Ozone exposure decreases the effect of a deep inhalation on forced expiratory flow in normal subjects

S. K. Kjærgaard, O. F. Pedersen, M. R. Miller, T. R. Rasmussen, J. C. Hansen, and L. Mølhave. Ozone exposure decreases the effect of a deep inhalation on forced expiratory flow in normal subjects. *J Appl Physiol* 96: 1651–1657, 2004. First published December 19, 2003; 10.1152/japplphysiol.00507.2003.—Sixteen healthy non-smoking subjects (7 women), 21–49 yr old, were exposed in a climate chamber to either clean air or 300 parts/billion ozone on 4 days for 5 h each day. Before each exposure, the subjects had been pretreated with either oxidants (fish oil) or antioxidants (multivitamins). The study design was double-blind crossover with randomized allocation to the exposure regime. Full and partial flow-volume curves were recorded in the morning and before and during a histamine provocation at the end of the day. Nasal cavity volume and inflammatory markers in nasal lavage fluid were also measured. Compared with air, ozone exposure decreased peak expiratory flow, forced expiratory volume in 1 s, and forced vital capacity (FVC), with no significant effect from the pretreatment regimens. Ozone decreased the ratio of maximal to partial flow at 40% FVC by 0.08 ± 0.03 (mean ± SE, analysis of variance: P = 0.018) and at 30% FVC by 0.10 ± 0.05 (P = 0.070). Ozone exposure did not significantly increase bronchial responsiveness, but, after treatment with fish oil, partial flows decreased more than after vitamins during the histamine test, without changing the maximal-to-partial flow ratio. The decreased effect of a deep inhalation after ozone exposure can be explained by changes in airway hysteresis relative to parenchymal hysteresis, due either to ozone-induced airway inflammation or to less deep inspiration after ozone, not significantly influenced by multivitamins or fish oil.

SPIROMETRY; AIRWAY INFLAMMATION; MAXIMAL-TO-PARTIAL FLOW RATIO; BRONCHOCONSTRICITION; MULTIVITAMINS; FISH OIL.

Subjects

Sixteen subjects were recruited among 100 subjects (50 men and 50 women) chosen from the department’s registry of test subjects. They answered a health questionnaire, which formed the basis for the decision on whether they could participate or not. The chosen subjects were invited to an information meeting and a health check by a medical doctor. This included spirometry with measurement of BR, namely, skin prick test with the most common allergens (from pollen, domestic animals, dust mites, and molds). A positive skin prick test was defined as any observable papule larger than the saline control. Subjects were excluded if they were smokers, failed the health check, had a medical history of asthma and allergy, had positive skin prick tests, had FEV<sub>1</sub> or FVC <80% of predicted, or had >10% reduction of FEV<sub>1</sub> after inhalation of <1.70 mg histamine chloride histamine by the Yan method (43). Subjects who had conditions that were considered not compatible with an exposure study were also rejected. Among the 16 subjects, 7 were women, and ages ranged from 21 to 49 yr. The study was approved by the local ethics committee (approval no. 1998/4139), and all subjects signed a written consent.

Pretreatments

The exposure design was a double-crossover double-blind study with randomized allocation to the exposure regime. For 2 wk before each exposure, the subjects were treated with either fish oils or antioxidants. The fish oil treatment consisted of 5 capsules each containing 333 mg of omega-3 fatty acids ~166 mg eicosapentaenoic acid (EPA) (Bio-Marin, PharmaNord, Vejle, Denmark), matching the content of 50–75 g of herring, mackerel, or salmon. Additionally, they received three placebo tablets. The antioxidant amounts of neutrophils and inflammatory mediators in bronchoalveolar lavage (BAL) fluid have been found in subjects exposed to ozone (39), which leads to reduced FVC and FEV<sub>1</sub>, inflammation in the airways, and increased bronchial responsiveness (BR) (24, 34).

From maximum expiratory flow (V<sub>max</sub>)-volume (MEFV) curves performed after partial and then maximal inspiration, the ratio between the maximal flow on the two maneuvers at fixed lung volumes [maximal-to-partial flow ratio (M/P)] can be measured. During the test, the patient forcibly and completely exhales from lung volumes in the breathing range [a so-called partial maneuver (P)] and then rapidly inhales to total lung capacity (TLC) followed by a forcible and complete exhalation [a so-called maximal maneuver (M)]. M/P are normally higher after induced bronchoconstriction, because the bronchoconstriction can be at least partially reversed by the relaxing effect of a deep inspiration on bronchial tone, and this effect is maintained during the subsequent expiration (14).

### Materials and Methods

#### Subiects

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treatment consisted of three multivitamin tablets, each containing antioxidants: vitamin C (200 mg), vitamin E (d-α-tocopherol acetate, 90 mg = 90 IU), beta carotene (7.5 mg), selenium (62.5 μg), B vitamins, and minerals (bio-antioxidant version 2.1, PharmaNord). Additionally, five placebo capsules were given in this treatment. All tablets and capsules were taken at the same time during the evening.

Ozone Exposure

The subjects were exposed to either clean air or 300 parts/billion (ppb) of ozone for 4 days, 5 h each day, in a climate chamber. There was a 6-wk pause between the fish oil and vitamin studies, but only 1 wk between the studies in ozone and clean air. During each 5-h exposure, temperature and humidity were kept constant, and the subjects performed bicycle exercise for 15 min at a heart rate corresponding to a doubling of the ventilation. Ozone was generated by an ozone generator (BMT-802, Meerbusch, Germany). During the exposure day, the concentration of ozone was continually monitored by a photometric O₃ analyzer (API-M400, Instrumatic, Denmark).

Measurements

A number of physiological and subjective parameters were measured in the morning before exposure and then again in the afternoon after exposure (clean air or ozone). Those relevant for the present study are as follows.

Antioxidant status and cells measured in serum and nasal lavage.

Serum samples were obtained by venipuncture. Nasal lavage (NL) was performed by installation of 5 ml sterile isotonic saline in each nostril with the subject bending the head backward. The saline was recovered after 10 s, and volume was measured by differential weighing. DL-Dithiothreitol (7 mM) was then added to dissolve aggregated mucus, and cells were separated by centrifuging. The antioxidant status of serum measured as TEAC was not influenced by ozone, irrespective of pretreatment with either vitamins or fish oil.

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Statistics

If not otherwise stated, the data were analyzed by using a general linear model for repeated measurements, and, if not otherwise stated, the results are presented as means ± SE.

RESULTS

Chamber Variables

The results are presented as means ± SD. Over the entire period of ozone exposure, the concentration was 301.2 ± 2.3 ppb, with a minimum of 271 and a maximum of 322 ppb. On the clean air days, the values were 0.0 ± 1.1 ppb. The temperature was 22.1 ± 0.2°C on the ozone days and 22.1 ± 0.2°C on the clean air days. Correspondingly, the relative humidity was 45.3 ± 1.3 and 45.1 ± 1.7%, respectively.

Serum and NL Fluid

The antioxidant status of serum measured as TEAC was not significantly changed by exposure (P > 0.05), but, in the NL, there was a significant fall in the concentration of urate and TEAC (P < 0.05), due to a tendency for the FEV₁-to-FVC ratio (FEV₁/FVC) to decrease (P < 0.065). M/P also decreased (P = 0.05), due to a tendency for maximal flows to decrease (FEV₁/FVC, P = 0.044) or remain unchanged (F₃₀/FVC, P = 0.984) and partial flows to increase (FEV₁/FVC, P = 0.077; F₃₀/FVC, P = 0.034).

Nasal Cavity Dimensions

There was a significant decrease in nasal cavity dimensions during the day (minimum cross-sectional area: P = 0.011, nasal cavity volume from 0 to 5 cm: P < 0.001) with no influence of ozone exposure or pretreatment (P > 0.05) (Table 1).

Lung Function Changes During the Day

The results are shown in Table 2. Irrespective of ozone exposure and pretreatment, there was a decrease in most lung function parameters during the day, most significantly for FVC, FEV₁, and PEF (P < 0.001 for each of them). There was a tendency for the FEV₁-to-FVC ratio (FEV₁/FVC) to decrease (P = 0.065). M/P also decreased (P = 0.05), due to a tendency for maximal flows to decrease (F₃₀/FVC, P = 0.044) or remain unchanged (F₃₀/FVC, P = 0.984) and partial flows to increase (F₃₀/FVC, P = 0.077; F₃₀/FVC, P = 0.034).

The Effect of Ozone and Pretreatment

Irrespective of the pretreatment regimen, there was a larger fall on ozone days than on air days (Table 2) in FEV₁ (157 ± 55 ml, P = 0.012) and in FVC (220 ± 63 ml, P = 0.003), with...
this effect is in $P$. Difference in maximal instantaneous flow ($F$, maximal $fl$) for the different pretreatment regimens became marked after exercise, but was not in different between exposure groups ($\beta_{MEFV}$ curve decreased significantly). Monitoring after exposure showed that the PEF value returned to normal very quickly. Pretreatment regimen. Monitoring after exposure showed that the PEF value returned to normal very quickly.

Table 1. Baseline and change of antioxidant status in nasal lavage and of nasal cavity dimensions during exposure to clean air and ozone for the different pretreatment regimens

| Treatment | Exposure | Baseline (0 ppb ozone) | Change (300 ppb ozone) | P Value: Oxidant Effect
|-----------|----------|------------------------|------------------------|------------------------
| Urate, mmol/l | Air day | 8.44 ± 0.94 | 5.69 ± 0.90 | 9.44 ± 0.83 | −1.06 ± 0.83 | 0.001
| TEAC, mmol/l | Vitamins | 9.81 ± 0.86 | 3.13 ± 0.73 | 9.87 ± 0.96 | −2.47 ± 1.41 | 0.667
| | Fish oil | 26.30 ± 3.33 | 11.03 ± 4.81 | 18.90 ± 2.53 | 5.22 ± 4.19 | 0.009
| $A_{min}$, cm$^2$ | Vitamins | 1.26 ± 0.10 | −0.12 ± 0.06 | 1.36 ± 0.10 | −0.12 ± 0.08 | 0.770
| | Fish oil | 1.26 ± 0.10 | −0.14 ± 0.06 | 1.41 ± 0.12 | −0.20 ± 0.07 | 0.426
| $V_{0.5}$, cm$^3$ | Fish oil | 12.00 ± 0.55 | −1.36 ± 0.40 | 12.61 ± 0.50 | −1.38 ± 0.39 | 0.610

Values are means ± SE. ppb, Part billion; TEAC, trolox equivalent antioxidant capacity; $A_{min}$, minimum cross-sectional area; $V_{0.5}$, nasal cavity volume of the first 5 cm into the nasal cavity. Upper P value for each parameter indicates the statistical significance of the ozone effect; the lower P value indicates whether this effect is influenced by the pretreatment.

no influence on the FEV1/FVC ($P = 0.421$). There was no difference in maximal instantaneous flows measured on the maximal or partial flow-volume curves. PEF measured on the MEFV curve decreased significantly only in the group pretreated with fish oil, and this was the only measured effect of pretreatment. The change in PEF monitored with the PEF meters during exposure is shown in Fig. 1. It was statistically different between exposure groups ($P = 0.007$), with the effect becoming marked after exercise, but was not influenced by the pretreatment regimen. Monitoring after exposure showed that the PEF value returned to normal very quickly.

Table 2. Baseline lung function and change during exposure to clean air and ozone for the different pretreatment regimens

| Lung Function | Exposure | Baseline (0 ppb ozone) | Change (300 ppb ozone) | P Value: Oxidant Effect
|--------------|----------|------------------------|------------------------|------------------------
| FVC, liters | Air day | 4.99 ± 0.23 | −0.174 ± 0.077 | 4.95 ± 0.22 | −0.388 ± 0.070 | 0.003
| | Ozone day | 4.95 ± 0.22 | −0.254 ± 0.064 | 4.98 ± 0.24 | −0.479 ± 0.110 | 0.952
| FEV1, liters | Air day | 4.12 ± 0.13 | −0.121 ± 0.039 | 4.09 ± 0.14 | −0.256 ± 0.040 | 0.012
| | Ozone day | 4.13 ± 0.14 | −0.164 ± 0.034 | 4.12 ± 0.15 | −0.343 ± 0.081 | 0.594
| FEV1/FVC | Air day | 83.6 ± 2.0 | −0.37 ± 0.06 | 83.4 ± 1.7 | −1.57 ± 0.98 | 0.421
| | Ozone day | 84.3 ± 1.9 | −0.73 ± 0.72 | 83.9 ± 2.1 | −0.76 ± 1.09 | 0.532
| PEF, l/s | Air day | 9.91 ± 0.53 | −0.635 ± 0.111 | 9.64 ± 0.50 | −0.537 ± 0.148 | 0.039
| | Ozone day | 9.50 ± 0.51 | −0.364 ± 0.063 | 9.77 ± 0.52 | −0.899 ± 0.167 | 0.018
| $F_{40%FVC}$, l/s | Air day | 3.71 ± 0.22 | −0.013 ± 0.073 | 3.58 ± 0.21 | −0.142 ± 0.083 | 0.199
| | Ozone day | 3.77 ± 0.24 | −0.077 ± 0.075 | 3.65 ± 0.23 | −0.185 ± 0.099 | 0.890
| $F_{50%FVC}$, l/s | Air day | 3.59 ± 0.27 | 0.044 ± 0.254 | 3.59 ± 0.27 | 0.315 ± 0.221 | 0.395
| | Ozone day | 3.87 ± 0.34 | 0.039 ± 0.280 | 3.56 ± 0.27 | 0.384 ± 0.189 | 0.501
| M | Air day | 2.66 ± 0.18 | 0.014 ± 0.066 | 2.56 ± 0.17 | 0.098 ± 0.092 | 0.733
| | Ozone day | 2.78 ± 0.18 | 0.038 ± 0.080 | 2.66 ± 0.19 | −0.070 ± 0.087 | 0.464
| P | Air day | 2.56 ± 0.23 | 0.071 ± 0.200 | 2.53 ± 0.22 | 0.415 ± 0.215 | 0.161
| | Ozone day | 2.73 ± 0.23 | 0.062 ± 0.217 | 2.51 ± 0.23 | 0.466 ± 0.193 | 0.546
| M/P | Air day | 1.058 ± 0.042 | −0.022 ± 0.066 | 1.025 ± 0.044 | −0.102 ± 0.043 | 0.018
| | Ozone day | 0.974 ± 0.046 | −0.070 ± 0.037 | 1.051 ± 0.042 | −0.149 ± 0.042 | 0.090
| 40% FVC | Air day | 1.087 ± 0.052 | −0.035 ± 0.082 | 1.054 ± 0.051 | −0.106 ± 0.060 | 0.070
| | Ozone day | 0.998 ± 0.041 | −0.069 ± 0.045 | 1.097 ± 0.051 | −0.187 ± 0.051 | 0.597

Values are means ± SE. FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; FEV1/FVC, ratio of FEV1 to FVC; PEF, peak expiratory flow; F, maximal flow from the flow-volume curve, $F_{40%FVC}$, $F_{50%FVC}$, F at 40% FVC, $F_{40%FVC}$, F at 30% FVC; M, flow after maximal inspiration; P, flow after partial inspiration; M/P, ratio of M to P. Upper P value for each parameter indicates the statistical significance of the ozone exposure; the lower P value indicates whether this effect is influenced by the pretreatment.
histamine was smaller after exposure to ozone than after air by significantly larger after pretreatment. This decrease was not influenced by ozone. It was significantly larger after pretreatment with fish oil than after vitamins (P < 0.002) but only for the partial flows. After histamine inhalation, the M/P40%FVC increased significantly by 0.23 ± 0.06 (P = 0.002). This is a combined estimate for air and ozone days (see Fig. 2). Table 3 shows that both ozone and pretreatment had a borderline influence on that increase. In the evening before the histamine provocation test, the M/P was significantly smaller after ozone than after air (see above), but after the largest dose of histamine there was no difference (P = 0.41). After ozone, therefore, inhalation of histamine increased the M/P40%FVC more than after air (0.11 ± 0.05, P = 0.066). There was no effect of pretreatment on the M/P.

DISCUSSION

Factors Influencing the M/P

Whereas a fall in FEV1 and FVC after exposure to ozone is a well-recognized phenomenon (2, 4, 18, 19, 24, 41), the influence of ozone on the M/P has not been previously described. During the day, M/P decreased, but it decreased more when exposed to ozone than when exposed to air.

The M/P is determined by the wave-speed flow equation, which states that the Vmax = A^2/(ρ Caw)^0.5 (10) or

\[ \text{M/P} = \left( A_d/A_p \right)^{0.5} \left( \text{Caw}_d/\text{Caw}_p \right)^{0.5} \]

where A is airway cross-sectional area, Caw is airway compliance at the choke point (CP), which is the flow-determining point in the airway during a forced expiration, and ρ is the gas density, which is identical under the two circumstances and cancels out by the division. The lung elastic recoil pressure (PeL,L) and the upstream frictional pressure loss from the alveolus to CP (Pfr) are indirect determinants of the maximal flow, because PeL,L − Pfr influences the distending pressure at CP and, therefore, A and Vmax. A deep inspiration diminishes the PeL,L (3, 11, 12), in both normal and asthmatic subjects. There is, furthermore, recent evidence that a deep inspiration increases the A and the Caw at CP (33). The direction of change in the M/P depends on the relative changes in A, Caw, and PeL,L − Pfr. Profound changes in all parameters by a deep breath can occur without changing M/P, if the effect of decreased PeL,L − Pfr and increased A and Caw balance out each other. Therefore, a decreased M/P is not synonymous with a decreased ability to dilate narrowed airways, because the effect might as well be due to relative changes of PeL,L.

The Effect of Ozone

Ozone exposure may influence the effect of a deep inspiration via all of the parameters above. Hazucha et al. (19) have found that ozone exposure increases PeL,L at a given lung volume. If this is so, both before and after a deep inspiration, the M/P may not be changed. In the present study, a deep inhalation did not cause any significant change in the maximum flow after ozone exposure, although Table 2 indicates insignificant increases in partial flows compared with unchanged maximal flows, leading to the significant decrease of the M/P. This can be explained by larger decrease in PeL,L and/or a smaller increase in airway area with the deep breath. In other words, the direction of change of the M/P depends on the relative hysteresis of airway area and lung elastic recoil, determined by the volume history, as described for the relationship between dead space and lung volume by Froeb and Mead (16). The decreased ability of a deep inspiration to increase flow is a matter of intense research, which is illustrated in recent reviews (5, 6, 9, 13). Most interest is focused on the airway smooth muscle, which, if contracted, will relax during a deep inspiration. If the relaxation does not last long enough, the effect of a deep inspiration will be minimal (elastic behavior of the muscles); if it lasts longer (during the entire expiration time), then the effect will be more marked (plastic behavior). The balance between elastic and plastic behavior.

![Fig. 1. Change of peak expiratory flow during exposure to clean air and 300 parts/billion (ppb) ozone. As there was no effect from the 2 pretreatment regimens, the values for the 2 treatments have been averaged. Values are means ± SE. After exercise, the 2 curves are clearly separated (see text).](http://jap.physiology.org/)

![Fig. 2. Change in maximal-to-partial flow ratio (M/P) at 40% forced vital capacity during exposure to either clean air or 300 ppb ozone. Values are means ± SE. During the day, M/P decreased significantly more on ozone than on air (P = 0.018). After histamine, there was a significant increase in M/P, but, at the maximum dose, there was no difference between air and ozone exposure (see text).](http://jap.physiology.org/)
may be influenced by inflammation (13), which may, therefore, change the M/P. The possible mechanisms involved have been presented by Ingram (22), based on the analysis of Froeb and Mead (16). In subjects with asthma, higher levels of inflammatory markers were found to be associated with lower M/P (22). In the present study, we found a smaller M/P at 40% FVC after ozone exposure than after air, which might indicate an effect of inflammation in the airways influencing the volume history of A and Caw and/or in the parenchyma influencing the volume history of Pel.1 — Pfr.

Before jumping to the conclusion that the smaller M/P after ozone is explained by inflammation, other possible causes should be explored. The main reason for ozone-induced decrease of FEV1 and FVC is believed to be decreased inspiratory capacity (IC) and, consequently, decreased TLC without any increase in residual volume (RV) or functional residual capacity (19). Because the effect could be partly blocked by the inhalation of lidocaine, the mechanism behind this was suggested to be through stimulation of airway receptors and/or nerve endings, leading to an involuntary inhibition of full inspiration. Pretreatment with atropine seems to attenuate the flow responses of ozone but does not significantly affect IC (1), indicating that the effect is not purely vagal.

Unfortunately, the design of the study did not allow us to include measurements of TLC. Such measurement would have told us whether FVC became smaller due to decreased IC and/or increased RV. If a decreased IC were the only effect, one would expect a parallel change in FEV1 and FVC, so that the FEV1/FVC percentage would not change, just as we actually found. To explore this finding further, we examined the slopes of the MEFV curves between 40% and 30% FVC. Ozone did not influence the slopes of the maximal and partial curves differently. Similarly, the intercept of the lines through the two points and the volume axis for maximal and partial curves were not different, indicating no change of RV. A decreased IC is, therefore, a likely explanation for the ozone-induced changes in FEV1 and FVC, but is inconsistent with a decrease of the M/P, because this ratio will remain unchanged if both slope and intercept remain unchanged. The reason for this inconsistency may be of a statistical nature.

If the point of full inflation on the maximum loop occurs at a lower volume, it is a question whether this will influence the effect of a deep inspiration. In our study, the fall in FVC on ozone days was 220 ml. If the main effect of a deep inhalation depends on coming very close to TLC then that might explain the smaller M/P flow after ozone. Brown and Mitzner (5) summarize previous research and conclude that, in airways with little smooth muscle tone, even moderate lung inflation readily distends airways to their maximal size. This was shown to be true in experimental animal models, even when airways were made edematous. If this is true also for our healthy subjects, the fact that the inspiration only reaches TLC — 220 ml should not mean much.

### Evidence for Inflammation

Many studies (18, 19, 24, 26) have found evidence of inflammation. Therefore, it is likely that it is not reflex inhibition of inspiration that alone explains our findings. The airways may also directly be influenced by ozone. Increased bronchomotor tone alone is not likely, because, as previously mentioned, this would rather increase the M/P. Inflammatory changes causing edema of the airway wall are a more likely possibility. This may also explain a statistically significant increase in RV, although small, after ozone in the study of Hazucha et al. (19), who did not pay attention to this. Animal experiments have shown that exposure to ozone increases the amount of epithelial lining fluid (8), inactivates surfactant by edema fluid (32), and increases the ratio of wet lung weight to body weight (29). Others have found characteristic lesions in the region of terminal bronchioles and central acinar alveoli marked by peribronchiolar edema (17). All of these effects may be related to inflammation and diminish the effect of a deep inspiration.

The next point to consider is whether our study showed evidence of inflammation. In the present study, we could not
perform BAL to examine for inflammatory markers in BAL fluid. We did not consider induced sputum to be a possibility, because another purpose of the study (to be reported elsewhere) was to measure subjective effects of ozone. We chose instead to measure the NL fluid. There is, however, conflicting evidence of whether findings in NL reflect findings in BAL. Koren et al. (23) exposed normal volunteers to 0.4 parts/million ozone for 2 h and found a six- to eightfold increase in polymorphonuclear neutrophils in both NL fluid and BAL fluid. This was present immediately postexposure and still detectable after 18 h. This is in contrast to a study of smokers and nonsmokers (15), where a number of inflammatory markers were increased in BAL fluid, reaching a maximum 18 h after the ozone exposure, but the findings in NL fluid did not mirror these findings in BAL fluid. In our study, we did not find any inflammation markers in the NL fluid, so there is no direct evidence of inflammation.

We did, indeed, find that ozone exposure caused a significant fall in the urate concentration in NL fluid, in agreement with Mudway et al. (31), who found a similar decrease of urate in NL fluid after exposure to 200 parts/million ozone for 2 h with intermittent exercise. Urate protects membranes and linolenic acid from ozone-induced oxidation (27). The decrease of the urate concentration may, therefore, be due to urate being oxidized by ozone. We found a similar significant pattern for TEAC, but with an almost significantly larger fall during ozone exposure than during air when pretreated with fish oil (see Table 1), supporting the hypothesis that fish oil increases the oxidative stress. For economical reasons, NL was done only immediately after exposure in our study, and this may be too early to detect the manifestations of inflammation (15). Our findings, therefore, do not exclude that inflammation was initiated. Furthermore, inflammatory markers are more common in subjects with bronchial hyperresponsiveness (39), and such subjects were excluded from the present study. Our measurements of nasal cavity dimensions during ozone exposure did not reveal any change in mucosal congestion. This confirms a study by McBride et al. (25), who used posterior rhinomanometry to examine nasal function in asthmatic subjects before and after ozone exposure and found no change.

BR

We did not find a significant increase in BR following exposure to ozone, contrary to the findings of other studies (24, 34). FEV₁ corresponding to a dose of 0.5-mg histamine was smaller after ozone than after air by -200 ml, which is almost identical to the difference before histamine. It, therefore, can be attributed to the lower level at the beginning of the test and not to increased BR. However, we excluded from our study any subjects with a fall of >10% in FEV₁ with a maximal dose of 1.70 mg histamine. Because an increase in BR is mostly seen in subjects with asthma or with bronchial hyperresponsiveness, this may be the reason why our subjects showed no significant increase in BR.

The Effect of Pretreatment

We expected that pretreatment with vitamins and fish oil would give a larger contrast, if the ozone effect is due to oxidative stress, because vitamins are antioxidative and fish oils create oxidative stress (35). Studies by Samet et al. (37) have shown that, after a diet low in antioxidants, supplementary antioxidants (250 mg vitamin C, 50 IU α-tocopherol + vegetable cocktail daily for 2 wk) increased FEV₁ and FVC after ozone exposure of healthy human subjects compared with placebo treatment. It is quite possible that the initial antioxidant status is important for the outcome.

Animal experiments and clinical intervention studies indicate that omega-3 fatty acids, which are found in fish oil, have anti-inflammatory properties and so might be useful in the management of inflammatory and autoimmune diseases (7, 40). These findings are supported by an epidemiological study showing that increased intake of fish is associated with higher lung function in adults (38). The equivalent dose of omega-3 fatty acids or EPA was not reported, but, in experimental studies, up to 4 g of EPA have been given. Longitudinal data do not support the beneficial effects of EPA (36). The effect of fish oil is brought about by modulation of the amount and types of eicosanoids produced and also by eicosanoid-independent mechanisms, which include actions on intracellular signaling pathways, transcription factor activity, and gene expression. If the ozone effect is due to inflammation, it might be that the anti-inflammatory effect of fish oils attenuated the ozone effect rather than increased it, as we had originally anticipated. Ideally, a third session, in which subjects were depleted in vitamins, should have been included. In that case, a better contrast with regard to inflammatory effects might have been obtained, but that would have made the study much more expensive.

We found, however, in accordance with our original hypothesis, an interaction effect of pretreatment on the PEF measured on the flow-volume curves: a decrease of PEF was found on ozone days with pretreatment with fish oil, but not on days with vitamin pretreatment. However, as no effect was found in the other parameters measured on the flow-volume curve, we did not put too much emphasis on this finding. It is interesting to note that pretreatment with fish oil increased the histamine response in the partial maneuvers, independent of ozone. Table 1 shows that ozone influences the antioxidant status by causing a fall in both urate and TEAC and that treatment with fish oil gave an almost significantly more pronounced fall in TEAC than treatment with vitamins. This points to increased oxidative stress as a possible mechanism behind the observed findings.

In conclusion, exposure to 300 ppb of ozone in a climate chamber decreased both FEV₁ and FVC without changing the FEV₁/FVC percentage. In addition, ozone reduced the effect of a deep inspiration on maximal flow. Despite failing to detect either raised levels of inflammatory markers in NL, inflammatory changes in the airway and lung may still explain the reduced bronchodilator effect of deep inspiration, although an impaired inspiration to TLC may play a part. There was indication of increased oxidative stress after pretreatment with fish oil compared with vitamins, but this caused no systematic change in lung function, although PEF decreased more during ozone than during air exposure, and maximum flows decreased more during histamine provocation when the subjects were pretreated with fish oil than with vitamins.

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