency (f_R) increased, while tidal volume (V_T) and alveolar volume (V_A) decreased with 0.30 ppm O_3 exposure. Significant interactions of O_3 and ambient heat were obtained for f_R and V_A, while FVC and $FEV_{1.0}$ displayed a trend toward an O_3/temperature interaction. Although expired ventilation ($\dot{V}_E$) increased, the interactions could not be ascribed to a greater O_3 effective dose in the 35°C exposures. However, subjective discomfort increased with both O_3 and heat exposure, such that three subjects ceased exercise prematurely when O_3 and ambient heat were combined. We conclude that accentuation of subjective limitations and certain physiological alterations by ambient heat coinciding with photochemical oxidant episodes, is likely to result in more severe impairment of exercise performance, although the mechanisms remain unclear.

Index Terms: air pollution; exertion; forced expiratory flow rates; respiratory function tests; thermal stress; vital capacity

INTRODUCTION

Ozone (O_3), a major component of photochemical oxidant pollution, induces pulmonary function impairment and respiratory discomfort, which are exacerbated by exercise (2,4,6,11,12). The enhanced pulmonary ventilation incurred during exercise may potentiate response to O_3 not only by increasing the absolute amount of O_3 inhaled, but also by increasing the uniformity of ventilation and compromising nasal "scrubbing" (6,30). Pulmonary function impairment has been observed to be more closely related to total effective dose (ED), defined as the simple product of O_3 concentration, average expired ventilation ($\dot{V}_E$), and exposure duration, than to O_3 concentration, alone (2,8,24).

Photochemical smog episodes normally coincide with high ambient temperatures. In addition to inducing cardiovascular changes, ambient heat stress during exercise also stimulates $\dot{V}_E$, with the magnitude apparently dependent on exercise intensity, exposure duration, and ambient temperature (1,17,20,21). The enhanced ventilation elicited by heat stress during exercise may increase the ED and thus, pulmonary function impairment. To simulate natural conditions and to determine the extent to which ambient heat stress exacerbates photochemical oxidant effects, several laboratory studies have included a combination of heat and pollutant exposure (7,8,10). In subjects exercising at 40% of maximum oxygen consumption ($\dot{V}_{O_2}max$) for 30 minutes during a two hour exposure to 0.40 ppm O_3[1], Folinsbee et al. (8) noted a diminution of forced vital capacity (FVC) with exposure to heat, but the combined effect of O_3 and ambient heat stress was not synergistic.

Previous studies which have combined O_3 and heat stress, have included exercise of short duration and/or low intensity. The purpose of the present investigation was to examine the effect of ambient heat on O_3 effects during

prolonged continuous, moderately heavy, exercise designed to simulate aerobic training.

METHODS

Subject characterization. Ten Caucasian females, all local residents for at least the previous nine months, served as subjects. Their average anthropometric and fitness characteristics were: age, 22.9 ± 2.5 years; height, 163.3 ± 3.6 cm; weight, 56.3 ± 5.0 kg; percent body fat, 22.4 ± 3.9; and $\dot{V}_{O_2}$ max, 2.79 ± 0.27 $\ell \cdot min^{-1}$. Institutional Human Use Committee approval and signed informed consent were obtained. All subjects were currently participating in regular competitive or personal aerobic training programs involving at least four exercise sessions per week. Each was asked to maintain her normal exercise patterns for the duration of the study. None smoked, and all demonstrated pulmonary function values within normal limits, although one had a history of asthma.

During each subject's first laboratory session, the workload at which she would ride during the experimental protocols was determined. While the subject rode an electronically braked bicycle ergometer (Quinton) at a pedal frequency of approximately 70 rpm, the workload was adjusted every 10 minutes until steady state $\dot{V}_E$ approached 55 $\ell \cdot min^{-1}$, BTPS. In a subsequent session, $\dot{V}_{O_2}$max was determined via a continuous, stepwise protocol. Following a four minute warmup at 400 $kpm \cdot min^{-1}$, the workload was increased 200 $kpm \cdot min^{-1}$ every two minutes until voluntary exhaustion. Body composition, via hydrostatic weighing, and baseline pulmonary function were also assessed.

Following baseline characterization, each subject completed five consecutive days of a progressively intensified heat acclimation regimen to minimize habituation effects and to attenuate failure to complete experimental protocols

involving heat. Further, to maintain heat acclimation, subjects were encouraged to perform their regular exercise training during the heat of the day at least twice a week for the duration of the experiment, which was conducted from July to October. During this period, peak one hour O_3 levels at a local area air quality monitoring station ranged from 0.02 to 0.10 ppm.

Experimental design. The six treatments included in the experimental design are depicted in Figure 1. Subjects were exposed orally to each of three air mixtures, including filtered air (FA), 0.15 ppm, and 0.30 ppm O_3 at both 24°C and 35°C ambient conditions. Each subject attempted the six one-hour exposures while riding the bicycle ergometer continuously at a workload designed to elicit a $\dot{V}_E$ of approximately 55 $\ell \cdot min^{-1}$.

Figure 1

Exposures were randomly delivered, with at least three days between to avoid development of O_3 tolerance. In a recent study (3) we noted a nonsignificantly greater forced expiratory volume in one second ($FEV_{1.0}$) impairment in six individuals exposed within 3 to 6 days to either 0.20 or 0.35 ppm after exposure to 0.35 ppm (-8.5%), compared with exposure at the same concentrations without prior exposure to 0.35 ppm (-7.6%).

Circadian rhythm effects were attenuated by scheduling all exposures at approximately the same time of day. The random order of exposures was not revealed to the subjects, and olfactory detection of O_3 before the exposures was prevented by having subjects wear a noseclip upon approaching the O_3 delivery system. Following each exposure, subjects completed a symptom questionnaire and reported whether or not they believed they had received O_3.

Pulmonary function measurements. Immediately prior to and following each experimental protocol, the subjects performed a brief series of pulmonary function tests. Forced vital capacity was measured on a Collins 10-liter Stead-

Wells spirometer assembly of the Basic Clinical Spirometer Module, No. 3000. Forced expiratory volume in one second and forced expiratory flow during the middle half of FVC ($FEF_{25-75\%}$) were obtained from spirometry tracings. Residual volume (RV) was measured on the same system utilizing the O_2 rebreathing method (28), with initial and equilibrium N_2 values determined on a Collins N_2 analyzer. Both FVC and RV maneuvers were performed at least twice, but not more than four times, to obtain values within 100 mℓ. Total lung capacity (TLC) was calculated as the sum of FVC and RV.

O_3 administration and monitoring. The O_3 generating system has been described in detail elsewhere (6). Basically, ozonized air from a Sander Ozonizer, type II, was combined with FA to achieve the appropriate O_3 concentration, which was then administered to the subject orally via a low resistance, Teflon coated, Hans-Rudolph respiratory valve. Expired air was expressed via Teflon tubing to a 5 ℓ stainless steel mixing and sampling chamber and then to a Parkinson-Cowan (PC) gas meter, type CD-4. After sampling and volume measurements, expired gas was returned to the distal portion of the O_3 mixing tube, and with ozonized air not inhaled by the subject, exhausted via Flexaust CWC neoprene hose to a laboratory ventilation outlet.

Inhaled O_3 concentration, in ppm, was monitored intermittently by drawing a sample of air from the inspiratory side of the Hans-Rudolph valve through Teflon tubing to a Dasibi O_3 meter. The Dasibi monitor was calibrated by the ultraviolet absorption photometric method at the University of California, Davis, Primate Research Center.

Environmental control and monitoring. All exposures were conducted in an environmental chamber measuring 3.0 m x 2.4 m x 3.7 m. Ambient temperature in the environmental chamber was thermostatically controlled by a JUMO mercury

thermoregulator attached to a Chrysler room air conditioner and a Titan floor heater. Dry-bulb and wet-bulb temperatures were recorded every 10 minutes from a Reuter Stokes Wibget Heat Stress Monitor (Model #RSS-211D). A Dayton industrial grade floor fan, set at 2.03 $m \cdot s^{-1}$, was employed to disperse temperature gradients in the environmental chamber and to facilitate evaporative cooling. The JUMO and Wibget systems were placed on a plexiglass shelf between the subject and the fan, such that the ambient environment near the subject was closely regulated.

Exercise measurements. Exercise ventilatory and metabolic measurements were made for one minute at 10 minute intervals throughout each exposure. $\dot{V}_E$ was obtained from a Hewlett-Packard 7402A strip chart recorder connected to a potentiometer within the PC meter. Respiratory frequency (f_R) was obtained from the $\dot{V}_E$ tracing and, following correction to BTPS, tidal volume (V_T) was calculated as $\dot{V}_E/f_R$. Expired gas was collected from the mixing chamber by a semi-automated sampling method incorporating a manually rotated three-way valve system (29), and O_2 and CO_2 concentrations were analyzed by Applied Electrochemistry S-3A and Beckman LB-2 analyzers, respectively. Expired air temperature was monitored by a Yellow Springs Instruments Telethermometer thermistor in the inlet side of the PC meter.

Alveolar ventilation ($\dot{V}_A$) was determined on seven subjects from the end tidal fraction of CO_2 ($FETCO_2$) measured at the mouth for one minute at 10-minute intervals. Gas samples were drawn directly from the mouthpiece to the Beckman LB-2 CO_2 analyzer and recorded on the second channel of the Hewlett-Packard recorder. $FETCO_2$ was corrected to alveolar values according to the equation advanced by Jones et al. (14). $\dot{V}_A$ was calculated using the corrected end tidal CO_2 fraction ($FETCO_2corr$) by the following equation:

$$\dot{V}_A = [\dot{V}_E(BTPS) \times FECO_2]/FETCO_2corr,$$

where: $\dot{V}_E(BTPS)$ is the minute volume corrected to BTPS, and $FECO_2$ is the expired CO_2 fraction. Functional dead space volume (V_D) was calculated by the following equation:

$$V_D = V_T - (\dot{V}_A/f_R).$$

Alveolar volume (V_A) was calculated by the following equation:

$$V_A = V_T - V_D,$$

while deadspace minute volume ($\dot{V}_D$) was calculated as:

$$\dot{V}_D = V_D \cdot f_R.$$

Heart rate (HR) was determined from a 6-second tracing on a Sanborn Visocardette electrocardiograph. Rectal temperature (T_{rec}) was monitored on a Yellow Springs Instruments Telethermometer from a rectal probe inserted to a depth of 12 cm.

Statistical procedures. The mean of at least two pulmonary function values from each subject measured pre- and postexposure were converted to BTPS. The preexposure value was subtracted from the postexposure value to determine the magnitude and direction of the treatment effect for each protocol. The differences in exercise measurements, including $\dot{V}_E(BTPS)$, $\dot{V}_{O_2}$, f_R, V_T, $\dot{V}_A$, $\dot{V}_D$, V_D, V_D/V_T, V_A, HR, and T_{rec}, from the 10th to the last minute of exposure, were calculated similarly.

A two-way analysis of variance (ANOVA), with repeated measures across O_3 concentration and ambient temperature, was performed on the pulmonary function and exercise data. Upon obtaining a significant main effect or interaction, a matched pair t test and Bonferroni inequality correction (19) were applied to determine between which protocols significant differences existed. The Bonferroni correction is applicable in repeated measures designs, and is com-

parable to most sensitive pairwise comparison tests under the conditions of this experimental design. All statistical tests were performed assuming a significance level of $p < 0.05$.

RESULTS

Three subjects were unable to complete all of the experimental protocols when heat and/or O_3 were present. Each of these subjects terminated exercise prematurely in the presence of 0.30 ppm O_3 and heat, while one also failed to complete the corresponding moderate temperature exposure and another also did not finish the 0.15 ppm O_3 exposure in the presence of heat. Although the duration of these exposures ranged from 38 to 53 minutes, data from these exposures were included in all statistical analyses.

Exercise ventilatory and metabolic responses. The group mean ventilatory and metabolic responses for the 10th and final minute of exercise for each of the six treatment protocols are presented in Table 1. The average $\dot{V}_E$ over all conditions was maintained at 54.1 $\ell \cdot min^{-1}$. This reflects a mean $\dot{V}_{O_2}$ of 1.85 $\ell \cdot min^{-1}$, or 66.3% of $\dot{V}_{O_2}max$.

Table 1

As O_3 concentration increased, f_R increased while V_T reciprocally decreased ($p < 0.0001$). Furthermore, there was an interactive effect of O_3 and temperature on f_R. However, this interaction was not evident at the 0.30 ppm O_3 level. Alveolar volume was reduced during O_3 exposure, with a significant interaction of temperature and O_3 concentration. Dead space volume was not significantly altered by O_3 inhalation or ambient heat. While the change in $\dot{V}_E$ during exercise was not affected by O_3, there was a significant elevation of $\dot{V}_E$ with time due to heat. However, neither $\dot{V}_A$ nor $\dot{V}_D$ were significantly changed in any experimental condition. Both the average exposure $\dot{V}_{O_2}$

and the change in $\dot{V}_{O_2}$ during exercise were decreased as O_3 concentration increased.

The change in HR from the 10th to the final minute of exposure was enhanced in the presence of heat, but reduced as O_3 concentration increased. A significant elevation of T_{rec} was measured in the hot conditions.

Pulmonary function responses. The pre- and postexposure pulmonary function group mean values are presented in Table 2. Decrements in FVC, $FEV_{1.0}$, $FEF_{25-75\%}$, and TLC were significantly related to O_3 concentration ($p < 0.004$). There was no statistically significant effect of O_3 on RV. Results of ANOVA and posterior tests are presented in Table 3. Although there was a trend toward pulmonary function decrement in several responses following exposure to 0.15 ppm O_3, in no case were they significantly different from those measured following FA exposure.

Table 2

Table 3

The only significant temperature effect among pulmonary function variables was obtained for FVC, although RV showed a trend toward higher values in the heat ($p < 0.10$). Near significant ($p < 0.10$) interactions between O_3 and temperature were determined for FVC and $FEV_{1.0}$.

Subjective responses to O_3 and temperature. The results of the symptom questionnaire are reported in Table 4. Statistical analysis showed that the number of subjective complaints increased with rising O_3 concentration. In addition, at every level of O_3 exposure, the presence of heat significantly intensified the subjects' discomfort. However, the combined effects of O_3 and heat stress on subjective complaints were not interactive. Among the symptoms included under "other" were (in order of frequency): pain on inspiration (13 responses), dizziness or lightheadedness (8 responses), dry mouth (7 responses), and leg fatigue (2 responses). In spite of the use of a noseclip

Table 4

upon entering the environmental chamber to prevent the detection of O_3 odor, approximately half of the subjects identified the presence of O_3 in the 0.15 ppm exposures, while all of the subjects correctly identified the presence of O_3 when exposed to 0.30 ppm.

DISCUSSION

It is recognized that photochemical smog episodes coincide with high ambient temperatures. Yet, only a few investigations of O_3 effects during exercise have included heat, an important environmental stressor, in an attempt to simulate natural conditions. Results of the present investigation reveal that ambient heat stress, typical of that experienced during a photochemical smog alert, may enhance O_3 effects among females exercising continuously at a moderately heavy workload for one hour.

O_3 effects. In this study, pulmonary capacities and flows were altered by oral exposure to O_3 during exercise for one hour. FVC and TLC were significantly reduced upon exposure to 0.30 ppm O_3, while RV was unchanged. While some researchers have attributed the decrement in FVC to an elevation in RV following O_3 administration (2,4,12,15), it is more likely that a decreased inspiratory volume contributed to the alterations noted in the present study. As shown in Table 2, FVC and TLC decreased in a similar fashion, while the average RV over both temperature conditions remained relatively unchanged as O_3 concentration increased. Others have also found no effect of O_3 on RV (6,8). Significant reductions in FVC and TLC, without a concurrent increase in RV following O_3 exposure, are consistent with stimulation of respiratory irritant receptors, resulting in reflex bronchoconstriction, shallow breathing, and coughing upon deep inhalation (16,26). All of these factors may diminish the ability to inhale deeply. In the present study, most subjects reported

pain on inspiration and coughing following 0.30 ppm O_3 exposure which, despite verbal encouragement, may have prevented a full inspiratory effort during the pulmonary function maneuvers.

Exposure to 0.30 ppm O_3 was associated with significant decrements in $FEV_{1.0}$ and $FEF_{25-75\%}$. Decreases in maximal expiratory flow values are assumed to reflect increased airway resistance (27). However, it has also been shown that a submaximal inspiration may result in relatively greater decreases in expiratory flow values (5). Since airway resistance was not measured in this study, it is difficult to determine the relative contributions of bronchoconstriction and compromised inspiratory volume to reduced maximal expiratory flows. It is unlikely that expiratory flows were limited by decreased effort on the part of the subjects, in that little difference in the ratio of $FEV_{1.0}$/FVC occurred with O_3 exposure. Thus, it appears that reduced inspiratory volume consequent to O_3 exposure was the principal determinant of impaired expiratory flow.

The total ED, defined as the simple product of O_3 concentration, average $\dot{V}_E$, and exposure duration, has been observed to be more closely related than O_3 concentration alone, to pulmonary function impairment incurred by young adult males (2,8). However, there is a paucity of literature on the responses of females to O_3 exposure during exercise. Lauritzen and Adams (15) exposed women to 0.30 ppm for one hour during continuous exercise at a moderately heavy intensity ($\dot{V}_E$ = 46 $\ell \cdot min^{-1}$). Although our subjects were exposed to a 15% greater ED, they were consistently less sensitive to O_3 than the subjects in the previous study (Table 5). This disparate sensitivity cannot be related to any difference in anthropometry or fitness level, as the two groups were homogeneous with regard to these characteristics. Upon comparison to data on

males inhaling 0.30 ppm O_3 for one hour (2), the females in these two studies appeared to be more sensitive, even though the males received a larger ED by virtue of their greater absolute exercise workload ($\dot{V}_E$ = 63 $\ell \cdot min^{-1}$). While Lauritzen and Adams (15) have hypothesized that the greater pulmonary function impairment noted in females at a similar O_3 ED is due in part to lung size differences, variations in group sensitivity cannot be dismissed (18).

In protocol 3 (0.30 ppm, moderate temperature), the change in pre- and postexposure $FEV_{1.0}$ ranged from -2.0% to -49.0%. Similar ranges in variation among subjects have been observed by others, but no plausible explanation has been advanced (2,6,24). In the present study, the greatest pulmonary function decrements due to O_3 were demonstrated by the subject with a history of asthma, although the associated symptoms were not typical of those experienced in an asthmatic episode. Individual variation in sensitivity probably contributed to the lack of statistical significance in pulmonary function changes following the 0.15 ppm O_3 (24°C) exposure. For example, in this exposure, $FEV_{1.0}$ varied by +3.5% to -30.6%, although a trend toward pulmonary function decrement at this concentration was apparent.

Others have reported the rapid, shallow breathing pattern noted with O_3 exposure during exercise (2,6,8,15), which has been ascribed to vagal hyperirritability consequent to stimulation of irritant receptors (16). Alveolar volume was significantly reduced by O_3 exposure, while trends toward a decrease in $\dot{V}_A$ and increased $\dot{V}_D$ were observed. These findings may partially explain the unusual attenuated rise in $\dot{V}_{O_2}$ with O_3 exposure, which was also observed by Folinsbee et al. (8). However, further examination revealed no O_3 effect on $\dot{V}_{O_2}$ among the subjects who completed the 0.30 ppm exposures. Thus, the previous finding may be a statistical artifact, in that those subjects who

exercised for less than 60 minutes did not display a demonstrable metabolic "drift." While not reported elsewhere, the drop in HR drift with O_3 is consistent with the pulmonary "chemoreflex" syndrome described by Tomori and Korpáš (26), in which stimulation of various lung receptors by low concentrations of irritants or certain drugs initiates bradycardia, as well as tachypnea, hypotension, and somatomotor inhibition.

As O_3 concentration increased, subjects reported more subjective discomfort, including cough, pain on inspiration, throat tickle, dizziness, and nausea. Upon questioning, the three subjects who did not complete the one hour exposures with 0.15 or 0.30 ppm O_3 felt that exercise was limited mostly by nausea or leg fatigue, rather than respiratory distress alone. These somatic symptoms are typical of the pulmonary chemoreflex syndrome described above.

Temperature effects. Heat exposure was related to decreases in FVC independent of O_3 concentration, although post hoc analysis revealed only a near significant difference between the protocols at 0.30 ppm O_3 due to temperature. Folinsbee et al. (8) also noted this phenomenon, which is unexpected because bronchodilation is assumed to occur when breathing warm air (23)[2]. Stacy et al. (25) noted an increase in FVC following a 4-hour exposure to heat (30°C) which included two 15-minute moderate intensity exercise bouts. Subjects in this study and that of Folinsbee et al. experienced longer exercise periods and greater thermal stress during heat exposures than those in the study of Stacy et al. The greater subjective discomfort observed in the present study and by Folinsbee et al. (8) may have diminished inspiratory effort and, subsequently, FVC.

In the present study O_3 exposure and ambient heat stress induced an interactive effect on V_A and f_R, while near significant interactions ($p < 0.10$)

were observed for FVC and $FEV_{1.0}$. Greater $\dot{V}_E$ incurred in the heat exposures might be expected to augment the ED at any level of O_3, although the 1.5% higher average $\dot{V}_E$ measured during exercise in the heat in this study did not result in a significantly larger ED. Although heat-induced hyperpnea during exercise has been observed in several laboratories (1,17,20,22), it is not of thermoregulatory importance in humans (13). Possible mechanisms include (1) sensitization of peripheral chemoreceptors by heat (21), and (2) hyperventilation as a consequence of the relative anaerobiosis in compromised working muscle as cutaneous blood flow increases for heat dissipation (17).

The effect of ambient heat stress on $\dot{V}_E$ in this study, and hence O_3 effects (as determined by ED), may have been attenuated by the heat acclimation regimen undertaken by the subjects prior to the experimental protocols. Although heat stress was evidenced by increases in HR and T_{rec}, $\dot{V}_E$ was enhanced by only 1.5% in the heat exposures. Other studies involving similar exercise intensity and duration, report a 12-16% greater $\dot{V}_E$ among unacclimated subjects in hot relative to moderate conditions (1,17). Following heat acclimation, Adams et al. (1) noted a return of $\dot{V}_E$ toward baseline values, although acclimation did not abolish hyperpnea in the heat. However, in acclimated female subjects exercising for 70 minutes at 70% $\dot{V}_{O_2}max$, Paumer (20) noted a 6% greater $\dot{V}_E$ in hot-dry conditions relative to moderate conditions.

Although exercise in the heat did not increase metabolic demand as reflected by $\dot{V}_{O_2}$, three subjects were unable to continue during the 35°C exposures (protocols 5 and 6), while only one subject could not complete a moderate exposure (protocol 3). Thus, O_3 and heat stress together were more likely to induce premature cessation of exercise. Among the three subjects who failed to complete the entire 60 minute protocols in one or both of the 0.30 ppm O_3 ex-

posures, all exercised longer in the absence of heat stress. A comparison of their pulmonary function responses in protocols 3 and 6 (0.30 ppm O_3, 24°C and 35°C, respectively) is presented in Table 6. Although of shorter duration and hence, lower ED in every case, O_3 exposure in the heat induced a greater mean pulmonary function impairment than in the cooler exposure. Thus, termination of exercise was not related to a particular ED level when ambient heat stress was included.

Tab. 6

At any O_3 concentration, there were more subjective complaints in the hot exposure. The excess symptoms reported in the heat, with the exception of "dry mouth", were more typical of those experienced during O_3 exposure alone. Hence, heat appeared to intensify O_3-induced symptoms, although the interaction was not statistically significant. The near significant interactions of O_3 and temperature on FVC and $FEV_{1.0}$ observed in the present study underline the importance of considering the ambient environment when applying the results of laboratory studies on O_3 effects during exercise.

Decrements in $\dot{V}_{O_2}max$ and work performance following O_3 administration (9) have been attributed in part to respiratory discomfort, rather than to any physiological restrictions. The accentuation of subjective limitations and certain physiological alterations by ambient heat coinciding with photochemical oxidant episodes, is likely to result in more severe impairment of exercise performance, although the mechanisms remain unclear.

FOOTNOTES

1. All O_3 concentrations referenced to buffered potassium iodide have been multiplied by a factor of 0.8 to convert to the ultraviolet photometric standard employed in the present study.
2. Although the source of inspired air in the present study was from outside of the environmental chamber, there was no difference in the temperature of the inspired air and ambient room temperature.

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Table 1. Mean Ventilatory and Metabolic Responses during Exercise

Measurement	Filtered Air				0.15 ppm O_3				0.30 ppm O_3			
	24°C		35°C		24°C		35°C		24°C		35°C	
	10th min	Final min	10th min	Final min	10th min	Final min	10th min	Final min	10th min	Final min	10th min	Final min
f_R, breaths·min^{-1}	29.3 ±2.4	32.5 ±4.8	29.4 ±4.2	37.5 ±7.9	29.2 ±4.6	34.6 ±4.9	30.1 ±4.7	36.9 ±5.0	29.0 ±4.7	43.6 ±8.9	30.3 ±3.8	42.2 ±8.2
V_T, ℓ·breaths^{-1}	1.81 ±0.12	1.69 ±0.19	1.75 ±0.21	1.61 ±0.20	1.80 ±0.28	1.56 ±0.23	1.77 ±0.23	1.57 ±0.17	1.81 ±0.20	1.29 ±0.19	1.73 ±0.18	1.38 ±0.21
V_D, mℓ·breath^{-1}*	323 ±52	312 ±47	297 ±22	293 ±41	333 ±59	317 ±59	283 ±23	281 ±24	276 ±63	225 ±48	298 ±30	285 ±43
V_A, mℓ·breath^{-1}*	1484 ±144	1413 ±225	1486 ±240	1344 ±207	1511 ±235	1254 ±210	1517 ±239	1333 ±176	1587 ±171	1036 ±176	1422 ±188	1111 ±229
$\dot{V}_E$, ℓ·min^{-1},BTPS	52.3 ±5.0	54.4 ±6.7	50.8 ±4.2	58.7 ±9.8	51.6 ±5.2	53.6 ±6.4	52.7 ±6.4	57.6 ±6.8	51.9 ±5.8	54.9 ±6.3	52.2 ±6.4	58.5 ±8.7
$\dot{V}_{O_2}$, ℓ·min^{-1}	1.85 ±0.17	1.96 ±0.19	1.80 ±0.06	1.93 ±0.14	1.84 ±0.14	1.89 ±0.14	1.81 ±0.19	1.85 ±0.15	1.84 ±0.17	1.87 ±0.16	1.77 ±0.19	1.83 ±0.15
HR, beats·min^{-1}	148 ±11	160 ±11	150 ±11	167 ±13	148 ±13	157 ±14	153 ±12	171 ±14	148 ±12	152 ±12	151 ±12	162 ±14
T_{rec}, degrees, C	37.4 ±0.3	38.3 ±0.3	37.3 ±0.2	38.4 ±0.2	37.3 ±0.4	38.1 ±0.2	37.3 ±0.2	38.4 ±0.4	37.5 ±0.3	38.1 ±0.2	37.3 ±0.4	38.3 ±0.4

Values are group means, plus or minus one standard deviation

*Alveolar ventilation was determined on only seven subjects

Table 2. Pre- and Postexposure Group Means of Pulmonary Function Measurements

Variable	Filtered Air				0.15 ppm O_3				0.30 ppm O_3			
	24°C		35°C		24°C		35°C		24°C		35°C	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
FVC, ℓ	4.023 ±0.55	3.962 ±0.54	4.000 ±0.50	3.900 ±0.59	4.051 ±0.52	3.892 ±0.49	4.052 ±0.52	3.847 ±0.52	4.004 ±0.54	3.455 ±0.77	4.104 ±0.49	3.287 ±0.90
$FEV_{1.0}$, ℓ	3.215 ±0.41	3.233 ±0.43	3.190 ±0.32	3.234 ±0.39	3.239 ±0.40	3.093 ±0.51	3.195 ±0.39	3.115 ±0.50	3.203 ±0.36	2.674 ±0.70	3.259 ±0.33	2.582 ±0.95
$FEF_{25-75\%}$, $\ell \cdot s^{-1}$	3.383 ±0.56	3.629 ±0.66	3.437 ±0.55	3.738 ±0.54	3.438 ±0.54	3.344 ±0.88	3.407 ±0.58	3.540 ±0.93	3.445 ±0.53	2.776 ±1.02	3.480 ±0.59	2.756 ±0.95
RV, ℓ*	0.904 ±0.20	0.950 ±0.27	0.889 ±0.30	0.965 ±0.27	0.941 ±0.36	0.985 ±0.32	0.919 ±0.28	1.019 ±0.31	0.906 ±0.29	0.940 ±0.30	0.886 ±0.26	0.989 ±0.27
TLC, ℓ	5.030 ±0.79	5.029 ±0.75	4.986 ±0.77	4.983 ±0.75	5.134 ±0.86	4.970 ±0.80	5.043 ±0.84	4.994 ±0.79	5.020 ±0.78	4.628 ±0.88	5.050 ±0.71	4.689 ±0.90

Values are group means, plus or minus one standard deviation

*Residual volume analysis was completed on seven subjects only.

Table 3. Results of ANOVA and Posterior Tests of Mean Differences for Pulmonary Function Variables

Variable	O_3 Effect, F Ratio	Temperature Effect, F Ratio	O_3-Temperature Interaction, F Ratio	Significant† Posterior Test Results, Protocol Comparisons
FVC	14.95*	6.12†	2.88‡	4 vs 6, 5 vs 6
$FEV_{1.0}$	21.64*	0.31	2.73‡	1 vs 3, 4 vs 6, 5 vs 6
$FEF_{25-75\%}$	18.43*	0.44	1.78	1 vs 3, 4 vs 6, 5 vs 6
RV	0.11	4.11‡	0.56	NA
TLC	18.70*	1.00	0.25	2 vs 3

* $p < 0.001$

† $p < 0.05$

‡ $p < 0.10$

Table 4. Number of Subjects Reporting Symptoms Following Exposure

Symptom	Filtered Air		0.15 ppm O_3		0.30 ppm O_3	
	24°C	35°C	24°C	35°C	24°C	35°C
Shortness of breath	1	2	2	4	8	8
Cough	0	1	3	5	8	9
Excessive sputum	3	5	4	4	5	7
Throat tickle	1	3	1	4	5	6
Raspy throat	1	3	1	4	3	6
Wheezing	0	0	0	1	1	4
Congestion	0	0	1	1	3	4
Headache	0	1	1	1	1	2
Nausea	0	0	0	1	4	6
Other*	2	4	5	4	6	9
Total Symptoms	8	19	18	29	44	61
Number of subjects believing they received O_3	0	2	4	5	10	10

*Symptoms listed under "other" include (in order of frequency): pain on inspiration, dizziness, dry mouth, and leg fatigue.

Table 5. Comparison of Pulmonary Function Responses of Subjects in Present Study to Those in Similar Studies

Variable	Present Study	Lauritzen and Adams (15)	Adams et al. (2)
Effective dose, ppm·ℓ	960	830	1110
% Δ FVC	-14.0 ± 12.0	-16.0 ± 10.1	- 5.9 ± 6.5
% Δ $FEV_{1.0}$	-17.4 ± 15.1	-24.2 ± 15.7	- 8.2 ± 7.2
% Δ $FEF_{25-75\%}$	-21.5 ± 22.0	-28.9 ± 16.5	-15.8 ± 10.8

Values are mean percent change $\left[\frac{\text{post- minus preexposure}}{\text{preexposure}} \times 100\right]$, plus or minus one standard deviation.

Table 6. Mean Pulmonary Function Percent Changes Among Three Subjects Who Terminated Exercise Early During 0.30 ppm O_3 Exposure at 24°C and/or 35°C

Protocol	Exercise Duration, min	Effective Dose, ppm·ℓ	FVC	$FEV_{1.0}$	$FEF_{25-75\%}$
0.30 ppm, 24°C	57.7	942	-24.3	-27.9	-30.1
0.30 ppm, 35°C	44	754	-29.5	-34.8	-38.9

Mean percent change values calculated as in Table 5.

FIGURE LEGEND

Fig. 1. Description of experimental design.

AMBIENT TEMPERATURE	AIR MIXTURE: FA	AIR MIXTURE: 0.15 ppm O_3	AIR MIXTURE: 0.30 ppm O_3
24°C	1	2	3
35 °C	4	5	6

Physiological Effects of NO_2 and NO_2 plus O_3 Consequent to Heavy, Sustained Exercise

PRELIMINARY REPORT

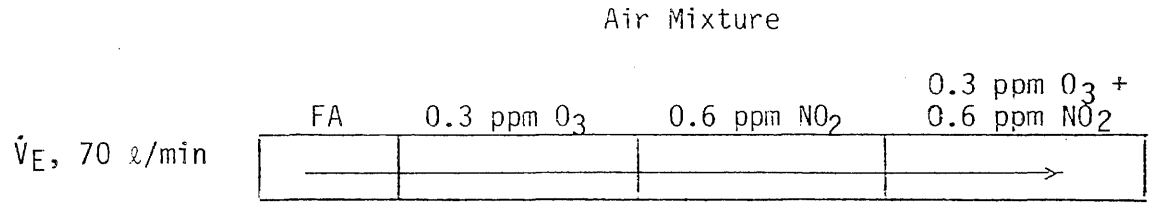
Introduction and Abbreviated Review of Literature. There have been numerous laboratory studies of humans exposed to O_3, but comparatively few entailing exposure to NO_2. When combined with ultraviolet radiation, hydrocarbons and oxygen in a warm stagnant environment, NO_2 plays a major role in the genesis of photochemical air pollution. As a result, NO_2 and O_3 can exist at high ambient levels simultaneously. This, combined with the lack of a provision for effecting smog alerts based on combined O_3 and NO_2 concentrations, creates a need to define how O_3 and NO_2 elicit acute physiological responses in concert.

NO_2 elicits similar short-term effects as O_3 but, in laboratory exposures of healthy young adult males, only at concentrations above 1.5 ppm, which is more than twice the maximum 1 h concentration observed in the Los Angeles Basin in recent years. An early report by Rokaw et al (1968) demonstrated that 10 min of moderate exercise in 1 h exposures elicited increased airway resistance in most subjects exposed to 1.5 ppm NO_2. These observations suggest that similar to O_3, the effects of NO_2 inhalation on pulmonary function are enhanced by a metabolically induced increase in ventilation during exercise. However, Hackney et al (1978) observed no significant change in pulmonary function in young adult males exposed to 1 ppm NO_2 for 2 h, with light intermittent exercise (IE). The effect of residence in an area of routinely high ambient NO_2 concentrations was not addressed in this study. Folinsbee et al (1978) exposed 15 young adult males to 0.62 ppm NO_2 for 2 h, with continuous exercise of from 15 to 60 min, which necessitated a minute ventilation of 33 ℓ/min. No changes in pulmonary function, cardiovascular or metabolic responses were observed when compared to filtered air (FA) control exposure. However, it should be noted that the total ventilation for the 60 min group would have been only about 2½ times that for a 2 h exposure at rest.

There have been few laboratory studies of subjects exposed to NO_2 with other ambient pollutants (Horvath, 1980). Folinsbee et al (1981) exposed 10 young adult males to 0.50 ppm O_3 plus 0.50 ppm NO_2 for 2 h, with 30 min of continuous exercise (which increased pulmonary ventilation to ~40 ℓ/min) during the first half of the second hour. Pulmonary function changes were similar to those observed in a previous study of young adult males exposed to 0.5 ppm O_3 alone. Kagawa (1983) also exposed young adult males for 2 h, with light IE, at 0.15 ppm O_3 and 0.15 ppm O_3 plus 0.15 ppm NO_2. He found marginally greater airway resistance response for this pollutant combination than that observed for O_3 alone. He suggested that the apparently enhanced toxicity of O_3 plus NO_2 might be due to the formation of a more irritating pollutant, such as HNO_3.

Statement of Research Objectives. From review of previous studies entailing NO_2 exposure, we observed that no investigation had been reported in which prolonged (>60 min), heavy exercise had been utilized. We envisioned that greater exercise intensities than previously employed, in combination with exposures of 1 h or longer, might well elicit significant pulmonary function effects at near maximum ambient levels (i.e., ~0.7 ppm), as has been demonstrated with O_3 inhalation at 0.20 ppm and lower (Adams & Schelegle, 1983; McDonnell et al, 1983).

The purpose of the present study was two-fold. (1) To determine the effects, if any, of exposure to 0.6 ppm NO_2 on pulmonary function (PF) consequent to heavy, sustained exercise ($\dot{V}_E$ = 70 ℓ/min); and (2) to determine the combined effects of exposure to O_3 (0.3 ppm) and NO_2 (0.6 ppm) on PF under the same exercise conditions. The experimental design to accomplish these objectives is depicted below:



The subjects are exposed to FA, 0.3 ppm O_3, 0.6 ppm NO_2 and 0.3 ppm O_3 plus 0.6 ppm NO_2 in four separate, randomly assigned 1 h protocols. Exercise work loads are set such that $\dot{V}_E$ is approximately 70 ℓ/min for each subject. It was hypothesized that: (1) 0.6 ppm NO_2 alone will result in statistically significant PF impairment; and (2) 0.6 ppm NO_2 combined with 0.3 ppm O_3 will have an additive effect on PF impairment to that effected by 0.3 ppm O_3 alone.

Statement of Research Protocol and Methodology.

1. Subject description and characterization. Ten healthy young adult males will be solicited as subjects. Each will be screened for clinically normal PF and for absence of history of significant allergies. Prior to initiating the four experimental protocols, each subject will complete an orientation session, in which PF and basic anthropometry, including body composition via hydrostatic weighing, will be measured. To attenuate habituation effects, all subjects will complete at least 60 min bicycle ergometer exercise at varied submaximal work loads while breathing FA. On another pre-experimental occasion, the subject's $\dot{V}_{O_{2max}}$ will be determined utilizing a multistage, progressive increment protocol (Adams et al, 1981).

2. Pulmonary function measurement. Immediately prior to each experimental protocol, a short battery of PF tests will be administered. Duplicate determinations of forced, maximal expiration will be recorded on a Collins

modular office spirometer, Model No. 3000, with x-y recorder and a residual volume module. An on-line data acquisition system includes a software package interfacing the spirometer module linear potentiometer output voltage (associated with volume changes) and the A-D converter for reading into a Digital Equipment Corporation (DEC) LSI 11/2 microcomputer. In addition to measurement of forced vital capacity (FVC), forced expiratory volume in 1 s ($FEV_{1.0}$), and forced expiratory flow rate in the middle half of FVC (FEF_{25-75}), other PF on-line computer determinations include maximal peak flow, FEV at two and three seconds, flow at 25, 50, and 75% of FVC, resting V_T, inspiratory reserve volume (IRV), and expiratory reserve volume (ERV). RV is also determined on-line by an O_2 rebreathing method, with initial and equilibrium N_2 readings taken on a Collins N_2 analyzer.

Duplicate forced expiratory maneuvers and RV determinations are consummated within 12 min following each protocol. A software package data filing system effects average PF pre- and postexposure determinations, as well as the absolute and percent changes.

3. O_3 and NO_2 administration and monitoring. Specific air mixtures during all experimental protocols are effected through a blow-by obligatory mouthpiece inhalation exposure procedure. Air is filtered through a Mine Safety Appliances C-B-R filter and drawn through a Rotron CHE-1 pump at a flow of approximately 600 $\ell \cdot min^{-1}$. From the exhaust port of the Rotron CHE-1, the air is pumped into a 0.91-m long Teflon-lined aluminum tube, and thence undergoes turbulent mixing at the tangential junction of a 5.1-cm diameter aluminum tube. Concentrated O_3, created from silent arc discharge (Sander Ozonizer, Type II) of compressed gaseous O_2, is introduced proximal to the turbulent mix. Atmospheric levels of NO_2 are produced by injecting into the airstream 351

ppm NO_2 in nitrogen at a controlled flow rate, using a micrometering valve and a Fisher and Park flow meter. The atmospheric levels of NO_2 are also introduced proximal to the turbulent mix. At the distal end of the 0.91-m tube, the air mix is directed from an exhaust port to a Teflon-coated Hans-Rudolph respiratory valve, via a 0.91-m length of Fluoroflex Teflon tubing.

Subatmospheric pressures generated during the inspiratory phase of breathing result in the flow of the pollutant-air mixture into the respiratory valve. Positive expiratory pressures shut the fenestrations on the diaphragm on the inspiratory side of the valve, allowing flow of expired air unidirectionally into a 5 stainless steel mixing and sampling chamber to an Alpha Technologies Turbotachometer ventilation module (Model VVM-2). Expired air is then routed into the distal portion of the mixing tube, and along with the volume of pollutant-containing air not inspired by the subject, exhausted via a 10-cm diameter Flexaust CWC Neoprene hose to the laboratory ventilation exhaust outlet.

Inspiratory O_3 concentration in the mixing chamber is monitored by continuous samples drawn through 0.64-cm diameter Teflon tubing connected to a Dasibi O_3 meter, while that for NO_2 is determined on a Thermo Electron NO_2 meter. The digital reading of O_3 concentration in ppm is compared periodically to that determined by the UV absorption photometric method (DeMore et al, 1976) at the University of California, Davis, Primate Center. The reading of NO_2 concentration is done on a Thermo Electron 14 B/E NO_X analyzer, calibrated using a Thermo Electron 102 precision calibrator.

4. Exercise measurements. All exposures are conducted in an environmental chamber 3.0 m wide by 2.4 m high by 3.7 m long, in which dry bulb temperature and relative humidity are maintained within 21-25°C and 45-60%, respectively. To facilitate convective and evaporative cooling during exercise, an

appropriate airflow is directed at the subject's frontal aspect via an industrial grade floor fan.

To assess possible effects of O_3 and/or NO_2 inhalation on selected exercise parameters, an on-line computerized data acquisition system effects a print-out of one minute average values for $\dot{V}_E$, HR, V_T, f_R, $\%O_2$, and $\%CO_2$ in expired gas, expired gas temperature, and $\dot{V}O_2$ every minute. Data acquisition instruments interfaced to the DEC LSI 11/2 microcomputer, include a LB-1 CO_2 analyzer, an Applied Electrochemistry S-3A O_2 analyzer, an Alpha Technologies turbotachometer ventilation module, an electrocardiograph with R-wave detector, and a temperature thermistor located in the expired gas line.

Subjective symptoms are monitored at 5, 30, 45, and 58 min by having the subjects point to an ordinal scale to rate their perception of the existence and, if so, the severity of symptoms called out by the investigator. Immediately following completion of the postexposure PF test battery, the subject again indicates the existence and, if so, the severity of symptoms, also stating whether he believed that he received O_3 and/or NO_2.

5. Statistical procedures. A data filing system software package computes the mean of at least duplicate PF measurements pre- and postexposure, while the values from the tenth minute and the last minute of exercise for $\dot{V}O_2$, HR, $\dot{V}_E$, f_R, and V_T are utilized to determine the exposure effect.

Upon completion of the project, the percent change for the six PF and five exercise respiratory metabolism parameters will be analyzed for statistical significance by one-way ANOVA. Upon obtaining a significant F ratio, a paired t post hoc test, with Bonferroni correction, will be applied (Miller, 1981) to determine which particular mean values are significantly different from others.

Current Status of Research Project

Unforeseen problems in acquiring the NO_2 analyzer and calibrating system, together with the design and fabrication of a reliable and safe delivery system, delayed initiation of experimental exposures until late May. Nonetheless, a complete data set on five subjects is included in this preliminary analysis. A sixth subject has been characterized and has initiated exposures, while the remaining four subjects have been identified. They will be characterized later this month, and should complete their exposures by late August.

Preliminary Results (5 subjects).

The subject's anthropometry, pulmonary function, and $\dot{V}O_{2max}$ are given in Table 1. Mean pulmonary function and exercise ventilatory responses to the four exposures are shown in Table 2. For reference, the subject's mean values at 10 min for $\dot{V}O_2$, $\dot{V}_E$, f_R, and V_T were 2.71 ℓ/min, 71.8 ℓ/min, 37 breaths/min, and 1.95 liters, respectively. Since the subject sample is small, no statistical analyses have been attempted. However, it seems apparent from study of Table 2, that the mean values are essentially similar for RV, $\dot{V}O_2$, and $\dot{V}_E$. It is also apparent that the only notably disparate responses for FVC, $FEV_{1.0}$, FEF_{25-75}, f_R, and V_T, are for the comparisons of FA (or NO_2 alone) to O_3, and to NO_2 and O_3 in combination. Further, comparison of response to the O_3 exposure and to NO_2 plus O_3, reveal no notable disparity.

Mean subjective rated perceived exertion (RPE), symptom number and severity at 58 min of each exposure are given in Table 3. These data appear to mirror that for PF, in that there are no appreciable differences for the comparisons between the FA and NO_2 exposures, nor between the O_3 and the NO_2 plus O_3 exposures. However, there are substantial differences between both the O_3 and NO_2 plus O_3 exposures compared to the FA and NO_2 exposures.

Comments

The present preliminary data set does not evidence any substantive support for the notion that 1 h exposure to 0.60 NO_2 while exercising continuously at work intensities eliciting $\dot{V}_E$ ~70 ℓ/min, will effect PF impairment as compared to FA. Further, PF impairment and altered exercise ventilatory pattern changes observed for the NO_2 plus O_3 exposure did not differ from those observed for O_3 alone, when compared to FA and to NO_2 alone. Nonetheless, continued investigation of the possible adverse effects of NO_2 at ambient smog alert concentrations, and of its possible additive effects to those well documented for O_3 alone, when inhaled in combination, remains promising. This assertion is based primarily on the advantages inherent in assessing airway resistance effects more directly than can be implied from routine PF tests. The development of a capability for measuring airway resistance via whole body plethysmography (ala DuBois, 1956), as well as peripheral airway impact via the forced oscillatory technique (Pimmel et al, 1978) will improve our capability to uncover evidence of any physiological effect of NO_2 and NO_2 plus O_3 in human subjects engaged in heavy, sustained exercise.

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Table 1. Subject anthropometric, pulmonary function, and $\dot{V}_{O_{2max}}$ data.

Subject, #	Age, yrs	Height, cm	Weight, kg	Body Fat, %	$\dot{V}_{O_{2max}}$, $\ell \cdot min^{-1}$	FVC, ℓ	$FEV_{1.0}$ ℓ	$FEF_{25-75\%}$, $\ell \cdot s^{-1}$	RV ℓ
1	23	178	68.4	10.0	4.27	5.05	4.62	6.28	1.28
2	30	180	65.0	8.5	4.50	6.17	4.81	4.14	1.66
3	27	179	69.1	8.5	3.64	5.44	4.55	4.82	0.93
4	22	180	63.0	7.0	3.36	5.19	4.61	5.28	1.97
5	22	190	80.1	10.0	4.75	7.67	5.63	4.15	1.66
$\bar{x}$	24.8	181.4	69.1	8.8	4.10	5.90	4.85	4.93	1.50
SD	$\pm$3.6	$\pm$4.9	$\pm$6.6	$\pm$1.3	$\pm$0.59	$\pm$1.1	$\pm$0.45	$\pm$0.90	$\pm$0.40

Table 2. Summary of percent change in pulmonary function, exercise ventilatory pattern, and respiratory metabolism.

Protocol	FVC	RV	$FEV_{1.0}$	FEF_{25-75}	f_R	V_T	$\dot{V}_E$	$\dot{V}O_2$
FA	-1.86	+6.76	+0.12	+3.53	+11.3	-5.2	+4.9	+5.3
	(±0.93)	(±8.2)	(±3.41)	(±10.7)	(±13.2)	(±7.31)	(±8.74)	(+5.72)
NO_2	-.09	+6.4	+1.15	+5.38	+22.4	-12.4	+7.15	+2.3
	(±3.5)	(±6.8)	(±4.8)	(±17.6)	(±9.1)	(±1.33)	(±7.33)	(±10.0)
O_3	-16.0	+4.6	-24.4	-42.6	+37.2	-22.0	+6.2	+4.3
	(±6.0	(±28.4)	(± 9.5)	(±11.4)	(±18.0)	(± 7.8)	(±7.18)	(+5.5)
$NO_2 + O_3$	-14.1	-7.4	-24.9	-39.1	+53.7	-31.0	+5.6	+2.8
	(±4.7)	(±10.0)	(±10.2)	(±19.5)	(±16.2)	(±5.4)	(±4.1)	(±4.33)

Values are mean percent changes; values in parentheses are ± 1 standard deviation. FVC, forced vital capacity (liters); RV, residual volume (liters); $FEV_{1.0}$, forced expiratory volume in 1.0 s (liters) FEF_{25-75}, forced expiratory flow during middle half of FVC (liters·s^{-1}); f_R, respiratory frequency (breaths·min^{-1}); V_T, tidal volume (liters); $\dot{V}_E$, minute ventilation (liters·min^{-1}); $\dot{V}O_2$, oxygen consumption (liters·min^{-1}).

Table 3. Subjective symptoms.

Variable	FA	0.6 ppm NO_2	0.3 ppm O_3	0.6 ppm NO_2 + 0.3 O_3
RPE	12.6 (± 1.14)	12.2 (±1.3)	14.3 (±1.72)	14.2 (±2.77)
No. of Symptoms	1.0 (± 1.22)	1.2 (±1.3)	4.2 (±2.59)	4.0 (±1.58)
Symptom Severity Total	3.0 (± 2.74)	2.2 (±2.59)	41.5 (±40.3)	37.0 (±30.7)

Values are means, with those in parentheses being ± 1 standard deviation.
RPE, rated perceived exertion.