Effects of NO_2 alone and in combination with O_3 on young men and women

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ADAMS, WILLIAM C., KATHERINE A. BROOKES, AND EDWARD S. SCHELEGLE. *Effects of NO_2 alone and in combination with O_3 on young men and women.* J. Appl. Physiol. 62(4): 1698–1704, 1987.—Previous studies of 2 h of exposure to NO_2 at high urban atmospheric levels (i.e., 0.50–1.0 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 on exposure to 0.60 ppm NO_2 and/or enhance the effects of exposure to 0.30 ppm O_3, 40 aerobically trained young adults (20 males and 20 females) completed 1 h of continuous exercise at work rates eliciting a mean minute ventilation of 70 and 50 l/min for the males and females, respectively. Exposures to filtered air, 0.60 ppm NO_2, 0.30 ppm O_3, and 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 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, exercise ventilatory response, 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.

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

NITROGEN DIOXIDE plays a major role in the genesis of 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 (4, 25, 28). In many respects, the toxic effects of NO_2 are similar to those of O_3 (25), 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 (5, 11, 27), 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, Swann, Keenan, and Phillips (unpublished observations) suggested that 10 min of moderate exercise during 1 h exposure to 1.5 ppm NO_2 elicited increased airway resistance (Raw) in most subjects. However, Hackney and colleagues (15) 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 minute ventilation ($\dot{V}E$) of 2.5 times that at rest.

Studies of the possible interaction of NO_2 with other ambient pollutants in healthy humans are limited (16), and apparently only three in combination with O_3 have incorporated exercise. Hackney et al. (14) employed light intermittent exercise in the exposure of adult males to 0.20 ppm O_3[1] and 0.30 ppm NO_2 for 2 h and found minimal changes in pulmonary function. Folinsbee et al. (10) 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 (11). Kagawa (19) 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 and Adams (21) observed significantly greater pulmonary function response to the same absolute inhaled O_3 dose in six aerobically trained young adult females compared with their male counterparts. This enhanced sensitivity was still evident when female subjects exercised at a reduced $\dot{V}E$ proportional to their maximum O_2 uptake ($\dot{V}O_{2\,max}$). Others have suggested that females demonstrate a greater acute response to O_3 inhalation whether at rest (18) or while engaged in exercise at the same relative intensity as males (6, 20). Horvath et al. (17), 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

[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 (7).

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symptoms than their male counterparts.

The primary purpose of this investigation was to determine the effects of 1 h of 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 $\dot{V}O_{2\,max}$), evidenced enhanced responses on 1 h of 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 (2) and absence of history of significant allergies. All were nonsmokers and none had resided in a high air pollution area within the previous 3 mo.

Before initiating the four 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 (FA). On another preexperimental occasion, the subjects' $\dot{V}O_{2\,max}$ was determined utilizing a progressive increment protocol (1). Group subject characterization data obtained in these orientation sessions are summarized in Table 1.

Experimental design. Subjects were exposed in random order for 1 h each to FA, 0.30 ppm O_3, 0.60 ppm NO_2, and 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 $\dot{V}E$ was ~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 before 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 analog-to-digital 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 mean forced expiratory flow between 25 and 75% of FVC (FEF_{25-75}).

Thoracic gas volume (Vtg) and Raw were measured in 20 female and 10 male subjects using constant-volume whole-body plethysmography (8, 9). Pre- and postexposure maneuvers consisted of five trials for each set. Valid data sets for all exposures were obtained for 17 females and 8 males. Specific airway resistance (sRaw) was calculated as the product of Raw and Vtg.

Exercise measurements. To assess possible effects of O_3 and/or NO_2 inhalation on selected exercise parameters, an on-line computerized data acquisition system delivered printouts of 1-min average values for $\dot{V}E$, heart rate (HR), tidal volume (VT), respiratory frequency (f), percent of O_2 and CO_2 in expired gas, expired gas temperature, and O_2 uptake ($\dot{V}O_2$) every minute. Data acquisition instruments interfaced to the DEC LSI 11/2 microcomputer, included a LB-1 CO_2 analyzer, an Applied Electrochemistry S-3A O_2 analyzer, an Alpha Technologies turbotachometer ventilation 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, 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 follows: 0, not present; 5, minimal; 10, mild; 20, moderate; 30, severe; and 40, incapacitating. Immediately after completion of the postexposure pulmonary function test battery, subjects provided written comments on discomforts experienced during the exposure and indicated whether or not they believed that they received O_3 and/or NO_2.

TABLE 1. *Group anthropometric, pulmonary function, and $\dot{V}O_{2\,max}$ data*

	Age, yr	Ht, cm	Wt, kg	Fat, % body wt	FVC, liters	$FEV_{1.0}$, liters	$\dot{V}O_{2max}$, l/min	$\dot{V}E_{max}$, l/min BTPS
				Females				
Mean ± SD	21.4±1.5	168.3±5.7	60.6±6.0	21.8±5.2	4.71±0.29	3.83±0.30	3.20±0.41	106.8±21.9
Min	19	154	48.4	12.3	4.15	3.05	2.62	65.2
Max	25	179	73.2	33.3	5.24	4.30	4.44	140.8
				Males				
Mean ± SD	22.7±3.3	181.6±5.0	74.9±6.8	13.1±4.8	6.41±0.98	5.02±0.68	4.43±0.55	149.8±24.4
Min	18	169	63.0	7.0	5.05	4.09	3.36	120.0
Max	30	193	88.1	23.4	7.94	6.76	5.29	208.0

FVC, forced vital capacity; $FEV_{1.0}$, forced expiratory volume at 1 s; $\dot{V}O_{2max}$, maximum O_2 uptake; $\dot{V}E_{max}$, maximum minute ventilation; Min, minimum value of sample; Max, maximum value of sample.

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TABLE 2. *Group pre- and postexposure values for pulmonary function and sRaw*

Variable	Filtered Air		0.30 ppm O_3		0.60 ppm NO_2		0.30 ppm O_3 + 0.60 ppm NO_2	
	Preexposure	Postexposure	Preexposure	Postexposure	Preexposure	Postexposure	Preexposure	Postexposure
FVC, liters								
Females	4.549±0.29	4.443±0.31	4.540±0.32	3.899±0.61	4.563±0.29	4.484±0.29	4.562±0.28	3.843±0.55
Males	6.375±0.88	6.315±0.93	6.307±1.10	5.315±1.04	6.279±1.00	6.275±0.91	6.218±0.95	5.255±0.97
$FEV_{1.0}$, liters								
Females	3.686±0.32	3.606±0.29	3.735±0.30	2.978±0.67	3.740±0.34	3.666±0.37	3.683±0.32	2.895±0.55
Males	4.918±0.64	4.837±0.74	4.945±0.63	3.769±0.75	4.929±0.66	4.930±0.57	4.870±0.62	3.794±0.95
FEF_{25-75}, l/s								
Females	3.670±0.85	3.663±0.93	3.818±0.94	2.885±1.26	3.813±0.88	3.809±1.05	3.744±1.05	2.577±1.18
Males	4.340±0.87	4.307±1.11	4.631±0.80	2.980±0.93	4.483±0.97	4.577±1.11	4.459±0.98	3.243±1.4
sRaw, $l \cdot cmH_2O \cdot l^{-1} \cdot s$								
Females	6.84±2.1	6.90±2.0	6.87±1.6	7.92±2.1	6.74±1.5	6.69±1.7	6.95±2.1	7.46±2.3
Males	6.98±0.9	7.15±1.4	6.63±1.0	7.96±1.7	7.20±1.3	6.93±1.0	7.15±1.5	7.44±1.7

Values are means ± SD for pre- and postexposure. FEF_{25-75}, forced expiratory flow rate between 25 and 75% of forced vital capacity; sRaw, specific airway resistance. See Table 1 legend for other abbreviations.

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 (5). 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 N_2 at a controlled flow rate using a micrometering valve and a Fisher and Park flowmeter, 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 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 mixing chamber was monitored by continuous samples drawn through 0.64-cm-diam Teflon tubing connected to a Dasibi O_3 meter, whereas that for NO_2 was determined on a Thermo Electron 14 B/E NO_x analyzer. The digital reading of O_3 concentration in ppm was compared periodically with that determined by the ultraviolet absorption photometric method (7). 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. Values for sRaw 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 10th min and the last minute of exercise for $\dot{V}O_2$, $\dot{V}E$, f, and VT 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 with 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), which included two within factors (NO_2 and O_3) with repeated measures, and one grouping factor (gender). On obtaining a significant F ratio for main effects due to a gas mixture, a gender, or an interaction, a paired t post hoc test, with Bonferroni correction (24), was applied 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 sRaw are given in Table 2, with group mean percent change for $FEV_{1.0}$ and sRaw depicted in Fig. 1. ANOVA and post hoc analyses results for pulmonary function responses 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 NO_2 and NO_2 plus O_3 exposures. There were no statistically significant differences in pulmonary function parameters for comparisons 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 sRaw values observed between FA and O_3, NO_2 and O_3, and O_3 and O_3 plus NO_2 exposures. No statistically significant differences were observed between males and females for pulmonary function or sRaw responses to any air mixture exposure.

Exercise responses. Group pre- and postexposure data for exercise ventilatory and respiratory metabolism parameters are given in Table 3. ANOVA and post hoc analyses 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

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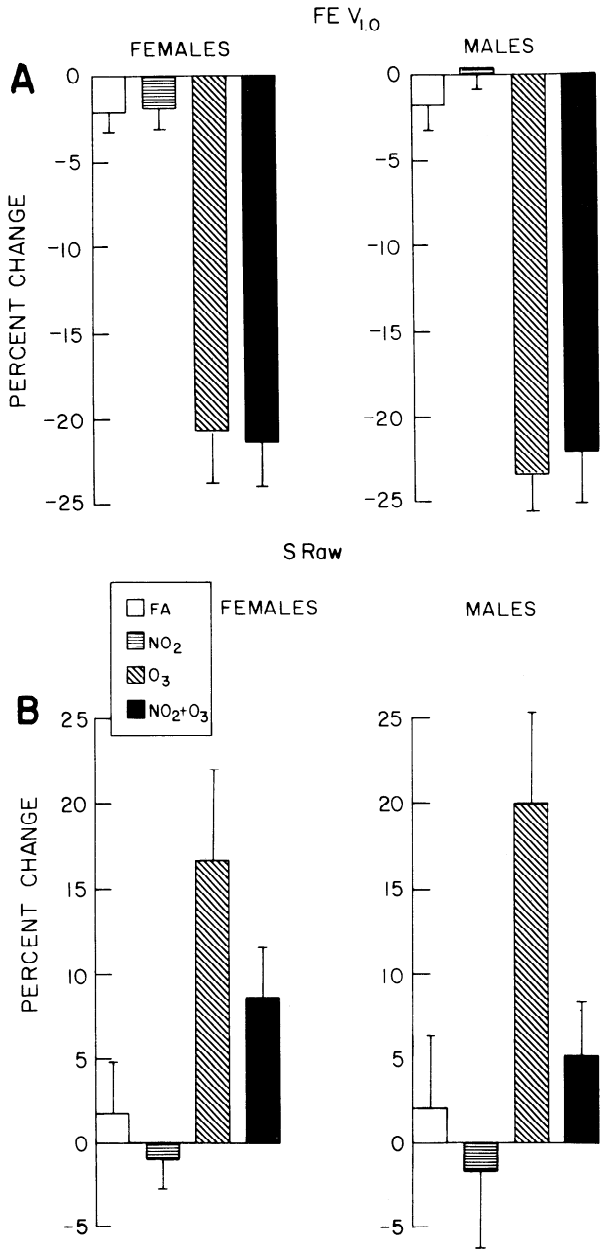

FIG. 1. Mean percent change from preexposure in (*A*) forced expiratory volume in 1 s ($FEV_{1.0}$) and (*B*) specific airway resistance (sRaw) for each exposure condition. *Bars* represent means ± SE. FA, filtered air.

f and VT when comparing values for FA to O_3, FA and O_3 plus NO_2, NO_2 and O_3, and 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 and VT. A significant interaction of gender with changes in f and VT revealed different ventilatory pattern responses in males and females. Post hoc analyses indicated that the males demonstrated a significantly greater f for all gas mixtures, but greater decrement in VT 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 4. The number and severity of symptoms reported were significantly greater ($P < 0.05$) for the O_3 and the O_3 plus NO_2 exposures, compared with both the FA and NO_2 exposures. There were no significant differences between values obtained for the O_3 and the NO_2 plus O_3 exposures, nor for females and males across all exposures.

DISCUSSION

Results of this study indicate that 1 h of heavy continuous exercise while exposed to a high ambient level of NO_2 (0.60 ppm) has no effect on pulmonary function, sRaw, exercise ventilatory pattern, or subjective symptoms response. Further, except for a smaller increase in sRaw, the combination of 0.60 ppm NO_2 plus 0.30 ppm O_3 had no effect on these parameters beyond that observed on exposure to 0.30 ppm O_3 alone. 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. (unpublished observations) demonstrated an increase in Raw in subjects exposed to 1.5 ppm NO_2. Von Nieding and Wagner (30) 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. (15) 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 of 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, sRaw, 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 (22), 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.

Limited acute sRaw dose-response data were summarized by Colucci (3), who found that a three- to fourfold greater concentration of NO_2 was required to elicit the same effect as that induced by O_3. Von Nieding and Wagner (30), however, suggest a much greater ratio, in that thoracic resistance was significantly elevated on 2 h of exposure to 0.1 ppm O_3 with light intermittent exercise, as well as on similar exposure to 5.0 ppm NO_2, but not at 1.5 or 2.5 ppm. Von Nieding and Wagner's observations, together with ours and those of Linn and associates (22), utilizing 75 min of exposure to 4.0 ppm NO_2, are in apparent agreement with the significantly enhanced oxidative effects of O_3 compared with NO_2

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TABLE 3. *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 + 0.60 ppm NO_2	
	Preexposure	Postexposure	Preexposure	Postexposure	Preexposure	Postexposure	Preexposure	Postexposure
f, 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
V_T, liters								
Females	1.50±0.22	1.42±0.25	1.50±0.22	1.18±0.20	1.45±0.22	1.37±0.23	1.53±0.21	1.16±0.18
Males	2.25±0.48	2.08±0.37	2.29±0.44	1.62±0.28	2.15±0.28	1.97±0.32	2.13±0.39	1.55±0.31
$\dot{V}_E$, 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±6.8	69.9±8.6	68.7±4.9	71.8±6.4	67.0±6.3	72.0±8.9
$\dot{V}_{O_2}$, l/min								
Females	1.94±0.26	2.00±0.27	1.90±0.20	1.96±0.26	1.92±0.29	2.00±0.29	1.99±0.28	2.06±0.31
Males	2.74±0.32	2.82±0.29	2.71±0.32	2.77±0.35	2.79±0.39	2.88±0.31	2.65±0.30	2.74±0.30

Values are means ± SD for average percent change between 10th min and last minute. f, respiratory frequency; V_T, tidal volume; $\dot{V}_E$, minute ventilation; $\dot{V}_{O_2}$, O_2 uptake.

TABLE 4. *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
No. 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 are means ± SD for each exposure. See text for details.

found in animal studies. Veninga et al. (29) report limited comparative biologic 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 (13) observed similar effects elicited by an ~20-fold greater concentration of NO_2 than O_3.

A lack of statistically significant pulmonary function and sRaw response on exposure to peak 1-h ambient concentrations of NO_2 or higher, does not, however, preclude the possibility of deleterious effects in the peripheral airways where animal studies accent NO_2's impact (25, 28). Forced expiratory flow and sRaw responses reveal primarily central airway, rather than peripheral airway effects (26). 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.

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 sRaw due to the formation of the acid reaction product, HNO_3 (19). Mautz et al. (23) 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 with 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 that might account for the observed synergism.

There have been few laboratory studies of humans exposed to NO_2 in combination with other air pollutants that have incorporated exercise. Hackney et al. (14) reported that the pulmonary function impairment resulting from 2 h of exposure to 0.20 ppm O_3 and 0.30 ppm NO_2 with light intermittent exercise was no greater than that observed for 0.20 ppm O_3 alone. When utilizing 0.40 ppm O_3 plus 0.30 NO_2, they found the changes to be equivalent to those observed for 0.40 ppm O_3 alone. Similarly, Folinsbee et al. (10) observed no difference in responses to 2 h of exposures to 0.40 ppm O_3 and 0.50 ppm NO_2 compared with those observed in an earlier study (11) for subjects exposed to 0.40 ppm O_3 alone at a similar mean protocol ventilation. In the present study a total inhaled effective dose of NO_2 for males ~2.5 times that employed by Folinsbee et al. (10) was utilized. Nonetheless, no significant additive effect of 1 h of 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 on exposure to 0.30 ppm O_3 was observed. In fact, a significantly lower percent change in sRaw for NO_2 plus O_3 than for O_3 alone was observed. Further, this reduced increase in sRaw for NO_2 plus O_3, compared with O_3 alone, was consistent for both males and females (Table 2 and Fig. 1). This observation is in contrast to the similar increases in Raw for NO_2 plus O_3 and O_3 alone by Folinsbee and colleagues (10, 11) and a slightly greater decrease in airway conductance observed for NO_2 plus O_3 exposure than for O_3 alone by Kagawa (19). A satisfactory explanation for this discrepancy is not readily apparent.

Several investigators have suggested that females are more sensitive to the same O_3 concentration and exposure time product whether at rest (18) or during exercise when $\dot{V}_E$ is proportional to percent $\dot{V}_{O_2\,max}$ (6, 20). Lauritzen and Adams (21) studied the responses of 1 h of 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

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the same percent $\dot{V}O_{2\,max}$ (resulting in ~25% lower $\dot{V}E$ for females), differences in pulmonary function response between the groups were reduced but not entirely negated. Horvath et al. (17), however, found no significant difference in pulmonary function and subjective symptom responses between 10 females and 10 males exposed to the following: *1)* 0.27 ppm peroxyacetyl nitrate, *2)* 0.48 ppm O_3, and *3)* 0.48 ppm O_3 plus 0.27 ppm peroxyacetyl nitrate for 2 h with light intermittent exercise. The total inhaled dose was ~11% 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 on 1 h of exposure to FA, NO_2, O_3, and O_3 plus NO_2, with mean $\dot{V}E$ ~30% lower for the females.

The consensus of the above studies is 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 practical importance of this observation is that if females and males of similar relative fitness were to undergo exposure at a similar O_3 concentration while exercising at the same percent $\dot{V}O_{2\,max}$ (which would be the case in many 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 work load and $\dot{V}E$ as the male, she would be expected to demonstrate a greater response to O_3 (21) for reasons not readily apparent.

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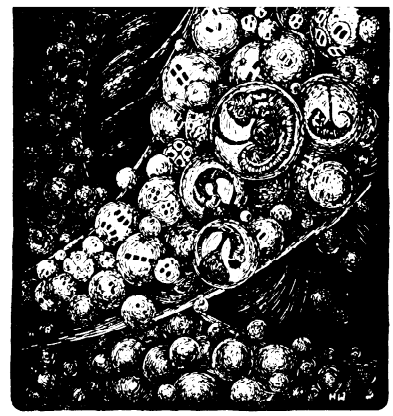

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