Double-Blind Intervention Trial on Modulation of Ozone Effects on Pulmonary Function by Antioxidant Supplements

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The aim of this study was to investigate whether the acute effects of ozone on lung function could be modulated by antioxidant vitamin supplementation in a placebo-controlled study. Lung function was measured in Dutch bicyclists (n = 38) before and after each training session on a number of occasions (n = 380) during the summer of 1996. The vitamin group (n = 20) received 100 mg of vitamin E and 500 mg of vitamin C daily for 15 weeks. The average ozone concentration during exercise was 77 μg/m³ (range, 14–186 μg/m³). After exclusion of subjects with insufficient compliance from the analysis, a difference in ozone exposure of 100 μg/m³ decreased forced expiratory volume in 1 second (FEV₁) 95 ml (95% confidence interval (CI) –265 to –53) in the placebo group and 1 ml (95% CI –94 to 132) in the vitamin group; for forced vital capacity, the change was –125 ml (95% CI –384 to –36) in the placebo group and –42 ml (95% CI –130 to 35) in the vitamin group. The differences in ozone effect on lung function between the groups were statistically significant. The results suggest that supplementation with the antioxidant vitamins C and E confers partial protection against the acute effects of ozone on FEV₁ and forced vital capacity in cyclists. Am J Epidemiol 1999; 149:306–14.

Acute effects of ozone on lung function have been studied in exercising subjects. Most of these studies were conducted under controlled laboratory conditions (1–7). All of these chamber studies showed that lung function decreased after exposure to high levels of ozone (160–700 μg/m³). However, low levels of ambient ozone (<120 μg/m³) were also negatively related to lung function (8) in exercising cyclists.

In addition to decrements in lung function, which occur within 1 hour after acute ozone exposure, an increase in levels of inflammatory mediators such as neutrophils and prostaglandins was shown in bronchoalveolar lavage within 1 hour (9, 10), 6 hours (11), 18 hours (9, 10), and 24 hours (11) of exposure. This increase in inflammatory mediators was different in time for each mediator (9–11). Antioxidants, such as vitamin C and E, could modulate the airway response to ozone by reducing the recruitment of inflammatory cells into the airway lumen (12). Since lung function decrements after ozone exposure occur earlier in time than the increase in inflammatory cells, another hypothesis could explain the modulation of ozone effects on lung function: Maximal inspiratory capacity is reduced through stimulation of the neural receptors in the upper airways by cyclooxygenase products of arachidonic acid which are released upon ozone exposure (13). Vitamins C and E have been shown to affect this arachidonic acid metabolism, but the role of these vitamins in this mechanism is not fully understood (14–16).

Few experimental studies have investigated a possible protective role of antioxidants in the acute effects of ozone. One study showed that supplementation with vitamin C and a combination of vitamins C and E protected against decrements in lung function in subjects exposed to ozone concentrations of 600 μg/m³ (14). Another study did not show a protective role of vitamin E alone in lung function after exposure to 1,000 μg/m³ of ozone (17).

We explored possible modulation of the acute effects of ambient ozone by antioxidants in a placebo-controlled study, supplementing cyclists with the antioxidant vitamins C and E and measuring lung function on a number of occasions in the Netherlands during the summer of 1996.
MATERIALS AND METHODS

Members of two bicycling clubs in Apeldoorn and Nijmegen, located in the eastern part of the Netherlands, were asked to participate in the study. Both amateur and recreational cyclists from these clubs participated. A recreational club in Malden, also located in the eastern part of the Netherlands, volunteered to participate after an advertisement was placed for new subjects. From these three clubs, 46 nonsmoking subjects volunteered and were randomly assigned to the placebo group or the vitamin group. An earlier study (8) of 23 cyclists with an average of 12 measurements per cyclist suggested an effect of ozone at relatively low levels. Therefore, the number of subjects in our study (n = 46) was designed to allow us to detect an effect of ozone in the control group or the absence of an effect in the vitamin group.

From May 20, 1996, to the end of August 1996, lung function was measured in each subject on a number of occasions, before and after each training session or competitive race. Most training sessions and races took place in rural areas in the late afternoon or early evening, when ozone levels tend to peak. The exact times of each exercise period were recorded for each participant for calculation of individual ozone exposure (mean ozone concentration during exercise and 8-hour mean ozone concentration before the postexercise lung function measurement) and duration of exercise.

Daily concentrations of both ozone and particulate matter with a 50 percent cutoff diameter of 10 μm (PM₁₀) were obtained from the nearest monitoring stations (Loenen and Wageningen) of the National Air Quality Monitoring Network, which is operated by the National Institute of Public Health and the Environment (Bilthoven, The Netherlands). Meteorologic data were obtained from the nearest station (Deelen) operated by the Royal Netherlands Meteorological Institute (The Bilt, The Netherlands). Airborne pollen concentrations were obtained from the Department of Lung Diseases, Academic Hospital (Leiden, The Netherlands).

At the beginning of the study, subjects completed a questionnaire that asked about chronic respiratory symptoms and other relevant characteristics. The vitamin group (n = 20) received a daily dose of 500 mg of vitamin C and 100 mg of vitamin E in two capsules, starting 1 week before the measurements, for a total duration of 15 weeks (May–August). Subjects were instructed to stop taking their own vitamin supplements (three subjects used daily multivitamins, vitamin C, or vitamin E) at least 1 week before the start of the study. The vitamin capsules and placebos were handmade, were visually identical, and were each given a code which was not known to the involved researchers and subjects. At the end of the study, the subjects returned the remainder of the capsules (a supply for 20 weeks was given) so compliance could be estimated. Besides counting of the capsules, an additional measure of compliance was evaluated: Subjects were asked at the end of the study if they had stopped taking the capsules for more than 1 week consecutively. Plasma was collected before supplementation and at the end of supplementation for determination of α-tocopherol and vitamin C levels as additional markers of compliance.

Blood specimens were collected in Vacutainer tubes (Becton-Dickinson, Rutherford, New Jersey) containing ethylenediaminetetraacetic acid, stored in a box on ice, and centrifuged within 5 hours to obtain plasma. Aliquots were then stored at −80°C until the time of analysis. Concentrations of α-tocopherol were measured in each subject at baseline and after supplementation by reverse-phase high performance liquid chromatography in one run. The method was adapted from that of Hess et al. (18). The column was a prepackaged 25-cm x 4.6-mm Vydac 201TP54, C₁₈, 300 Å (Vidac, Hesperia, California). The mobile phase consisted of methanol-tetrahydrofuran-water in the following configuration (percent volume/volume/volume): 0 minutes at 89:2:9; 10 minutes at 98:2:0; 20 minutes at 97:3:0; 30 minutes at 90:10:0; and 40 minutes at 90:10:0 with a flow rate of 1 ml/minute. Detection after separation was executed using an ultraviolet light detector (UV1000; Thermo Separation Products, San Jose, California) for determination of tocopherol levels at wavelengths of 325 nm for 0–9 minutes and 292 nm for 9–40 minutes. Plasma vitamin C was analyzed at baseline and at the end of the intervention period by a modification of the method developed by Roe and Kuether (19).

As a measure of exertion, continuous heart rate measurements were made on a number of different occasions in all subjects during training sessions and competitive races, using Polar heart rate devices (Polar Electro Oy, Kempele, Finland). With these measures of mean heart rates during exercise, the volumes of inhaled air were estimated according to the equation used in the study by Colucci (20) for subjects with an age range of 21 to 60 years.

Lung function was measured before and after cycling with a heated pneumotachometer (Jaeger, Wuerzburg, Germany). The flow-volume and volume-time curves were observed on the attached computer for all maneuvers during measurement; all data were automatically saved on the computer. Ambient temperature at the location of testing was measured and controlled for during the measurements by entering the...
temperature into the computer. Following calibration with a 1-liter syringe, the researchers measured their own lung function twice at the same time as the cyclists, to check for abnormalities in the equipment. All lung function tests were conducted indoors, at most 60 minutes before and on average 17 minutes after (range, 5–60 minutes) the exercise. Pre- and postexercise lung function were measured on the same pneumotachometer. Measurements were performed according to the guidelines of the European Respiratory Society (21); subjects were seated in an upright posture, with a fixed mouthpiece adjusted for the individual’s height, and a nose clip was used. For each measurement, subjects had to perform at least three technically acceptable forced maneuvers, of which two were reproducible (according to 1993 European Respiratory Society criteria).

In data analysis, maximum values of the three maneuvers comprising each measurement were used for each subject. The following lung function parameters were considered: forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC), peak expiratory flow (PEF), and maximal midexpiratory flow (MMEF). Before data analysis, subjects had to meet two criteria in order to be included in the individual regression analysis, for prevention of the occurrence of unstable regression coefficients. First, the subject had to have at least four observations—that is, four lung function measurements before and after exercise (pre- and postexercise lung function, respectively). Second, each subject had to have a range in ozone or PM₁₀ exposure of >40 μg/m³. All subjects fulfilled this second criterion for ozone exposure during exercise and for 8-hour mean ozone exposure. Six subjects had a range of PM₁₀ exposures of <40 μg/m³ and were excluded from the analysis of PM₁₀ concentrations in relation to lung function. PM₁₀ concentrations were calculated as 24-hour mean concentrations previous to the postexercise lung function measurement. In addition, PM₁₀ concentrations with lag times of 1 and 2 days were calculated. Twenty-four-hour mean PM₁₀ concentrations were missing for 3 days, but all subjects still had at least four observations for the analysis of PM₁₀ in relation to lung function.

Data were analyzed using SAS software, version 6.12 (SAS Institute, Inc., Cary, North Carolina). For each subject, delta lung function (postexercise lung function minus preexercise lung function), as the dependent variable, was regressed on ozone concentration (in μg/m³; 1 μg/m³ of ozone = 0.5 parts per billion) during exercise. We also considered, for each subject, postexercise lung function as the dependent variable with the previous 8-hour mean ozone concentration as the independent variable, because exposure to ozone prior to exercise could have affected the preexercise lung function levels. The resulting individual regression coefficients were pooled in both analyses, and medians and means (with standard errors) were calculated for the total group and for the control (placebo) and vitamin groups separately. In addition, weighted group means were calculated with the inverse of the variance of the individual regression coefficients used as the weight. Ninety-five percent confidence intervals around the median regression coefficients were calculated using a nonparametric method published by Campbell and Gardner (22). Differences between the vitamin group and the control group were tested for statistical significance (p < 0.05) with the Wilcoxon rank-sum test.

The following potential confounders were taken into account in the regression analyses: ambient temperature, absolute humidity, PM₁₀ concentration with lag times of 0, 1, and 2 days, pollen concentrations (Poaceae (grass) and Betula (birch)), and an interaction term of ozone x PM₁₀ (a dummy variable for PM₁₀ with a cutoff point at the median x the ozone concentration). Pearson coefficients for correlations between the independent variables were calculated. There were too few subjects with self-reported asthma (n = 4) and/or inhalant allergies (n = 8) to analyze this subgroup separately. Therefore, we examined whether the regression coefficients were affected when asthmatic or allergic subjects were excluded from analyses. The effect of insufficient compliance was considered by excluding subjects who returned too few or too many capsules (equivalent to >110 percent or <80 percent of the planned cumulative dose, respectively). In addition, subjects who reported that they had not taken the capsules for 3 consecutive weeks or more were excluded.

RESULTS

Of the 46 study subjects, eight were excluded from the analyses; five subjects had less than four measurements, one subject dropped out immediately because he could not swallow the capsules, and two subjects were not able to perform reproducible lung function measurements.

The 38 remaining subjects had a total of 380 lung function measurements (i.e., pre- and postexercise lung function measurements) with a range of 5–19 measurements per subject. Three subjects were female, with one female subject in the control group. Eight subjects reported a doctor-diagnosed allergy to pets, house dust mites, or pollen; four subjects reported doctor-diagnosed asthma. A slightly higher number of allergic (n = 5) and asthmatic (n = 3) subjects were allocated to the vitamin group than to the control group (three allergic subjects and one asthmatic subject). The
TABLE 1. Mean values and ranges for pertinent characteristics and measurements in a study of antioxidants' modulation of the effects of air pollution on lung function among 38 Dutch cyclists, 1996

<table>
<thead>
<tr>
<th>Characteristic or measurement</th>
<th>Control group (n = 18) Mean</th>
<th>Range</th>
<th>Vitamin group (n = 20) Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.1</td>
<td>17–58</td>
<td>33.8</td>
<td>16–59</td>
</tr>
<tr>
<td>Baseline lung function</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1* (liters)</td>
<td>4.71</td>
<td>3.58–6.05</td>
<td>4.58</td>
<td>2.73–7.23</td>
</tr>
<tr>
<td>FVC* (liters)</td>
<td>5.86</td>
<td>4.18–7.01</td>
<td>5.75</td>
<td>3.89–7.98</td>
</tr>
<tr>
<td>No. of observations</td>
<td>10.2</td>
<td>5–19</td>
<td>9.9</td>
<td>5–17</td>
</tr>
<tr>
<td>Compliance (%)†</td>
<td>96.8</td>
<td>70–130</td>
<td>86.1</td>
<td>53–101</td>
</tr>
<tr>
<td>Ozone concentration during exercise (μg/m³)‡</td>
<td>75.4</td>
<td>17–181</td>
<td>78.4</td>
<td>14–186</td>
</tr>
<tr>
<td>8-hour mean ozone concentration (μg/m³)‡</td>
<td>84.2</td>
<td>32–199</td>
<td>88.2</td>
<td>33–199</td>
</tr>
</tbody>
</table>

* FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity.
† Compliance for each subject was expressed as percentage of the number of capsules which he/she should have taken.
‡ 1 μg/m³ = 0.5 parts per billion.

prevalence of respiratory symptoms and the use of vitamin supplements prior to the start of the study did not differ between the groups.

Table 1 shows mean age, baseline FEV1 and FVC, numbers of measurements made, compliance (assessed by counting the number of returned capsules), ozone concentration during exercise, and 8-hour mean ozone concentration. There was no difference in means or medians (results not shown) between the groups, except for compliance: The control group had somewhat higher compliance (97 percent vs. 86 percent). Of these 38 subjects, 33 had data on plasma α-tocopherol levels before and after supplementation. The mean baseline plasma α-tocopherol level was 27.2 (standard error 1.17) mmol/liter, and the mean plasma vitamin C level was 81.2 (standard error 2.9) μmol/liter; neither value differed between the two study groups. After supplementation, the mean of the individual changes for plasma α-tocopherol was 3.8 percent (range, −12 percent to 19 percent) in the control group (n = 15) and 48.4 percent (range, −2 percent to 134 percent) in the vitamin group (n = 18). For plasma vitamin C, the mean individual changes were −4.3 percent (range, −58.1 percent to 37.3 percent) in the control group and 4.1 percent (range, −18 percent to 49 percent) in the vitamin group.

Figure 1 shows the mean 1-hour maximum ozone concentration for each day between May 1, 1996, and the end of August 1996. The average ozone concentration during exercise was 77 μg/m³ (range, 14–186 μg/m³). Mean ambient temperature during exercise was 17°C, with a range of 10–25°C. The 8-hour mean temperature was 19°C, with a range of 12–28°C. Mean absolute humidity was 13 mg/liter of air, with a range of 9–20 mg/liter of air. The average 24-hour mean PM10 concentration was 41 μg/m³, with a range of 13–144 μg/m³. The Pearson correlation coefficient for the correlation of 8-hour mean ozone concentration with 8-hour mean temperature was 0.59; that for the correlation with 8-hour mean absolute humidity was 0.28, and that for 24-hour mean PM10 concentration was 0.37. The mean duration of exercise, 104 minutes (range, 20–216 minutes), did not differ between the two groups. Heart rate measurements were made on 315 different occasions; the average heart rate for training sessions was 141 beats per minute (range, 83–182 beats per minute), and that for competitive races was 173 beats per minute (range, 156–187 beats per minute). There was again no difference between the two groups. Mean estimated volumes of inhaled air were 55 liters/minute during training sessions and 92 liters/minute during races (20).

Table 2 compares ozone effects in the vitamin group with those in the control group for both delta lung function and postexercise lung function. There was a significant negative effect of ozone on delta FEV1 and delta FVC but not on delta PEF and delta MMEF. Ozone exposure was also negatively associated with postexercise FEV1, FVC, and PEF. Further analyses
TABLE 2. Median regression coefficients for the regression of ozone on delta lung function and postexercise lung function in 38 Dutch cyclists and in the control (n = 18) and vitamin (n = 20) groups separately, 1996

<table>
<thead>
<tr>
<th>Lung function parameter</th>
<th>Total group</th>
<th>Control group</th>
<th>Vitamin group</th>
<th>Study group</th>
<th>Total group</th>
<th>Control group</th>
<th>Vitamin group</th>
<th>Study group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta lung function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV,*</td>
<td>-0.99</td>
<td>-1.03</td>
<td>-1.11</td>
<td>-0.99</td>
<td>-1.05</td>
<td>-1.07</td>
<td>-0.97</td>
<td>-0.95</td>
</tr>
<tr>
<td>FVC*</td>
<td>-1.14</td>
<td>-1.24</td>
<td>-3.42</td>
<td>-1.14</td>
<td>-1.05</td>
<td>-4.01</td>
<td>-3.80</td>
<td>-4.36</td>
</tr>
<tr>
<td>PEF*</td>
<td>-1.14</td>
<td>-1.03</td>
<td>-2.86</td>
<td>-1.03</td>
<td>-1.10</td>
<td>-0.43</td>
<td>-1.69</td>
<td>-0.28</td>
</tr>
<tr>
<td>MMEF*</td>
<td>-1.11</td>
<td>-2.04</td>
<td>-4.43</td>
<td>-2.04</td>
<td>-1.10</td>
<td>-4.43</td>
<td>-4.32</td>
<td>-2.99</td>
</tr>
<tr>
<td>Postexercise lung function†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV*</td>
<td>-0.68</td>
<td>-0.68</td>
<td>-0.43</td>
<td>-0.68</td>
<td>-0.85</td>
<td>-0.43</td>
<td>-1.69</td>
<td>-0.28</td>
</tr>
<tr>
<td>PEF*</td>
<td>-8.05</td>
<td>-8.05</td>
<td>-8.07</td>
<td>-8.05</td>
<td>-6.08</td>
<td>-8.07</td>
<td>-6.64</td>
<td>-7.29</td>
</tr>
</tbody>
</table>

* FEV*, forced expiratory volume in 1 second; FVC, forced vital capacity; PEF, peak expiratory flow; MMEF, maximal midexpiratory flow.† Median coefficient for the regression of postexercise lung function minus preexercise lung function on ozone during exercise, in ml per mg/m² of ozone for FEV, and FVC and in ml/second per mg/m² of ozone for PEF and MMEF.‡ Median coefficient for the regression of postexercise lung function on 8-hour mean ozone level, in ml per mg/m² of ozone for FEV, and FVC and in ml/second per mg/m² of ozone for PEF and MMEF.

...were concentrated on FEV, and FVC, which were significantly different from the null value (results not shown).
TABLE 3. Median regression coefficients (in ml per μg/m³ of ozone) for the regression of postexercise forced expiratory volume in 1 second (FEV₁) and forced vital capacity (FVC) on 8-hour mean ozone levels in a group of Dutch cyclists, by study group, after several different adjustments, 1996

<table>
<thead>
<tr>
<th>Adjustment/exclusion and lung function parameter</th>
<th>Total group (n = 38)</th>
<th>Study group</th>
<th>Control (n = 18)</th>
<th>Vitamin (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjustment for 24-hour mean PM₁₀*</td>
<td>Median 95% CI</td>
<td></td>
<td>Median 95% CI</td>
<td>Median 95% CI</td>
</tr>
<tr>
<td>FEV₁</td>
<td>-0.71 -1.64 to -0.35</td>
<td>-1.36 -2.80 to -0.35</td>
<td>-0.65 -1.64 to 1.20</td>
<td></td>
</tr>
<tr>
<td>FVC</td>
<td>-1.33 -2.06 to -0.30</td>
<td>-1.89 -2.75 to -0.82</td>
<td>-0.11 -1.93 to 0.74†</td>
<td></td>
</tr>
<tr>
<td>Adjustment for 8-hour mean ambient temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁</td>
<td>-1.25 -1.94 to -0.20</td>
<td>-1.58 -2.63 to -0.44</td>
<td>-0.08 -3.21 to 2.06</td>
<td></td>
</tr>
<tr>
<td>FVC</td>
<td>-1.89 -2.66 to -0.46</td>
<td>-1.89 -3.03 to -1.31</td>
<td>-0.37 -4.41 to 0.97</td>
<td></td>
</tr>
<tr>
<td>Exclusion of insufficient compliers‡ (unadjusted)</td>
<td>(n = 28)</td>
<td>(n = 14)</td>
<td>(n = 14)</td>
<td></td>
</tr>
<tr>
<td>FEV₁</td>
<td>-0.67 -0.95 to 0.24</td>
<td>-0.95 -2.65 to -0.53</td>
<td>-0.01 -0.94 to 1.32†</td>
<td></td>
</tr>
<tr>
<td>FVC</td>
<td>-1.00 -1.30 to -0.36</td>
<td>-1.25 -3.84 to -0.36</td>
<td>-0.42 -1.30 to 0.35†</td>
<td></td>
</tr>
</tbody>
</table>

* PM₁₀, particulate matter with a 50 percent cutoff diameter of 10 μm.
† Vitamin group differs significantly from control group (p < 0.05, Wilcoxon rank-sum test).
‡ See "Materials and Methods."

Figure 2 shows the distribution of the crude regression coefficients for the regression of 8-hour mean ozone level on postexercise FEV₁ in all subjects (part A) and in good compliers only (part B). Figure 3 shows the same distributions for FVC in all subjects (part A) and in good compliers (part B). These figures illustrate that the distribution of regression coefficients for the regression of ozone level on FEV₁ and FVC was shifted upwards in the vitamin group compared with the control group and that this became clearer after exclusion of the insufficient compliers.

Figure 2. Boxplots for the regression of 8-hour mean ozone level on postexercise forced expiratory volume in 1 second (ml per μg/m³) in vitamin-using cyclists and control cyclists, The Netherlands, 1996. Shown are the median value (center line in box), the mean value (+), the 25th and 75th percentiles, i.e., the interquartile range (borders of the box), the range of values (vertical lines), and outliers. (A) all subjects; (B) good compliers only.

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DISCUSSION

The results of this study show that relatively low concentrations of ozone were associated with a decrease in postexercise FEV1 and FVC. The effect was stronger in the control group receiving placebo treatment, suggesting that supplementation with vitamins C and E was able to partially counteract the acute effects of ozone on lung function in heavily exercising subjects.

Since the subjects were cycling in ambient air and ozone concentrations tend to be uniform over large areas in the Netherlands, we assumed that the level of ozone at the monitoring site was a good proxy measure for the level of ozone at the place where the subjects were cycling. The monitoring sites in our study were less than 50 km from the training or racing sites, and a correlation coefficient of 0.82 was observed at a distance of more than 100 km between two monitoring sites in the Netherlands (23). It is known that ozone concentrations are lower in urban areas than in more rural areas, but since our monitoring site was in a nonurban location and cycling took place in nonurban areas as well, there is no reason to believe that the level of ozone exposure among our cyclists was different from that at the monitoring network site.

Adjustment for ambient temperature and absolute humidity did not change the results considerably. Experimental studies showed only some potentiation of ozone effects with very high temperatures (>35°C) and high ozone concentrations (>600 μg/m3) (24, 25). Previously, we did not find an effect of temperature or absolute humidity under ambient conditions with an average temperature of 18°C, which was very comparable to that of the present study (8).

Adjustment for PM10 did not essentially change the results. Hoek et al. (23) also did not find an effect of low concentrations of PM10 on the relation between ozone and lung function during a summer in the Netherlands.

The effect of insufficient compliance (number of pills taken) was investigated. There was no effect in the control group, which is understandable, since an intake of fewer placebos is unlikely to change results. However, effects of ozone on lung function were no longer seen in the vitamin group when insufficient compliers were excluded.

Differences in plasma concentrations of α-tocopherol and vitamin C between baseline and the end of the study were taken as markers for group compliance. However, plasma vitamin C was not a good marker in this study, because these subjects were young and healthy and their
baseline levels of vitamin C were already in the plateau phase (68–85 µmol/liter) of the intake (26). Therefore, a higher supplemental intake would not lead to higher plasma levels in this group of subjects (27).

Plasma α-tocopherol level is considered a relatively good marker of vitamin E intake, because it is moderately reactive to vitamin E intake (28), although the absorption of vitamin E may be incomplete (20–80 percent) (26) and variable because absorption declines with increasing dose (29). Our results showed that there was a clear increase in plasma levels of α-tocopherol in the vitamin group, whereas this was not so in the placebo group. This was similar to our findings in a pilot study conducted in 1994 with daily supplementation of 75 mg of vitamin E and plasma α-tocopherol increases of 9 percent and 38 percent in the control and vitamin groups, respectively (30). The increase in plasma α-tocopherol in this study was also comparable to the findings of other supplementation studies (31, 32), although comparison can only be crude because the length and amount of supplementation differ between the studies.

Chatham et al. (14) found that supplementation with vitamin C (1 g just before ozone exposure) and vitamin E (800 mg daily) partly protected against the acute effects of ozone (600 µg/m³) on FEV₁ and FVC in 14 healthy adults. Preliminary results of a recent experimental study suggested that peak expiratory flow decrements from two sulfur dioxide (0.10 and 0.25 parts per million) challenges given for 10 minutes after 45 minutes of ozone exposure (240 µg/m³) were significantly smaller on the vitamin regimen (500 mg of vitamin C and 400 mg of vitamin E) than on the placebo regimen in exercising subjects (n = 6) with severe asthma (33). In a recent experimental study (34), healthy nonsmoking subjects were given daily supplementation with vitamin C (250 mg), α-tocopherol (100 mg), and vegetable cocktail. After 2 weeks, the subjects were exposed to ozone at 800 µg/m³ (0.4 parts per million) for 2 hours with moderate exercise. Preliminary results suggested that the lung function decrements were lower in the antioxidant group than in the placebo group. However, there was no change in the inflammatory endpoints between the groups (34).

In the summer of 1994, we performed a pilot study with a design similar to that of the present study among cyclists (n = 26) in the Netherlands. Half of the subjects received daily supplementation with a cocktail of vitamin C (650 mg), vitamin E (75 mg), and β-carotene (15 mg) for 3 months; the control group did not receive a placebo. Postexercise lung function was related to 8-hour mean ozone concentrations, which were slightly higher (101 µg/m³) than those in this study; the weighted mean regression coefficients were more negative in the control group and less negative in the vitamin group than in the present study (30). Street workers (n = 49) in Mexico City, Mexico, received a cocktail similar to that of the 1994 cyclists in a placebo-controlled study (35). Although the street workers were not exercising heavily, the mean 1-hour maximum ozone concentration was higher (110 parts per billion, equivalent to 163 µg/m³ for conditions in Mexico City), and the results suggest a beneficial effect of the antioxidants on FEV₁, FVC, and MMEF.

The results of the present study suggest that supplementation with vitamins C and E might protect against a decrement in lung function of about 2 percent for FEV₁ and 1.5 percent for FVC if there were a difference in ozone exposure of 100 µg/m³ in the good compliers. This percentage of protection is approximately equal to the effect of ozone in the control group and might be classified as a mild response to ozone, particularly in this group of healthy subjects (36).

In summary, the present study suggests that supplementation with the antioxidant vitamins C and E partially protects against the acute effects of low levels of ozone on FEV₁ and FVC in cyclists.

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