FINAL REPORT TO

STATE OF CALIFORNIA AIR RESOURCES BOARD

Title of Research Contract: Physiological Responses of Healthy Human Subjects Consequent to Inhalation of NO_2, O_3, and NO_2 plus O_3 During Heavy, Sustained Exercise

Contract Number: A4-070-33

Contract Period: 9 November 1984 - 9 November 1986

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Date of Report: 17 October 1986

ABSTRACT

The primary purposes of this research were to: (1) investigate whether healthy young adult females are more sensitive to high ambient concentration of nitrogen dioxide (NO_2), ozone (O_3), and NO_2 plus O_3 than their male counterparts; (2) determine the duration of enhanced responsiveness upon reexposure to O_3 at intervals of 1 to 5 days; and (3) study the physiologic mechanisms associated with pulmonary function and airway resistance effects observed upon O_3 exposure. Ninety-four healthy young adults, including 20 females, undertook 60 minutes of heavy bicycle ergometer exercise, which elicited an average minute ventilation volume of approximately 8 times greater than that at rest. Subjects were exposed via an obligatory mouthpiece inhalation system to filtered air and to O_3 concentrations of either 0.30 or 0.35 parts per million (ppm). In one study, subjects were also exposed to 0.60 ppm NO_2 and to 0.60 ppm NO_2 plus 0.30 ppm O_3. Measurement of standard pulmonary function tests, including airway resistance, pre- and postexposure, together with periodic observations of exercise ventilation, respiratory metabolism, and subjective symptoms of respiratory discomfort, were obtained. In one study, respiratory system impedance was measured to assess the relative effect of O_3 on central and peripheral airways.

It was observed that inhalation of 0.60 ppm NO_2 for 1 hour, while engaged in heavy, sustained exercise, does not elicit significant physiological effects evidenced by measurement techniques used in this study, nor evoke additive effects in combination with 0.30 ppm O_3 in healthy young adults. The possibility remains, however, that peripheral airway effects of NO_2 in combination with O_3 at these concentrations, as recently revealed in histological evidence in exercising rats by others, may have occurred. Young adult females were found to evidence similar responses to O_3 and to O_3 plus NO_2 inhalation as young adult males when their 1 hour exercise pulmonary ventilation rate was proportional to the gender difference in lung size.

In accord with previous work of others, we observed an enhanced responsiveness upon reexposure to 0.35 ppm O_3 within 24 hours. While this greater response may persist for 48 hours in some apparently more sensitive individuals, it is absent upon 72 hours or more after initial exposure. Further,

EXECUTIVE SUMMARY

PHYSIOLOGICAL RESPONSES OF HEALTHY HUMAN SUBJECTS CONSEQUENT TO INHALATION OF NO_2, O_3, AND NO_2 PLUS O_3 DURING HEAVY, SUSTAINED EXERCISE

STATE OF CALIFORNIA AIR RESOURCES BOARD

CONTRACT A4-070-33

Contractor: William C. Adams, PhD
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Date of Report: 17 October 1986

INTRODUCTION

Photochemical air pollution occurs widely, particularly in the heavily populated Los Angeles basin. Ozone is the principal component of concern because of its demonstrated biochemical and morphological effects on the lungs of animals acutely and chronically exposed to ambient smog alert levels, its association with an increased rate of hospital admissions for respiratory ailments, and an increasingly impressive documentation of physiological responses to acute laboratory exposures. From this evidence, governmental agencies have attempted to set appropriate standards of air quality.

There are still limited data relating specific levels of a given pollutant, upon acute exposure, to a particular physiological effect, especially during exercise when the total amount of pollutant inhalation in a given time is dependent both on the ambient concentration and the 5 to 15 times increased rate of ventilation volume effected upon engaging in moderate to very heavy physical activity, compared to that at rest. This observation is of practical importance because, in addition to vigorous voluntary exercise becoming increasingly prevalent in the adult population, primarily for improved health/fitness, there are still numerous outdoor occupations that require prolonged moderate activity, including construction labor, refuse collection, and postal delivery.

Those charged with setting appropriate air quality standards are concerned with the protection of all segments of the population, including those with chronic pulmonary disease. However, there are other more numerous subpopulation groups who may also be more sensitive to photochemical air pollution than the usually studied healthy young adult male. Indeed, we have recently observed suggestive evidence that young adult females are more sensitive to acute ozone exposure than their male counterparts.

Significant photochemical smog episodes, in which the ozone concentration is sufficient to effect acute physiological response, are frequently accompanied by ambient heat stress and/or other air pollutants of concern. Indeed, nitrogen dioxide plays a major role in the genesis of ozone and other constituents of the photochemical air pollution mix. It is also of concern due to its toxic oxidant effects, particularly on tissues of the respiratory tract. Further, in some respects, the toxic effects of nitrogen dioxide are similar to those of ozone, which suggests the potential for additive effects in humans exposed to concentrations existent in smog alert episodes.

It is now well documented that reexposure to ozone within 24 hours, following an initial exposure at a level eliciting significant physiological response, results in an enhanced effect. Though peak ambient concentrations of oxidants frequently occur at approximately 24 hour intervals over several consecutive days, photochemical smog episodes can and do occur with two or three days intervening. Thus, health authorities need information evaluating the potentially heightened toxic impact of these smog episodes 48 or more hours apart. By varying the time interval between consecutive ozone exposures, the presence of enhanced responsiveness and its corresponding time decay can be evaluated. This effort would also provide important information to those engaged in repeated laboratory exposure of a limited number of subjects, each acting as their own control, as this procedure reduces variability provided that the initial exposure enhanced response has fully dissipated.

There now exists an impressive body of knowledge concerning the adverse health effects of ozone, emanating from epidemiological evidence, as well as animal and human laboratory studies. However, there is a pressing need to

provide a more effective link between the evidence accrued from acute and chronic animal studies to the largely descriptive outline of acute response in humans. This task can be effectively addressed by investigating more sophisticated physiological mechanisms associated with the oft reported pulmonary function impairment and subjective symptoms of respiratory discomfort upon acute ozone exposure.

This research project was designed to investigate several fundamental issues identified above. In particular, we sought to examine: (1) The effects of exposures entailing heavy, sustained exercise at high ambient levels of nitrogen dioxide, alone and in combination with ozone; (2) Whether young adult females respond similarly to nitrogen dioxide and ozone exposure, alone and in combination, as do their male counterparts; (3) The duration of enhanced responsiveness upon reexposure to ozone at 1 to 5 day intervals; and (4) Underlying physiological mechanisms associated with pulmonary function impairment and respiratory discomfort upon exposure to ozone.

METHODOLOGY

Ninety-four healthy human subjects, age 18-34 years, including 20 females, undertook heavy bicycle ergometer exercise, which elicited an average minute ventilation volume of approximately 8 times greater than that at rest, for 60 minutes. Subjects were exposed via an obligatory mouthpiece inhalation system to filtered air, to ozone concentrations of either 0.30 or 0.35 parts per million (ppm). In one study, subjects were also exposed to 0.60 ppm nitrogen dioxide and to 0.60 ppm nitrogen dioxide plus 0.30 ppm ozone. All exposures, 420 in total, were conducted in moderate temperature and humidity conditions.

Measurement of standard pulmonary function tests, including airway resistance, pre- and postexposure, together with periodic observations of exercise

ventilation, respiratory metabolism, and subjective symptoms of respiratory discomfort, were obtained. In one study, respiratory system impedance was measured to assess the relative effect of ozone on central and peripheral airways.

RESULTS

A brief statement of the purpose, experimental design and principal observations of the four experimental studies follows:

1. Effects of Nitrogen Dioxide and Ozone, Alone and in Combination, on Exercising Males and Females. To test the hypothesis that heavy, sustained exercise would elicit pulmonary dysfunction upon exposure to 0.60 ppm nitrogen dioxide, and/or enhance the effects of exposure to 0.30 ppm ozone, 40 aerobically trained young adults - 20 males and 20 females - completed 1 hour of continuous exercise on four occasions. Workloads were set to elicit a greater minute ventilation volume for the males that was nearly proportional to the gender difference in lung size. Exposures to (1) filtered air, (2) 0.60 ppm nitrogen dioxide, (3) 0.30 ppm ozone, and (4) 0.60 ppm nitrogen dioxide plus 0.30 ppm ozone were randomly delivered via an obligatory mouthpiece inhalation system. Exposure effects were assessed by standard pulmonary function tests and exercise ventilatory and subjective symptoms responses. Statistical analysis revealed a significant effect of ozone on forced expiratory parameters, specific airway resistance, exercise ventilatory response (rapid, shallow breathing), and reported subjective symptoms of respiratory discomfort. In contrast, no significant effect of nitrogen dioxide was observed, nor did the combination of nitrogen dioxide and ozone elicit greater effects than exposure to ozone alone. Male and female group responses did not differ significantly for any exposure. Though inhalation of 0.60 ppm nitrogen dioxide for 1 hour, while engaged in heavy, sustained exercise, does not elicit significant physiological effects evidenced by measurement techniques used in this study, nor evoke additive effects in combination with 0.30 ppm ozone in healthy young adults, the possibility remains that peripheral airway effects of nitrogen dioxide in combination with ozone at these concentrations, as recently revealed in histological evidence by others in exercising rats, may have occurred.

2. The Duration of Enhanced Responsiveness upon Reexposure to Ozone. This study was designed to assess the effects of reexposure to 0.35 ppm ozone at intervals of 24, 48, 72 and 120 hours. Forty young adult male subjects were randomly assigned to one of four groups in ascending order of time to reexposure. Each subject exercised on a bicycle ergometer for 1 hour at a workload eliciting a mean minute ventilation volume of 60 ℓ/min on three occasions in the same order of exposure: filtered air, 0.35 ppm ozone, and 0.35 ppm ozone. In addition to standard pulmonary function measures, specific airway resistance, exercise ventilatory pattern and subjective symptoms of respiratory discomfort were assessed. Statistical analysis revealed significant differences for all groups between the filtered air responses and those for both of the two ozone exposures. However, when responses for the two ozone exposures

were compared, only the group reexposed after 24 hours demonstrated statistically significant differences upon reexposure. These differences ranged from 50 to 100 percent greater for pulmonary function and exercise ventilatory pattern responses than those observed upon initial ozone exposure. Analysis of reexposure data for the 48 hour reexposure group indicated a trend toward significantly enhanced responsiveness. Analyses for the 72 and 120 hours intervening groups revealed no significant differences upon reexposure to ozone. Our observations suggest that, the enhanced pulmonary function responsiveness noted upon reexposure to 0.35 ppm ozone within 24 hours may persist for 48 hours in some subjects, but is absent 72 hours or more after initial ozone exposure. Further, none of these ozone reexposure intervals resulted in diminution of responses (i.e., adaptation) observed by others following several consecutive days of exposure.

3. Indomethacin Pretreatment Reduces Ozone Induced Pulmonary Function Decrements in Human Subjects. In this investigation, we examined whether ozone-induced pulmonary function impairments could be reduced by ingestion of indomethacin (a prostaglandin synthetase inhibitor) in healthy human subjects. Fourteen college age males completed six 1 hour exposure protocols consisting of no drug, placebo, and indomethacin pretreatments, with filtered air and 0.35 ppm ozone exposures within each pretreatment condition. One hour of bicycle ergometer exercise, with workloads set to elicit a mean minute ventilation volume of 60 ℓ/min, was completed in each exposure. Exposure effects were assessed by standard pulmonary function tests, airway resistance, exercise ventilatory, and subjective symptoms of respiratory discomfort responses. Statistical analysis revealed pretreatment effects for pulmonary function tests, with the no drug vs indomethacin and the placebo vs indomethacin comparisons being significant. Indomethacin pretreatment also resulted in no significant difference between the filtered air and ozone exposure for tidal volume during exercise (i.e., the average depth of breathing), and significantly reduced subjective symptoms of respiratory discomfort observed in the no drug ozone exposure. These findings suggest that the inflammation process associated with airway epithelial damage upon acute ozone exposure, plays a predominant role in the development of pulmonary function impairment.

4. Atropine Pretreatment Blocks Ozone Induced Specific Airway Conductance Decrements in Human Subjects. The purpose of this investigation was to determine the degree to which neural components account for decreased airway conductance (the inverse of airway resistance) consequent to ozone exposure in healthy human subjects. This was accomplished by blocking neural input to bronchial smooth muscle via atropine pretreatment. In addition, respiratory system impedance was measured to assess the relative contribution of central and peripheral components to ozone-induced bronchoconstriction. Thirteen college age males completed six 1 hour exposure protocols consisting of no drug, atropine sham, and atropine, with filtered air and 0.35 ppm ozone exposures within each pretreatment condition. Atropine and atropine sham (quinine sulfate) were delivered via a nebulizer with dose metering device 20 min prior to the measurement of preexposure pulmonary function tests. One hour of bicycle ergometer exercise, with workloads set to elicit a mean ventilation volume of 60 ℓ/min, was completed during each exposure. Exposure effects were assessed by standard pulmonary function tests, airway conductance, respiratory system impedance, exercise ventilatory, and subjective symptoms of respiratory

discomfort. Atropine pretreatment abolished ozone-induced bronchoconstriction, with a 2.5, -11.4, and -15.0 percent change in specific airway conductance observed for the atropine, atropine sham, and no drug pretreatment, respectively. Forced expiratory flow over the middle half of vital capacity was reduced with atropine, but there was no significant effect on ozone-induced lung volume, exercise ventilatory or subjective symptoms of respiratory discomfort responses. Respiratory system impedance analysis indicated that ozone-induced bronchoconstriction is distributed between both central and peripheral airways, and that this effect is inhibited by atropine at both loci. These observations suggest that parasympathetic neural input to bronchial smooth muscle plays a predominant role in determining bronchoconstriction consequent to acute O_3 exposure.

CONCLUSIONS

From the results of the above studies, we have drawn the following conclusions.

1. One hour exposure of healthy young adult males and females to high urban ambient levels of nitrogen dioxide while engaged in heavy, sustained exercise, does not elicit physiologic effects revealed by routine pulmonary function tests.

2. Similar exposures of males and females at high ambient levels of nitrogen dioxide and ozone do not elicit physiologic effects revealed by routine pulmonary function tests beyond those observed at the same ozone concentration alone.

3. The synergistic peripheral airway lesions evidenced upon inhalation of combined high ambient levels of nitrogen dioxide and ozone in exercising rats is not evidenced in similar exposures of humans by available pulmonary tests of peripheral airway function.

4. Young adult females are not more sensitive to ozone inhalation if their rate of pulmonary ventilation is closely proportional to the gender difference in lung size (about 40% greater for males).

5. Preexposure within 24 hours to high urban ambient levels of ozone does not elicit significant pulmonary function response upon reexposure to high ambient levels of nitrogen dioxide, nor elicit any greater effect upon reexposure to similarly high nitrogen dioxide plus ozone levels beyond that observed with reexposure to ozone alone.

6. Preexposure to a high urban ambient level of ozone within 24 hours prior to reexposure at the same concentration enhances pulmonary function responses by 50 to 100 percent. This increased sensitization upon initial exposure to ozone may persist for 48 hours but is absent following a 72 hour interval before reexposure. These observations have implications for those charged with setting ozone health effect standards, as well as those who wish to conduct repeated laboratory exposures of human subjects.

7. Ozone induced pulmonary function impairment is associated with the release of mediators of lung inflammation, in that pretreatment with a drug which blocks the release of specific mediators of inflammation significantly reduces pulmonary function impairment, but without affecting airway resistance response.

8. Ozone induced bronchoconstriction is elicited predominantly by neural components innervating bronchial smooth muscle, in that pretreatment with a drug which blocks neural input to bronchial smooth muscle abolishes increased airway resistance (but without effect on lung volume response).

RECOMMENDATIONS

1. Pulmonary function tests particularly sensitive to peripheral airways effects in humans should be used to determine if the synergism observed in exercising animals exposed to high ambient concentrations of nitrogen dioxide and ozone elicit greater peripheral airway effects than ozone alone in exercising humans.

2. Pulmonary ventilation is increased 6 to 10 times that at rest in moderate to heavy exercise, and has been shown to reduce the ozone concentration at which pulmonary function effects are observed. 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 (i.e., 0.20 ppm).

3. The role of very high ventilation rates incurred by elite endurance athletes during prolonged exercise (i.e., 1 to 3 hours), such as marathon runners and race walkers, should be studied in combination with ozone exposures at concentrations at or below the current Federal Air Quality Standard (i.e., $\leq$0.12 ppm).

4. Reexposure to high ambient levels of ozone within 24 hours significantly enhances the physiologic effects observed upon initial exposure. Studies should be conducted to determine if exposure to high ambient levels of ozone within 24 hours prior to reexposure lowers the threshold ozone concentration at which significant pulmonary function effects are observed.

5. Healthy young adult females evidence greater physiologic response to the same total ozone dose than do their male counterparts. Preliminary evidence from our laboratory indicates that this gender difference does not appear related to lung size difference, per se. Thus, further investigation is warranted.

6. Further studies of the physiologic mechanisms of ozone's effects on the human respiratory system upon acute exposure should be conducted to assess more accurately the associated health risk. Of particular importance is investigation of the role that inflammatory mediators and/or cells play in ozone induced pulmonary function effects.

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16. Abstract (Limit: 200 words)

The main goal of this project was to study the role of exercise and gender in a person's sensitivity to the effects of nitrogen dioxide, ozone, or a combination of the two pollutants. Healthy young adult subjects engaged in 60 minutes of heavy bicycle ergometer exercise while inhaling pollutants through a mouthpiece. Major findings were:

1. Heavy exercise does not seem to increase subject sensitivity to inhaled NO_2 (0.6 ppm) or to a combination of NO_2 and O_3 (0.3 ppm) with respect to the parameters that were measured (pulmonary function tests, symptoms, and exercise ventilatory pattern).
2. Inflammation and neural responses are important underlying factors in the response to O_3 as measured by the above parameters.
3. Women and men, when exercising at the same relative instensity, do not show gender based differences in sensitivity to the effects of O_3, NO_2, or a combination of the two pollutants.
4. The duration of enhanced responsiveness upon reexposure to O_3 (0.35 ppm) appears to last up to 48 hours with variation between individuals.
5. Prior exposure to O_3 (0.3 ppm) does not affect the response to NO_2 (0.6 ppm) or to a combination of NO_2 and O_3 over that which can be attributed to O_3 alone.

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none of these consecutive O_3 exposure intervals resulted in diminution of responses (i.e., adaptation, or tolerance development) observed upon initial exposure.

It was demonstrated by drug pretreatment studies that pulmonary function impairment induced by 1 hour inhalation of 0.35 ppm O_3 is associated with lung inflammation mediators which are sensitive to indomethacin inhibition. Increased airway resistance associated with bronchoconstriction was not affected by indomethacin pretreatment, but was blocked by atropine pretreatment, which did not affect O_3-induced lung volume changes. These observations reveal that O_3-induced pulmonary function impairment is associated with recognized indicators of at least temporary direct tissue damage, and demonstrate the dichotomy of lung volume and airway resistance responses.

ACKNOWLEDGEMENTS

In addition to completing two of four studies reported herein as part of his doctoral dissertation in physiology, Edward S. Schelegle has afforded a consistent, omnipresent contribution of invaluable proportion to the successful completion of this research. The expert and timely laboratory instrumentation technical support of Richard Fadling is gratefully acknowledged. Special appreciation is extended to Allan Siefkin, M.D., Director of the Pulmonary Function and Blood Gases Laboratory, University of California Davis Medical Center, who supplied and gave instruction in the use of the ultrasound nebulizer and provided clinical advice on appropriate atropine and indomethacin pretreatment dosages, as well as subject screening factors.

Andrew C. Jackson, Ph.D., Biomedical Engineering Department, Boston University, Boston, Mass., developed and supplied the impedance system pressure generator and attendant computer software. Peter J. Hunter, Ph.D., Department of Environmental Studies, U.C. Davis, provided the software optimization package for impedance data analysis and assisted in its effective operation on our laboratory microcomputer. On-line DEC LSI 11/2 computer data acquisition and reduction capability was developed primarily by Benson Cheung. Mike Miller, Ph.D., Statistical Services Division, U.C. Davis, provided helpful advice in the most effective utilization of statistical analyses. Messrs. Tim Duvall and Brian Tarkington, California Primate Research Center, U.C. Davis, provided routine calibration of the Dasibi O_3 analyzer.

Several U.C. Davis students provided capable laboratory assistance: Ms. Kim Filler, Ms. Candace Ireton, William Walby, and Ms. Anna Yanes. Ms. Kathi Brookes furnished able assistance in all studies, and assumed a major role in the first study as evidenced by coauthor designation. Brian Schonfeld also furnished adept assistance in all studies, and was principally responsible for one study reported herein, which constituted a major part of his Master's thesis. Almost 100 individuals served as subjects, volunteering significant time and effort. Finally, Mrs. Barbara Lyon and Ms. Sylvia Whitley have expertly and cheerfully deciphered the handwritten material provided them, which now constitutes a formidable documentation of collaborative research.

This report is submitted in fulfillment of Air Resources Board Interagency Agreement A4-070-33, "Human Physiological Response to Inhalation of NO_2, O_3, and NO_2 plus O_3 During Heavy, Sustained Exercise," by the Regents of the University of California, Davis, under the partial sponsorship of the California Air Resources Board. Work was accomplished as of 17 October 1986.

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SUMMARY AND CONCLUSIONS

Photochemical air pollution occurs widely in California, especially in the Los Angeles basin during the summer months. Its health effects, particularly in terms of O_3 inhalation, have been the subject of scientific scrutiny for more than two decades. Nonetheless, there are still limited data relating acute exposure of humans to specific levels of a given pollutant to a particular physiologic effect. This effort is confounded by a number of factors, including multiple pollutant inhalation (e.g., NO_2 plus O_3), group and individual variation in sensitivity, the effects of repeated exposure, and the enhancing effect of physical activity level due to exercise induced increase in pulmonary ventilaton rate. Further, there is a notable gap linking evidence from acute and chronic animal exposure studies to the pulmonary function impairment observed in humans exposed to ambient levels of O_3.

Using heavy, sustained exercise (minute ventilation volume 8 times that at rest) during 1 hour exposures to ambient smog alert levels of O_3, NO_2, and NO_2 plus O_3, we addressed four major questions: (1) Do healthy young adult females incur a greater response to NO_2, O_3, and NO_2 plus O_3 than do their male counterparts? (2) Is the physiologic response of exposure to NO_2, or to NO_2 plus O_3 enhanced by exposure to O_3 24 hours prior? (3) What is the effect of an initial O_3 exposure on reexposure of subjects 1 to 5 days later? (4) What are the major physiologic factors associated with O_3 induced pulmonary function impairment and increased airway resistance in humans?

Subjects were exposed via an obligatory mouthpiece inhalation system to filtered air and to O_3 concentrations of either 0.30 or 0.35 parts per million (ppm). In one study, subjects were also exposed to 0.60 ppm NO_2 and to 0.60 ppm NO_2 plus 0.30 ppm O_3. Measurement of standard pulmonary function tests, including airway resistance, pre- and postexposure, together with periodic observations of exercise ventilation, respiratory metabolism, and subjective symptoms of respiratory discomfort, were obtained. In one study, respiratory system impedance was measured to assess the relative effect of O_3 on central and peripheral airways.

Healthy young adult subjects exposed to 0.60 ppm NO_2 did not demonstrate significant physiologic effects, as assessed by measurement techniques

used in this study, nor evidence additive effects upon exposure to 0.60 ppm NO_2 in combination with 0.30 ppm O_3. Female subjects, who exercised at a workload inducing minute ventilation proportionally lower than males in accord with gender difference in lung size, demonstrated no greater response to NO_2, O_3, and NO_2 plus O_3. Further, preexposure to 0.30 ppm O_3 24 hours prior to 0.60 ppm NO_2 exposure did not elicit significant responses, nor enhance the effects observed upon exposure to O_3 plus NO_2 compared to those at 0.30 ppm O_3, alone.

In our study of the effect of time interval between two repeated exposures to 0.35 ppm O_3, we observed an enhanced responsiveness - as have others - upon reexposure within 24 hours. This enhanced physiologic response is evidenced in some subjects reexposed after 48 hours, but is absent upon reexposure intervals of 72 and 120 hours. Further, none of these O_3 reexposure intervals resulted in the adaptation effect observed by others following several consecutive days of exposure.

We demonstrated in our drug pretreatment studies that pulmonary function impairment induced following 1 hour exposure to 0.35 ppm O_3 is associated with lung inflammation mediators (effects largely blocked by indomethacin), whereas increased airway resistance associated with bronchoconstriction is not. Further, pretreatment with atropine blocked the airway resistance effect upon O_3 exposure and reduced forced expiratory flow rate responses, but did not alter forced vital capacity response.

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

1. One hour exposure of healthy young males and females to high urban ambient levels of NO_2, while engaged in heavy, sustained exercise, does not elicit physiologic effects revealed by routine pulmonary function tests.

2. Similar exposures of males and females at high ambient levels of NO_2 and O_3 do not elicit physiologic effects revealed by routine pulmonary function tests beyond those observed at the same O_3 concentration alone.

3. The synergistic peripheral airway lesions evidenced upon acute inhalation of combined high ambient levels of NO_2 and O_3 in exercising rats is not evidenced in similar exposures of humans by available pulmonary tests of peripheral airway function.

4. Young adult females are not more sensitive to O_3 inhalation provided that their rate of pulmonary ventilation is closely proportional to the gender difference in lung size (about 40% greater for males).

5. Preexposure within 24 hours to high urban ambient levels of O_3 does not elicit significant pulmonary function response upon reexposure to high ambient levels of NO_2, nor elicit any greater effect upon reexposure to similarly high NO_2 plus O_3 levels beyond that observed with reexposure to O_3 alone.

6. Preexposure to a high urban ambient level of O_3 within 24 hours prior to reexposure at the same concentration enhances pulmonary function responses by 50 to 100 percent. This increased sensitization upon initial exposure to O_3 may persist for 48 hours but is absent following a 72 hour interval before reexposure. These observations have implications for those charged with setting O_3 health effects standards, as well as those who wish to conduct repeated laboratory exposures of human subjects.

7. O_3 induced pulmonary function impairment is associated with the release of mediators of lung inflammation, in that pretreatment with a drug which blocks the release of specific mediators of inflammation significantly reduces pulmonary function impairment, but without affecting airway resistance response.

8. O_3 induced bronchoconstriction is elicited predominantly by neural components innervating bronchial smooth muscle, in that pretreatment with a drug which blocks neural input to bronchial smooth muscle abolishes increased airway resistance (but without effect on forced vital capacity).

RECOMMENDATIONS

1. Pulmonary function tests particularly sensitive to peripheral airways effects in humans should be used to determine if the synergism observed in exercising animals exposed to high ambient concentrations of NO_2 and O_3 elicit greater peripheral airway effects than O_3 alone in exercising humans.

2. Pulmonary ventilation is increased 6 to 10 times that at rest in moderate to heavy exercise, and has been shown to reduce the O_3 concentration at which pulmonary function effects are observed. Further investigations of subjects exercising continuously at intensities characteristic of increasingly popular aerobic training programs should be conducted at O_3 concentrations characteristic of those approaching the first stage smog alert level (i.e., 0.20 ppm).

3. The role of very high ventilation rates incurred by elite endurance athletes during prolonged exercise (i.e., 1 to 3 hours), 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).

4. Reexposure to high ambient levels of O_3 within 24 hours significantly enhances the physiologic effects observed upon initial exposure. Studies should be conducted to determine if exposure to high ambient levels of O_3 within 24 hours prior to reexposure lowers the threshold O_3 concentration at which significant pulmonary function effects are observed.

5. 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. Thus, studies should be conducted in which these parameters are carefully monitored at O_3 concentrations and effective doses that may fail to elicit statistically significant pulmonary function impairment.

6. Healthy young adult females evidence greater physiologic response to the same total O_3 dose than do their male counterparts. Preliminary evidence from our laboratory indicates that this gender difference does not appear related to lung size difference, per se. Thus, further investigation is warranted.

7. Further studies of the physiologic mechanisms of O_3's effects on the human respiratory system upon acute exposure should be conducted to assess more accurately the associated health risk. Of particular importance is investigation of the role that inflammatory mediators and/or cells play in O_3-induced pulmonary function effects.

Introduction[1]

Photochemical air pollution occurs widely, particularly in the heavily populated Los Angeles basin. Ozone (O_3) is the principal component of concern because of its demonstrated biochemical and morphological effects on the lungs of animals acutely and chronically exposed to ambient smog alert levels, its association with an increased rate of hospital admissions for respiratory ailments, and an increasingly impressive documentation of physiological responses to acute laboratory exposures.

Significant photochemical smog episodes, in which the O_3 concentration is sufficient to effect acute physiological response, are frequently accompanied by ambient heat stress and/or other air pollutants of concern. Indeed, nitrogen dioxide (NO_2) plays a major role in the genesis of O_3 and other constituents of the photochemical air pollution mix. It is also of concern due to its toxic oxidant effects, particularly on tissues of the respiratory tract. Further, in some respects, the toxic effects of NO_2 are similar to those of O_3, which suggests the potential for additive effects in humans exposed to concentrations existent in smog alert episodes.

Unfortunately, there are still limited data relating acute exposure to specific levels of a given pollutant to a particular physiological effect. This problem is compounded by numerous factors affecting human response, including group and individual sensitivity, the effects of repeated exposure, and the fact that exercise both increases the response at a given pollutant concentration and lowers the concentration level at which physiologic responses are observed.

During exercise, the total amount of pollutant inhalation in a given time is dependent both on the ambient concentration and the 5 to 15 times increased rate of pulmonary ventilation volume induced upon engaging in moderate to very heavy physical activity, compared to that at rest. This observation is of practical importance because, in addition to vigorous voluntary exercise becoming increasingly prevalent in the adult population, there are also numerous outdoor occupations that require prolonged moderate activity, including construction labor, refuse collection, and postal delivery.

It is now well documented that reexposure to O_3 within 24 hours, following an initial exposure at a level eliciting significant physiological

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[1]References substantiating specific and generalized statements in this introduction are identified in the individual manuscripts which follow, and which comprise the major portion of this report.

reponse, results in an enhanced effect. Though peak ambient concentrations of oxidants frequently occur at approximately 24 hour intervals over several consecutive days, photochemical smog episodes can and do occur with two or three days intervening. Thus, health authorities need information evaluating the potentially heightened toxic impact of these smog episodes 48 or more hours apart. By varying the time interval between consecutive O_3 exposures, the presence of enhanced responsiveness and its corresponding time decay can be evaluated.

Those charged with setting appropriate air quality standards are concerned with the protection of all segments of the population, including those with asthma or chronic obstructive pulmonary disease. However, there are other more numerous subpopulation groups who may also be more sensitive to photochemical air pollution than the most frequently studied group - the healthy young adult male. Indeed, we have observed suggestive evidence that young adult females are more sensitive to acute O_3 exposure than their male counterparts.

There now exists an impressive body of knowledge concerning the adverse health effects of O_3, emanating from epidemiological evidence, as well as animal and human laboratory studies. However, there is a pressing need to provide a more effective link between the evidence accrued from acute and chronic animal studies to the largely descriptive outline of acute physiologic response in humans. This task can be effectively addressed by investigating more sophisticated physiological mechanisms associated with the oft reported pulmonary function impairment and subjective symptoms of respiratory discomfort upon acute ozone exposure.

Theoretical Approach to the Problem. The intent of this project was to address important aspects of each of the general problem areas identified above. This was accomplished by advancing three proposals for study, using heavy, sustained exercise during 1 hour exposures to ambient concentrations of oxidants in the first stage alert range. Statements of the problem and research objective for each study follow.

A. Comparison of Young Adult Male and Female Responses Upon Exposure to NO_2, O_3, and NO_2 plus O_3 Consequent to Heavy, Sustained Exercise

1. Statement of the Problem. 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. There have been numerous laboratory studies of humans exposed to O_3, but comparatively few entailing exposure to NO_2. In fact, the damaging effects of NO_2 inhalation have been demonstrated in humans exposed to nonambient levels of NO_2 found in certain occupational conditions, as well as in laboratory studies of animals.

Short-term toxic effects of NO_2 have not been observed in healthy humans exposed to concentrations below 1.5 ppm (approximately twice the maximum ambient concentration observed in the Los Angeles basin). However, heavy prolonged exercise, which would dramatically increase the rate and total amount of NO_2 inhaled, has not been employed previously. Further, only light to moderate intermittent exercise has been used to study human response to NO_2 plus O_3 at ambient smog alert concentrations. No effects in healthy young adults, beyond those observed upon exposure to the same concentration of O_3 alone, have been observed.

Limited data from other laboratories, as well as studies in our laboratory, indicate that females experience 1.5 to 2 times the pulmonary function impairment seen in males exposed to the same total amount of O_3. Whether these limited, nondefinitive observed differences between males and females are real and, if so, can be attributed to a sex difference in lung size, exercise breathing pattern, or are secondary to some sex difference in neural/tissue sensitivity, remains to be investigated. Further, there exists the possibility that females may also be more sensitive to NO_2, and to NO_2 and O_3 in combination. An increasingly large number of adult females are engaging in moderate to heavy work entailed both in voluntary recreational exercise and industrial and government work applications. Increased pulmonary ventilation required for participation in these vigorous activities results in increased air pollution inhalation during exposure to photochemical smog.

2. Statement of Research Objective. The primary purpose of this study is to compare the acute toxic effects of exposure to NO_2, O_3, and NO_2 plus O_3 in healthy young adult males and females. Heavy, sustained exercise, not previously used in studying the effects of NO_2,

will be employed during exposures to concentrations observed in the ambient environment.

B. Effect of Prior O_3 Exposure on Physiologic Responses Following Reexposure to NO_2, O_3, and NO_2 plus O_3.

1. Statement of the Problem. It is well documented that reexposure to O_3 within 24 hours following an initial exposure at a level eliciting significant toxic response results in an enhanced response. Previous studies of the effects of NO_2 have utilized a single acute exposure, and consequently ignore the potential for greater changes in airway resistance with reexposure, as has been observed with O_3 upon reexposure within 24 hours following initial exposure to O_3. Since O_3 and NO_2 inhalation induce similar toxic efforts, it seems probable that the effects of NO_2 might be enhanced if preceded by exposure to O_3 24 hours before at a dose sufficient to elicit short-term toxic response. With such information, health authorities would be better able to evaluate the impact of a given concentration of NO_2 or O_3 during the photochemical smog season, when peak oxidant concentrations occur with 24 hours or more intervening.

Another aspect of the initial O_3 exposure hyperresponsiveness effect is the length of time required for the individual to return to the same level of response as that demonstrated initially. This is important for at least three reasons: (1) it enables health authorities to better evaluate the impact of a given concentration of O_3 during the photochemical smog season, when peak oxidant concentrations occur with 24 hours or more intervening; (2) repeated exposures of subjects in laboratory studies, with as few days intervening as possible, reduces subject attrition and the costs of subject recruitment and characterization; and (3) repeated exposures of the same subjects serving as their own controls, reduces statistical variability. At present, repeated laboratory O_3 exposures are separated by a variable period of time (usually 3 to 7 days) during which, it is assumed the initial exposure hyperresponsiveness "wears off." Howeover, the effectiveness of this recovery period cannot be evaluated without systematically describing the time decay of postexposure hyperresponsiveness. Data cur-

rently available regarding this question are extremely limited and equivocal.

C. Investigation of Physiologic Mechanisms Associated with O_3-Induced Pulmonary Impairment in Humans.

1. Statement of the Problem. The study of acute O_3 toxicity in humans has been largely dependent upon the detection of significant impairment in pulmonary function. In this connection, studies of human O_3 toxicity have focused on how O_3 concentration and increased pulmonary ventilation volume consequent to exercise affects pulmonary function impairment. Despite the important role this body of literature has played in determining air quality standards, the physiologic factors contributing to the observed impairment in pulmonary function following acute O_3 exposure have not been quantitatively studied. Clearly, study of these factors would provide a better understanding of the health risk incurred upon acute O_3 exposure and the efficacy of present air quality standards.

Acute exposure of animals to O_3 concentrations equal to those found in the ambient environment have been shown to result in an initial phase of epithelial cell damage, followed by a second phase of cell replacement and repair. This epithelial cell damage has been observed in both central and peripheral airways in cats. Primates exposed for 8 hours/day for 7 days to O_3 concentrations between 0.20 and 0.80 ppm, showed morphological lesions of the trachea, large bronchi and respiratory bronchioles. Since humans have similar bronchial anatomy and airway morphology, it seems reasonable to assume that human subjects may experience nearly identical O_3 induced lesions if similarly exposed.

Acute exposures of humans below the O_3 dose which elicits significant pulmonary function impairment have been shown to increase the reactivity of the airways to histamine and methacholine. How these changes in bronchial reactivity are related to changes in pulmonary function impairment at higher O_3 doses is unknown. Thus, it remains unclear to what extent the factors of cell damage and bronchial hypersensitivity are associated with significant changes in lung volumes, flows and airway resistance (Raw) following acute O_3 exposure in humans.

We contend that the available data on acute O_3 exposure in humans is consistent with a progressive increase in irritation and damage to airway epithelium with increasing O_3 dose. The initial manifestation of ths damage is the sensitization of both bronchial smooth muscle and lung C-fiber receptors prior to significant changes in baseline Raw and other pulmonary function. At higher O_3 exposure doses, the pulmonary function impairment is the result of direct damage to the airway epithelium and increased sensitization and stimulation of both C-fiber receptors and bronchial smooth muscle. Further, there is recent evidence suggesting that airway epithelium damage in humans is associated with inflammation products associated with arachidonic acid production. However, the relative contribution of airway epithelium damage, bronchial smooth muscle irritability and C-fiber receptor stimulation to changes in baseline Raw and other pulmonary function parameters in human subjects has yet to be elucidated.

2. Statement of Research Objective. Using drug pretreatments affecting bronchial smooth muscle (atropine) and selected inflammation mediators (indomethacin), the purpose of this investigation is to study the specific factors involved in O_3 induced changes in pulmonary function and airway resistance. Further, we propose to study the role that central and peripheral airway constriction play in increased airway resistance following O_3 exposure, utilizing recently developed models of forced oscillations at the mouth.

Design, Results, and Discussion of Individual Research Projects

Relevant details of the experimental design to accomplish the aforementioned research objectives, together with methodology, results and discussion, are presented in the four manuscripts submitted for publication in refereed scientific journals.

EFFECTS OF NITROGEN DIOXIDE AND OZONE, ALONE AND IN COMBINATION, ON EXERCISING MALES AND FEMALES

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ABSTRACT

Previous studies of 2-h exposure to nitrogen dioxide (NO_2) at high urban atmospheric levels (i.e., 0.50-1.0 parts per million, ppm), utilizing light to moderate exercise for up to 1 h have failed to demonstrate significant pulmonary dysfunction in healthy humans. To test the hypothesis that heavy, sustained exercise would elicit pulmonary dysfunction upon exposure to 0.60 ppm NO_2, and/or enhance the effects of exposure to 0.30 ppm ozone (O_3), forty aerobically trained young adults - 20 males and 20 females - completed 1 h continuous exercise at 62% $\dot{V}O_{2max}$, which necessitated minute ventilation ($\dot{V}_E$) of 70 ℓ/min and 50 ℓ/min for the males and females, respectively. Exposures to (1) filtered air (FA), (2) 0.60 ppm NO_2, (3) 0.30 ppm O_3, and (4) 0.60 ppm NO_2 plus 0.30 ppm O_3 were randomly delivered via an obligatory mouthpiece inhalation system. Treatment effects were assessed by standard pulmonary function tests and exercise ventilatory and subjective symptoms response. Two-way analysis of variance (ANOVA) with repeated measures and post-hoc analyses revealed a statistically significant ($P < 0.05$) effect of O_3 on forced expiratory parameters, specific airway resistance (S_{Raw}), exercise ventilatory response (rapid, shallow breathing), and reported subjective symptoms of respiratory discomfort. In contrast, no significant effect of NO_2 was observed, nor was there any significant interaction of NO_2 and O_3 in combination. There were no significant differences between male and female responses to gas mixture treatments. It was concluded that inhalation of 0.60 ppm NO_2 for 1 h while engaged in heavy, sustained exercise does not elicit effects evidenced by measurement techniques used in this study, nor evoke additive effects beyond those induced by 0.30 ppm O_3 in healthy young adults. Nonetheless, the possibility remains that peripheral airway effects of NO_2 in combination with O_3

at these concentrations, as recently revealed in histological evidence by others in exercising rats, may have occurred.

Index terms: air pollution effects; exercise ventilatory pattern; forced expiratory flow rates; forced expiratory volume; pulmonary function impairment

Nitrogen dioxide (NO_2) plays a major role in the genesis of ozone (O_3) and other constituents of the photochemical air pollution mix. It is also of concern to agencies charged with setting air pollution standards because of its toxic oxidant effects, particularly on tissues of the respiratory tract (3,28, 30,34,35). In many respects, the toxic effects of NO_2 are similar to those of O_3 (30), which suggests the potential for additive effects in humans exposed to concentrations existent in smog alert episodes.

Numerous investigators have demonstrated that the acute toxic effects of O_3 are enhanced with exercise, as opposed to exposures at rest of the same duration (4,10,15,17,33), but the possible potentiation of exercise hyperpnea on the acute toxic effects of NO_2 has not been comparatively well studied.

An early report by Rokaw and associates (32) suggested that 10 min moderate exercise during 1 h exposure to 1.5 ppm NO_2 elicited increased airway resistance (Raw) in most subjects. However, Hackney and colleagues (16) observed no significant change in pulmonary function in young males exposed to 1 ppm NO_2 for 2 h with light intermittent exercise. Folinsbee et al (12) also observed no changes in pulmonary function, cardiovascular or metabolic responses in young adult males exposed to 0.62 ppm NO_2 for 2 h with a mean $\dot{V}_E$ 2-1/2 times that at rest.

Studies of the possible interaction of NO_2 with other ambient pollutants in healthy humans are limited (18), and apparently only three in combination with O_3 have incorporated exercise. Hackney et al (15) employed light intermittent exercise in the exposure of adult males to 0.20 ppm O_3 and 0.30 ppm NO_2 for 2 h, and found minimal changes in pulmonary function. Folinsbee et al (9) exposed young adult males to 0.50 ppm NO_2 plus 0.40 ppm O_3 for 2 h, with a mean $\dot{V}_E$ twice that at rest. Observed pulmonary function changes were similar

to those observed in a previous study of young adult males exposed to 0.4 ppm O_3 alone (10). Kagawa (22) also exposed young adult males for 2 h, with light intermittent exercise, at 0.15 ppm O_3 and 0.15 ppm O_3 plus 0.15 ppm NO_2. He found marginally greater Raw response for this pollutant combination than that observed for O_3 alone.

Lauritzen & Adams (24) observed significantly greater pulmonary function response to the same absolute inhaled O_3 dose in six aerobically trained young adult females compared to their male counterparts. This enhanced sensitivity was still evident when female subjects exercised at a reduced $\dot{V}_E$ proportional to their $\dot{V}o_{2max}$. Others have suggested that females demonstrate a greater acute response to O_3 inhalation, whether at rest (21) or while engaged in exercise at the same relative intensity as males (5,23). Horvath et al (19), however, have recently reported that young adult females exposed to 0.48 ppm O_3 for 2 h, with light intermittent exercise, evidence no greater pulmonary function impairment or respiratory subjective symptoms than their male counterparts.

The primary purpose of this investigation was to determine the effects of 1 h exposure to 0.60 ppm NO_2, alone and in combination with 0.30 ppm O_3, on pulmonary function and subjective symptoms in subjects engaged in continuous, heavy exercise. Additionally, we sought to determine if young adult females, exercising at the same relative intensity as their male counterparts (i.e., 62% of $\dot{V}o_{2max}$), evidenced enhanced responses upon 1 h exposure to 0.60 ppm NO_2, 0.30 ppm O_3, and to the two gases combined.

METHODS

Subject description and characterization. Forty healthy young adults (20 males and 20 females) served as subjects. (Institutional Human Subjects Review

Committee approval and signed individual informed consent were obtained.) Subjects were screened for clinically normal pulmonary function and absence of history of significant allergies. None had resided in a high air pollution area within the previous three months.

Prior to initiating the four experimental protocols, each subject completed an orientation session in which base-line pulmonary function and basic anthropometry, including body composition via hydrostatic weighing, were obtained. To attenuate habituation effects, all subjects completed at least 45 min of bicycle ergometer exercise at varied submaximal work loads while breathing filtered air (FA). On another preexperimental occasion, the subjects' $\dot{V}O_{2max}$ was determined utilizing a progressive increment protocol (1). Subject characterization data obtained in these orientation sessions are given in Table 1 (females) and Table 2 (males).

Experimental design. Subjects were exposed for 1 h each, in random order, to FA, 0.30 ppm O_3, 0.60 ppm NO_2, and to 0.30 ppm O_3 plus 0.60 ppm NO_2. At least 5 days intervened between each exposure. Exercise work rates were set such that minute ventilation ($\dot{V}_E$) was approximately 70 ℓ/min for each male and 50 ℓ/min for each female subject. All exposures were conducted in a room in which dry bulb temperature and relative humidity were maintained within 21-25 °C and 45-60%, respectively. To facilitate convective and evaporative cooling during exercise, an airflow between 1.5 and 3 m/s was directed at the subject's frontal aspect via an industrial grade floor fan.

Pulmonary function assessment. Pulmonary function tests were administered immediately prior to and after each experimental protocol. Duplicate determinations of forced maximal expiration were obtained via a Collins modular office spirometer, Model No. 3000. An on-line data acquisition system included 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. Pulmonary function on-line computer determinations included measurements 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}).

Thoracic gas volume (V_{tg}) and Raw were measured in 20 female and 10 male subjects using constant volume whole body plethysmography (7,8). Pre- and postexposure maneuvers consisted of 5 trials for each set. Specific airway resistance (S_{Raw}) was calculated as the product of Raw and V_{tg}.

Exercise measurements. To assess possible effects of 0_3 and/or $N0_2$ inhalation on selected exercise parameters, an on-line computerized data acquisition system delivered print-outs of one minute average values for $\dot{V}_E$, heart rate (HR), tidal volume (V_T), respiratory frequency (f_R), $\%0_2$ and $\%C0_2$ in expired gas, expired gas temperature, and $\dot{V}0_2$ every minute. Data acquisition instruments interfaced to the DEC LSI 11/2 microcomputer, included a LB-1 $C0_2$ analyzer, an Applied Electrochemistry S-3A 0_2 analyzer, an Alpha Technologies turbotachometer ventilation module (VVM-2), an electrocardiograph with R-wave detector, and a temperature thermistor located in the expired gas line.

Subjective symptoms were monitored at 5, 30, 45, and 58 min by the subject pointing to an ordinal scale to rate their perception of the existence and, if so, the severity of the symptoms. The symptom severity scale was rated as: 0, not present; 5, minimal; 10, mild; 20, moderate; 30, severe; and 40, incapacitating. Immediately following completion of the postexposure pulmonary function test battery, the subject provided written comments on discomforts ex-

perienced during the exposure and indicated whether or not he/she believed that they received O_3 and/or NO_2.

O_3 and NO_2 administration and monitoring. Air mixtures during all experimental protocols were inhaled by subjects through a blow-by obligatory mouthpiece system described in detail elsewhere (4). In brief, FA, blended with appropriate concentrations of O_3 generated by a Sander Ozonizer (Type II) was introduced proximal to the turbulent mix. Atmospheric levels of NO_2 were 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, and thence introduced proximal to the turbulent mix. The air mix was directed from an exhaust port through a Teflon-coated Hans-Rudolph respiratory valve to the subject.

Expired gas was directed through a unidirectional 5 liter stainless steel mixing and sampling chamber to an Alpha Technologies Turbotachometer ventilation module (Model VVM-2). Expired air was then routed into the distal portion of the mixing tube and, along with the volume of pollutant-containing air not inspired by the subject, passed through a Barneby-Cheney QDF multistage filter assembly and thence to the laboratory ventilation exhaust outlet.

Inspiratory O_3 concentration in the mixing chamber was monitored by continuous samples drawn through 0.64-cm diameter Teflon tubing connected to a Dasibi O_3 meter, while that for NO_2 was determined on a Thermo Electron 14 B/E N_{ox} analyzer. The digital reading of O_3 concentration in ppm was compared periodically to that determined by the UV absorption photometric method (6). The reading of NO_2 concentration was also calibrated periodically using a Thermo Electron 102 precision calibrater.

Statistical analysis. Duplicate pulmonary function measurements from pre- and postexposures were corrected to BTPS and averaged. S_{Raw} values were calculated from an average of three of five trials. The preexposure values for each parameter were subtracted from the postexposure values, and then divided by the preexposure values to obtain percent changes representing the treatment effect for each protocol. Similarly, values from the tenth minute and the last minute of exercise for $\dot{V}o_2$, $\dot{V}_E$, f_R, and V_T were utilized to calculate percent change. Subjective symptoms severity scores for eight variables were added to obtain a total score. Values obtained at 5 min were compared to those obtained in the 58th min of each protocol.

All subjects completed the FA and NO_2 protocols, whereas three females and four males were unable to complete the O_3 exposure and five females and three males were incapable of finishing the O_3 plus NO_2 exposure. Data obtained on these subjects were included in all statistical analyses.

All data were analyzed via a two-way analysis of variance (ANOVA), with repeated measures, which included two within factors (NO_2, or not) and one grouping factor (gender). Upon obtaining a significant F ratio for main effects due to NO_2 or gender, or an interaction, a paired t post hoc test, with Bonferroni correction, was applied (29) to determine which particular mean values were significantly different from others.

RESULTS

Pulmonary function response. Group pre- and postexposure data for pulmonary function parameters and S_{Raw} are given in Table 3, with group mean percent change for FVC and $FEV_{1.0}$ depicted in Fig. 1 and S_{Raw} in Fig 2b. ANOVA and post hoc analyses results for pulmonary function, S_{Raw}, and exercise ventila-

Table 3
Figure 1
Figure 2

tory and metabolic variables are summarized in Table 4. The specific mean differences, as assessed via post hoc analysis, revealed that decrements in FVC, $FEV_{1.0}$, and FEF_{25-75} were significantly different ($P < 0.05$) when comparing values for FA and O_3, FA and NO_2 plus O_3, NO_2 and O_3, and for NO_2 and NO_2 plus O_3 exposures. There were no statistically significant differences in pulmonary function parameters when comparisons were made between FA and NO_2 exposures, as well as between O_3 and NO_2 plus O_3 exposures. A significant mean difference ($P < 0.05$) was obtained when comparing S_{Raw} values observed between FA and O_3, NO_2 and O_3, and for O_3 and O_3 plus NO_2 exposures. No statistically significant differences were observed between males and females for pulmonary function or S_{Raw} responses to any air mixture exposure.

Exercise responses. Group pre- and postexposure data for exercise ventilatory and respiratory metabolism parameters are given in Table 5, with group mean percent change for f_R depicted in Fig. 2b. As shown in Table 4, F ratios and post hoc analysis revealed that air mixture treatments had no significant effect on $\dot{V}_E$ and $\dot{V}O_2$. Significant differences ($P < 0.05$) were obtained in response to gas mixtures for f_R and V_T when comparing values for FA to O_3, FA and O_3 plus NO_2, NO_2 and O_3, and for NO_2 and NO_2 plus O_3 exposures. No significant difference was observed between FA and NO_2 exposures and between O_3 and NO_2 plus O_3 exposures for f_R and V_T. A significant interaction of gender on f_R and V_T F ratio revealed by post hoc analysis that the males demonstrated a significantly greater f_R for all gas mixtures, but greater decrement in V_T for the O_3 exposure only.

Subjective Symptom Response. A summary of the symptomatic response to treatments for females and males is given in Table 6. The number and severity

of symptoms reported were significantly enhanced in the 0_3 exposures and the 0_3 plus $N0_2$ exposures. No significant difference between values obtained for females and males was observed. A subsequent time series analysis revealed significant mean differences from 5th min values beginning at 30 min for symptom severity when comparing 0_3 exposures to FA or $N0_2$ exposures, or when comparing 0_3 plus $N0_2$ exposures to FA or $N0_2$ exposures. Differences for these exposures were significantly enhanced at 45 min, but not further significantly increased at 60 min.

DISCUSSION

Results of this study indicate that 1 h of heavy, continous exercise while exposed to a high ambient level of NO_2 (0.60 ppm) has no effect on pulmonary function, S_{Raw}, exercise ventilatory pattern, or subjective symptoms response. Further, the combination of 0.60 ppm NO_2 plus 0.30 ppm O_3 had no effect on these parameters beyond that observed upon exposure to 0.30 ppm O_3 alone, except to decrease S_{Raw}. Female group responses were not significantly different from those of males.

Results from previous studies of the possible potentiation of exercise hyperpnea on the acute toxic effects of NO_2 and NO_2 plus O_3 in healthy human subjects are equivocal. Utilizing 10 min of light intermittent exercise in conjunction with a 1 h exposure protocol, Rokaw et al (32) demonstrated an increase in Raw in subjects exposed to 1.5 ppm NO_2. Von Nieding and Wagner (37) also observed increased Raw with 2 h of light intermittent exercise at 5.0 ppm NO_2. These observations suggest that similar to O_3, the effects of NO_2 inhalation on

pulmonary function are enhanced with exercise. However, Hackney et al (16) observed no significant pulmonary function effects in subjects exposed for 1 h to 1.0 ppm NO_2 in protocols incorporating light intermittent exercise. Similarly, Folinsbee and associates (12) found no effect of 0.62 ppm NO_2 on pulmonary function, cardiovascular, or metabolic parameters in 2 h exposures incorporating light intermittent exercise.

To retest the hypothesis that an increase in ventilation induced by exercise might lower the threshold at which effects of NO_2 are observed, we implemented a 1 h continuous, high intensity protocol and administered a near peak ambient 1 h concentration, i.e., 0.60 ppm. Nonetheless, we observed no effect on pulmonary function, S_{Raw}, exercise ventilatory pattern, or subjective symptoms, even though a high total inhaled dose of NO_2 was utilized. A recent study by Linn and colleagues (26), which entailed 15 min of light exercise, 15 min of moderate exercise and 45 min of rest at 4.0 ppm NO_2, demonstrated similar "no effect" results in both healthy and asthmatic young adults. Some investigators, however, have suggested that asthmatics may constitute a more sensitive subpopulation upon exposure to NO_2.

It has been suggested that the combination of ambient levels of NO_2 and O_3 might result in a synergistic effect on pulmonary function impairment and S_{Raw} due to the formation of the acid reaction product, HNO_3 (22). Mautz et al (27) have recently reported a synergistic effect when rats, who exercised continuously while exposed for 3 h to 0.60 ppm NO_2 and 0.35 ppm O_3, demonstrated enhanced lung tissue lesions compared to exposures at the same concentrations of NO_2 and O_3, alone. Again, presence of nitric acid vapor was implicated as a combined air pollutant reaction product which might account for the observed synergism.

There have been few laboratory studies of humans exposed to NO_2 in combina-

tion with other air pollutants which have incorporated exercise. Hackney et al (15) reported that the pulmonary function impairment resulting from 2 h exposure to 0.20 ppm O_3 plus 0.30 ppm NO_2 with light intermittent exercise was no greater then that observed for 0.20 ppm O_3 alone. When utilizing 0.40 ppm O_3 plus 0.30 ppm NO_2, they found the changes to be equivalent to those observed for 0.40 ppm O_3, alone. Similarly, Folinsbee et al (9) observed no difference in 2 h exposures to 0.40 ppm O_3 and 0.50 ppm NO_2 compared to those observed in an earlier study (10) for subjects exposed to 0.40 ppm O_3, alone, at a similar mean protocol ventilation. The present study incorporated a total inhaled effective dose of NO_2 for males approximately 2-1/2 times that employed by Folinsbee et al (9). Nonetheless, results of this investigation revealed no significant additive effect of 1 h exposure, with heavy continuous exercise, at a high ambient concentration of NO_2, i.e., 0.60 ppm, in combination with 0.30 ppm O_3 beyond that observed upon exposure to 0.30 ppm O_3 alone. In fact, a significantly lower percent change in S_{Raw} for NO_2 plus O_3 than for O_3 alone was observed.

A near doubling of $FEV_{1.0}$ decrement following reexposure to 0.32-0.42 ppm O_3 within 24 h of initial exposure has been observed by several investigators (11,13,20,25). Since NO_2 and O_3 inhalation effects are similar in many respects (30) we conducted a pilot study on four female subjects used in this investigation to determine if 1 h exposure to 0.30 ppm O_3 24 h prior to reexposure to 0.60 ppm NO_2, 0.30 ppm O_3, and to 0.60 ppm NO_2 plus 0.30 ppm O_3, respectively, would elicit an enhanced pulmonary function response. The data in Table 7 reveals no change upon exposure to NO_2 24 h after preexposure to 0.30 ppm O_3, while that for the NO_2 plus O_3 exposure was not appreciably different from that

obtained upon reexposure to 0.30 ppm O_3 24 h following initial exposure. Similar observations for FVC, FEF_{25-75} , f_R and subjective symptom responses were obtained. Thus, it appears that 24 h preexposure to 0.30 ppm O_3 does not induce significant responses to 1 h exposure to 0.60 ppm NO_2, or elicit an enhanced response to NO_2 plus O_3 beyond that observed for reexposure to O_3 alone.

Our observations of no significant effect of 0.60 ppm NO_2 on pulmonary function, S_{Raw} and subjective symptom response in healthy young adults undertaking 1 h of heavy, continuous exercise, together with the recent "no effect" results of 75 min exposure to 4.0 ppm NO_2 of healthy and asthmatic young adults (26), appear to corroborate the enhanced oxidative effects of O_3 compared to NO_2 found in animal studies. Veninga et al (36) report limited comparative biological effects data on acute and prolonged exposures of several mammalian species to NO_2 and O_3 ranging from 10 to 30 times greater concentration of NO_2 to induce similar effects. Based on survival time of rats during exposure to relatively high, subacute concentrations and on cellular responses at lower levels, Freeman and associates (14) observed similar effects elicited by approximately a 20-fold greater concentration of NO_2 than O_3.

Limited acute S_{Raw} dose-response data were summarized by Colucci (2), who found that a 3 to 4-fold greater concentration of NO_2 was required to elicit the same effect as that induced by O_3. On the other hand, von Nieding and Wagner (37) have reported significantly elevated R_t upon 2-h exposure to 0.1 ppm O_3 with light intermittent exercise, as well as upon similar exposure to 5.0 ppm NO_2, but not at 1.5 or 2.5 ppm. Linn et al (1985) did not observe significantly increased S_{Raw} following 75 min exposure to 4.0 ppm NO_2 with 15 min each of mod-

erate and heavy exercise.

Failure of other investigators (9,12,15) as well as in this investigation, to demonstrate statistically significant pulmonary function and S_{Raw} response upon exposure to peak 1 h ambient concentrations of NO_2 does not preclude the possibility of deleterious effects in the peripheral airways where animal studies accent NO_2's impact (30,35). Forced expiratory flow and S_{Raw} responses reveal primarily central airway, rather than peripheral airway effects (31). Thus, it seems apparent that tests of peripheral airway response should be developed to elucidate what effects, if any, occur consequent to acute exposure to peak ambient NO_2 levels when engaged in heavy, sustained exercise. This contention gains further credence in view of Mautz et al's (27) recent observation of a synergistic peripheral airway lesions effect upon 3 h acute exposure of exercising rats to 0.6 ppm NO_2 plus 0.35 ppm O_3 compared to that elicited by exposure to O_3 alone.

Several investigators have suggested that females appear to be more sensitive to the same O_3 concentration, exposure time product, whether at rest (21) or during exercise when $\dot{V}_E$ is proportional to percent $\dot{V}O_{2max}$ (5,23). Lauritzen & Adams (24) studied the responses of 1 h exposure to O_3 of six aerobically trained females and a similar group of six males. When both groups exercised continuously at the same $\dot{V}_E$, percent pulmonary function impairment was significantly greater for the females. When comparisons were made at work rates entailing the same percent $\dot{V}O_{2max}$ (necessitating ~ 25 percent lower $\dot{V}_E$ for females), differences in pulmonary function response between the groups were reduced but not entirely negated. Horvath et al (19), however, found no significant difference in pulmonary function and subjective symptom responses between

ten females and ten males exposed to 1) 0.27 ppm peroxyacetyl nitrate (PAN), 2) 0.48 ppm O_3, and 3) 0.48 ppm O plus 0.27 ppm PAN for 2 h with light intermittent exercise. The total inhaled dose was approximately 11 percent less for the females. Similarly, in the present study of 20 female and 20 male subjects, we observed no significant group mean differences between the sexes in pulmonary function, exercise ventilatory pattern, and subjective symptom response upon 1 h exposures to FA, NO_2, O_3, and O_3 plus NO_2, with mean $\dot{V}_E$ approximately 30 percent lower for the females.

Taken together, the above studies suggest that healthy young adult females are no more sensitive to O_3 inhalation effects at ambient smog alert levels than their male counterparts, as long as $\dot{V}_E$ is nearly proportional to their smaller lung size and body weight. The latter is of practical importance, in that if a female and male of similar relative fitness were to undergo exposure at a similar O_3 concentration while exercising at the same percent $\dot{V}O_{2max}$ (which would be the case in body weight-bearing activities performed at the same speed), their responses to O_3 would be similar. However, if the female was required to perform at the same absolute workload and $\dot{V}_E$ as the male, she would be expected to demonstrate a greater response to O_3 (24) for reasons not readily apparent.

FOOTNOTE

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 this study.

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Table 1. Female subjects' anthropometric, pulmonary function, and VO2max data.

Subject #	Age, yr	Height, cm	Weight, kg	Fat, % body wt.	FVC, l	RV, l	$FEV_{1.0}$, l	VO2max, l/min	VEmax, l/min BTPS
1	25	164	53.9	21.0	4.56	1.01	3.89	2.70	115.1
2	20	170	64.6	25.8	4.84	1.04	3.72	3.28	128.6
3	22	170	61.3	17.1	4.42	0.92	3.70	2.91	101.5
4	22	179	59.4	22.8	4.66	0.94	3.76	2.98	119.0
5	22	164	56.0	15.3	4.58	1.17	3.76	2.98	95.6
6	22	171	73.2	26.5	4.78	0.97	3.69	4.44	140.8
7	21	168	61.4	21.2	4.83	1.30	3.67	3.25	86.4
8	20	174	63.5	18.6	5.21	1.36	4.30	3.58	112.8
9	21	175	67.2	30.4	5.24	0.94	4.12	2.92	83.9
10	22	154	55.7	21.4	4.68	0.71	3.66	3.24	93.4
11	21	168	64.8	19.5	4.88	1.13	3.68	3.57	124.2
12	23	164	55.6	21.1	4.15	0.93	3.47	3.18	105.1
13	21	175	65.1	28.4	4.74	0.80	4.03	2.62	65.2
14	21	169	60.4	19.6	4.76	0.65	4.15	3.15	100.6
15	23	165	55.1	14.8	4.42	0.95	3.05	3.19	97.6
16	22	165	55.9	22.6	4.36	1.02	3.76	3.21	70.1
17	19	168	70.1	33.3	4.64	0.77	3.74	3.15	92.6
18	19	170	60.5	23.6	4.84	1.00	4.30	3.78	137.0
19	23	160	48.4	12.3	4.45	0.85	3.86	3.16	135.2
20	20	173	60.7	19.8	5.21	1.17	4.21	2.72	130.4
$\bar{x}$	21.4	168.3	60.6	21.8	4.71	0.98	3.83	3.20	106.8
SD	1.5	5.7	6.0	5.2	0.29	0.18	0.30	0.41	21.9

FVC, forced vital capacity; RV, residual volume; $FEV_{1.0}$, forced expiratory volume at 1s; VO_{2max}, maximum O_2 uptake; V_{Emax}, maximum minute ventilation.

Table 2. Male subjects' anthropometric, pulmonary function, and VO2max data.

Subject #	Age, yr	Height, cm	Weight, kg	Fat, % body wt.	FVC, 1	RV, 1	$FEV_{1.0}$, 1	VO2max, 1/min	VEmax, 1/min BTPS
1	18	182	81.8	9.9	7.45	1.55	5.54	5.18	131.5
2	23	183	77.1	17.0	6.46	1.98	4.82	4.45	168.4
3	21	183	75.1	7.8	6.93	1.59	5.08	4.08	154.3
4	20	178	72.6	17.3	5.86	1.27	4.09	4.92	175.4
5	19	178	74.8	23.4	5.84	1.23	4.46	3.62	129.0
6	19	183	80.8	11.6	7.94	1.22	6.76	5.29	184.6
7	22	183	80.1	12.5	7.83	1.60	5.32	5.26	185.9
8	19	193	80.9	15.0	7.75	1.92	6.05	4.61	140.0
9	24	183	68.7	13.5	7.11	1.58	5.63	5.03	159.6
10	21	169	75.1	22.6	5.07	0.78	4.30	4.09	125.6
11	23	178	68.4	10.0	5.05	1.28	4.62	4.27	143.7
12	30	180	65.0	8.5	6.17	1.66	4.81	4.50	208.0
13	27	179	69.1	8.5	5.44	0.93	4.55	3.64	132.0
14	22	180	63.0	7.0	5.19	1.97	4.61	3.36	152.8
15	22	190	80.1	10.0	7.67	1.66	5.63	4.75	150.0
16	28	180	82.6	9.5	6.73	1.21	4.74	4.42	122.6
17	24	180	72.7	13.0	6.22	1.04	5.28	4.36	120.0
18	22	187	88.1	19.8	5.88	1.18	4.50	4.21	139.8
19	23	185	77.3	14.3	6.51	1.22	5.46	3.97	149.4
20	28	178	65.5	10.0	5.20	0.99	4.18	4.58	123.2
$\bar{x}$	22.7	181.6	74.9	13.1	6.41	1.39	5.02	4.43	149.8
SD	±3.3	±5.0	±6.8	±4.8	±0.98	±0.35	±0.68	±0.55	±24.4

See Table 1 for abbreviations identification

Table 3. Group pre- and postexposure values for pulmonary function and specific airway resistance.

Variable	Filtered Air		0.30 ppm O_3		0.60 ppm NO_2		0.30 ppm O_3 plus 0.60 ppm NO_2	
	Preexp.	Postexp.	Preexp.	Postexp.	Preexp.	Postexp.	Preexp.	Postexp.
FVC, liters								
females	4.549 ±.29	4.443 ±.31	4.540 ±.32	3.899 ±.61	4.563 ±.29	4.484 ±.29	4.562 ±.28	3.843 ±.55
males	6.375 ±.88	6.315 ±.93	6.307 ±1.10	5.315 ±1.04	6.279 ±1.00	6.275 ±.91	6.218 ±.95	5.255 ±.97
$FEV_{1.0}$, liters								
females	3.686 ±.32	3.606 ±.29	3.735 ±.30	2.978 ±.67	3.740 ±.34	3.666 ±.37	3.683 ±.32	2.895 ±.55
males	4.918 ±.64	4.837 ±.74	4.945 ±.63	3.769 ±.75	4.929 ±.66	4.930 ±.57	4.870 ±.62	3.794 ±.95
FEF_{25-75}, ℓ/s								
females	3.670 ±.85	3.663 ±.93	3.818 ±.94	2.885 ±1.26	3.813 ±.88	3.809 ±1.05	3.744 ±1.05	2.577 ±1.18
males	4.340 ±.87	4.307 ±1.11	4.631 ±.80	2.980 ±.93	4.483 ±.97	4.577 ±1.11	4.459 ±.98	3.243 ±1.4
SRAW, liters·[cmH2O] / [ℓ/s]								
females	6.82 ±2.0	6.89 ±1.9	7.31 ±2.8	8.40 ±2.8	6.68 ±1.5	6.79 ±1.6	7.89 ±3.5	8.83 ±4.7
males	7.34 ±1.3	7.89 ±2.4	7.13 ±1.8	9.20 ±3.0	7.58 ±1.8	7.66 ±2.6	9.49 ±4.2	10.62 ±6.1

Values represent the pre- and postexposure means and the corresponding ± 1 standard deviation. FEF_{25-75}, forced expiratory flow rate over the middle half of FVC; SRAW, specific airway resistance; other abbreviations as in Table 1.

Table 4. F ratios and specific significant mean differences for post hoc analysis for pulmonary functions, specific airway resistance, and exercise ventilatory and metabolism variables.

Variable	FA--NO2 O3--O3+NO2 F RATIO	SEX F RATIO	FA--O3 NO2--O3+NO2 F RATIO	SPECIFIC SIGNIFICANT MEAN DIFFERENCES (P<0.05)
FVC	0.06	0.14	76.8	FA--O3;NO2--O3+NO2 FA-O3+NO2;NO2-O3
$FEV_{1.0}$	0.63	0.01	109.5	FA--O3;NO2--O3+NO2 FA-O3+NO2;NO2-O3
FEF_{25-75}	0.43	0.03	102.2	FA--O3;NO2--O3+NO2 FA-O3+NO2;No2-O3
S_{RAW}	7.00	0.59	13.4	FA--O3;NO2-O3 O3-O3+NO2
fR	0.82	4.24	83.5	FA--O3;NO2--O3+NO2 FA-O3+NO2;NO2-O3
VT	0.34	5.13	82.3	FA--O3;NO2--O3+NO2 FA-O3+NO2;NO2-O3
$\dot{V}E$	0.24	3.81	1.6	NA
$\dot{V}O2$	1.48	0.10	0.35	NA

f_R, respiratory frequency; V_T, tidal volume; $\dot{V}_E$, expired minute ventilation; $\dot{V}O_2$, oxygen uptake. Other abbreviations as in Tables 1 and 3.

Table 5. Group pre- and postexposure values for exercise ventilatory and respiratory metabolism.

Variable	Filtered Air		0.30 ppm O_3		0.60 ppm NO_2		0.30 ppm O_3 plus 0.60 ppm NO_2	
	Preexp.	Postexp.	Preexp.	Postexp.	Preexp.	Postexp.	Preexp.	Postexp.
fR, breaths/min								
females	33.6 ±4.5	36.9 ±4.6	33.0 ±4.8	43.3 ±6.6	34.8 ±9.0	37.5 ±7.4	33.7 ±5.8	46.0 ±9.1
males	31.8 ±5.3	36.1 ±5.3	31.0 ±4.8	44.1 ±6.9	32.4 ±4.4	37.4 ±6.7	32.4 ±5.6	48.0 ±9.2
VT, liters								
females	1.50 ±.22	1.42 ±.25	1.50 ±.22	1.18 ±.20	1.45 ±.22	1.37 ±.23	1.53 ±.21	1.16 ±.18
males	2.25 ±.48	2.08 ±.37	2.29 ±.44	1.62 ±.28	2.15 ±.28	1.97 ±.32	2.13 ±.39	1.55 ±.31
$\dot{V}E$, ℓ/min								
females	49.3 ±4.7	50.6 ±5.2	48.7 ±3.8	50.2 ±5.6	48.9 ±4.7	50.1 ±4.9	50.8 ±5.6	51.9 ±6.0
males	69.2 ±6.7	71.7 ±5.9	66.6 ±6.6	69.9 ±8.6	68.7 ±4.9	71.8 ±6.4	67.0 ±6.3	72.0 ±8.9
$\dot{V}O2$, ℓ/min								
females	1.94 ±.26	2.00 ±.27	1.90 ±.20	1.96 ±.26	1.92 ±.29	2.00 ±.29	1.99 ±.28	2.06 ±.31
males	2.74 ±.32	2.82 ±.29	2.71 ±.32	2.77 ±.35	2.79 ±.39	2.88 ±.31	2.65 ±.30	2.74 ±.30

Values represent average percent change between 10th minute and last minute means, and the corresponding ± 1 standard deviation. See Table 4 for abbreviations identification.

Table 6. Subjective symptom response to treatments.

Variable	Filtered Air	0.30 ppm O_3	0.60 ppm NO_2	0.30 ppm O_3 plus 0.60 ppm NO_2
Number of symptoms				
females	1.3 ±1.5	4.1 ±2.0	1.6 ±1.7	4.5 ±1.3
males	1.2 ±1.6	3.9 ±1.5	2.7 ±3.5	4.4 ±2.5
Symptom severity				
females	8.7 ±11.5	61.0 ±50.9	9.0 ±10.8	62.0 ±51.1
males	9.6 ±14.9	52.2 ±57.6	14.0 ±20.0	58.2 ±55.4

Values represent the mean for each exposure with corresponding ± 1 standard deviations. See text for details.

Table 7. Effects of reexposure to NO_2, O_3, and NO_2 plus O_3 24-h following prior exposure to O_3 on $FEV_{1.0}$.

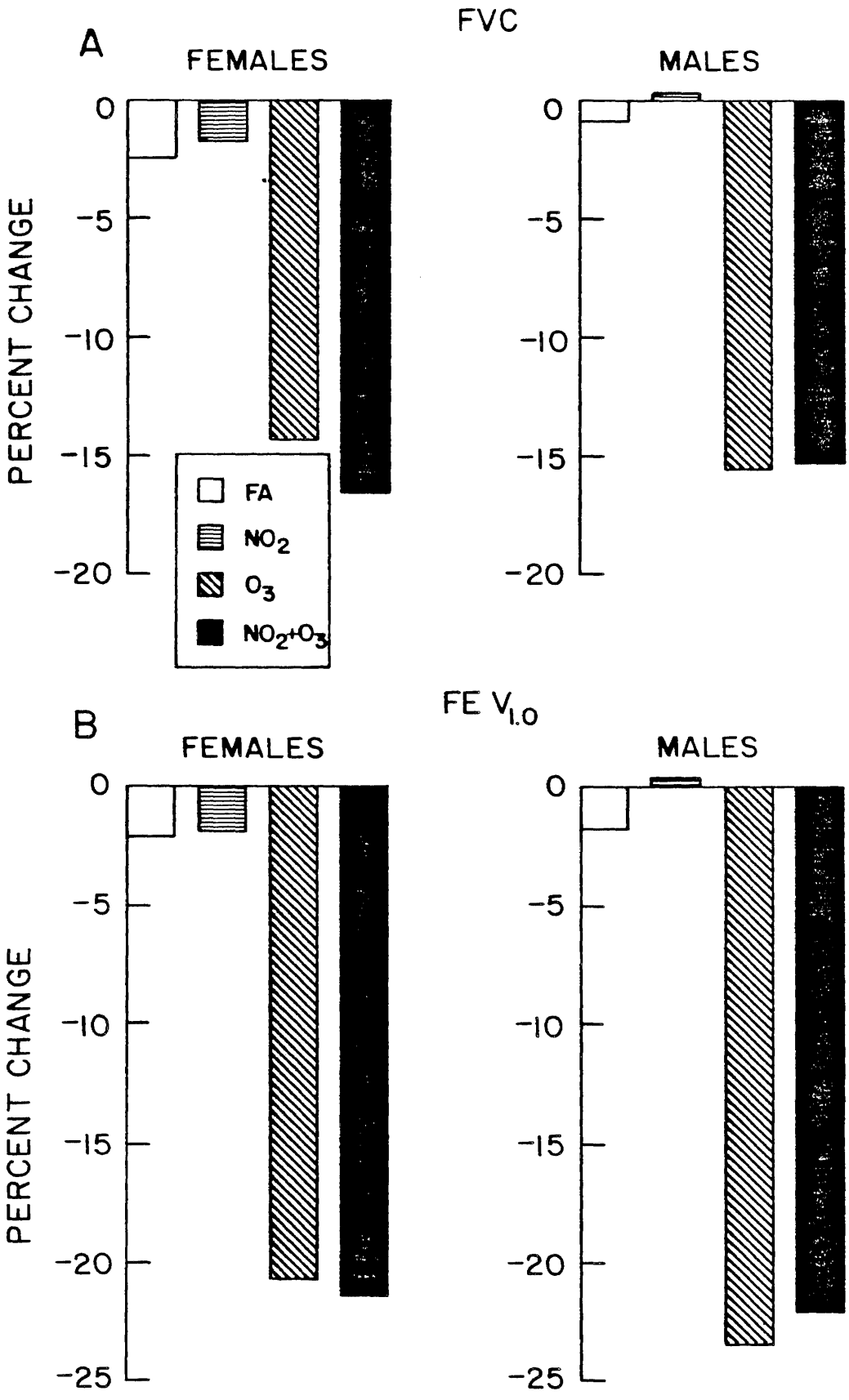
EXPOSURE CONDITION	$FEV_{1.0}$, liters PREEXPOSURE	POSTEXPOSURE	% Change
0.30 ppm O_3	3.74 ±0.05	3.35 ±0.20	-10.3, ±6.3
0.60 ppm NO_2	3.67 ±0.11	3.69 ±0.11	+ 0.6 ±0.5
0.30 ppm O_3	3.77 ±0.12	3.32 ±0.21	-10.7, ±6.5
0.30 ppm O_3	3.64 ±0.16	2.87 ±0.30	-21.0, ±9.8
0.30 ppm O_3	3.67 ±0.20	3.37 ±0.14	- 8.1, ±3.9
0.60 ppm NO_2 + 0.30 ppm O_3	3.64 ±0.17	2.95 ±0.28	-18.7, ±10.2

Values are group means for four females, plus or minus 1 standard deviation.

FIGURE LEGENDS

FIG. 1. Mean percent change from preexposure in (A) forced vital capacity (FVC), and (B) forced expiratory volume in 1s ($FEV_{1.0}$) for each exposure condition.

FIG. 2. Mean percent change (A) for last min vs. 10th min of exercise in respiratory frequency (f_R), and (B) from post- vs preexposure in specific airway resistance (S_{Raw}) for each exposure condition.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PERCENT CHANGE
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0
-5
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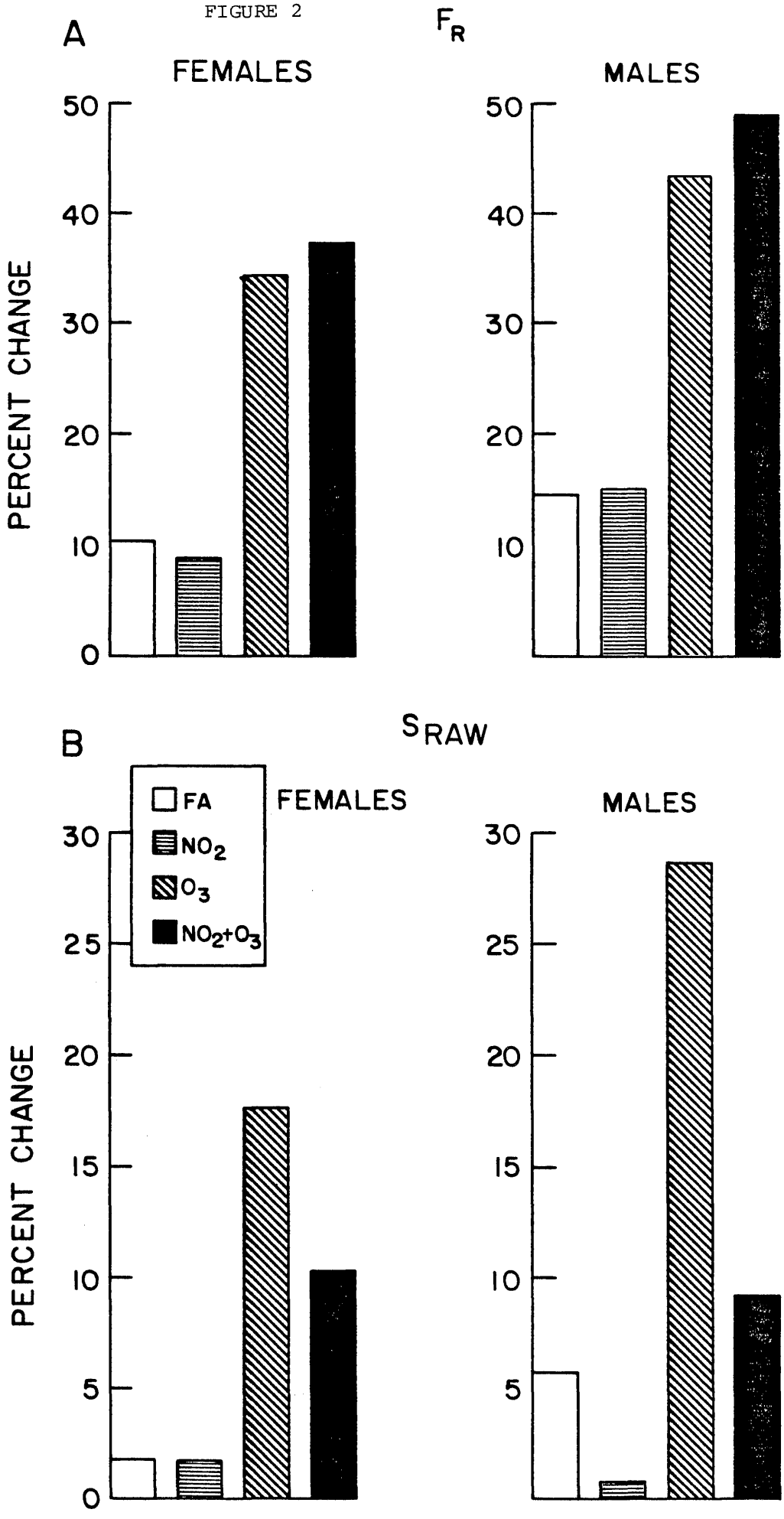
-20
-25

FIGURE 2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THE DURATION OF ENHANCED RESPONSIVENESS UPON REEXPOSURE TO OZONE

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ABSTRACT

It has been repeatedly observed that ozone (O_3) reexposure within 24 h elicits enhanced pulmonary function responses. Limited observations upon reexposure to O_3 at intervals between 24 h and several days have yielded divergent results. The present study was designed to assess the effects of reexposure to 0.35 ppm O_3 at intervals of 24, 48, 72, and 120 h. Forty young adult male subjects were randomly assigned to one of four groups in ascending order of time to reexposure (Groups 1-4). Each exercised on a bicycle ergometer for 60 min at a work load eliciting a mean ventilation of 60 ℓ/min on three occasions--protocols 1, 2 and 3 (P1, P2, and P3), respectively--in the same order: filtered air (FA), 0.35 ppm O_3, and 0.35 ppm O_3. In addition to standard pulmonary function measures, specific airway resistance (sRaw) via whole body plethysmography, exercise ventilatory pattern (fR and VT), and subjective symptoms (SS) were assessed. Statistical analysis revealed significant differences for all groups between the FA responses and those for the two O_3 exposures for forced vital capacity (FVC), forced expiratory volume in 1s ($FEV_{1.0}$), sRaw, fR, VT, and SS ($p < .05$). When the two O_3 exposures (P2 and P3) were compared, only Group 1 (24 h) responses showed statistically significant differences upon reexposure: $FEV_{1.0}$, -16.1 vs. -30.4%, $p < .003$; sRaw, 20.5 vs. 34.5%, $p < .05$; fR, 44.2 vs. 65.3%, $p < .001$; and SS, $p < .015$. Analysis of O_3 reexposure data for Group 2 (48 h) indicated a trend toward significantly enhanced responsiveness: sRaw, 13.4 vs. 23.7%, $p < .10$; fR, 40.4 vs. 53.2%, $p < .10$; and VT, -24.2 vs. -31.7%, $p < .10$. Statistical analyses for groups 3-4 (72-120 h intervening) revealed no significant differences upon reexposure to O_3. Our observations demonstrated that, for the total O_3 inhaled dose used in this study (~1200 ppm-ℓ), the enhanced pulmonary function responsiveness noted upon

reexposure to O_3 within 24 h may persist for 48 h, but is absent 72 h after initial O_3 exposure.

air pollution; exercise ventilatory pattern; forced expiratory flow rates; ozone reexposure; pulmonary function impairment

INTRODUCTION

In controlled laboratory exposures to ozone (O_3), the primary constituent of photochemical air pollution, several investigators have demonstrated transient changes in pulmonary function, including decrements in forced vital capacity (FVC) and flow rates ($FEV_{1.0}$, FEF_{25-75}); bronchial hyperreactivity to histamine or cholinergic challenge; and subjective symptoms of respiratory discomfort (2,3,4,8,14,15,16,21,22). Evidence of acute lung inflammation in animals and humans has also been observed (11,19,28,29,31). Repeated O_3 exposure for 3-5 consecutive days has consistently produced a tolerance development or "adaptation" effect, usually resulting in a near complete reversal of initially observed decrements in FVC and forced expiratory volume in 1s ($FEV_{1.0}$), along with diminished symptoms of respiratory discomfort (12,14,16,21,22,24).

Previous studies examining adaption to O_3 have yielded three basic observations. First, the development and persistence of O_3 adaptation appears dependent on an individual's initial O_3 sensitivity, with more sensitive subjects requiring a longer time to fully adapt while maintaining the adaptation for a shorter time, usually less than 7 days (21,22). Second, O_3 adaptation appears to be dose or concentration specific; i.e., repeated daily exposure to a low [O_3] or inhaled dose does not protect against the acute exposure effects at a higher O_3 dose or concentration (17). Finally, most daily O_3 reexposure regimens have demonstrated both enhanced pulmonary function and subjective symptom responsiveness after the second consecutive day of exposure (12,14,16, 21,22,24). However, in one study (18) an enhanced $FEV_{1.0}$ decrement of only 1.4% was observed. In another, published only as an abstract (30), adaptation upon reexposure to 0.48 ppm O_3 two, three and four days following initial exposure was suggested.

The evidence for enhanced responsiveness upon O_3 reexposure beyond 24 h is sparse. Adams and Schelegle (3) noted--in a non-systematic, retrospective analysis--that six subjects reexposed to either 0.20 or 0.35 ppm O_3[1] within 3-6 d (mean= 4.5 d) after previous exposure to 0.35 ppm O_3 had a nonsignificantly greater $FEV_{1.0}$ impairment (-8.5%) compared with exposure to the same concentrations without prior exposure to 0.35 ppm O_3 (-7.6%). However, Bedi and colleagues (4), recently observed enhanced responsiveness upon reexposure to 0.45 ppm O_3 48 h after initial exposure, suggesting that hyperresponsiveness upon O_3 reexposure may persist beyond 24 h.

Peak ambient concentrations of oxidants frequently occur at approximately 24 h intervals over several consecutive days in some areas during several months of the year. However, photochemical smog episodes can and do occur with two or three days intervening. Thus, health authorities need information evaluating the potentially heightened toxic impact of these smog episodes 48-72 h apart. By varying the time interval between consecutive O_3 exposures, the presence of enhanced responsiveness and its corresponding time decay can be evaluated. Also, since repeated laboratory exposure of a limited number of subjects, each acting as their own control, reduces variability and permits use of repeated measures statistical designs, an optimum time interval between the repeated exposures yielding similar pulmonary function, exercise ventilatory pattern, and subjective symptom responses needs to be assessed. The purpose of this study was to examine systematically the hyperresponsiveness and its persistence associated with O_3 reexposure ranging from 24 to 120 h following initial exposure.

METHODS

Subjects. Forty young adult males, 19-35 yrs of age, who were nonsmokers and free from asthma or allergic rhinitis symptoms, served as subjects. (Institu-

tional Human Use Committee approval and signed individual informed consent were obtained.) Their basic anthropometry, and pulmonary function data are summarized in Table 1. Each subject was randomly assigned to one of four groups (G-1 to G-4) in ascending time to reexposure: 24, 48, 72, or 120 h, respectively. All subjects demonstrated pulmonary function within normal limits, and none lived in a high air pollution environment during the 6 mo prior to the study.

Table 1

Each subject initially completed two introductory sessions involving practice of pulmonary function tests, airway resistance (Raw) measurement via whole body plethysmography, and exercising on a Quinton bicycle ergometer (Model 845) for 30-60 min while breathing filtered air (FA) through the obligatory mouthpiece inhalation system employed in the study. In a subsequent session, basic anthropometry, including body composition via hydrostatic weighing, and determination of maximum O_2 uptake were obtained according to procedures described elsewhere (2,6).

Experimental design. Each subject completed three 60 min protocols (P1, P2, P3) while exposed once to FA (P1) and twice to 0.35 ppm O_3 (P2, P3) according to the design shown in Figure 1. This design permitted assessment of the rate of decay in hyperresponsiveness upon O_3 reexposure without the subject being aware of the purpose of the study. The exercise intensity was set to elicit a minute ventilation ($\dot{V}E$) of approximately 60 ℓ/min ($\bar{x}$ = 57.3 ℓ/min). To assess each subject's pulmonary function, exercise ventilatory pattern, and subjective symptom severity (SS score) responses throughout the protocol, the 60 min exposure was divided into six 10 min cycles. At the end of each cycle, while remaining seated on the ergometer and no longer pedalling, the subject performed two forced expiratory maneuvers on a Collins 10-L Stead-Wells spirometer (clinical module, model 3000). Within 2.5 min, the subject resumed pedal-

Figure 1

ling and returned to the inhalation system. Thus, the 60 min exposure was spread over approximately 72 min.

Pulmonary function measurements. A short battery of pulmonary function tests was administered immediately before, between each 10 min cycle, and immediately after exercise. An on-line data acquisition system interfaced 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. Usually, an average of two trials for FVC, $FEV_{1.0}$, and forced expiratory flow rate over the middle half of FVC (FEF_{25-75}) was utilized in subsequent analyses.

In addition to the forced expiratory tests, Raw and thoracic gas volume (Vtg) were measured in a whole body plethysmograph at a panting frequency of 1.75 Hz by a modified DuBois technique (9,10). In brief, a Collins variable pressure whole body plethysmograph along with a Vertek digital pneumotachometer (model VR4000) and three Validyne transducers (model CD15) to monitor changes in mouth flow rate ($\dot{V}$), box pressure (Pb), and mouth pressure (Pm) were connected on-line with the aforementioned microcomputer. The computer algorithm of SooHoo and Brown (33), modified for the LSI 11/2, was utilized. This algorithm minimized the drift and hysteresis of the $\dot{V}$-Pb loops by taking the derivatives of $\dot{V}$ and Pb, thereby reducing the loops to a series of essentially parallel lines from which the slope can be easily calculated using linear regression. The identical technique is employed when Pm is plotted against Pb, enabling on-line calculation of Vtg (33). Specific airway conductance (sGaw) was calculated as the reciprocal of Raw multiplied by Vtg (sGaw= 1/Raw x Vtg), and sRaw was calculated as the product of Raw and Vtg. A storage oscilloscope, Tektronix model 5111-A, enabled the visual display of the plethysmographic maneuvers. Because

of our inability to isolate the plethysmograph system from random electrical noise and various vibrations, a low pass digital filter (5.0 Hz) was built into the algorithm. This low pass filter effectively reduced the mean standard error (MSE) about the calculated regression lines.

Exercise measurements. All exposures were conducted in a 3.0x 2.4x 3.7m environmental chamber in which temperature (21-25°C) and relative humidity (40-60%) were controlled. To facilitate convective and evaporative cooling during exercise, an industrial grade floor fan provided airflow aimed at the subject's frontal surface. An on-line data acquisition system printed out one minute values for $\dot{V}E$, tidal volume (VT), respiratory frequency (fR), %O2 and %CO2 in expired air, expired gas temperature, and O2 uptake ($\dot{V}O2$) during each cycle. Instruments interfaced with the LSI 11/2 included a Beckman LB-2 CO2 analyzer, an Applied Electrochemistry S-3A O2 analyzer, an Alpha Technologies turbotachometer ventilation measurement module, and a temperature thermistor located in the expired gas line.

To evaluate specific subjective symptoms and total symptom severity, the subjects pointed to an ordinal scale to rate their perception as to the existence and severity of certain symptoms identified on a tape recorder. The symptom severity scale was rated as follows: not present=0; minimal=5; mild=10; moderate=20; severe=30; incapacitating=40. A total symptom severity score was defined as the sum of the individual symptom scores during a particular 10 min cycle. Immediately following the postexposure pulmonary function tests, the subject completed a questionnaire evaluating the difficulty of the exercise bout compared to the previous one (P2 and P3 only), and whether or not the exposure involved O_3 inhalation.

O_3 administration and monitoring. Specific air mixtures during all experi-

mental protocols were inhaled by subjects through an obligatory mouthpiece system described in detail elsewhere (6). O_3 concentration was routinely determined by sampled air from the inspiratory side of the Hans-Rudolph valve through a 0.64 cm-Id Teflon tube connected to a Dasibi O_3 meter. The Dasibi digital reading of $[O_3]$ in ppm was compared periodically during the study with that determined by the UV photometric absorption method (7) at the University of California, Davis, Primate Research Center (no change in calibration was noted).

Statistical methods. Duplicate pulmonary function measurements, corrected to BTPS, were averaged for pre-, post-, and within exposure values. The plethysmographic measures were calculated from an average of three of five trials. The postexposure value was subtracted from the preexposure value to obtain differences representing the treatment effect for each protocol. For exercise measurements, values from the 8-10th and final min were averaged, and the absolute differences were calculated similarly. For the SS scores, differences between the 10th and final min scores were calculated.

Seven subjects failed to complete one or both of the O_3 exposures, terminating after 30-50 min of exposure. Of these seven subjects, three were from G-1, two from G-2, and one each from G-3 and G-4. The mean exposure times for these subjects were 43, 38, 50 and 50 min for G-1 to G-4, respectively. Data from these subjects were included in all statistical analyses.

A two-way analysis of variance (ANOVA) with repeated measures across protocol and time during exposure (pre/post) was performed on the data. When a significant F value due to group, protocol, or an interaction effect was obtained, Scheffé post hoc analyses were done to determine between which specific groups and/or protocols significant differences existed. The Scheffé method of multiple comparisons is applicable to repeated measures designs, especially when

all possible comparisons are of interest, and is comparable to other pairwise comparison tests under the conditions of this experimental design (27).

RESULTS

Pulmonary function response. Pre- and postexposure group mean values for the pulmonary function data are presented in Table 2. Results of the ANOVA procedure are included in Table 3. Across all groups, decrements in FVC, $FEV_{1.0}$, and FEF_{25-75}, as well as reciprocal changes in sRaw and sGaw, were significantly nificantly related to an O_3 treatment effect ($p < .0001$). Though G-1 did exhibit slightly larger decrements in FVC and $FEV_{1.0}$ upon initial O_3 exposure, there were no statistically significant differences between groups. However, the primary question in this study concerned the effect of exposure repetition and the time interval between consecutive exposures; thus, the im portant comparison was between group responses to the first and second O_3 treatments, i.e., P2 and P3.

The significant protocol (O_3 treatment) main effects listed in Table 3 were further evaluated by post hoc analysis and are summarized in Table 4. Group 1 showed significant hyperresponsiveness upon O_3 reexposure, as demonstrated by the significant difference ($p < .05$) in the P2-P3 comparison, for all pulmonary function variables. The enhanced pulmonary function responses for G-2 (48 h interval) upon reexposure to O_3 were numerically greater than for those observed for G-3 and G-4, but did not reach statistical significance ($p > .05$). In G-3 and G-4, the pulmonary function responses seen with reexposure to O_3 did not differ significantly from those observed upon initial O_3 exposure. These results are graphically illustrated, in absolute change (pre- to postexposure) for $FEV_{1.0}$ and sRaw, in Figures 2A and B, respectively.

Exercise ventilatory and metabolic responses. The group mean exercise ventilatory and metabolic responses for the 10th and final minute of exercise are presented in Table 5. The average $\dot{V}E$ over the three protocols for the 8-10th min of each 10 min cycle for G-1 to G-4 was 61.5, 60.7, 59.0, and 57.9 ℓ/min, respectively. As shown in Table 3, O_3 treatment effects upon $\dot{V}E$ and $\dot{V}O2$ were absent. The group main effect for $\dot{V}E$ indicated that, compared to G-1 and G-2, G-3 exhibited a significantly larger increase in $\dot{V}E$ during the exposures. However, this was a consistent finding in G-3 across all exposures and thus, appears to be an exercise effect, rather than a treatment effect. The group main effect for VT noted in Table 3, upon post hoc analysis, indicated that G-1 exhibited a greater reduction in this variable than either G-3 or G-4 upon initial or repeat O_3 exposure.

Table 5

Upon exposure to O_3 (i.e., P2 and P3), fR increased and VT reciprocally decreased ($p < .0001$). Post hoc analyses (Table 4) showed significant hyperresponsiveness upon O_3 reexposure in G-1, G-2, and G-4 for both fR and VT. In G-3, fR and VT responses upon reexposure were not significantly different. A graphical representation of the absolute change in fR upon initial and repeat O_3 exposure is depicted in Figure 2C.

Figure 2C

Subjective symptoms response. Group mean values for the 10th and final minute symptom severity (SS) scores are presented in Table 5. Statistical analysis showed a significant difference between the groups in how they subjectively rated each exposure, as well as a significant O_3 treatment effect (Table 3). The group effect, via post hoc analysis, indicated that G-1 reported a significantly higher SS score than either groups 2, 3, or 4 upon O_3 reexposure (i.e., P3). This result paralleled the enhanced pulmonary function decrements observed in this group (see Figs. 2A, B, and D). The SS score during P3 for G-2 tended to be slightly higher than upon initial O_3 exposure (P2),

Figure 2D

but the difference was not statistically significant. Figure 2D graphically illustrates the final minute mean SS scores for each of the groups for the two O_3 exposures. Finally, nine subjects in G-1 compared to three, two, and two subjects in groups 2, 3, and 4, respectively, perceived the second O_3 exposure to be more difficult than the initial O_3 exposure.

DISCUSSION

In this study we examined the effect of varied intervals of reexposure to 0.35 ppm O_3 (24-120 h after initial exposure). We were particularly interested in determining the persistence of hyperresponsiveness beyond 24 h, and the time interval between successive O_3 exposures which yield similar pulmonary function, exercise ventilatory pattern, and subjective symptom responses.

Our results clearly demonstrated significantly enhanced pulmonary function, exercise ventilatory pattern (↑fR/↓VT), and SS responses upon O_3 reexposure within 24 h at the inhaled dose used in this study (~1200 ppm-ℓ). Further, there was a trend toward significantly enhanced pulmonary function and exercise ventilatory pattern responses upon reexposure within 48 h. However, in this group (G-2), the SS score upon reexposure was not significantly different from initial O_3 exposure (see Figs. 2A and D). In groups 3-4 (reexposure intervals of 72 and 120 h, respectively), the P2 and P3 responses were similar, though G-4 did show a greater exercise ventilatory pattern response upon reexposure.

In assessing the reproducibility of individual acute responses to O_3 exposure, McDonnell and coworkers (25) noted that FVC and $FEV_{1.0}$ were highly reproducible at concentrations between 0.18-0.40 ppm O_3, even when exposures were separated by as long as several months. Our results of repeated exposure to 0.35 ppm O_3 demonstrated no significant differences in lung function para-

meters when two O_3 exposures were separated by 72-120 h (see Tables 2 and 4, and Figs. 2A, and B). Further, the initial and repeat O_3 exposure delta $FEV_{1.0}$ values for groups 3 and 4 were highly correlated when the data were pooled (r= 0.92).

As suggested by the data in Fig. 2C, reproducible results for fR and VT may require a time interval longer than 120 h, although G-3 (72 h reexposure interval) did not demonstrate a significantly enhanced exercise ventilatory pattern response upon reexposure to O_3. The final minute SS scores for G-3 and G-4 for the P2 and P3 exposures yielded a pooled data correlation coefficient of 0.69.

Our results of enhanced pulmonary function impairment upon reexposure to 0.35 ppm O_3 24 h following initial exposure were in accord with observations from numerous studies of subjects exposed to high ambient O_3 concentrations (0.32-0.42 ppm) for 2 to 3 h with light intermittent exercise (IE) (12,14,16,18, 21,22,24). Further, the magnitude of enhanced $FEV_{1.0}$ decrement observed in this study (from -16.4% to -30.0% for G-1) was similar to the mean of that observed in four studies (14,16,21,24) in which the total inhaled O_3 dose was closest to ours (1110 ppm-ℓ vs. 1200 ppm-ℓ), i.e., from -18.7 to -27.2%.

A reduction in the preexposure pulmonary function measures with several consecutive daily O_3 exposures has been observed previously (15,21). Similarly, in the present study, our G-1 subjects also demonstrated a small, but statistically significant ($p < 0.05$), reduction in the preexposure FVC and $FEV_{1.0}$ values (P2-P3 comparison; paired t-test). The mean reductions in these preexposure values were 0.062 ℓ and 0.122 ℓ for FVC and $FEV_{1.0}$, respectively, and represented only a small portion of the heightened pulmonary function decrements observed during P3 in this group. Further, the relationship between the P2-P3

$FEV_{1.0}$ preexposure decrement in G-1 was poorly related to the enhanced $FEV_{1.0}$ impairment observed after O_3 reexposure (r= -0.07).

Investigators who have assessed subjective symptoms of respiratory discomfort, report that 24 h reexposure values were greater than those upon initial exposure to O_3 and, in general, paralleled the enhanced impairment in pulmonary function upon reexposure (12,14,16,24). Our results for G-1 (24 h reexposure interval) were consistent with these previously reported observations.

Evidence for enhanced responsiveness upon O_3 reexposure beyond 24 h is sparse and, in part, conflicting. An early study, reported as an abstract (30), noted no enhanced responsiveness after 24 h to 0.48 ppm O_3 for 2 h during light IE in four subjects. Further, equivalent size subject groups reexposed at 48, 72, and 96 h evidenced adaptation (i.e., reduced pulmonary function impairment compared to initial exposure values). In contrast, Bedi et al (4), utilizing a similar 2 h light IE exposure to 0.45 ppm O_3, observed significantly greater pulmonary function impairment upon reexposure at 48 h (the decrement in $FEV_{1.0}$ was -13.3% upon initial exposure and -22.8% upon rexposure). Folinsbee and Horvath (15) have recently reported observations of repeated 1 h exposures to 0.25 ppm O_3, entailing continuous exercise at a mean $\dot{V}E$ of 63 ℓ/min, at intervals varying from 12 to 72 h. Subjects reexposed at 12 and 24 h demonstrated significantly enhanced pulmonary function and SS responses, while those reexposed at 48 h revealed no signifiant enhancement of SS and only a nonsignificant trend toward an enhanced $FEV_{1.0}$ decrement (-18.6 to -21.8%; $p > 0.05$). Similarly, in the present study, G-2 (48 h reexposure interval) showed a similarly enhanced $FEV_{1.0}$ response upon reexposure (i.e., -14.4 and -20.6%), though it failed to reach statistical significance.

Enhanced pulmonary function impairment and SS response upon reexposure to O_3 within 24 h, though statistically significant, is not universal. Farrell et al (12) observed that three of 14 subjects reported greater SS upon initial exposure to O_3. This was accompanied by a greater initial pulmonary function decrement than upon reexposure 24 h later. Similarly, Folinsbee et al (14) found that two of eight subjects felt better upon reexposure to 0.42 ppm O_3 24 h after their initial exposure to the same O_3 concentration. In our study, nine of ten subjects in G-1 reported greater SS responses upon reexposure 24 h after initial exposure, with each also demonstrating an enhanced $FEV_{1.0}$ decrement upon reexposure. However, G-2 individual subject $FEV_{1.0}$ and SS responses upon reexposure 48 h after initial O_3 exposure were similar in direction in only four of ten cases.

Disparate individual subject sensitivity upon initial O_3 exposure is well documented (2,6,13,25), and has been advanced as an important associative factor in tolerance development and persistence after several consecutive daily O_3 exposures (21). That senstivity upon initial O_3 exposure does not appear systematically related to enhanced pulmonary function impairment beyond 24 h was evidenced in the G-2 individual subject $FEV_{1.0}$ data given in Table 6. There was a similar lack of relationship in the G-1 (24 h reexposure interval) individual subject $FEV_{1.0}$ data.

Recent observations of Folinsbee and Horvath (15) reveal that enhanced pulmonary function responsiveness upon O_3 reexposure is evidenced within 12 h and persists through 24 h, with correspondingly greater subjective discomfort in most subjects. Our results confirm this hyperresponsiveness in most subjects for 24 h (G-1) and, in agreement with others (4,15), its persistence in some individuals for 48 h (G-2), though generally in the absence of heightened subjec-

tive discomfort. At an O_3 reexposure interval of 72 h, neither Folinsbee and Horvath (15) nor G-3 in our study demonstrated significantly different responses from those evidenced upon initial exposure. We observed similar nonsignificant differences upon reexposure 120 h after initial exposure (G-4). The latter was in accord with our earlier retrospective analysis of O_3 reexposure 3-6 d ($\bar{x}$= 4.5 d) following initial exposure resulting in no significant difference in $FEV_{1.0}$ decrement (3).

There are a number of possible factors which could account for enhanced responsiveness following initial O_3 challenge, including possible mediators of airway inflammation (19,28,29) and/or effectors stimulating neural transmission (5,13). Recent pathologic and biochemical evidence points to an acute postexposure airway inflammation response as a probable cause for airway hyperreactivity after O_3 exposure as assessed via cholinergic challenge (11,19,28,29). Animal (dog) studies (11,19,28,29) have shown significant increases in mucosal epithelial cells, and neutrophils indicative of airway inflammation, in bronchoalveolar lavage fluid after exposure to 3.0 ppm O_3. Direct evidence of airway inflammation in humans is limited. However, the data of Seltzer and colleagues (31), in which ten human subjects were exposed to either 0.40 or 0.60 ppm O_3, support the hypothesis associating airway hyperreactivity with a localized, postexposure airway epithelial inflammation response. Except for the lack of an increase in epithelial cells recovered in bronchoalveolar lavage fluid, their results were strikingly similar to those reported in the dog studies, i.e., neutrophilic infiltration, accompanied by significantly elevated concentrations of PGE-2, PGF-2α, and TxB-2. Additionally, addressing the topic of disparate human sensitivity to O_3, Seltzer et al (31) observed that subjects unresponsive to methacholine challenge following O_3 exposure showed little signs of airway in-

flammation, with the increase in neutrophils recovered from bronchoalveolar lavage being only 8.0% versus 30.8% ($p < .01$) in more responsive subjects.

More evidence associating enhanced pulmonary function decrements and airway hyperreactivity with acute airway inflammation can be found in anti-inflammatory drug studies (28,29). Pilot studies in our laboratory on two subjects who repeated the G-1 (24 h reexposure interval) protocols at least 21 d following the initial exposure sequence while taking oral indomethacin (50 mg tid), demonstrated markedly reduced FVC decrements, both upon initial and repeat O_3 exposure. As shown in Table 7, a similar trend was noted for both fR and SS score during the indomethacin exposures. However, modest increases in sRaw, similar to those observed after O_3 but without indomethacin, were still present, though indomethacin did attenuate the hyperresponsiveness in sRaw observed in subject 1 during P3 without the drug. Similarly, O'Byrne and coworkers (29) reported that indomethacin inhibited the bronchial hyperreactivity but not the neutrophil influx in dogs after exposure to 3.0 ppm O_3. Also, they (28) observed that drug induced neutropenia inhibited bronchial hyperreactivity to ACh following a 2 h exposure to 3.0 ppm O_3, as well as blocking the neutrophil influx into the airways. However, a significant increase in bronchoalveolar lavage recovered epithelial cells was reported in this study. These observations seem to affirm the involvement of airway epithelial damage, neutrophil infiltration, and cyclooxygenase products of arachidonic acid metabolism with the observed pulmonary function decrements, bronchial hyperreactivity, and subjective discomfort associated with acute and with repeated O_3 exposure in humans.

Table 7

Mechanisms responsible for enhanced pulmonary function decrements upon O_3 reexposure within 48 h in humans have yet to be fully elucidated. Observations from Dimeo et al (8) demonstrated that bronchial reactivity to histamine chal-

lenge upon O_3 reexposure after 24 h was similar to that upon initial exposure. However, Kulle et al (22) noted significant bronchial hyperreactivity to methacholine after the second and third consecutive days of exposure to 0.32 ppm O_3. Additionally, they (22) observed different durations of persistence after five consecutive days of exposure for spirometric measures (adaptation lost between 4 and 7 days) and bronchial reactivity to methacholine (adaptation still present after 7 days). Taken together, these results suggest that more than one mechanism may be involved. Since hyperresponsiveness appears to be greatest 12-24 h following initial O_3 exposure (15), with a partial decay after 48 h, and a near complete absence 72 h following initial exposure, the airway inflammation scheme, as outlined by Holtzman et al (19) and O'Byrne et al (28) appears plausible. Also the results from Seltzer et al (31) support the presence of this reaction scheme in humans, though its persistence beyond 3 h after exposure has yet to be assessed.

Evidence from animal studies (26) indicates that persistence of airway inflammation may be as long as 96 h, since these authors observed a significantly heightened mucosal neutrophil count in guinea pigs persisting from 6 to 96 h after initial O_3 exposure. Therefore, another O_3 challenge within 12-48 h, coupled with an already inflamed airway epithelium, may result in greater epithelial desquamation, enhanced neutrophil infiltration, heightened inflammatory mediator release, greater stimulation of vagal C afferents and irritant receptors, and a reduced quantity and thickness of the mucous layer potentially leading to greater oxidant permeability (1,5,19,20,23,28,32). These O_3 toxicity effects, known to occur with a single acute exposure, may be heightened upon reexposure within 48 h, accounting for the enhanced pulmonary function decrements, subjective symptoms, and exercise ventilatory pattern changes observed in this

study and routinely by other investigators at 24 h reexposure intervals (14,15, 16,21,22,24).

The present data highlight the need to demonstrate an association between descriptive pulmonary function decrements and subjective irritation with cellular and biochemical markers of individual O_3 toxicity and sensitivity, i.e., neutrophil influx, epithelial desquamation, and inflammatory mediator content in bronchoalveolar lavage fluid, in addition to blood concentrations of these purported mediators and possible products of lipid peroxidation. This would permit better understanding of the temporal sequence of events associated with O_3 exposure and reexposure within a short time period. Finally, our observations suggest a need for increased concern for persons particularly responsive to O_3, since they may be at increased risk of enhanced response when ambient photochemical smog episodes are separated by up to 48 h.

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.

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TABLE 1. Subject characterization

	AGE (y)	HT (cm)	WT (kg)	FVC (ℓ)	FEV-1.0 (ℓ/s)	FEF_{25-75} (ℓ/s)	RV (ℓ)
GROUP 1	25.6	180	73	5.68	4.85	5.40	1.37
	3.8	4	6	0.58	0.59	1.59	0.30
GROUP 2	24.2	178	73	5.61	4.72	5.03	1.33
	5.2	4	9	0.77	0.58	1.14	0.29
GROUP 3	21.4	180	71	5.71	4.69	4.73	1.44
	1.5	9	9	0.88	0.64	1.34	0.42
GROUP 4	24.1	180	73	5.55	4.36	3.92	1.39
	3.8	6	14	0.62	0.51	0.93	0.23

Values are group means, $\pm$ 1 S.D. FVC, forced vital capacity; $FEV_{1.0}$, forced expiratory volume in 1 sec; FEF_{25-75}, forced expiratory flow rate over the middle half of FVC; RV, residual volume.

TABLE 2. Pulmonary Function Responses

		GROUP 1			GROUP 2		
		P1	P2	P3	P1	P2	P3
FVC	Pre	5.68 ± 0.6	5.71 ± 0.7	5.65 ± 0.7	5.61 ± 0.8	5.56 ± 0.8	5.55 ± 1.0
(ℓ)	Post	5.82 ± 0.6	4.95 ± 1.0	4.11 ± 1.0	5.61 ± 0.8	5.03 ± 1.2	4.70 ± 1.6
$FEV_{1.0}$	Pre	4.85 ± 0.6	4.77 ± 0.6	4.66 ± 0.6	4.72 ± 0.6	4.69 ± 0.5	4.71 ± 0.7
(ℓ/s)	Post	4.97 ± 0.6	4.00 ± 0.9	3.23 ± 1.1	4.80 ± 0.6	4.00 ± 0.9	3.75 ± 1.2
FEF_{25-75}	Pre	5.40 ± 1.6	5.29 ± 1.6	4.97 ± 1.5	5.03 ± 1.1	5.05 ± 1.0	5.15 ± 1.3
(ℓ/s)	Post	5.81 ± 1.6	4.23 ± 1.9	3.26 ± 1.9	5.50 ± 1.2	4.03 ± 1.2	3.90 ± 1.2
sRaw	Pre	8.69 ± 3.7	7.38 ± 2.7	7.73 ± 2.8	7.54 ± 2.3	7.91 ± 2.6	6.69 ± 1.8
(cm·sec)	Post	9.10 ± 3.6	8.73 ± 2.6	10.25 ± 3.5	7.56 ± 2.1	8.92 ± 2.9	8.26 ± 2.4

		GROUP 3			GROUP 4		
		P1	P2	P3	P1	P2	P3
FVC	Pre	5.68 ± 0.9	5.67 ± 0.9	5.70 ± 0.8	5.55 ± 0.6	5.50 ± 0.6	5.47 ± 0.6
(ℓ)	Post	5.72 ± 0.9	5.23 ± 1.0	5.22 ± 1.1	5.60 ± 0.6	4.89 ± 1.0	4.61 ± 1.2
$FEV_{1.0}$	Pre	4.69 ± 0.6	4.65 ± 0.7	4.65 ± 0.7	4.36 ± 0.5	4.38 ± 0.5	4.33 ± 0.5
(ℓ/s)	Post	4.89 ± 0.7	4.22 ± 0.9	4.10 ± 1.0	4.58 ± 0.5	3.88 ± 1.1	3.66 ± 1.0
FEF_{25-75}	Pre	4.75 ± 1.4	4.65 ± 1.4	4.67 ± 1.5	3.97 ± 0.9	4.04 ± 1.0	4.07 ± 1.0
(ℓ/s)	Post	5.35 ± 1.6	4.05 ± 1.7	4.17 ± 1.9	4.39 ± 1.1	3.64 ± 1.6	3.43 ± 1.5
sRaw	Pre	8.57 ± 1.8	7.95 ± 1.8	7.88 ± 1.9	8.33 ± 2.5	8.08 ± 2.8	7.69 ± 2.5
(cm·sec)	Post	7.72 ± 2.0	8.44 ± 2.3	8.50 ± 2.3	8.02 ± 2.7	9.43 ± 2.9	9.07 ± 2.1

Values are group means ± 1 S.D. sRaw, specific airway resistance; see Table 1 for other abbreviations.

TABLE 3. Summary of Two-Way ANOVA Analysis

VARIABLE	GROUP EFFECT F-Ratio	PROTOCOL EFFECT F-Ratio
FVC	0.91	41.0**
$FEV_{1.0}$	1.34	51.5**
FEF_{25-75}	1.63	45.9**
sRaw	3.59*	31.7**
SS	3.51*	36.0**
V_T	3.47*	73.2**
f_R	2.19	62.2**
$\dot{V}_E$	4.81*	0.08
$\dot{V}_{O_2}$	0.53	0.39

See Tables 1, 2, and 5 for abbreviations. *Significant at $P < 0.05$. **Significant at $P < 0.0001$.

Table 4. Summary of post-hoc analyses for specific significant mean differences: O_3 protocol effects.

Variable	Specific Significant Mean Differences			
	Group 1	Group 2	Group 3	Group 4
FVC	P1-P2, P1-P3, P2-P3	P1-P2, P1-P3	P1-P2, P1-P3	P1-P2, P1-P3
$FEV_{1.0}$	P1-P2, P1-P3, P2-P3	P1-P2, P1-P3	P1-P2, P1-P3	P1-P2, P1-P3
$FEF_{25-75\%}$	P1-P2, P1-P3 P2-P3	P1-P2, P1-P3	P1-P2, P1-P3	P1-P2, P1-P3
sRaw	P1-P2, P1-P3 P2-P3	P1-P2, P1-P3 P2-P3*	P1-P2, P1-P3	P1-P2, P1-P3
f_R	P1-P2, P1-P3 P2-P3	P1-P2, P1-P3 P2-P3*	P1-P2*,P1-P3	P1-P2*,P1-P3 P2-P3*
V_T	P1-P2, P1-P3 P2-P3	P1-P2, P1-P3 P2-P3*	P1-P2, P1-P3	P1-P2, P1-P3 P2-P3
SS score	P1-P2, P1-P3 P2-P3	P1-P2, P1-P3	P1-P2, P1-P3	P1-P2, P1-P3

See Tables 1, 2, and 5 for abbreviations. All differences are significant at the $p < 0.05$ level, except when noted by an asterisk ($p < .10$).

TABLE 5. Exercise Ventilatory Pattern, Metabolism, and Subjective Symptom Responses

		GROUP 1			GROUP 2		
		P1	P2	P3	P1	P2	P3
$\dot{V}_E$	10th	62.8 ± 5	61.6 ± 3	61.1 ± 3	62.9 ± 6	60.9 ± 7	60.1 ± 7
(ℓ/min)	Final	63.0 ± 4	63.2 ± 4	60.7 ± 3	63.2 ± 6	62.4 ± 6	63.7 ± 8
V_T	10th	1.97 ± 0.2	1.92 ± 0.2	1.85 ± 0.2	1.93 ± 0.4	1.90 ± 0.4	1.89 ± 0.3
(ℓ)	Final	1.79 ± 0.2	1.37 ± 0.2	1.09 ± 0.2	1.84 ± 0.4	1.44 ± 0.4	1.29 ± 0.5
f_R	10th	32.4 ± 5	32.7 ± 3	32.8 ± 5	33.4 ± 7	32.0 ± 5	32.0 ± 5
(1/min)	Final	35.9 ± 6	46.0 ± 8	54.3 ± 11	35.3 ± 6	44.3 ± 10	49.2 ± 16
$\dot{V}O_2$	10th	2.63 ± 0.3	2.58 ± 0.3	2.54 ± 0.3	2.54 ± 0.4	2.49 ± 0.3	2.49 ± 0.3
(ℓ/min)	Final	2.65 ± 0.4	2.61 ± 0.4	2.56 ± 0.4	2.62 ± 0.4	2.54 ± 0.4	2.55 ± 0.3
SS score	10th	3.5 ± 5	3.0 ± 6	2.4 ± 2	5.5 ± 6	2.0 ± 2	1.0 ± 3
	Final	8.0 ± 12	57.4 ± 35	84.9 ± 33	8.7 ± 9	37.0 ± 39	42.3 ± 51

		GROUP 3			GROUP 4		
		P1	P2	P3	P1	P2	P3
$\dot{V}_E$	10th	57.8 ± 3	57.9 ± 4	57.6 ± 4	58.4 ± 6	56.7 ± 5	58.0 ± 5
(ℓ/min)	Final	61.6 ± 2	60.8 ± 4	61.0 ± 4	58.7 ± 5	58.7 ± 6	60.7 ± 6
V_T	10th	1.90 ± 0.2	1.82 ± 0.2	1.86 ± 0.3	1.78 ± 0.4	1.83 ± 0.2	1.81 ± 0.3
(ℓ)	Final	1.78 ± 0.2	1.47 ± 0.2	1.44 ± 0.2	1.71 ± 0.3	1.57 ± 0.3	1.38 ± 0.3
f_R	10th	30.5 ± 3	31.9 ± 4	31.4 ± 5	32.8 ± 6	31.4 ± 4	32.5 ± 4
(1/min)	Final	35.1 ± 5	41.4 ± 6	43.4 ± 7	35.1 ± 5	38.1 ± 8	44.7 ± 8
$\dot{V}O_2$	10th	2.50 ± 0.2	2.35 ± 0.2	2.35 ± 0.2	2.42 ± 0.2	2.37 ± 0.3	2.39 ± 0.2
(ℓ/min)	Final	2.48 ± 0.2	2.39 ± 0.2	2.41 ± 0.2	2.47 ± 0.2	2.45 ± 0.2	2.46 ± 0.2
SS score	10th	7.0 ± 12	2.5 ± 5	3.0 ± 5	1.0 ± 2	0.5 ± 1	1.5 3
	Final	8.0 ± 10	33.5 ± 22	30.5 ± 26	4.0 ± 6	27.2 ± 30	37.2 37

Values are group means ± 1 S.D. $\dot{V}_E$, expired minute ventilation; V_T, tidal volume; f_R, respiratory frequency; $\dot{V}O_2$, oxygen consumption; SS, symptom severity.

TABLE 6. Group 2 (48 h) Individual Subject's FEV 1.0 Percent Change Responses to the O_3 Treatments

SUBJ NO.	P2	P3
1	-21.43% (34 min)	-21.10% (30 min)
2	- 1.08	- 3.41
3	-53.52	-59.07
4	-21.82	-42.26
5	-25.65	-15.88
6	-11.18	-20.04
7	+ 5.45	+ 5.44
8	+ 0.94	-34.22 (50 min)
9	-17.07	-19.06
10	+ 0.98	+ 4.05
$\bar{x}$	-14.4%	-20.6%
SD	$\pm$17.7	$\pm$ 20.4

Numbers in parentheses represent total exercise time for subjects not completing the entire 1 h exposure.

Table 7. The effect of indomethacin pretreatment on FA and O_3 inhalation responses in two subjects.

Parameter	SUBJECT 1						SUBJECT 2					
	without (indomethacin)			with (indomethacin)			without (indomethacin)			with (indomethacin)		
	FA	O_3	O_3	FA	O_3	O_3	FA	O_3	O_3	FA	O_3	O_3
FVC	+4.9	- 8.8	-16.6	+1.9	- 1.3	- 2.4	-2.7	-13.4	-28.4	-4.2	- 1.8	+ 5.2
sRaw	-5.0	+41.6	+70.7	+3.6	+33.3	+22.8	+16.5	+36.2	+42.3	+16.6	+32.8	+30.0
f_R	-5.4	+21.2	+37.1	-9.4	+12.8	+23.0	+14.9	+64.9	+87.8	+27.9	+32.3	+28.6
SS score	0	34	67	0	0	0	0	50	100	0	12	22

See Tables 1, 2, and 5 for abbreviations. Values for FVC, sRaw, and f_R are calculated percent changes from preexposure values. Values for SS score represent the total postexposure reported symptom severity score. Each exposure within a series was separated by 24 h, with the indomethacin series initiated at least 21 days following completion of the original experimental series.

FIGURE LEGENDS

FIG. 1. Description of experimental design

FIG. 2. Absolute changes in: (A) Forced expiratory volume at 1.0s (FEV-1.0), (B) specific airway resistance (sRaw), and (C) respiratory frequency (f_R) for each group (G-1, G-2, G-3, G-4) for the two O_3 protocols (P2, P3). (D), post-exposure symptom severity score for groups 1-4, P2 and P3.

** Significant at $P < 0.05$ for P2 vs P3 responses; * Significant at $P < 0.10$ for P2 vs P3 responses.

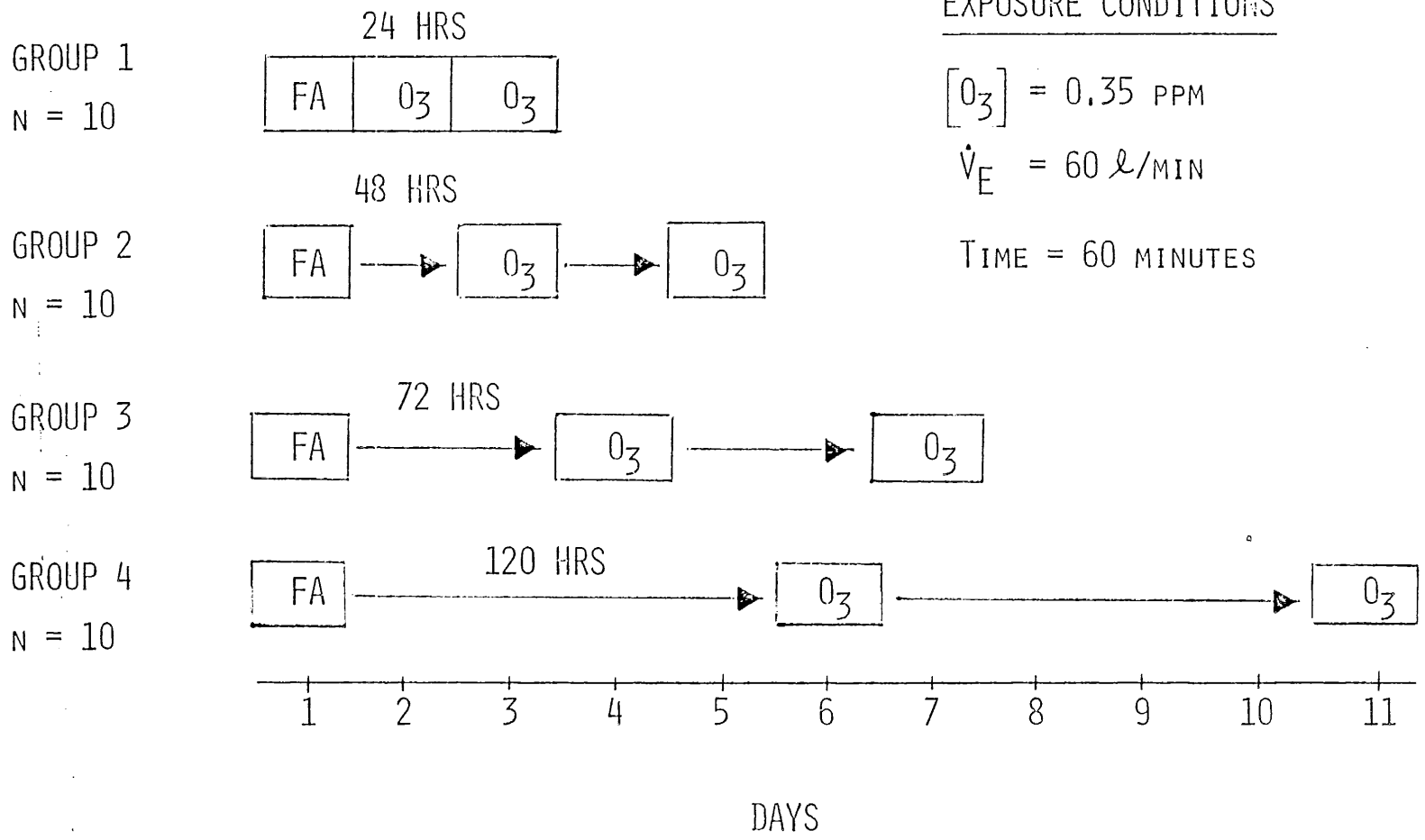
EXPOSURE CONDITIONS
$[O_3]$ = 0.35 PPM
$\dot{V}_E$ = 60 ℓ/MIN
TIME = 60 MINUTES
GROUP 1
N = 10
24 HRS
FA
O_3
O_3
GROUP 2
N = 10
48 HRS
FA
O_3
O_3
GROUP 3
N = 10
72 HRS
FA
O_3
O_3
GROUP 4
N = 10
120 HRS
FA
O_3
O_3
1 2 3 4 5 6 7 8 9 10 11
DAYS

FIGURE 1

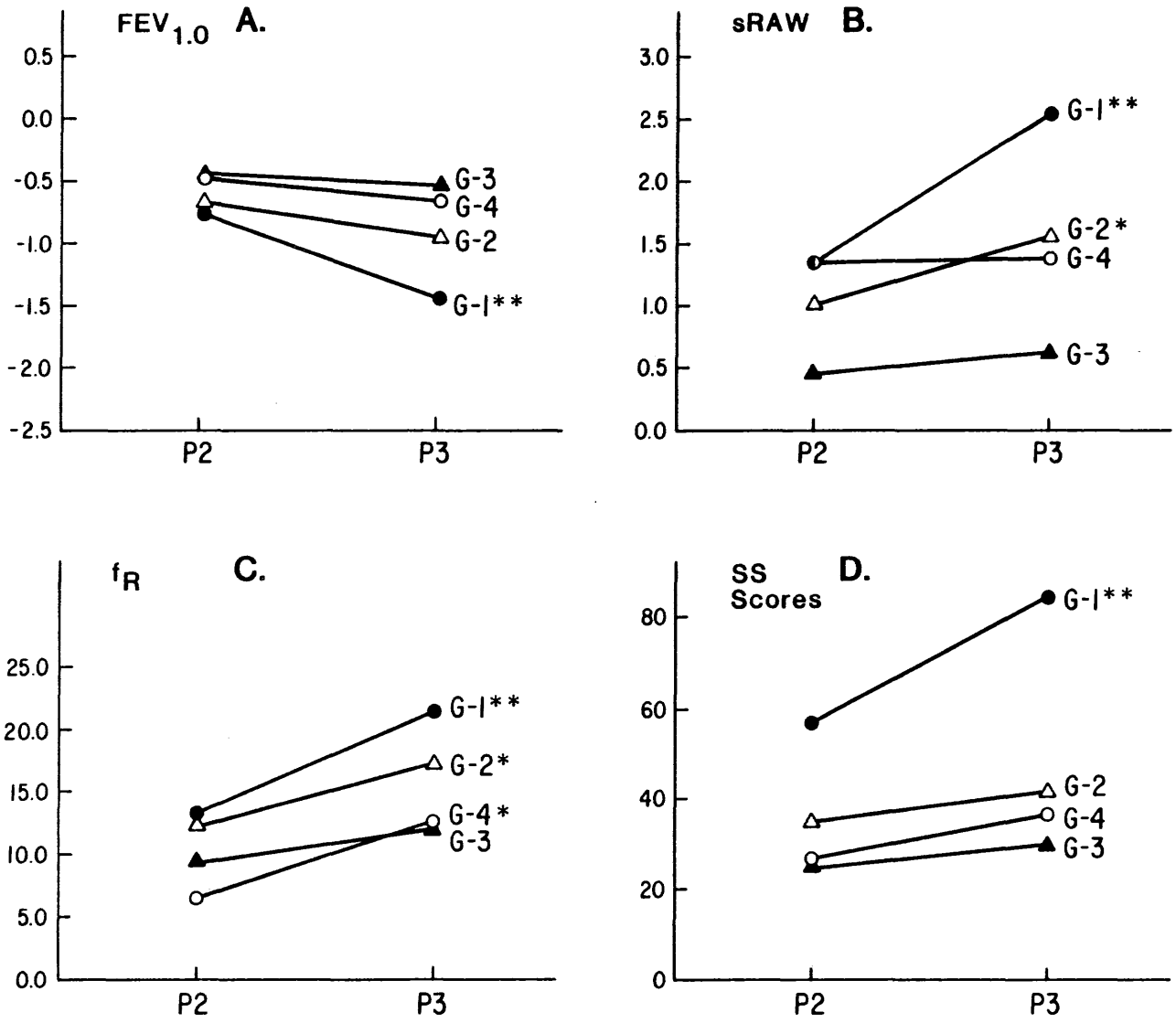
FEV1.0 A.
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
G-3
G-4
G-2
G-1**
P2
P3
sRAW B.
3.0
2.5
2.0
1.5
1.0
0.5
0.0
G-1**
G-2*
G-4
G-3
P2
P3
fR C.
25.0
20.0
15.0
10.0
5.0
0.0
G-1**
G-2*
G-4*
G-3
P2
P3
SS Scores D.
80
60
40
20
0
G-1**
G-2
G-4
G-3
P2
P3

FIGURE 2

Indomethacin Pretreatment Reduces Ozone Induced Pulmonary Function Decrements in Human Subjects

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Short Running Head: Reduced Ozone Inhalation Effects in Humans via Indomethacin Pretreatment

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ABSTRACT

We studied whether ozone-induced pulmonary function decrements could be inhibited by the prostaglandin synthetase inhibitor, indomethacin, in healthy human subjects. Fourteen college age males completed six 1 h exposure protocols consisting of no drug, placebo, and indomethacin (Indocin SR 75 mg every 12 h for 5 days) pretreatments, with filtered air and ozone (0.35 ppm) exposures within each pretreatment. Pretreatments were delivered weekly in random order in a double blind fashion. Ozone and filtered air exposures, separated by 72 h, were delivered in random order in a single blind fashion. Exposures consisted of 1 h exercise on a bicycle ergometer with workloads set to elicit a mean minute ventilation of 60 ℓ/min. Statistical analysis revealed significant ($p<0.05$) across pretreatment effects for FVC, $FEV_{1.0}$, and FEF_{25-75} with no drug vs indomethacin and placebo vs indomethacin comparisons being significant. These findings suggest that cyclooxygenase products of arachidonic acid, which are sensitive to indomethacin inhibition, play a prominent role in the development of pulmonary function decrements consequent to acute ozone exposure.

KEY WORDS: Cyclooxygenase product inhibition, forced expiratory flow rates, forced expiratory volume, ozone induced pulmonary function impairment.

INTRODUCTION

Ozone is the predominant oxidant found in photochemical air pollution prevalent in numerous urban areas. Short-term laboratory exposures (1-2 h) to ambient concentrations of ozone, utilizing exercising human subjects have focused primarily on acute pulmonary function effects. The primary pulmonary function responses observed have included decreased forced vital capacity (FVC) and forced expiratory volume in 1 sec ($FEV_{1.0}$), and an increase in specific airway resistance (SRaw) (1,2,3). Exposure protocols utilizing moderate to heavy levels of exercise, have shown an increase in respiratory frequency (f_R) and a decrease in tidal volume (V_T), without significant effect on pulmonary minute ventilation ($\dot{V}_E$) (1,2,3,4).

Observed pulmonary function impairment and changes in exercise ventilatory pattern, have been accompanied by reported subjective symptoms of cough, shortness of breath, and pain on deep inspiration (1,3,4). In addition, acute laboratory exposure to ozone has been shown to result in bronchial hyperresponsiveness to nonspecific stimuli in healthy subjects (5,6,7). In their study on bronchial hyperresponsiveness, consequent to ozone exposure, Seltzer et al (7) found significant increases in the concentrations of prostaglandins E_2, $F_{2\alpha}$, and thromboxane B_2 in bronchoalveolar lavage fluid. Prostaglandins E_2 and $F_{2\alpha}$ have been found to stimulate pulmonary neural afferents which initiate several responses characteristic of acute ozone exposure (8,9). These findings suggest that the release of prostaglandins in the lung consequent to acute ozone exposure may be involved in routinely observed pulmonary function decrements, and, perhaps in altered exercise ventilatory pattern and reported subjective symptomology.

We undertook the present study to determine whether pretreatment with indomethacin, an inhibitor of the cyclooxygenation of arachidonic acid to prostaglandins, prevents the onset and/or severity of ozone-induced pulmonary function, exercise ventilatory, and subjective symptom responses.

METHODS

Subject description and characterization. Fourteen healthy young adult males served as subjects. (Institutional Human Subjects Review Committee approval and signed individual informed consent were obtained.) Subjects were screened for clinically normal pulmonary function and absence of history of asthma, severe allergic rhinitis, aspirin intolerance, kidney disease and liver disease. None had resided in a high air pollution area within the previous three months.

Prior to initiating the six experimental protocols, each subject completed an orientation session in which base-line pulmonary function and basic anthropometry, including body composition via hydrostatic weighing, were obtained. To attenuate habituation effects, subjects completed at least 45 min of bicycle ergometer exercise at varied submaximal work loads while breathing filtered air. On another prexperimental occasion, the subject's $\dot{V}O_{2max}$ was determined utilizing a progressive increment protocol (2). Individual and group subject characterization data obtained in these orientation sessions are summarized in Table 1.

Experimental design. Each subject underwent three experimental pretreatments in random order: (1) a no drug control, (2) a placebo control, and (3) an indomethacin pretreatment (Indocin 75, Merck, Sharp & Dohme). Within each pretreatment, subjects were exposed for 1 h to both filtered air and 0.35 ppm ozone in random order. Subjects underwent a separate pretreatment each week,

with filtered air and ozone exposures being separated by 72 h. Subjects were instructed to take the placebo or indomethacin capsules twice daily with morning and evening meals, beginning 1 day prior to the first exposure, and to continue taking them twice daily through the day of second exposure for that week's preteatment. Placebo and indomethacin pretreatments were delivered in a double-blind manner, whereas the filtered air and ozone exposures were administered in single-blind fashion.

Exercise work rates were set such that minute ventilation ($\dot{V}_E$) was approximately 60 ℓ/min. All exposures were conducted in a room in which dry bulb temperature and relative humidity were maintained within 21-25 °C and 45-60%, respectively. To facilitate convective and evaporative cooling during exercise, an airflow betwen 1.5 and 3 m/s was directed at the subject's frontal aspect via an industrial grade floor fan.

Pulmonary function assessment. Pulmonary function tests were administered immediately prior to and after each experimental protocol. Duplicate determinations of forced maximal expiration were obtained via a Collins modular office spirometer, Model No. 3000. An on-line data acquisition system included 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. Pulmonary function on-line computer determinations imcluded measurements of FVC, $FEV_{1.0}$, and forced expiratory flow rate in the middle half of FVC (FEF_{25-75}).

Thoracic gas volume (V_{tg}) and Raw were measured using constant volume

whole body plethysmography (10,11). Pre- and postexposure maneuvers consisted of 5 trials for each set. Specific airway resistance (SRaw) was calculated as the product of Raw and V_{tg}.

Exercise measurements. To assess possible effects of ozone inhalation on selected exercise parameters, an on-line computerized data acquisition system delivered print-outs of one minute average values for $\dot{V}_E$, heart rate (HR), V_T, f_R, $\%0_2$ and $\%C0_2$ in expired gas, expired gas temperature, and $\dot{V}_{0_2}$ every minute. Data acquisition instruments interfaced to the DEC 11/2 microcomputer, included a Beckman LB-1 $C0_2$ analyzer, an Applied Electrochemistry S-3A 0_2 analyzer, an Alpha Technologies turbotachometer ventilation module (VVM-2), an electrocardiograph with R-wave detector, and a temperature thermistor located in the expired gas line.

Subjective symptoms were monitored at 5, 30, 45, and 58 min by the subject entering rated perceived exertion (RPE) and subjective symptom scores on an adding machine after listening to a tape, which asked specific questions concerning RPE and subjective symptom occurrence and severity. This procedure was followed to reduce possible experimenter bias which might be introduced by knowledge of the gas mixture being utilized during exposures. Subjects were first asked to rate feelings of exertion limited to the chest (chest RPE) and legs (leg RPE), and then to overall feelings of exertion (overall RPE). All questions concerning RPE were answered on a Borg scale (12). The next five questions involved specific subjective symptoms which the subject may or may not have felt during the exposures. The subjective symptoms scored included cough, shortness of breath, throat tickle, pain on deep inspiration, and an integrated rating of overall symptoms. Subjective symptoms were rated using

the following symptom severity scale: 0, not present; 5, minimal; 10, mild; 20, moderate; 30, severe; and 40, incapacitating. Immediately following completion of the postexposure pulmonary function test battery, the subject provided written comments on discomforts experienced during the exposure and indicated whether or not they believed that they received ozone.

Ozone administration and monitoring. Air mixtures during all experimental protocols were inhaled by subjects through a blow-by obligatory mouthpiece system described in detail elsewhere (4). In brief, filtered air, blended with appropriate concentrations of ozone generated by a Sander Ozonizer (Type II), was directed through a Teflon-coated Hans-Rudolph respiratory valve to the subject.

Expired gas was directed through a unidirectional 5 liter stainless steel mixing and sampling chamber to an Alpha Technologies Turbotachometer VVM-2 ventilation module. Expired air was then routed into the distal portion of the mixing tube and, along with the volume air mixture not inspired by the subject, passed through a Barneby-Cheney QDF multistage filter assembly and thence to the laboratory ventilation exhaust outlet.

Inspiratory ozone concentration in the exposure system was monitored by continuous samples drawn through 0.64-cm diameter Teflon tubing connected to a Dasibi ozone meter. The digital reading of ozone concentration in parts per million (ppm) was compared periodically to that determined by the UV absorption photometric method (13).

Statistical analysis. Duplicate pulmonary function measurements from pre- and postexposures were corrected to BTPS and averaged. S_{Raw} values were

calculated from an average of three of five trials. The preexposure values for each parameter were subtracted from the postexposure values, and then divided by the preexposure values to obtain percent changes representing the treatment effect for each protocol. Similarly, values from the tenth minute and the last minute of exercise for $\dot{V}_{O_2}$, $\dot{V}_E$, f_R, and V_T were utilized to calculate percent change. RPE and subjective symptom severity scores were analyzed as the absolute change between the values reported at 5 min and 58 min of the exposure.

Two subjects were unable to complete the no drug treatment ozone exposure, and one was unable to complete the placebo ozone exposure. Data from these subjects' responses to all these exposures were included in the statistical analyses.

All data were analyzed via a two-way analysis of variance (ANOVA) with repeated measures (14), which tested for within drug pretreatment effects (filtered air vs ozone comparison) and across drug pretreatment effects (i.e., no drug vs placebo, no drug vs indomethacin, and placebo vs indomethacin comparisons). Upon obtaining a significant F ratio for main effects due to across or within treatment effects, Tukey's pairwise comparison of cell means (15) was applied to determine which particular mean values were significantly different from others.

RESULTS

Indomethacin. All subjects tolerated the indomethacin pretreatment dose used quite well, with reports only of minor increased incidence of flatulence.

Pulmonary function response. Pre- and postexposure data for pulmonary function parameters, SRaw and exercise ventilatory and metabolic data are given in Table 2, with mean percent change for FVC, $FEV_{1.0}$, FEF_{25-75}, and

Table 2

SRaw depicted in Fig. 1. ANOVA and post hoc analyses results for pulmonary function, SRaw, and exercise ventilatory and metabolism data are summarized in Table 3. Significant across and within pretreatment effects were obtained for FVC, $FEV_{1.0}$ and FEF_{25-75} ($p<0.05$). Post hoc analysis revealed significant within pretreatment effects (filtered air vs ozone) for all three pulmonary function parameters, and significant across pretreatment effects for the no drug vs indomethacin and the placebo vs indomethacin comparisons. Analysis of SRaw data resulted in significant pretreatment effects for the no drug pretreatment only, with no significant across pretreatment effects being observed. V_{tg} showed no significant change either across or within within pretreatment.

Fig.

Table 3

Exercise responses. Group mean percent change for f_R and V_T are depicted in Fig. 2. As shown in Table 3, statistical analysis revealed no significant within or across pretreatment effects for $\dot{V}_E$ and $\dot{V}_{O_2}$. Significant within pretreatment effects on f_R were obtained for all pretreatments, but no significant differences were observed for across pretreatment comparisons. Analysis of V_T data revealed significant within pretreatment effects only for the no drug and placebo pretreatments, and a near significant ($p<0.08$) across pretreatment effect for the no drug vs indomethacin comparison.

Fig. 2

Subjective symptom response. A summary of the symptomatic responses is given in Table 4. Chest RPE data revealed significant within pretreatment effects for the no drug and placebo pretreatments, as well as a significant across pretreatment effect for the no drug vs indomethacin comparison. No

Table 4

significantly different comparisons were found for leg RPE. Overall RPE data showed a significant within pretreatment effect for the no drug pretreatment, only. All within pretreatment comparisons were significant for throat tickle, pain on deep inspiration, and over-all symptom severity. Throat tickle data showed a significant across pretreatment comparison for the no drug vs indomethacin comparison. Analysis of cough and shortness of breath data revealed significant within pretreatment effects for the no drug and placebo pretreatments, with the cough data also showing a significant across pretreatment effect for the no drug vs indomethacin comparison.

DISCUSSION

In the present study, indomethacin pretreatment was shown to reduce significantly ($p<0.05$) ozone induced decrements in FVC, $FEV_{1.0}$ and FEF_{25-75} when compared to both no drug and placebo controls (Table 3; Fig. 1). These reduced pulmonary function decrements were associated with less marked reductions in V_T, chest RPE and symptoms of cough, shortness of breath and throat tickle, as indicated by within pretreatment comparisons of filtered air vs ozone exposure and significant across pretreatment comparisons between no-drug control and indomethacin. These findings suggest that cyclooxygenase products of arachidonic acid, which are sensitive to indomethacin inhibition, play at least a partial role in the development of pulmonary function decrements, reduced exercise V_T, and associated subjective symptoms consequent to acute ozone exposure.

Because our study design required several exposures to ozone, it is possible that adaptation or sensitization to ozone's toxic effects may have influenced our results. However, 4 to 7 days intervened between our sub-

jects' ozone exposures, which would be sufficient time not to elicit either an adaptive or sensitization response (16,17). Furthermore, pretreatments were randomized in order to reduce order effects. Finally, our data evidenced no order or repeated exposure effects, as indicated by the reproducibility of pulmonary function and exercise ventilatory pattern data between the no drug and placebo controls (Table 2).

Close inspection of the subjective symptom response data in Table 4 reveals a consistent trend for the placebo pretreatment mean values for the ozone exposures to lie numerically between those for the no drug and indomethacin pretreatments. This resulted in no statistically significant across pretreatment effects for the no drug vs placebo and the placebo vs indomethacin comparisons of subjective symptom responses. Hence, a partial placebo effect appears evident. Subjects were aware that one of the pill pretreatments might alleviate ozone induced responses, which may have contributed to the placebo effect trend in subjective symptom responses. However, no such trend was observed in forced expiratory measurements (Table 2, Fig. 1), suggesting that forced expiratory decrements are not systematically related to feelings of subjective discomfort within the range observed in this study.

Acute ozone exposure has been shown to result in short term airway inflammation in both animals and humans (18,19,20). This inflammatory response has been shown to involve both the infiltration of neutrophils into the airways (7,18, 19,20) and the release of cyclooxygenase products of arachidonic acid in the lung (7). The dose of indomethacin given in this study (1 mg/kg every 12 h for 3-6 days) would be expected to result in a mean plasma concentration of 1.9-2.3 μg/ml (21,22). Plasma concentrations of indomethacin in this range have been shown to be sufficient to inhibit cyclooxygenase metabo-

lism from 50 to 90% in vivo (23,24,25,26). This may, in part, explain why only a partial reduction in pulmonary function decrements was observed in the present investigation. Another possibility is that some other mediators not affected by indomethacin pretreatment also contributes to ozone-induced pulmonary function decrements. However, since we did not measure other mediators of inflammation or cyclooxygenase products in the lung in the present study, the precise affect of indomethacin on arachidonic acid metabolism in this study is uncertain.

The cell of origin of cyclooxygenase products in the human lung during acute ozone exposure remains undetermined. Ozone-induced bronchial hyperresponsiveness in dogs has been shown to be inhibited by neutrophil depletion (27) and indomethacin pretreatment (28). These findings suggest that neutrophils which infiltrate the airway following acute ozone exposure (18,28) are the cells which release the cyclooxygenase products responsible for ozone induced bronchial hyperreactivity in dogs. However, given the rapid onset seen with ozone induced pulmonary function decrements in humans (2) and the observation that neutrophil infiltration does not peak until sometime after acute ozone exposure in animals (18,20), it would seen unlikely that the neutrophil is the source of the cyclooxygenase products that result in pulmonary function decrements consequent to ozone exposure. Indeed, the observation that human lung parenchyma tissue releases prostaglandin E_2 and $F_{2\alpha}$ when subjected to antigenic challenge in vitro (29), suggests that some cell type in the lung parenchyma itself might be responsible for the release of prostaglandins during acute ozone exposure, which may then contribute to the observed decrements in pulmonary function.

The mechanism by which the release of cyclooxygenase products in the lung leads to pulmonary function decrements in humans upon ozone exposure remains undefined. Available data indicates that ozone induced pulmonary function decrements and ventilatory pattern changes are neurally mediated (30,31). Hazucha et al (31) concluded that ozone inhalation stimulates airway receptors, which leads to an involuntary inhibition of full inspiration, reduction in FVC, and a concomitant decrease in maximal expiratory flow rates in humans. The observation that cyclooxygenase products stimulate neural afferents in the lung (8,9), combined with the observation of reduced ozone-induced pulmonary function decrements following indomethacin pretreatment in the present study, suggest that cyclooxygenase products released consequent to ozone-induced tissue damage stimulate neural afferents in the lung which result in observed pulmonary function decrements.

The significance of the present study is that it suggests that cyclooxygenase products, which are released as a result of airway inflammation, in some way contribute to the pulmonary function decrement observed consequent to acute ozone exposure. This association between airway inflammation and pulmonary function decrements suggests that acute ozone exposure to concentrations as low as 0.18 ppm, when combined with sufficiently high exercise minute ventilation (32), would result in airway inflammation within 1 h.

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

Subject	Age, yr	Ht, cm	Wt, kg	Fat, % Body Weight	FVC, ℓ	$FEV_{1.0}$, ℓ	SRaw ℓ·cmH_2O/ ℓ/sec	$\dot{V}_{O_{2max}}$, ℓ/min	$\dot{V}_{Emax}$, ℓ/min BTPS
1	21	185.4	86.0	19.7	6.41	4.97	6.25	4.66	157.9
2	22	179.1	69.3	13.4	5.85	4.68	7.14	4.20	157.3
3	26	179.1	87.0	18.0	6.37	5.47	5.26	4.80	197.8
4	22	177.8	64.8	13.4	6.52	5.65	7.04	5.03	182.9
5	21	177.8	62.9	13.9	5.83	4.58	6.28	4.41	137.6
6	18	171.4	69.0	15.6	5.79	4.26	7.28	4.88	122.3
7	28	172.7	70.9	16.0	5.13	4.17	7.44	4.09	157.4
8	24	190.5	90.2	22.9	8.27	5.33	8.50	4.90	167.9
9	19	185.4	75.1	13.6	6.41	5.03	5.76	4.03	126.8
10	21	177.8	62.4	11.6	5.26	4.44	5.15	3.11	105.6
11	23	193.0	86.6	14.3	6.62	5.43	8.40	4.53	137.8
12	32	177.8	65.3	11.6	6.02	4.68	7.75	4.77	221.8
13	34	175.3	73.9	14.6	6.32	5.07	8.24	4.21	136.5
14	23	177.8	73.6	12.6	6.08	4.81	8.41	3.92	116.0
$\bar{x}$	23.86	180.1	74.1	15.1	6.21	4.90	7.06	4.40	151.8
S.D.	±4.66	±6.27	±9.66	±3.19	±0.74	±0.46	±1.16	±0.52	±32.7

FVC, forced vital capacity; $FEV_{1.0}$, forced expiratory volume in 1 s; SRaw, specific airway resistance in liters times centimeter of water per liters/s; $\dot{V}_{O_{2max}}$, maximum O_2 uptake; $\dot{V}_{Emax}$, maximum minute ventilation.

Table 2. Group pre- and postexposure values for pulmonary function, specific airway resistance, and exercise respiratory parameters.

Variable	NO DRUG CONTROL				PLACEBO				INDOMETHACIN			
	Filtered Air		Ozone		Filtered Air		Ozone		Filtered Air		Ozone	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
FVC, liters	6.19 ±0.73	6.15 ±0.68	6.25 ±0.84	5.22 ±1.16	6.20 ±0.77	6.15 ±0.76	6.18 ±0.71	5.13 ±1.12	6.20 ±0.74	6.27 ±0.75	6.18 ±0.72	5.75 ±0.89
$FEV_{1.0}$, liters	4.90 ±0.50	4.95 ±0.41	4.91 ±0.61	3.62 ±0.76	4.88 ±0.40	4.85 ±0.42	4.90 ±0.42	3.64 ±0.74	4.84 ±0.54	5.00 ±0.48	4.83 ±0.53	4.32 ±0.59
FEF_{25-75}, ℓ/s	4.71 ±1.14	4.75 ±0.91	4.61 ±1.14	2.83 ±0.77	4.61 ±0.75	4.48 ±0.86	4.54 ±0.89	2.84 ±0.93	4.62 ±1.10	4.60 ±0.94	4.45 ±0.91	3.72 ±0.91
SRaw, liters•[cmH_2O] [ℓ/s]	7.66 ±1.78	7.53 ±1.88	7.36 ±1.44	8.57 ±2.19	6.91 ±1.46	7.18 ±1.23	7.54 ±1.70	8.35 ±2.04	7.53 ±1.84	7.16 ±1.35	7.54 ±1.70	8.35 ±2.04
f_R, breaths/min	31.00 ±3.44	34.14 ±7.63	30.64 ±3.46	47.00 ±13.40	30.29 ±3.63	34.86 ±6.00	30.93 ±3.50	45.60 ±12.70	30.50 ±4.27	33.93 ±5.15	31.43 ±3.41	42.90 ±13.30
V_T, liters	1.84 ±0.23	1.81 ±0.27	1.88 ±0.23	1.34 ±0.27	1.91 ±0.27	1.75 ±0.33	1.88 ±0.27	1.39 ±0.29	1.94 ±0.29	1.82 ±0.26	1.87 ±0.27	1.53 ±0.39
$\dot{V}_E$, ℓ/min	56.50 ±4.49	60.04 ±4.85	57.18 ±5.60	59.80 ±6.66	57.05 ±3.93	59.05 ±3.58	57.40 ±4.47	60.55 ±5.92	58.16 ±3.53	60.51 ±3.44	58.17 ±4.32	61.30 ±6.73
$\dot{V}_{O_2}$, ℓ/min	2.37 ±0.19	2.37 ±0.26	2.29 ±0.15	2.41 ±0.25	2.38 ±0.20	2.40 ±0.18	2.34 ±0.17	2.40 ±0.20	2.34 ±0.19	2.50 ±0.22	2.32 ±0.24	2.39 ±0.21

Values represent the pre- and postexposure means, with corresponding ± 1 standard deviation. F_R, respiratory frequency; V_T, tidal volume; $\dot{V}_E$, expired minute ventilation; $\dot{V}_{O_2}$, oxygen uptake. Other abbreviations as in Table 1.

Table 3. F ratios and specific significant mean differences for post hoc analysis for pulmonary function, specific airway resistance, and exercise ventilatory and metabolism variables.

Variable	Across Pretreatment F Ratio	Specific Significant Mean Differences ($p<0.05$)	Within Pretreatment F Ratio	Specific Significant Mean Differences ($p<0.05$)
FVC	11.90	ND-I; P-I	21.52	ND; P; I
$FEV_{1.0}$	10.98	ND-I; P-I	57.37	ND; P; I
FEF_{25-75}	3.63	ND-I; P-I	57.50	ND; P; I
S_{Raw}	1.60	NS	8.83	ND
f_R	0.97	NS	31.96	ND; P; I
V_T	2.79	NS	55.47	ND; P
$\dot{V}_E$	0.11	NS	0.06	NS
$\dot{V}_{O_2}$	0.86	NS	0.47	NS

ND, I, and P denote no drug, indomethacin and placebo pretreatments, respectively. NS indicates no statistically significant difference ($p>0.05$). Other abbreviations as in Tables 1 and 2.

Table 4. Subjective symptom response to treatments

		ND		P		I	
	Variable	FA	O_3	FA	O_3	FA	O_3
	RPE, Chest	1.29 ± 2.64	5.43 ± 4.07	1.15 ± 1.68	4.86 ± 3.82	1.71 ± 1.94	3.07 ± 2.95
	RPE, Legs	2.50 ± 3.08	3.79 ± 3.75	2.62 ± 3.23	4.43 ± 4.43	2.43 ± 3.55	3.21 ± 2.99
	RPE, Overall	1.93 ± 2.89	3.71 ± 3.24	1.93 ± 2.87	3.57 ± 3.84	2.00 ± 2.83	3.57 ± 3.13
SYMPTOMS	Cough	0.36 ± 1.34	13.71 ±11.77	0 0	8.57 ± 9.49	0 0	4.50 ± 9.22
SYMPTOMS	Shortness of Breath	0 0	11.36 ±12.70	0.36 ± 1.34	9.64 ±10.00	0 0	6.79 ± 9.73
SYMPTOMS	Throat Tickle	- 0.36 ± 1.34	9.29 ± 8.52	0.36 ± 1.34	5.29 ± 8.20	-0.71 ± 1.82	2.79 ± 3.75
SYMPTOMS	Pain on Deep Inspiration	0.36 ± 1.34	19.43 ±12.33	0 0	12.36 ±11.08	0 0	12.21 ±13.03
SYMPTOMS	Overall Symptom Severity	0.36 ± 1.23	17.29 ±12.06	0.36 ± 1.34	13.07 ±10.29	0 0	9.79 ±10.84

Values represent mean change from 5 to 58 min for each exposure, with corresponding ± 1 standard deviations. RPE, rated perceived exertion. See text for further details.

FIGURE LEGENDS

FIG. 1. Mean percent change from preexposure in (A) forced vital capacity (FVC), (B) forced expiratory volume in 1 s ($FEV_{1.0}$), (C) forced expiratory volume during the middle half of FVC (FEF_{25-75}), and (D) specific airway resistance (SRaw) for each exposure condition.

FIG. 2. Mean percent change for last minute vs 10th minute of exercise in (A) tidal volume (V_T) and (B) respiratory frequency (f_R) for each exposure condition.

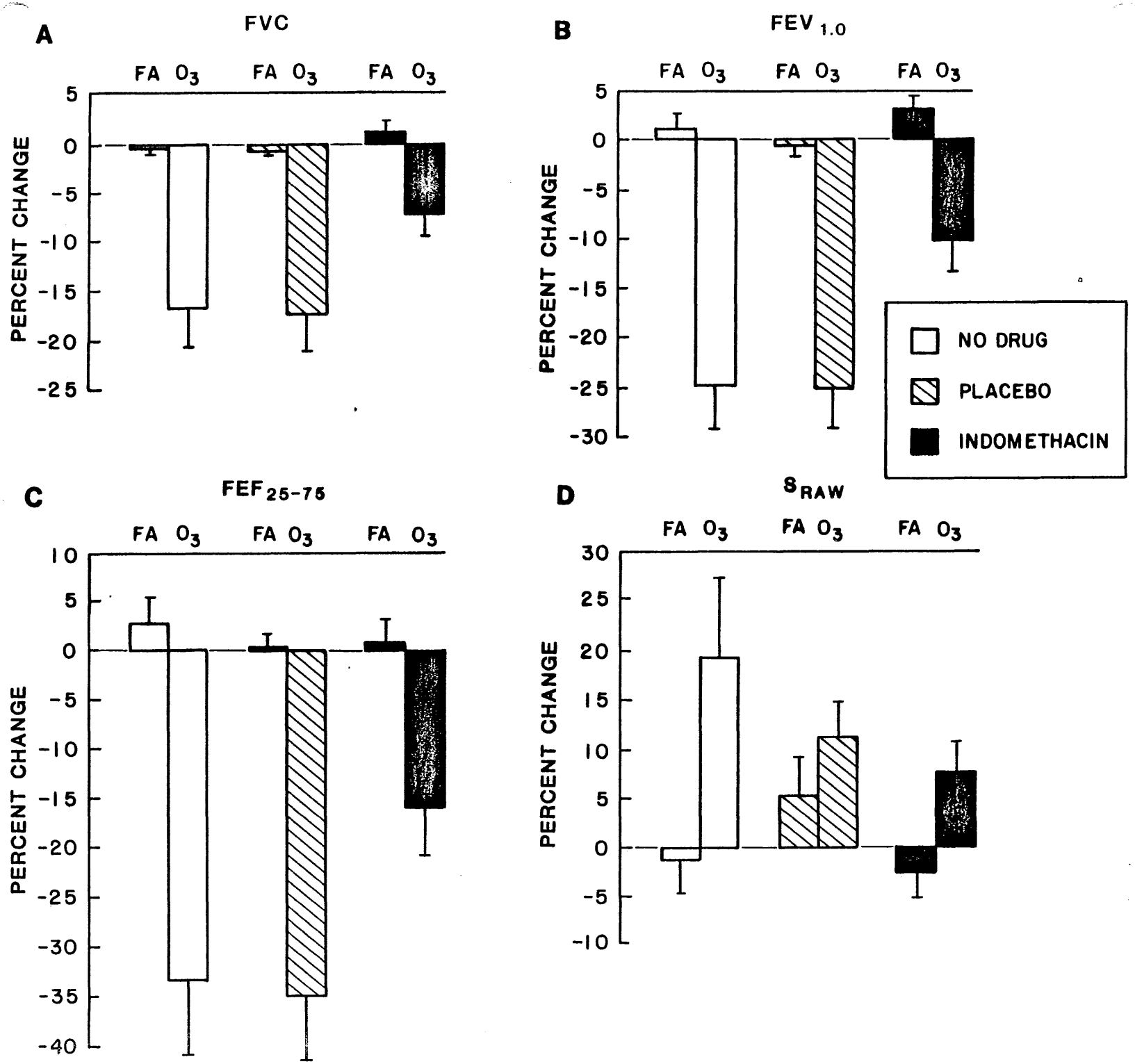
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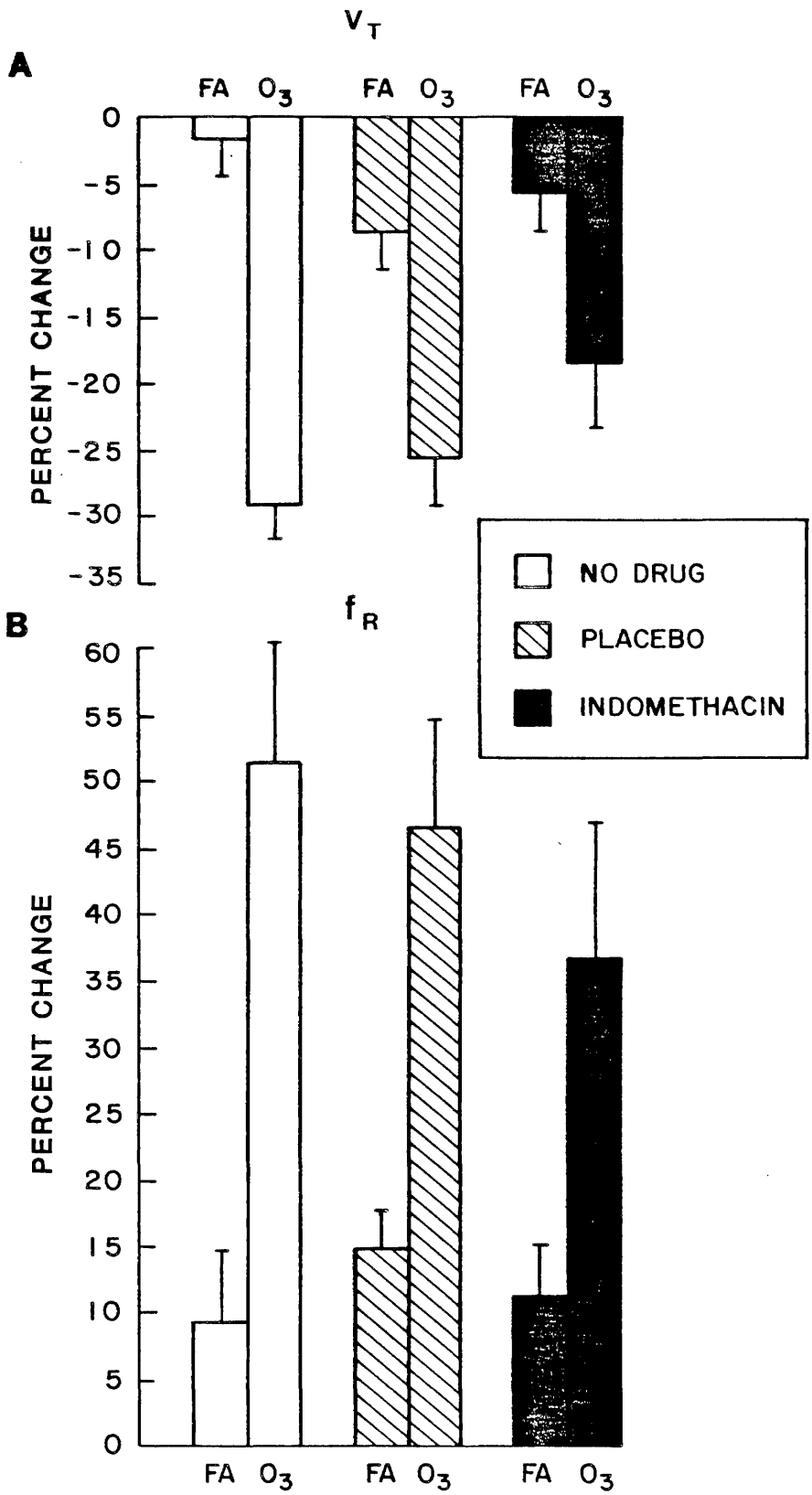
FIGURE 1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Atropine Pretreatment Blocks Ozone Induced Specific Airway Conductance Decrement in Human Subjects

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Short Running Head: Reduced Ozone Induced Airways Response via Atropine Pretreatment

This manuscript has been submitted for publication to the Journal of Applied Physiology

ABSTRACT

Thirteen healthy college-age males were studied to determine the contribution of neural and non-neural components to the bronchoconstriction induced by acute exposure to ambient concentrations of ozone (O_3). In addition, respiratory system impedance (Z_{RS}) of the three most sensitive subjects was measured and analyzed in an attempt to assess the relative contribution of central and peripheral components to O_3-induced bronchoconstriction. All subjects completed six 1 h exposure protocols consisting of no drug, atropine sham, and atropine, with filtered air (FA) and O_3 (0.35 ppm) exposures within each pretreatment. Pretreatments were delivered weekly in random order in a double blind fashion. O_3 and FA exposures, separated by 72 h, were delivered in random order in a single blind fashion. Exposures consisted of 1 h exercise on a bicycle ergometer with workloads set to elicit a mean ventilation of 60 ℓ/min. Atropine (~0.04 mg/kg) and atropine sham (~0.02 mg/kg quinine sulfate) were delivered via a nebulizer with dose metering device 20 min prior to the measurement of pre-exposure pulmonary functions. Statistical analysis revealed significant ($p<0.05$) across pretreatment effects for specific airway conductance (SGaw) and forced expiratory flow over the middle half of FVC (FEF_{25-75}), with no drug vs atropine and atropine sham vs atropine comparisons being significant. Atropine pretreatment abolished O_3-induced bronchoconstriction, with a 2.50, -11.4 and -15.0 percent change in SGaw observed for the atropine, atropine sham, and no drug pretreatment, respectively. Optimization techniques were utilized to determine the best fit for a lumped-six parameter mechanical model to the Z_{RS} data. This analysis suggests that O_3-induced bronchoconstriction is distributed between both central and peripheral airways, and that this effect is inhibited by atropine at both

loci. Further, these observations suggest that cholinergic post-ganglionic neural input to bronchial smooth muscle plays a predominant role in determining bronchoconstriction consequent to acute O_2 exposure.

Index terms: Bronchoconstriction; central and peripheral airways effects of ozone inhalation; respiratory system impedance; specific airway conductance

Ozone (0_3) is the major oxidant in photochemical smog found in numerous urban areas. Acute exposure of healthy human subjects to ambient 0_3 concentrations has been shown to result in moderate decrease in specific airway conductance (SGaw) (2,10,27). This decrease in SGaw has been shown to be transient in nature, lasting only 1-2 h post exposure. Following the return of SGaw to baseline values, an increase in airway hyperresponsiveness to non-specific stimuli has been observed in both animals (18,25,30) and humans (14,17, 35).

The underlying mechanism for the observed decrease in SGaw consequent to 0_3 exposure remains poorly defined. 0_3-induced airway hyperresponsiveness has been shown to involve: (1) a neural component, as demonstrated by blockade via vagal cooling (25) and atropine pretreatment (14,24) and, (2) a direct bronchial smooth muscle hyperresponsiveness as indicated by airway sensitivity to acetylcholine agonists (18,30,35).

Acute exposure of animals to ambient 0_3 concentrations has been shown to result in pathologic changes in the lung. The primary 0_3 induced pathologic lesion has been shown to occur at the level of the terminal bronchioles and the proximal alveoli (3,26,36,37). The lesions observed have included loss of type 1 pneumocytes, loss of cilia in terminal bronchioles, and infiltration of inflammatory cells (3). Functional changes associated with these lesions include altered mucociliary clearance (12,13,21), an increase in airway permeability (20), and an increase in pulmonary resistance which was only partially blocked by atropine treatment (38).

The present investigation was conducted to determine the degree to which neural and non-neural components are involved in the decrease in SGaw consequent to acute 0_3 exposure in humans. This was accomplished by blocking

cholinergic post-ganglionic input to bronchial smooth muscle via atropine pretreatment. In addition, respiratory system impedance (Z_{RS}) of the three most sensitive subjects was measured via forced oscillation, in order to evaluate changes in pulmonary mechanics consequent to O_3 exposure and atropine pretreatment.

METHODS

Subject description and characterization. Thirteen healthy young adult males served as subjects. (Institutional Human Subjects Review Committee approval and signed individual informed consent were obtained). Subjects were screened for clinically normal pulmonary function and absence of history of asthma, severe allergic rhinitis, aspirin intolerance, kidney disease and liver disease. None had resided in a high air pollution area within the previous three months.

Prior to initiating the six experimental protocols, each subject completed an orientation session in which baseline pulmonary function and basic anthropometry, including body composition via hydrostatic weighing, were obtained. To attenuate habituation effects, all subjects completed at least 45 min of bicycle ergometer exercise at varied submaximal work loads while breathing filtered air. $\dot{V}_{O_{2max}}$ was determined utilizing a progressive increment protocol (1). Individual and group subject characterization data obtained in these orientation sessions are summarized in Table 1.

Experimental design. Each subject underwent three experimental pretreatments in random order (1) a no drug control, (2) an atropine sham, (quinine sulfate, ~0.02 mg/kg), and (3) an atropine pretreatment (atropine sulfate, ~0.04 mg/kg). Within each pretreatment, subjects were exposed for 1 h to

both filtered air (FA) and 0.35 ppm O_3 in random order. Subjects completed a separate pretreatment each week, with FA and O_3 exposures being separated by 72 h. Atropine sham and atropine pretreatments were delivered 20 min prior to beginning pulmonary function measurements via an ultrasonic nebulizer (Mist-O-Gen) equipped with a dose metering device. The dose and time of atropine administration was such that it would result in peak bronchodilation at the time of pre-pulmonary function tests and then plateau for the remainder of the protocol (23,32). Atropine sham and atropine pretreatments were delivered in a double-blind manner, whereas FA and O_3 were delivered in a single-blind fashion.

Exercise work rates were set such that minute ventilation ($\dot{V}_E$) was approximately 60 ℓ/min. All exposures were conducted in a room in which dry bulb temperature and relative humidity were maintained within 21-25 °C and 45-60%, respectively. To facilitate convective and evaporative cooling during exercise, an airflow betwen 1.5 and 3 m/s was directed at the subject's frontal aspect via an industrial grade floor fan.

Pulmonary function assessment. Pulmonary function tests were administered immediately prior to and after each experimental protocol. Duplicate determinations of forced maximal expiration were obtained via a Collins modular office spirometer, Model No. 3000. An on-line data acquisition system included 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. Pulmonary function on-line computer determinations included measurements of forced vital capacity (FVC), forced expiratory volume in 1 s, and forced expiratory flow rate in the middle half of FVC (FEF_{25-75}).

Thoracic gas volume (V_{tg}) and Raw were measured using constant volume whole body plethysmography (8,9). Pre- and postexposure maneuvers consisted of 5 trials for each set. SGaw was calculated as the inverse of the product of Raw and V_{tg}.

Exercise measurements. To assess possible effects of 0_3 inhalation on selected exercise parameters, an on-line computerized data acquisition system delivered print-outs of one minute average values for $\dot{V}_E$, heart rate (HR), tidal volume (V_T), respiratory frequency (f_R), $\%0_2$ and $\%C0_2$ in expired gas, expired gas temperature, and $\dot{V}_{0_2}$ every minute. Data acquisition instruments interfaced to the DEC 11/2 microcomputer, included a Beckman LB-1 $C0_2$ analyzer, an Applied Electrochemistry S-3A 0_2 analyzer,an Alpha Technologies turbotachometer ventilation measurement module (VMM-2), an electrocardiograph with R-wave detector, and a temperature thermistor located in the expired gas line.

Subjective symptoms were monitored at 5, 15, 30, 45, and 58 min by the subject entering rated perceived exertion (RPE) and subjective symptom scores on an adding machine after listening to a tape, which asked specific questions concerning RPE and subjective symptom severity. This procedure was followed to reduce possible experimenter bias which might be introduced by the experimenter's knowledge of the gas mixture being utilized during exposures. Subjects were first asked to score feelings of exertion limited to the chest (chest RPE) and legs (leg RPE) and then to score overall feelings of exertion (overall RPE). All questions concerning RPE were answered on a Borg scale (4). The next five questions involved specific subjective symptoms which the

subject may or may not have felt during exposure protocols. The subjective symptoms scored included cough, shortness of breath, throat tickle, pain on deep inspiration and an integrated rating of overall symptoms. Subjective symptoms were scored using the following symptom severity scale: 0, not present; 5, minimal; 10, mild; 20, moderate; 30, severe; and 40, incapacitating. Immediately following completion of the postexposure pulmonary function test battery, the subject provided written comments on discomforts experienced during the exposure and indicated whether or not they believed that they received O_3.

O_3 administration and monitoring. Air mixtures during all experimental protocols were inhaled by subjects through a blow-by obligatory mouthpiece system described in detail elsewhere (5). In brief, FA, blended with appropriate concentrations of O_3 generated by a Sander Ozonizer (Type II), was directed through a Teflon-coated Hans-Rudolph respiratory valve to the subject.

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

Inspiratory O_3 concentration in the exposure system was monitored by continuous samples drawn through 0.64-cm diameter Teflon tubing connected to a Dasibi O_3 meter. The digital reading of O_3 concentration in parts per

million (ppm) was compared periodically to that determined by the UV absorption photometric method (6).

Respiratory System Impedance. Respiratory system impedance at the mouth was obtained using forced pressure oscillations of a discrete frequency Spectra (4-256Hz, by increments of 4Hz). The pressure generator and software used was developed and supplied by Andrew C. Jackson, Ph.D., Biomedical Engineering Department, Boston University, Boston, Mass. and is described in detail elsewhere (19,22). Pressures were measured using two Microswitch 163PC01D36 differential pressure transudcers ($\pm$5 in H_2^0, Quement Electronics, San Jose, Calif.) and a preamplifier manufactured by Dorchak Equipment, El Dorado Hills, Calif. Sinusoidal electrical signal waves for use by the pressure generator were supplied by a LSI 11/2 microcomputer, D to A converter and were amplified using a NAD 2155 stereo power amplifier. Raw data were analyzed and stored via the LSI 11/2 microcomputer. Five trials pre- and postexposure were obtained and an ensemble averaged to produce the arrays used for initial analysis.

A three-way valve (Warren E. Collins, Braintree, Mass.) was automated using a rotary solenoid and attached to the front of the pressure generator. This valve was normally open to the atmosphere, but when activated was open to the pressure generator. The valve was activated by the subject via a foot switch. This valve assembly allowed the subject to remain on the mouthpiece between measurement trials. Each measurement trial was initiated with the subject seated and breathing quietly into the mouthpiece. When the subject was ready, he was instructed to pause at the end of a normal expiration and apply pressure on the foot switch. To insure that the subject's glottis remained open during the maneuver, he was instructed to inspire slowly for the

duration of the maneuver (~12 sec). This inspiratory effort created a slight negative pressure in the Z_{RS} measurement system and allowed the subject to inspire a volume of approximately 0.5 - 1.0 liters.

The respiratory system impedance data was collected in order to complement the specific airway conductance data. Hence, the impedance data from only three of the most O_3 sensitive subjects (>15 percent impairment in SGaw for the atropine sham pretreatment) were analyzed for the atropine sham and atropine pretreatments. This analysis permitted investigation of the distribution of resistance changes in the lung consequent to O_3 exposure, and to what extent the central and peripheral components of bronchoconstriction are sensitive to atropine pretreatment. Optimization techniques were used to estimate the values of six parameters for a lumped-parameter mechanical model in which the airways are separated into central and peripheral components by a shunt pathway containing an airway wall compliance (19). The parameters optimized included central airway resistance (Rc) and innertance (Ic), airway wall compliance (Caw), peripheral resistance (Rp) and innertance (Ip), and respiratory system compliance (C_{RS}).

Due to noise in the data at low and high frequencies, only the data for frequencies 16 Hz through 100 Hz were analyzed. Optimizations were done using a DEC LSI 11/2 microcomputer, employing procedures described by Jackson and Watson (19).

Statistical analysis. Duplicate pulmonary function measurements from pre- and postexposures were corrected to BTPS and averaged. SGaw values were calculated from an average of three of five trials. The preexposure values for each parameter were subtracted from the postexposure values, and then

divided by the preexposure values to obtain percent changes representing the treatment effect for each protocol. Similarly, values from the tenth minute and the last minute of exercise for $\dot{V}o_2$, $\dot{V}_E$, f_R, and V_T were utilized to calculate percent change. RPE and subjective symptom severity scores are reported as the absolute change between the value report at 5 min and 58 min of the exposure.

Two subjects were unable to complete the no drug 0_2 exposures, and two other subjects were unable to complete the atropine 0_3 exposure. Data from these exposures were included in all statistical analyses.

The effect of atropine on baseline measurements was tested by comparing the pre-values for SGaw across pretreatments using a one-way analysis of variance (ANOVA) with repeated measures (7). Except for the impedance analysis, all other data were analyzed via a two-way ANOVA with repeated measures, which tested for within drug pretreatment effects (FA vs 0_3 comparison) and across drug pretreatment effects (i.e., no drug vs atropine sham, no drug vs atropine, and atropine sham vs atropine comparisons) (7). Upon obtaining a significant F ratio for main effects due to across or within treatment effects, Tukey's pairwise comparison of cell means (31) was applied to determine which particular mean values were significantly different from others.

Optimized parameters obtained from the analysis of the respiratory system impedance data were analyzed in a descriptive manner via calculation of means and standard deviations for the atropine sham and atropine pretreatment conditions.

RESULTS

Atropine Side Effects. The mean dose of atropine for the FA exposures

was 0.042 mg/kg, with a range of 0.034 to 0.056 mg/kg. For the 0_3 exposures, the mean atropine dose was 0.039 mg/kg, with a range of 0.034 to 0.046 mg/kg. All subjects reported the symptom of dry mouth with atropine inhalation, with 11 subjects also reporting transient dizziness varying in degree from mild to severe. Two subjects also reported mild headache during the atropine inhalation exposure.

Specific Airway Conductance and Thoracic Gas Volume. Pre- and postexposure data for SGaw, V_{tg}, forced expiratory parameters, and exercise ventilatory and metabolism data are given in Table 2. Mean percent change for SGaw is depicted in Figure 1C. ANOVA and post hoc analyses results for SGaw, V_{tg}, forced expiratory parameters, and exercise ventilatory and metabolism data are summarized in Table 3. Significant across and within pretreatment effects were obtained for SGaw ($p<0.05$). Post hoc analysis revealed a significant within pretreatment effect (FA vs 0_3) for the no drug pretreatment and significant ($p<0.05$) across pretreatment effects for the no drug vs atropine and atropine sham vs atropine comparisons for 0_3 exposures. No significant changes for V_{tg}, either within or across pretreatments, were observed.

Table 2

Fig. 1

Table 3

No significant effect of atropine pretreatment on baseline SGaw was observed. However, the mean SGaw baseline value for the atropine pretreatment was numerically greater than those for the atropine sham and no drug condition (Table 2).

Forced Expiratory Parameters. Mean percent changes for FVC and $FEV_{1.0}$ are depicted in Figure 1. Significant across and within pretreatment effects were obtained for FVC and FEF_{25-75} ($p<0.05$). All within pretreatment compari-

sons were significant for FVC and $FEV_{1.0}$. The no drug vs atropine sham across pretreatment comparison for FVC was significant for the 0_3 exposures. The no drug and atropine sham within pretreatment comparisons were significant for FEF_{25-75}, with the no drug vs atropine and atropine sham vs atropine across pretreatment for the 0_3 exposures also being significant.

Exercise Responses. As shown in Table 3, statistical analysis revealed no significant within or across pretreatment effects for $\dot{V}_{0_2}$. Analysis of $\dot{V}_E$ data revealed no significant within pretreatment effects, but did show a significant across pretreatment effect for the atropine sham vs atropine comparison for the FA exposures. All within pretreatment comparisons were significant for f_R and V_T, with no significant across pretreatments effects observed.

Subjective Symptom Response. Analysis of all RPE and subjective symptom parameters revealed no significant across pretreatment comparisons (Table 4). All within pretreatment comparisons were significant for RPE and subjective symptom parameters except for leg RPE, where the only significant within pretreatment comparison was with atropine.

Table 4

Respiratory Impedance Measurements. Individual and descriptive group data for the three subjects most sensitive to 0_3 are given in Table 5.

Table 5

DISCUSSION

In the present study, atropine pretreatment was shown to block 0_3-induced decrements in SGaw when compared to both no drug and atropine sham controls (Table 2, Fig. 1c). These findings suggest that cholinergic post-ganglionic efferents play a predominant role in eliciting bronchoconstriction consequent to acute 0_3 exposure, and that 0_3-induced pathologic lesions, if present, do not contribute significantly to this response.

Atropine pretreatment did not significantly affect FVC, exercise ventilatory and subjective symptom responses when comparisons were made across pretreatments. Similarly, $FEV_{1.0}$ responses were unaffected by atropine pretreatment, suggesting that O_3-induced decrements in $FEV_{1.0}$ are primarily a function of O_3-induced decrements in inspiratory capacity and not bronchoconstriction. In contrast, FEF_{25-75} responses paralleled SGaw responses with similar significant across pretreatment effects. These findings suggest that FEF_{25-75} is a better indicator of bronchoconstriction consequent to O_3 exposure than is $FEV_{1.0}$.

Our experimental design entailed several exposures to O_3, and it is possible that adaption or sensitization to O_3 toxic effects may have influenced our results. However, 4 to 7 days intervened between O_3 exposures, which appears to be sufficient time not to elicit either an adaptive or sensitization response (11). Further, pretreatments were randomized in order to reduce order effects. Finally, our data evidenced no order or repeated exposure effects, as indicated by the reproducibility of pulmonary function and exercise ventilatory pattern data between the no drug and atropine sham control conditions (Table 2).

Atropine pretreatment did not significantly alter baseline SGaw values, although a trend toward an increased SGaw for the atropine pretreatment did exist (Table 2). Two steps were taken to reduce the effect such a trend might have on interpretation of our data. First, within protocol effects were reported as a percent change from baseline for each protocol. Second, all pretreatments contained a FA control which provided comparison between FA and O_3 responses within each pretreatment. Given these experimental design con-

straints, we conclude that there were no significant differences between FA and 0_3 protocols within the atropine pretreatment for SGaw, whereas significant differences ($p<0.05$) within pretreatments did exist for the atropine sham and no drug pretreatments.

Parameters derived from measurement of respiratory system impedance (Z_{RS}) have been shown to be sensitive to small airway changes in rats exposed to 0.64 ppm 0_3 for 7 to 22 days (22) and in asymptomatic smokers (15). Z_{RS} data were analyzed in the present study using a lumped-parameter mechanical model, in which the airways were separated into central and peripheral components by a short pathway containing an airway wall compliance (19). This model was originally proposed by Mead (28) to explain frequency dependence of compliance, and has been successfully used by others to fractionate Z_{RS} into central and peripheral components (33,39). In the present study, Z_{RS} was analyzed in the frequency range of 16-100 Hz. There existed a gradual increase in the real component of Z_{RS} between 40-100 Hz in all subjects, suggesting an incongruity in the resistance x innertance time constants between the peripheral and central airways (29). Hence, we modified the Mead model (28) to include a central and peripheral airway innertance (19).

Due to the extensive analysis time required to optimize the Z_{RS} to the chosen model, only the Z_{RS} data of three subjects has been completely analyzed for the atropine and atropine sham pretreatments (Table 5). Due to the variability within and between these three subjects, it is not possible to draw any definite conclusions as to the degree to which central and peripheral components may contribute to the observed changes in SGaw with 0_3 exposure.

Acute exposure to O_3 has been shown to elicit several responses having neural components (14,16,24,25). Rapid, shallow breathing in dogs consequent to acute O_3 exposure has been shown to be blocked by vagal cooling (25). Further, O_3-induced bronchial hyperresponsiveness has been blocked by vagal cooling in dogs (24) and atropine pretreatment in both dogs (25) and human subjects (14). Recently, Hazucha et al (16) have demonstrated that FVC impairment consequent to acute O_3 exposure is abolished by inhalation of lidocaine mist, which suggests that lidocaine sensitive afferents are stimulated during acute O_3 exposure, resulting in an involuntary decrease in inspiratory capacity. The observation in the present study that bronchoconstriction consequent to acute O_3 exposure is abolished by atropine pretreatment suggests that O_3-induced bronchoconstriction is also neurally mediated.

The specific stimuli and neural afferents involved in the bronchoconstriction observed consequent to acute O_3 inhalation have yet to be elucidated. O_3-induced bronchial hyperresponsiveness has been shown to be associated with the process of airway inflammation in both animals (18,30) and human subjects (35). Recently, we have shown that pretreatment with indomethacin, an inhibitor of the cyclooxygenase pathway of arachidonic acid metabolism, significantly reduces FVC decrements consequent to acute O_3 exposure, but does not significantly affect O_3-induced bronchoconstriction in human subjects (34). This observation suggests that a dichotomy exists in the stimuli and neural afferents, which results in lung volume decrements and in bronchoconstriction consequent to acute O_3 exposure. Further research is required to define the specific neural stimulus which elicits O_3-induced bronchoconstriction and the relationship between this stimulus and O_3-induced lung tissue changes before comprehensive evaluation of health risks based on pulmonary function response, alone, can be made.

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

Subject	Age, yr	Ht, cm	Wt, kg	Fat, % Body Weight	FVC, ℓ	$FEV_{1.0}$, ℓ	SGaw, $\ell \cdot \frac{1}{[cmH_2O]/[\ell/s]}$	$\dot{V}_{O_{2max}}$, ℓ/min	$\dot{V}_{Emax}$, ℓ/min BTPS
1	21	185.4	86.0	19.7	6.41	4.97	.154	4.66	157.9
2	22	179.1	69.3	13.4	5.85	4.68	.113	4.20	157.3
3	26	179.1	87.0	18.0	6.37	5.47	.200	4.80	197.8
4	22	177.8	64.8	13.4	6.52	5.65	.132	5.03	182.9
5	21	177.8	62.9	13.9	5.83	4.58	.138	4.41	137.6
6	18	171.4	69.0	15.6	5.79	4.26	.147	4.88	122.3
7	28	172.7	70.9	16.0	5.13	4.17	.135	4.09	157.4
8	24	190.5	90.2	22.9	8.27	5.33	.106	4.90	167.9
9	19	185.4	75.1	13.6	6.41	5.03	.181	4.03	126.8
10	21	177.8	62.4	11.6	5.26	4.44	.183	3.11	105.6
11	23	193.0	86.6	14.3	6.62	5.43	.112	4.53	137.8
12	32	177.8	65.3	11.6	6.02	4.68	.120	4.77	221.8
13	34	175.3	73.9	14.6	6.32	5.07	.087	4.21	136.5
$\bar{x}$	24.7	180.2	74.1	15.3	6.21	4.90	.139	4.43	154.6
S.D.	±4.79	±6.5	±10.0	±3.23	±0.77	±0.48	±.033	±0.52	±32.3

FVC, forced vital capacity; $FEV_{1.0}$, forced expiratory volume in 1 s; SGaw, specific airway conductance; $\dot{V}_{O_{2max}}$, maximum O_2 uptake; $\dot{V}_{Emax}$, maximum minute ventilation.

Table 2. Group pre- and postexposure values for plethysmographic, pulmonary function, and exercise respiratory parameters.

Variable	NO DRUG CONTROL				ATROPINE SHAM				ATROPINE			
	Filtered Air		Ozone		Filtered Air		Ozone		Filtered Air		Ozone	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
SGaw, $\frac{1}{\ell \cdot [cmH_2O]}$ [ℓ/s]	.139 ± .033	.140 ± .032	.150 ± .031	.125 ± .029	.151 ± .029	.146 ± .033	.150 ± .037	.134 ± .041	.181 ± .039	.190 ± .033	.175 ± .040	.178 ± .039
V_{tg}, liters	4.12 ±0.79	4.31 ±0.88	4.05 ±0.80	4.25 ±0.70	3.91 ±0.77	4.03 ±0.79	3.97 ±0.80	4.23 ±1.00	4.20 ±0.80	4.34 ±0.81	4.03 ±0.94	4.29 ±0.88
FVC, liters	6.19 ±0.73	6.15 ±0.68	6.25 ±0.84	5.22 ±1.16	6.25 ±0.82	6.22 ±0.77	6.22 ±0.81	4.97 ±1.27	6.25 ±0.84	5.93 ±1.24	6.29 ±0.82	5.25 ±1.18
$FEV_{1.0}$, liters	4.90 ±0.50	4.95 ±0.41	4.91 ±0.61	3.62 ±0.76	4.92 ±0.52	4.87 ±0.43	4.84 ±0.56	3.59 ±0.72	5.22 ±0.56	4.99 ±0.99	5.26 ±0.55	4.32 ±0.92
FEF_{25-75}, ℓ/s	4.71 ±1.14	4.75 ±0.91	4.61 ±1.14	2.83 ±0.77	4.60 ±0.93	4.48 ±1.03	4.51 ±0.91	3.06 ±0.99	5.51 ±0.83	5.44 ±1.09	5.60 ±0.95	4.94 ±1.52
f_R, breaths/min	31.00 ±3.44	34.14 ±7.63	30.64 ±3.46	47.00 ±13.40	30.64 ±4.50	33.86 ±6.71	31.07 ±5.21	49.40 ±3.90	31.71 ±5.95	37.57 ±8.91	31.21 ±4.73	47.10 ±11.70
V_T, liters	1.84 ±0.23	1.81 ±0.27	1.88 ±0.23	1.34 ±0.27	1.89 ±0.23	1.80 ±0.29	1.86 ±0.31	1.30 ±0.31	1.90 ±0.27	1.75 ±0.31	1.87 ±0.21	1.38 ±0.27
$\dot{V}_E$, ℓ/min	56.50 ±4.49	60.04 ±4.85	57.18 ±5.60	59.80 ±6.66	57.20 ±5.09	59.48 ±6.40	56.76 ±5.91	62.21 ±6.31	59.24 ±6.32	63.41 ±6.95	57.67 ±6.01	62.50 ±5.79
$\dot{V}_{O_2}$, ℓ/min	2.37 ±0.19	2.37 ±0.56	2.29 ±0.15	2.41 ±0.25	2.29 ±0.25	2.43 ±0.19	2.25 ±0.20	2.33 ±0.17	2.29 ±0.19	2.38 ±0.26	2.25 ±0.20	2.32 ±0.18

Values represent the pre- and postexposure means, with corresponding ± 1 standard deviation. V_{tg}, thoracic gas volume; F_R, respiratory frequency; V_T, tidal volume, $\dot{V}_E$, expired minute ventilation; $\dot{V}_{O_2}$, oxygen uptake. Other abbreviations as in Table 1.

Table 3. F ratios and specific significant mean differences for post hoc analysis of plethysmographic, pulmonary function, and exercise ventilatory and metabolism variables.

Variable	Across Pretreatment F Ratio	Specific Significant Mean Differences ($p<0.05$)	Within Pretreatment F Ratio	Specific Significant Mean Differences ($p<0.05$)
SGaw	8.85	ND-A; AS-A	9.38	ND
V_{tg}	0.12	NS	3.11	NS
FVC	4.09	ND-AS	14.53	ND; AS; A
$FEV_{1.0}$	1.89	NS	23.86	ND; AS; A
FEF_{25-75}	5.42	ND-A; AS-A	20.16	ND;AS
f_R	1.36	NS	34.56	ND; AS; A
V_T	1.34	NS	128.30	ND; AS; A
$\dot{V}_E$	3.60	ND-AS	0.06	NS
$\dot{V}_{O_2}$	0.29	NS	0.09	NS

ND, A, and AS denote no drug, atropine and atropine sham pretreatments, respectively. NS indicates no statistically significant difference ($p>0.05$). Other abbreviations as in Tables 1 and 2.

Table 4. Subjective symptom response to treatments

		NO DRUG		ATROPINE SHAM		ATROPINE	
Variable		FA	O_3	FA	O_3	FA	O_3
RPE, Chest		1.29 ± 2.64	5.43 ± 4.07	1.71 ± 2.55	4.21 ± 4.08	1.57 ± 1.91	3.79 ± 3.72
RPE, Legs		2.50 ± 3.08	3.79 ± 3.75	1.93 ± 3.38	4.07 ± 4.01	2.79 ± 2.86	4.21 ± 3.49
RPE, Overall		1.93 ± 2.89	3.71 ± 3.24	1.71 ± 3.41	4.21 ± 4.00	1.79 ± 2.08	3.86 ± 3.13
SYMPTOMS	Cough	0.36 ± 1.34	13.71 ±11.77	-0.07 ± 2.27	11.86 ±13.52	0 0	9.71 ±10.83
	Shortness of Breath	0 0	11.36 ±12.70	-0.14 ± 0.53	12.43 ±12.10	0 0	12.00 ±13.46
	Throat Tickle	- 0.36 ± 1.34	9.29 ± 8.52	0 0	11.07 ±11.30	0 0	9.07 ±11.78
	Pain on Deep Inspiration	0.36 ± 1.34	19.43 ±12.33	0 0	18.43 ±12.37	0 0	19.14 ±13.20
	Overall Symptom Severity	0.36 ± 1.34	17.29 ±12.06	-0.07 ± 0.28	16.00 ±10.19	0 0	14.00 ±12.83

Values represent mean change from 5 to 58 min for each exposure, with corresponding ± 1 standard deviations. RPE, rated perceived exertion. See text for further details.

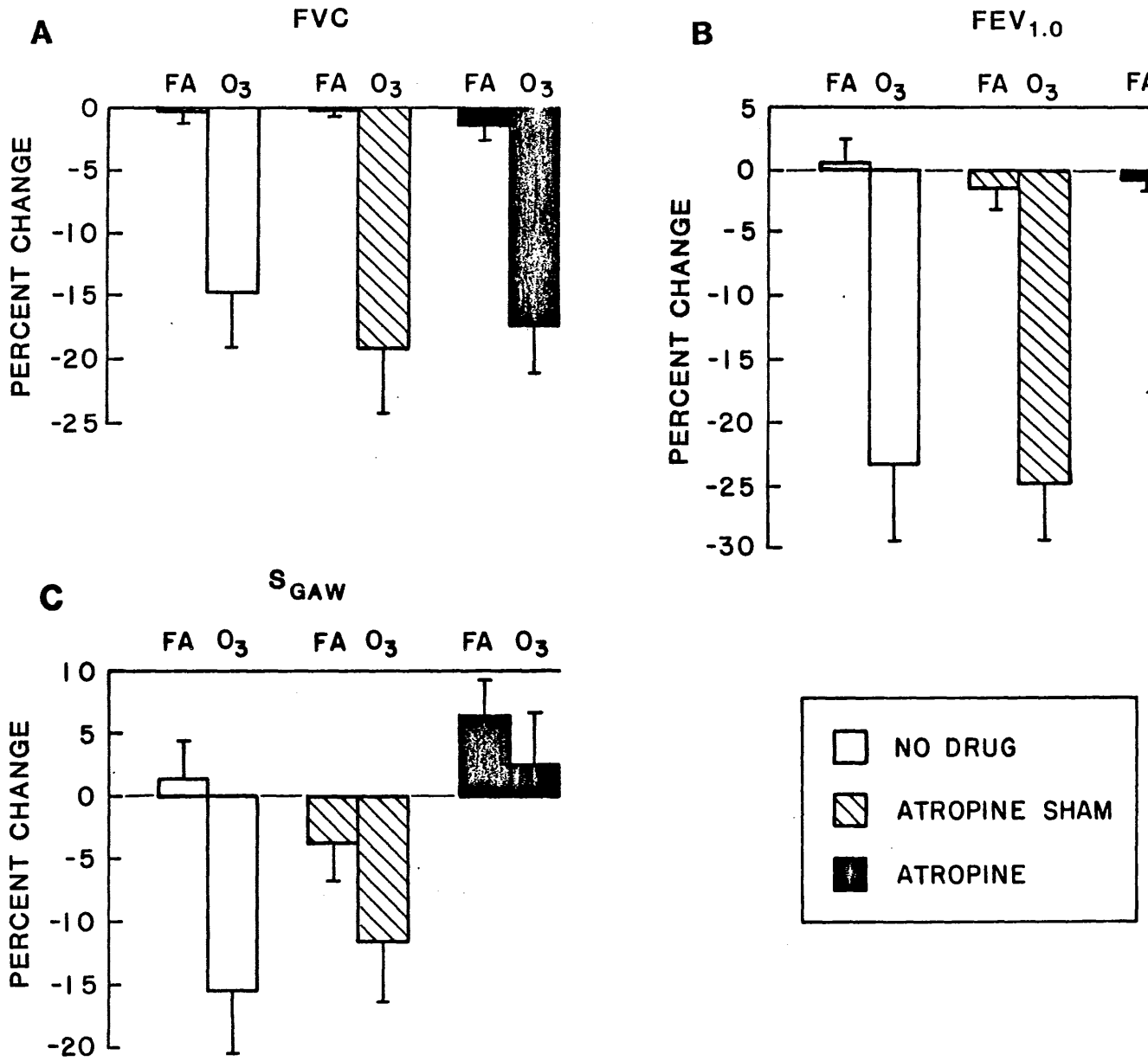
Table 5. Optimized impedance parameters for 6 parameter model.

		ATROPINE SHAM				ATROPINE			
		Filtered Air		Ozone		Filtered Air		Ozone	
Variable	Subject #	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Rc, cm H_2O/L/sec	1	3.09	1.71	.47	1.66	2.04	2.03	2.64	4.15
	9	1.19	1.10	1.34	1.18	1.19	1.45	1.08	1.09
	12	2.00	1.63	1.59	1.29	2.05	2.49	1.90	1.79
	$\bar{x}$	2.09	1.48	1.13	1.38	1.76	1.99	1.87	2.34
	±S.D.	0.95	0.33	0.59	0.25	0.49	0.52	0.78	1.60
Ic, cm H_2O/L/sec x 10^{-2}	1	1.69	1.36	0.74	1.17	1.51	1.58	1.07	0.81
	9	1.21	0.92	1.52	1.52	1.52	1.52	1.54	1.42
	12	1.50	1.68	1.40	1.10	1.84	1.53	1.60	1.59
	$\bar{x}$	1.47	1.32	1.22	1.26	1.62	1.54	1.40	1.27
	±S.D.	0.24	0.38	0.42	0.23	0.19	0.03	0.29	0.41
Caw, L/cm H_2O x 10^{-4}	1	1.95	0.86	0.58	0.90	2.48	3.34	0.51	0.46
	9	3.41	0.60	4.03	3.75	3.26	3.67	3.16	2.64
	12	3.00	2.80	1.73	0.85	3.79	2.82	2.56	3.24
	$\bar{x}$	2.79	1.42	2.11	1.83	3.18	3.28	2.08	2.11
	±S.D.	0.75	1.20	1.76	1.66	0.66	0.43	1.39	1.46
Rp, cm H_2O/L/sec	1	0.90	3.19	2.80	2.14	0.76	0.65	0.81	0.26
	9	1.52	1.57	1.35	1.50	1.72	1.54	1.55	1.48
	12	1.59	2.26	1.48	2.26	0.99	0.45	0.90	0.95
	$\bar{x}$	1.34	2.34	1.88	1.97	1.16	0.88	1.09	0.90
	±S.D.	0.38	0.81	0.80	0.41	0.50	0.58	0.40	0.61
Ip, cm H_2O/L/sec x 10^{-3}	1	4.69	8.34	11.8	7.96	5.97	6.92	12.4	12.6
	9	5.40	9.08	3.93	4.14	4.27	3.81	4.27	5.02
	12	4.01	4.41	5.38	7.99	3.41	3.79	3.77	3.89
	$\bar{x}$	4.70	7.28	7.04	6.70	4.55	4.84	6.81	7.17
	±S.D.	0.70	2.51	4.19	2.21	1.30	1.80	4.84	4.74
C_{RS}, L/cm H_2O x 10^{-2}	1	1.16	1.03	5.47	1.27	2.62	2.42	0.84	1.28
	9	1.19	1.29	0.86	0.85	0.58	0.74	0.65	0.73
	12	3.35	1.22	1.13	0.79	0.79	1.35	1.55	1.37
	$\bar{x}$	1.90	1.18	2.49	0.97	1.33	1.50	1.01	1.13
	±S.D.	1.26	0.13	2.59	0.26	1.12	0.85	0.47	0.35

Rc is central airway resistance; Ic is central airway innertance; Caw is airway compliance; Rp is peripheral airway resistance; Ip is peripheral airway innertance; C_{RS} is respiratory system compliance.

FIGURE LEGEND

FIG. 1. Mean percent change from preexposure in (A) forced vital capacity (FVC), (B) forced expiratory volume in 1s ($FEV_{1.0}$) and (C) specific airway conductance (SGaw) for each exposure condition.


A
FVC
FA O3 FA O3 FA O3
0
-5
-10
-15
-20
-25
PERCENT CHANGE
B
FEV1.0
FA O3 FA O3 FA O3
5
0
-5
-10
-15
-20
-25
-30
PERCENT CHANGE
C
SGAW
FA O3 FA O3 FA O3
10
5
0
-5
-10
-15
-20
PERCENT CHANGE
NO DRUG
ATROPINE SHAM
ATROPINE

GLOSSARY OF TERMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
Caw	Airway Wall Compliance
C_{RS}	Respiratory System Compliance
FA	Filtered Air
FEF_{25-75}	Forced Expiratory Flow Between 25-75 Percent of FVC
$FEV_{1.0}$	Forced Expiratory Volume at 1 sec
F_R	Respiratory Frequency
FVC	Forced Vital Capacity
HR	Heart Rate
I_C	Central Airway Inertance
IE	Intermittent Exercise
I_P	Peripheral Airway Inertance
NO_2	Nitrogen Dioxide
O_3	Ozone
PAN	Peroxyacetyl Nitrate
P_b	Box Pressure
PC	Parkinson-Cowan
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
Pm	Mouth Pressure.
ppm	Parts per Million
ppm·L	Parts per Million x Liters
Raw	Airway Resistance
R_C	Central Airway Resistance
R_P	Peripheral Airway Resistance
RPE	Rating of Perceived Exertion
RV	Residual Volume
SD	Standard Deviation
SO_2	Sulfur Dioxide
SGaw	Specific Airway Conductance
SRaw	Specific Airway Resistance
SS	Subjective Symptoms
TLC	Total Lung Capacity
T_XB_2	Thromboxane B_2
$\dot{V}$	Mouth Flow Rate
$\dot{V}_E$	Expiratory Ventilation Rate
$\dot{V}_{O_2}$	Oxygen Uptake
$\dot{V}_{O_2max}$	Maximal Oxygen Uptake Rate
V_T	Tidal Volume
V_{tg}	Thoracic Gas Volume
Z_{RS}	Respiratory System Impedance