

# Effect of Vitamin C on Transient Increase of Bronchial Responsiveness in Conditions Affecting the Airways

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## INTRODUCTION

The recent growth in our knowledge of the metabolic properties of vitamin C has provided clues for investigating its effectiveness in conditions of increased bronchial reactivity (BR). The term increased BR describes the exaggerated bronchoconstrictive response to a variety of physical or chemical stimuli, typically shown by asthmatic patients.<sup>1-4</sup> The mechanisms by which vitamin C may counteract increased BR include its ability to shift prostaglandin biosynthesis from constrictor PGF<sub>2α</sub> to dilator PGE<sub>2</sub>,<sup>5</sup> to promote nonenzymatic histamine degradation,<sup>6</sup> to stimulate catecholamine biosynthesis,<sup>7</sup> to decrease smooth muscle contractility,<sup>8,9</sup> and to maintain the lung redox state.<sup>10</sup> Acute and short-term effects of vitamin C on increased BR of asthmatic patients have been investigated, with conflicting results.<sup>11-14</sup> These contrasts might depend on the great interindividual variability in the pathologic manifestations of asthma: airway inflammation<sup>15</sup> and damage of bronchial epithelium.<sup>16</sup>

We reasoned that vitamin C, by its metabolic properties, could better display its protective effect in conditions in which increased BR is of recent onset. Actually, the extensive use of nonspecific bronchial challenges in recent years has shown that several conditions, besides asthma, may induce a shift in BR. These include viral infections of the upper respiratory tract,<sup>17-20</sup> atopic rhinitis,<sup>21-23</sup> exposure to air-borne pollutants,<sup>24-26</sup> and cigarette smoking.<sup>27,28</sup> Although the increase in BR in these conditions is usually milder than that observed in asthma, they share some pathogenetic mechanisms in common with asthma, such as airway inflammation,<sup>15,20,29-31</sup> with release of mediators and toxic oxygen radicals. To explore this hypothesis, we performed a series of investigations on the effects of large doses of vitamin C on lung function and bronchial reactivity to histamine in subjects with viral infections of the upper respiratory tract,<sup>32</sup> in patients with seasonal allergic rhinitis,<sup>33</sup> in policemen with occupational exposure to urban air pollutants,<sup>34</sup> and in heavy cigarette smokers.<sup>35</sup>

## PHYSIOLOGIC MEASUREMENTS

Lung function tests were measured following the protocol proposed by the American Thoracic Society,<sup>36</sup> using a computerized water-sealed spirometer

(BAIRES, Biomedin, Padova, Italy), which simultaneously recorded the time-volume and flow-volume curves. Forced expiratory volume in 1 s ( $FEV_1$ ), forced vital capacity (FVC), peak expiratory flow (PEF), and maximal midexpiratory flow ( $MEF_{50}$ ) were calculated from the best of five reproducible expiratory curves (*i.e.* whose FVC did not differ by more than 5%). Predicted values for FVC and  $FEV_1$  were obtained from Quanjer<sup>37</sup> and those for  $MEF_{50}$  from Knudson *et al.*<sup>38</sup>  $FEV_1$  and PEF were used to detect changes in central airway patency, as these tests detect only great increases in airway resistance. Because peripheral airways contribute to less than 25%<sup>39</sup> of total airflow resistance, a large increase in their resistance would not affect overall airway resistance. As it has been shown that small airway obstruction may be recognized by reduced flows at low lung volumes,<sup>40</sup>  $MEF_{50}$  was used to detect constrictive responses in this segment of the airways, such as after exposure to noxious fumes and in cigarette smokers.<sup>41</sup>

Bronchial reactivity to inhaled histamine was measured following the standardized procedure proposed by the American Academy of Allergy.<sup>2</sup> The test consisted of delivering nebulized histamine in doubling concentrations (from 1 to 64 mg/mL) by a compressed air breath-actuated dosimeter (MB3 MEFAR, Brescia, Italy). Each concentration was inhaled by taking five slow vital capacity breaths from the dosimeter.  $FEV_1$  and  $MEF_{50}$  were measured 2 min after each dose and were stopped when  $FEV_1$  had dropped by 20% or after the highest histamine concentration had been reached.  $FEV_1$  was used as the response index of the large airway,  $MEF_{50}$  as that of the peripheral airway. The dose/response relationship was then constructed by plotting each histamine concentration (in log mg/mL) against the relative percent change from the baseline of the response index ( $FEV_1$  or  $MEF_{50}$ , depending on the case). The bronchial responsiveness was expressed as the concentration causing a 15 or 20% decrease in  $FEV_1$  from its baseline value ( $PC_{15}$  or  $PC_{20}FEV_1$ )<sup>1-4</sup> or a 25% drop in  $MEF_{50}$  ( $PC_{25}MEF_{50}$ ).<sup>2</sup>

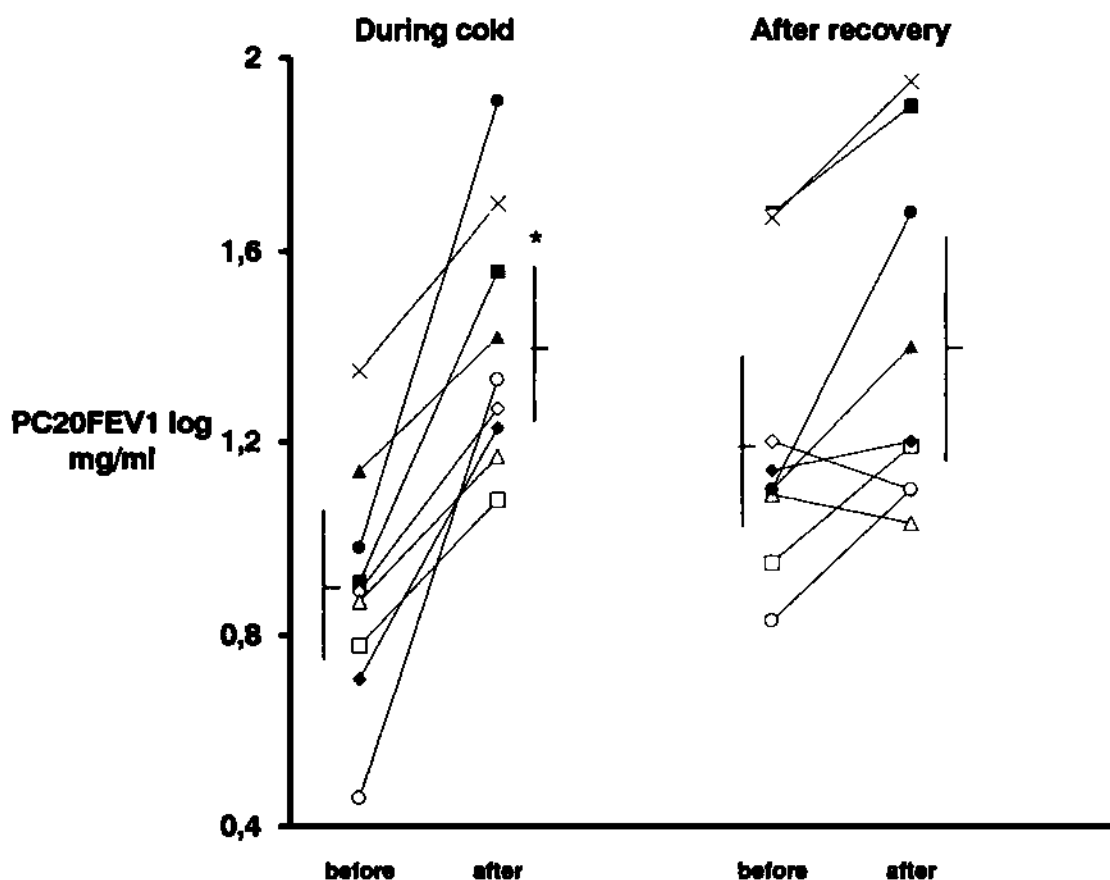
## STUDY 1: UPPER AIRWAY INFECTION

### *Patients and Methods*

The study was conducted on 10 otherwise healthy volunteers who were members of the hospital staff. We included five men and five women, age range 18 to 55 years, who complained of symptoms of a common cold associated with cough, had normal spirometry, and no history of asthma. The subjects were examined during disease, on two consecutive days, and after recovery, 6 weeks after the onset of symptoms. On each occasion, histamine bronchial responsiveness ( $PC_{20}FEV_1$ , log mg/mL) was measured twice, one hour apart. On the first day, within 5 days from the onset of cold, the reproducibility of  $PC_{20}$  was assessed by comparing the values of the first challenge with those obtained one hour later. On the following day and after recovery from disease,  $PC_{20}$  was measured before and one hour after oral intake of 2 g of vitamin C. One-way analysis of variance was used to assess the variability of  $PC_{20}$  before treatment and after the intake of vitamin C. The 95% confidence intervals of mean  $PC_{20}$  before and after ascorbic acid were compared to verify the validity of the significant changes.

*Results and Comments*

A woman was excluded from the study as she had whooping cough with a raised titer of serum antibodies. The reproducibility of PC<sub>20</sub> was very good, the values of the first measurement being closely related to those of the second ( $r = .96, p < .001$ ). The values of PC<sub>20</sub> before and after vitamin C, during cold and after recovery, are shown in FIGURE 1. During cold, baseline PC<sub>20</sub> was significantly lower than after recovery ( $F = 5.23, p < .05$ ) and was remarkably increased after vitamin C ( $F=17, p<.01$ ). The increase in PC<sub>20</sub> was very similar to what occurred spontaneously six weeks after the onset of the disease. After recovery, vitamin C produced no further improvement in PC<sub>20</sub> ( $F = 1.7, p > .05$ ). At this time, however,

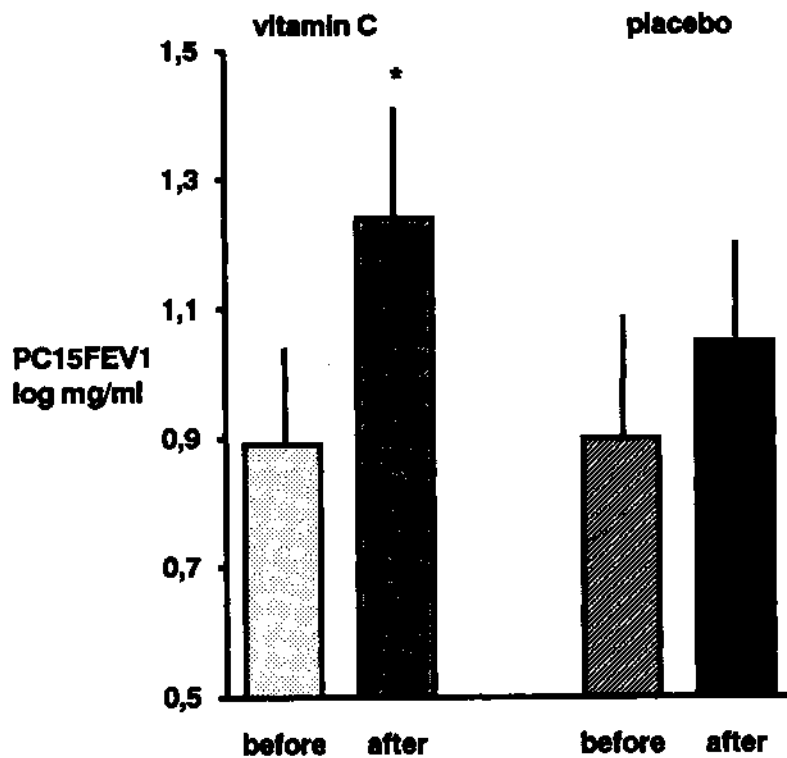


**FIGURE 1.** Values of PC<sub>20</sub>FEV<sub>1</sub> before and after treatment with vitamin C, during upper respiratory tract infection and after recovery.

any further increase in PC<sub>20</sub> would have been of trivial clinical importance, because all the subjects, except one, were totally asymptomatic and had normal responsiveness.

*Conclusion*

The results of this study indicate that vitamin C may protect against the transient increase in bronchial responsiveness caused by viral infections of the upper respiratory tract in nonasthmatic subjects.



**FIGURE 2.** Mean values of PC<sub>15</sub>FEV<sub>1</sub> before and after treatment with vitamin C and placebo.

## STUDY 2: ALLERGIC RHINITIS

### *Patients and Methods*

The study was conducted on 16 volunteers with seasonal allergic rhinitis. We included 12 men and 4 women, ranging in age from 16 to 46 years, who had normal lung function tests and a negative history for bronchial asthma. The subjects were examined soon after the onset of rhinitic symptoms, for two consecutive days. On both occasions, bronchial responsiveness to inhaled histamine (PC<sub>15</sub>FEV<sub>1</sub> log mg/mL) was measured before and one hour after oral intake of 2 g vitamin C or placebo, following a double-blind, crossover design. Plasma ascorbate was measured at baseline on the first day and one hour after treatment on both study days. Analysis of variance for a two-period crossover trial<sup>42</sup> was used to compare the changes in PC<sub>15</sub> after placebo with those after vitamin C.

### *Results*

Mean levels of plasma ascorbate were 8.84 mg/L (SEM .82) at baseline and increased to 17.5 mg/L (1.64) ( $p < .001$ ) after ingestion of vitamin C. Mean values of PC<sub>15</sub> before and after ingestion of vitamin C or placebo are shown in FIGURE 2. PC<sub>15</sub> improved after both active and placebo treatment, but the increase after vitamin C was significantly greater than after placebo ( $p < .001$ ).

### *Conclusion*

The results of this study indicate that vitamin C may protect against the transient increase in bronchial responsiveness induced by seasonal allergic rhinitis. In-

creased bronchial responsiveness in patients with allergic rhinitis is supposed to represent preclinical bronchial asthma.<sup>11</sup> Clinical experience with ascorbic acid in asthma is conflicting, with some authors reporting a beneficial effect<sup>11-12</sup> and others reporting no effect on BR.<sup>13-14</sup> Our results suggest that vitamin C may be effective when the increase in bronchial responsiveness induced by allergen exposure is mild and of recent onset.

### STUDY 3: AIR POLLUTION

#### *Subjects and Methods*

The study was conducted in December, when the highest concentration of pollutants is reached in urban air. Twenty volunteers, 15 men and 5 women, ranging in age from 24 to 37 years, were selected from the 30 policemen who directed heavy car traffic in the city center within 500 m from a monitoring station. The subjects had normal lung function tests, no documented history of asthma, and no respiratory tract infection in the six weeks preceding the study. The effects of acute (study 1) and short-term (study 2) treatment with high doses of vitamin C on lung function changes induced by exposure to urban air pollution were investigated against placebo, following a double-blind, randomized crossover design.

#### *Study A*

The policemen reported to the laboratory on two different days, eight days apart. On each occasion, lung function tests, histamine bronchial responsiveness, plasma ascorbate, and carboxyhemoglobin (HbCO%) were measured before and after two hours of directing traffic. On each day, after baseline measurements, the subjects were administered oral vitamin C, 2 g, or placebo, given in random order, following a double-blind crossover design. MEF<sub>50</sub> was used, instead of the conventional FEV<sub>1</sub> to assess the bronchial response to histamine challenge. PC<sub>25</sub>MEF<sub>50</sub>, log mg/mL, was used as an index of bronchial responsiveness.

#### *Study B*

After each acute study, the subjects received four bags containing the daily dose of vitamin C (2 g) or placebo, in the same order as in study A, to be taken in the morning as a single oral dose for four consecutive working days (from day 2 to 5 and from 9 to 12); a two-day washout period was allowed between each

**TABLE 1.** Ranges of the Daily Average and Peak Concentration of Pollutants in the Study Period

Pollutant		24-hour average	Peak
SO <sub>2</sub>	mcg/mol	142–159	271–397
Total particulate	mcg/mol	322–453	
CO	ppm	1.5–4.8	3.1–17.9
NO <sub>2</sub>	mcg/mol	139–178	219–408

treatment. To evaluate the effects of exposure on lung function during active and placebo treatment, the policemen were instructed to self-assess the peak expiratory flow (PEF) before and after duty, using the Mini-Wright portable peak flow meter. PEF measurement provides a simple, quantitative, reproducible measure of central airway obstruction, as it correlates well with FEV<sub>1</sub> measured with the spirometer.<sup>43</sup>

Data were analyzed by means of the multivariate analysis of variance (MANOVA) for repeated measures,<sup>44</sup> In each model a mixed effect on lung function was tested, with subjects as random effect and treatment (vitamin C/placebo), or trial order and exposure (before/after duty) as cross-mixed effects. The changes in PC<sub>25</sub>MEF<sub>50</sub> after exposure and vitamin C or placebo were compared using the Wilcoxon's signed rank test.

### *Results and Comments*

Exposure estimates, see TABLE 1, showed that NO<sub>2</sub> and total suspended particulate (TSP) were the predominant pollutants during the study period, with 24-hour averages always exceeding the recommended air quality standards.<sup>45</sup> Experimental studies showed that exposure to TSP may cause bronchoconstriction either in asthmatic or in normal subjects, by stimulating histamine release,<sup>45,46</sup> whereas NO<sub>2</sub> acts also by inhibiting the release of bronchodilating PGE<sub>2</sub><sup>47</sup> and by inducing the production of oxidant radicals, which seem to be responsible for morphological changes in the peripheral airway.<sup>30,48</sup> Mean values of plasma ascorbate, HbCO%, MEF<sub>50</sub>, and PC<sub>25</sub>MEF<sub>50</sub> before and after duty on a vitamin C or placebo day, and mean values of PEF before and after duty during active and placebo treatment for four days are reported in FIGURE 3. One of the subjects refrained from the study after the first day and was not included in the analysis.

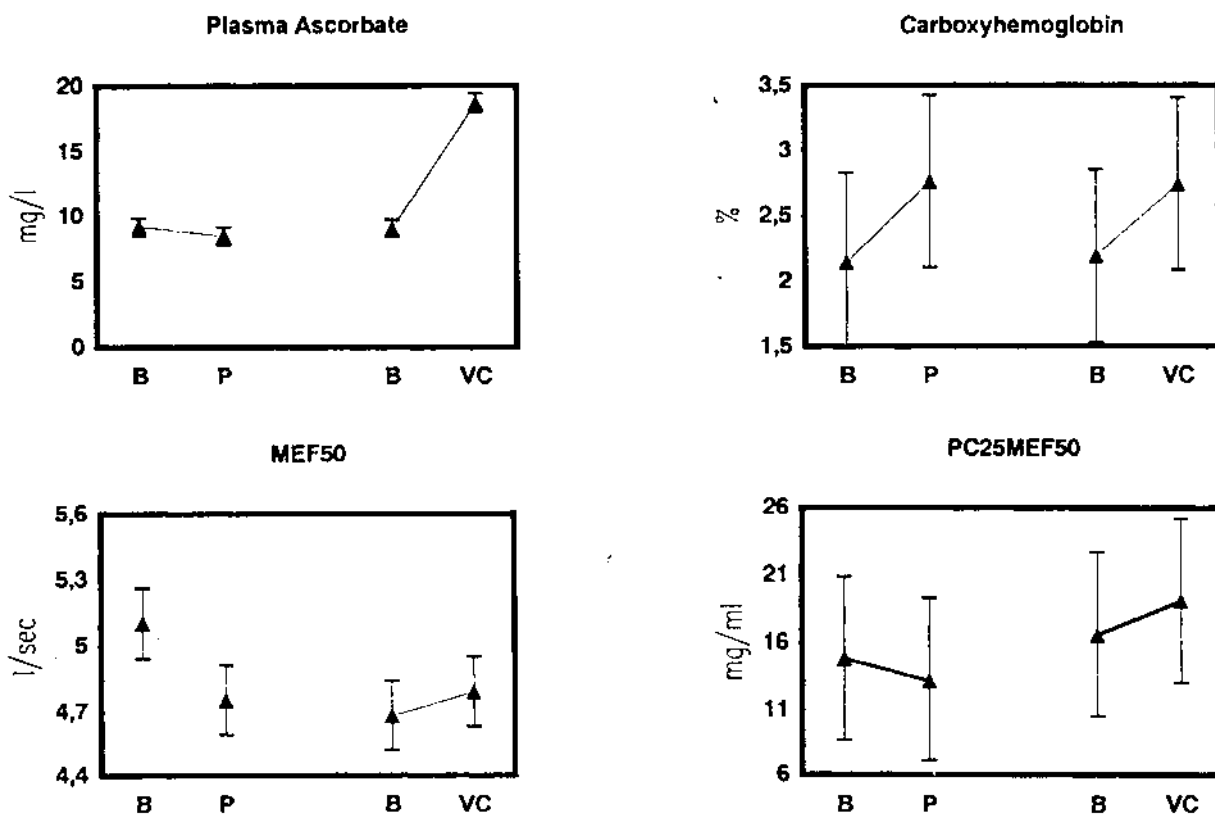
#### *Study A*

HbCO% was raised significantly after duty on both study days. Plasma ascorbate was increased after ingestion of vitamin C and was slightly but significantly decreased after placebo, suggesting increased consumption of reducing agents induced by pollutants. This hypothesis is supported by the experimental evidence that lung tissue content of ascorbic acid decreased after exposure to NO<sub>2</sub>.<sup>49</sup> MEF<sub>50</sub> was significantly decreased after exposure on the placebo day, whereas FVC and FEV<sub>1</sub> remained nearly unchanged. This finding suggests that peripheral airways were the main site affected by airborne pollutants. Vitamin C prevented the decrease in MEF<sub>50</sub>. Bronchial responsiveness was not significantly changed after duty. As PC<sub>25</sub>MEF<sub>50</sub>, however, was slightly decreased with placebo and increased with vitamin C, the effect of active treatment was seen to be significant.

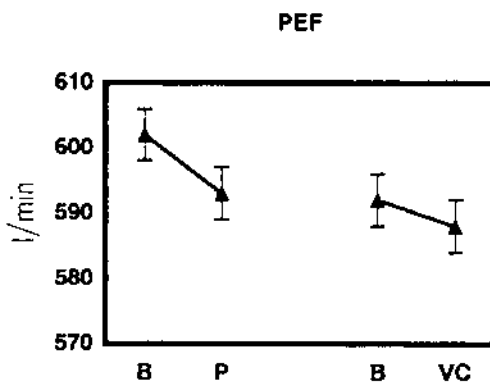
#### *Study B*

The results of PEF self-assessment before and after duty for four days showed that during placebo treatment the mean values of PEF were slightly but significantly decreased after exposure and were not significantly affected by exposure during vitamin C treatment.

STUDY A



STUDY B



**FIGURE 3.** Study A: Mean values of plasma ascorbate, carboxyhemoglobin, MEF<sub>50</sub>, and PC<sub>25</sub>MEF<sub>50</sub> before (B), after exposure plus placebo (P), and after exposure plus vitamin C (VC). Study B: Values of PEF (mean of four days) before and after duty, during treatment with placebo and vitamin C.

*Conclusion*

The results of this study suggest that short-term exposure to urban air pollution produces some decrease in lung function, which may be counteracted by pretreatment with vitamin C. These findings are in agreement with the results obtained by others after experimental exposure to NO<sub>2</sub><sup>50</sup> and ozone.<sup>51</sup>

## STUDY 4: HEAVY CIGARETTE SMOKERS

### *Subjects and Methods*

The study was conducted on seven smokers who smoked more than 20 cigarettes a day, who had normal lung function tests, and who had no history of asthma. Control consisted of seven sex- and age-matched healthy nonsmokers.

The acute effect of 2 g of vitamin C was investigated against placebo given in double-blind randomized crossover fashion on two consecutive days. On each day, lung function measurements and histamine bronchial challenge were performed before and after oral intake of active or placebo treatment. Baseline serum ascorbate was determined on day one. PC<sub>25</sub>MEF<sub>50</sub> (log mg/mL) was used as an index of bronchial responsiveness. The effect of one week of treatment with vitamin C (1 g/daily) was assessed in smokers, with an open design. Student's *t* test for unpaired data was used to compare smokers with nonsmokers; linear regression analysis was used to assess the relationship between vitamin C status and PC<sub>25</sub>MEF<sub>50</sub>; and two-way analysis of variance was used to assess the effect of active and placebo treatment in smokers and nonsmokers.

### *Results and Comments*

In agreement with prior findings,<sup>7-41</sup> smokers had significantly lower plasma ascorbate, MEF<sub>50</sub>, and PC<sub>25</sub>MEF<sub>50</sub> than nonsmokers. Ascorbate deficiency in smokers depends<sup>52,53</sup> on increased consumption of reducing agents due to oxidants and free radicals contained in tobacco smoke, on nicotine-induced increase in biosynthesis of catecholamines and serotonin, and on inadequate dietary intake. No significant influence of vitamin C or placebo on bronchial responsiveness was observed in nonsmokers. By contrast, in smokers, PC<sub>25</sub>MEF<sub>50</sub> was significantly lowered after acute treatment with vitamin C, but not with placebo, and was further decreased after one week of treatment with vitamin C (see FIG. 4). Moreover, before treatment, PC<sub>25</sub>MEF<sub>50</sub> was negatively related with vitamin C status ( $r = .85$ ,  $p < .001$ ). These findings suggested that ascorbate deficiency attenuated rather than increased bronchial responsiveness. Although the worsening of BR after vitamin C was rather unexpected, in view of its metabolic properties, possible explanations for these findings are offered by the results of experimental studies. These show that scorbutic guinea pigs have decreased conversion of histidine to histamine and lower sensitivity to histamine,<sup>54</sup> and that these abnormalities may be reversed by treatment with vitamin C.<sup>55</sup> It is thus possible that in our ascorbate-deficient smokers, treatment with vitamin C restored histamine sensitivity.

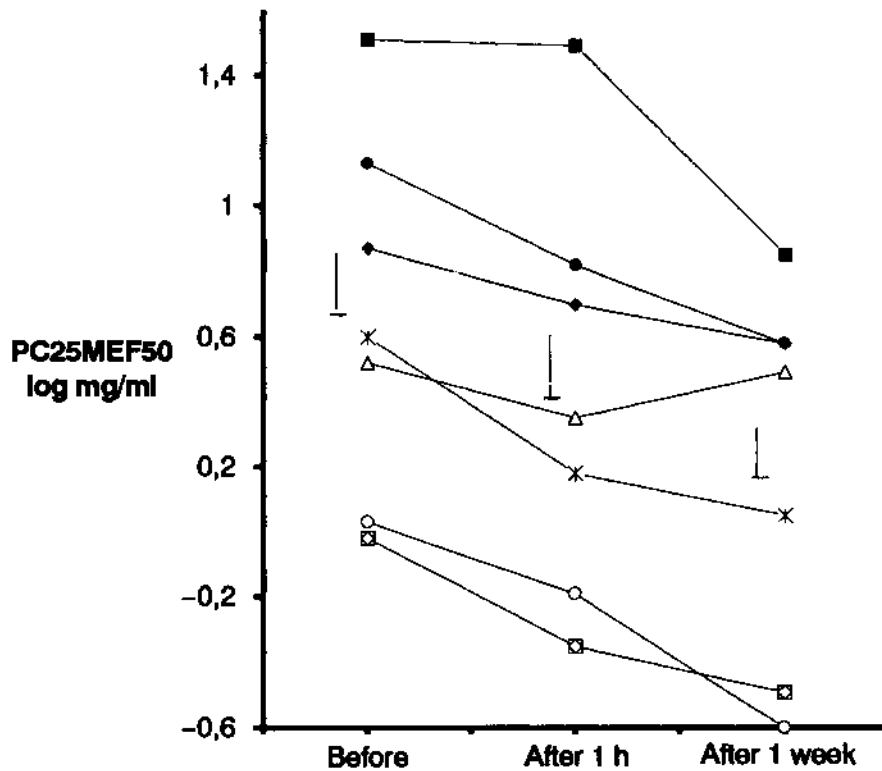
### *Conclusions*

The results of this study show that heavy cigarette smokers have ascorbate deficiency and small airway disease. Acute or short-term treatment with vitamin C produced worsening in airway responsiveness. This effect might depend on restoration of airway sensitivity to inhaled irritants.



## GENERAL CONCLUSIONS

The results of our studies suggest that vitamin C may attenuate the transient increases in bronchial tone or in bronchial reactivity induced by viral infection of the upper respiratory tract, seasonal allergic rhinitis, or exposure to urban air pollution. The mechanisms by which vitamin C may protect the airways are acceleration of histamine breakdown, shift in the cyclooxygenase pathway from constrictor  $\text{PGF}_{2\alpha}$  toward dilator  $\text{PGE}_2$ , decrease in smooth muscle contractility, and scavenging of oxidant radicals. Based on our results, chronic treatment with high doses of vitamin C may be expected to improve symptoms of airway irritability, offer protection against airway and lung damage induced by heavy air pollution in industrialized areas, and improve the prognosis of chronic obstructive lung disease.



**FIGURE 4.** Values of  $\text{PC}_{25}\text{MEF}_{50}$  before and after acute and short-term treatment with vitamin C.

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## DISCUSSION

D. ROE (*Cornell University, Ithaca, NY*): You said the mechanism might be a change in the rate of histamine breakdown. Did you actually look at that or the effect of vitamin C on bronchoconstriction induced by histamine releases?

C. BUCCA (*University of Turin, Turin, Italy*): No, unfortunately, I did neither.

D. MENZEL (*University of California at Irvine*): Based on the sensitivity to carbachol, there is evidence that a difference in the acetylcholine sensitivity or the receptor frequency is important in the overall sensitivity to oxidants. In this case, you used histamine. Have you attempted to use carbachol to see if there is any segregation within the subject population?

BUCCA: The use of histamines was due to the fact that histamine has a very short action, and so you can measure twice in a brief time following the challenge. There is a strong correlation, however, between the bronchial response to histamine and to methacholine or acetylcholine.

MENZEL: So, do you believe that there were no unusually responsive individuals within the policeman/policewoman study population?

BUCCA: There were subjects in whom the responsiveness changed after exposure. This may suggest that NO<sub>2</sub> has an immediate irritating effect and maybe an irreversible effect on peripheral airways with long-term chronic exposure.

J. MASON (*Human Nutrition Research Center, Boston, MA*): Inasmuch as

airway hyperresponsivity is the primary cause of symptoms in bronchial asthma, did you have the opportunity to study a population that specifically is known to have bronchial asthma?

BUCCA: We did not study asthmatics, because there are several papers, with contrasting results, dealing with the effect of vitamin C in asthmatics. We think that asthma is a heterogeneous disease, and probably some subjects have different degrees of airway inflammation. Some have chronic changes that can be hardly improved by short-term treatment.

S. N. MEYDANI (*Human Nutrition Research Center, Boston, MA*): One of the speculations you had for the mechanism of the vitamin C effect was conversion of PGF<sub>2α</sub> to PGE<sub>2</sub>. Are there data in the literature or from your own work to support that?

BUCCA: Yes, in Fann *et al.*<sup>5</sup> in *Prostaglandins*.

C. L. KRUMDIECK (*University of Alabama at Birmingham*): I am puzzled by the fact that the name of Linus Pauling hasn't been mentioned. It is beginning to look like he was right, after all. Shouldn't we be ready to acknowledge that fact?

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*Editors and Conference Organizers*

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