

# Lipid Peroxidation and 5-Lipoxygenase Activity in Chronic Obstructive Pulmonary Disease

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We studied the urinary excretion of the isoprostane 8-iso-prostaglandin  $F_{2\alpha}$  as an index of *in vivo* oxidant stress, and the production of leukotriene (LT)  $B_4$  ( $LTB_4$ ) by neutrophils in subjects with chronic obstructive pulmonary disease (COPD) and normal subjects. Overnight urinary excretion of the isoprostane was significantly higher in patients with COPD than in control subjects, and  $LTB_4$  production by challenge of neutrophils obtained from patients with COPD was also significantly higher than that observed in control neutrophils. Treatment with a standardized polyphenol extract caused a significant decrease in isoprostane excretion, accompanied by a statistically significant increase of  $Pa_{O_2}$ . Furthermore, changes in  $FEV_1$  significantly correlated with the changes in isoprostane urinary excretion observed from enrollment to the end of treatment. The results of this study suggest that enhanced oxidative stress in subjects with COPD is paralleled by the increased ability of neutrophils to synthesize the chemotactic factor  $LTB_4$ , and may ultimately contribute to the infiltration/activation of neutrophils into the airways of subjects with COPD. Antioxidant treatment in subjects with COPD is effective in reducing oxidant stress as shown by the decrease of urinary isoprostane, a reduction that correlates with the severity of the disease, as indicated by changes in  $Pa_{O_2}$  and  $FEV_1$ .

**Keywords:** leukotriene  $B_4$ ; natural polyphenols; neutrophils; oxidative stress; urinary isoprostanes

Chronic obstructive pulmonary disease (COPD) ranks sixth among the causes of mortality throughout the world, and it is expected to rank third by 2020. COPD is characterized by a slowly progressive and irreversible decrease of  $FEV_1$  and is associated with airway inflammation characterized by an increased number of neutrophils, macrophages, and  $CD8^+$  T-lymphocytes (1). Whereas clinical manifestations and progression of COPD relate to several risk factors, such as  $\alpha_1$ -antitrypsin deficiency, recurrent infections, air pollution, and lower birth weight, the most important appears to be cigarette smoking (2–4). Cigarette smoke has been shown to contain very significant amounts of oxidant molecules (5), and considerable evidence now links COPD, increased oxidative stress, and reactive oxygen species (6). Reactive oxygen species are highly unstable compounds with odd electron number, capable of initiating the oxidation of biological structures, and there is evidence they may contribute to amplification of the inflammatory response, typical of COPD airways, through the activation of inflammatory gene transcription (7).

$F_2$ -isoprostanes are a family of free radical catalyzed prostaglandin  $F_2$ -isomers formed *in situ* from the fatty acid backbone

esterified in membrane phospholipids (8). They are released in response to cellular activation and have been detected in human plasma and urine (9). Increased excretion of  $F_2$ -isoprostanes in urine has been found in association with several pathologies, including diabetes (10), hypercholesterolemia (11), cigarette smoking (12), and COPD (13). Due to their mechanism of formation, specific structural features, and chemical stability, urinary excretion of a representative  $F_2$ -isoprostane, such as 8-iso-prostaglandin  $F_{2\alpha}$  (now known as  $iPF_{2\alpha}$ -III) (14) is considered a reliable index of oxidant stress and ensuing lipid peroxidation *in vivo* (15).

Metabolism of arachidonic acid through the 5-lipoxygenase (5-LO) pathway leads to the formation of 5-hydroperoxy-eicosatetraenoic acid and leukotriene (LT)  $A_4$  ( $LTA_4$ ). This unstable allylic epoxide can be further converted by secondary enzymes into LTs ( $LTB_4$  and cysteinyl LTs  $LTC_4$ ,  $LTD_4$ , and  $LTE_4$ ), a family of potent biologically active compounds synthesized by specific cell types, such as eosinophils, neutrophils, and mast cells, as well as by transcellular biosynthetic mechanisms involving different cell types (16).  $LTB_4$  is a potent chemotactic and chemokinetic factor for human neutrophils and it significantly enhances neutrophil adhesion to endothelial cells (17). Full activity of 5-LO is dependent on the presence of lipid hydroperoxides with both the isolated enzyme (18) and intact cells (19) and, therefore, on the oxidant–antioxidant balance of a given cell.

In light of the potential relationship between COPD, oxidant stress, and 5-LO activity, the aim of our study was to evaluate the urinary excretion of  $F_2$ -isoprostanes as an index of *in vivo* oxidant stress and the production of  $LTB_4$  by peripheral blood neutrophils in patients with COPD versus normal subjects. The effects of antioxidant treatments with a polyphenol rich skin extract from selected *Vitis vinifera* grapes *in vivo* were also studied.

## METHODS

### Subjects

Differences in basal excretion of  $iPF_{2\alpha}$ -III were studied in 12 subjects with COPD in stable condition and 10 age-matched healthy control subjects (Table 1). The diagnosis of moderate COPD was consistent with the American Thoracic Society (ATS) classification of disease severity (the NHLBI and World Health Organization Global Initiative for Chronic Obstructive Lung Disease [GOLD]) (20) and established on the basis of clinical history, physical findings, and spirometry (21). Bronchodilator reversibility testing was performed to exclude an asthmatic component and resulted in a 12% lower  $FEV_1$  (200 ml) when compared with basal values in all subjects with COPD. The subjects included did not have a bronchial infection during the month preceding the study, and subjects with COPD were free from exacerbations during 6 months preceding the enrollment.

The use of oral bronchodilators was not permitted 1 week before and during the study, whereas inhaled short-acting bronchodilator drugs and inhaled long-acting bronchodilator agents were not permitted for at least 6 and 12 hours, respectively, before each respiratory test. Moreover, no antioxidant drugs were permitted for 4 weeks before and during the study.

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**TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE SUBJECTS PARTICIPATING IN THE FIRST STUDY, AIMED AT EVALUATING THE URINARY EXCRETION OF 8-ISO-PROSTAGLANDIN F<sub>2α</sub> AND THE PRODUCTION OF 5-LIPOXYGENASE METABOLITES BY PERIPHERAL BLOOD NEUTROPHILS OF CONTROL SUBJECTS (N = 10) AND SUBJECTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE (N = 12)**

Characteristics	Subjects with COPD*	Control Subjects
Age, yr	66.7 ± 6.5	64.6 ± 5.8
Weight, kg	67.2 ± 8.5	71.3 ± 7.6
Height, cm	166 ± 11	171 ± 9
Sex, M/F	11/1	8/2
Pa <sub>O<sub>2</sub></sub> , mm Hg	76.7 ± 8.1	88.4 ± 7.5
PcO <sub>2</sub> , mm Hg	39.9 ± 4.3	40.6 ± 3.2
FEV <sub>1</sub> , L	1.5 ± 0.46	2.8 ± 0.32
FVC, L	2.7 ± 1.03	3.4 ± 1.1
FEV <sub>1</sub> , % pred	58 ± 12	89 ± 10
FVC, % pred	81.9 ± 23	101 ± 18
FEV <sub>1</sub> /FVC, %	57.9 ± 12	82.1 ± 12
Smoking history, packs/yr	48 ± 20	—

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; pred = predicted.

Values are expressed as means ± SD.

\* 9/12 subjects were under treatment with long-acting β<sub>2</sub>-agonist plus anticholinergic, plus topical steroid; 3/12 subjects were under treatment with long-acting β<sub>2</sub>-agonist and topical steroid.

Healthy volunteers matched for age and sex to enrolled subjects with COPD were all lifelong nonsmokers. All subjects were required to collect overnight urine samples for 3 consecutive nights and to store it at 4°C until conferred at the S. Paolo hospital.

Blood (40 ml) was collected from 6 of 12 subjects with COPD and from 5 of 10 control subjects, and neutrophils were obtained by dextran sedimentation and centrifugation over a discontinuous Percoll gradient as previously described (22).

A second group of 11 patients with COPD in stable condition (10 moderate and 1 severe) was enrolled to evaluate the effect of a short-term (6 weeks) treatment with tablets containing a dry extract from selected *Vitis vinifera* grapes (Zantox; Dolphin Society, Milan, Italy) standardized at 180 mg of polyphenols per tablet (three times a day). Subjects with COPD were all current smokers (Table 2) who were not affected by hypertension, diabetes or heart failure, and they were asked to collect overnight urine samples the day before and at the end of the treatment period. Urine samples were stored at -20°C until analysis. Respiratory parameters were evaluated before and at the end of the treatment.

All subjects continued their pharmacologic treatment (inhaled corticosteroid, long-acting β<sub>2</sub>-agonist, and anticholinergic, as listed in Tables 1 and 2), except as required for testing at the beginning and at the end of the study. One subject failed to collect the appropriate urine samples and was excluded from the study.

A group of nine age- and sex-matched control subjects was also administered the standardized extract according to the same protocol as that used for subjects with COPD.

The study was approved by the ethical committee of the S. Paolo Hospital and was conducted according to the rules of the declaration of Helsinki. Written informed consent was obtained from all subjects participating in the study.

#### iPF<sub>2α</sub>-III Analysis

Urinary iPF<sub>2α</sub>-III was purified with a double-extraction protocol using columns for solid-phase extraction, and quantified as previously described (23, 24).

#### LTB<sub>4</sub> Production by Isolated Human Neutrophils

Neutrophils were suspended in phosphate-buffered saline, primed with granulocyte-macrophage colony-stimulating factor 1 nM for 30 minutes), and challenged with formylmethionylleucylphenylalanine (0.1 μM, at

**TABLE 2. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE SUBJECTS WITH COPD (N = 11) AND CONTROL SUBJECTS (N = 9) PARTICIPATING IN THE SECOND STUDY, AIMED AT EVALUATING THE EFFECTS OF A SHORT-TERM (6 WEEKS) TREATMENT WITH A STANDARDIZED EXTRACT OF THE SKIN OF SELECTED *VITIS VINIFERA* GRAPES, RICH IN ANTIOXIDANT POLYPHENOLS, ON 8-ISO-PROSTAGLANDIN F<sub>2α</sub> URINARY EXCRETION AND ON PULMONARY FUNCTIONS**

Characteristics	Subjects with COPD*	Control Subjects
Age, yr	66.9 ± 6.6	62.8 ± 6.7
Weight, kg	72.7 ± 12.3	74.3 ± 10.1
Height, cm	168 ± 9	173 ± 10
Sex, M/F	9/2*	7/2
Pa <sub>O<sub>2</sub></sub> , mm Hg	76.7 ± 8.5	89.8 ± 1.2
PcO <sub>2</sub> , mm Hg	40.8 ± 5.5	40.2 ± 2.1
FEV <sub>1</sub> , L	1.41 ± 0.32	3 ± 0.4
FVC, L	2.42 ± 0.46	3.9 ± 0.55
FEV <sub>1</sub> , % pred	55.3 ± 13.4	99 ± 15
FVC, % pred	77.8 ± 7.5	106 ± 13
FEV <sub>1</sub> /FVC, %	57 ± 11.1	82.1 ± 12
Smoking history, packs/yr	61 ± 26	—
Actual smoking, cigarettes/d	15 ± 7	—

For definition of abbreviations see Table 1.

Values are expressed as means ± SD.

\* 9/11 subjects were under treatment with long-acting β<sub>2</sub>-agonist, plus anticholinergic, plus topical steroid; 2/11 subjects were under treatment with long-acting β<sub>2</sub>-agonist plus topical steroid.

37°C for 10 minutes) in the presence or absence of α-tocopherol (10–100 μM). LTB<sub>4</sub> and its ω-oxidized metabolites were analyzed as previously described (25).

#### Statistical Analysis

The distribution of iPF<sub>2α</sub>-III was markedly skewed and values were expressed as median and 25–75 percentiles. Within and between group differences were analyzed by nonparametric tests (Signed-Rank test and Rank-Sum Test, respectively). PaO<sub>2</sub> and LTB<sub>4</sub> values were expressed as mean ± SE, except where otherwise stated, and analyzed by analysis of variance and paired and unpaired Student's *t* test, as appropriate. Linear correlation between variables was expressed as the correlation coefficient *r* and the significance was set at *p* < 0.05; in this case iPF<sub>2α</sub>-III values were log-transformed before analysis. The statistical analysis was performed using the SPSS program (Statistical Package for the Social Sciences for Windows, version 7.0; Chicago, IL).

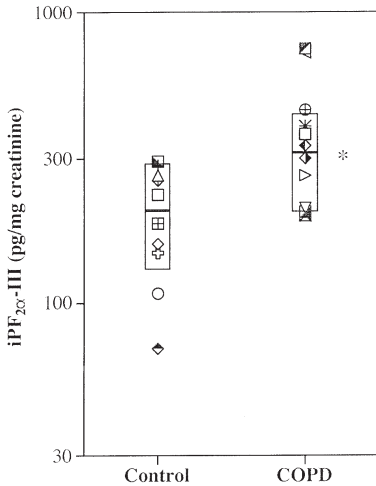
#### RESULTS

All subjects with COPD had a significant airway obstruction with a marked reduction in FEV<sub>1</sub> (1.5 ± 0.46 L) and FEV<sub>1</sub>/FVC (57.9 ± 12%) when compared with control subjects (Table 1).

Analysis of overnight urinary excretion of iPF<sub>2α</sub>-III over 3 consecutive days gave reproducible results, showing an average SD, relative to the three different determinations, of 20.3 ± 10.9% (mean ± SD).

Comparing the average of the three measurements, iPF<sub>2α</sub>-III was significantly higher in subjects with COPD than in control subjects (330, 207–447 and 212, 139–280 pg/mg creatinine, median and 25–75 percentiles, respectively) (*p* < 0.05) (Figure 1), in agreement with published data (13). No correlation was found between age, sex, FEV<sub>1</sub>, or FEV<sub>1</sub>/FVC and the urinary concentrations of iPF<sub>2α</sub>-III.

Given that the activity of leukocyte 5-LO is dependent on the presence of lipid hydroperoxides (18, 19) and that the increased excretion of F<sub>2</sub>-isoprostanes is believed to reflect an enhanced lipid peroxidation in subjects with COPD (26, 27), we evaluated the production of the main 5-LO metabolite in neutrophils, namely

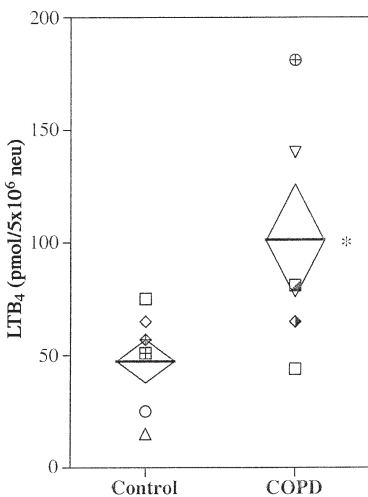


**Figure 1.** Urinary excretion of 8-iso-prostaglandin  $F_{2\alpha}$  (iPF $_{2\alpha}$ -III) in subjects with COPD (n = 12) and control subjects (n = 10). Overnight urines were collected on 3 consecutive days, and the average of three separate measurements was used for each subject. iPF $_{2\alpha}$ -III was measured by double extraction followed by specific enzyme immunoassay (EIA) as described in METHODS. \*p < 0.05.

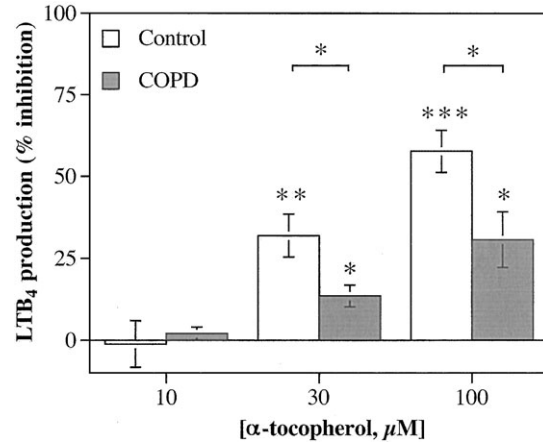
LTB $_4$  and its  $\omega$ -oxidized products 20-OH-LTB $_4$  and 20-COOH-LTB $_4$ , after receptor-mediated activation of peripheral blood neutrophils. LTB $_4$  production by formylmethionylleucylphenylalanine challenge of neutrophils obtained from subjects with COPD was significantly higher than that observed in control neutrophils ( $102.5 \pm 28.8$  and  $48 \pm 10.8$  pmol/ $5 \times 10^6$  neutrophils, subjects with COPD and control subjects, respectively; p < 0.05) (Figure 2). Furthermore, whereas LTB $_4$  production by neutrophils from control subjects was concentration dependently inhibited by the lipophilic antioxidant  $\alpha$ -tocopherol (Figure 3), neutrophils from subjects with COPD were less sensitive to this treatment, as the observed inhibition was less pronounced than in healthy control subject neutrophils ( $58 \pm 6$  and  $31 \pm 8\%$  at  $100 \mu\text{M}$   $\alpha$ -tocopherol, healthy subjects and subject with COPD, respectively, p < 0.05) (Figure 3).

No significant correlation was found between age, sex, FEV $_1$ , or FEV $_1$ /FVC and the LTB $_4$  production by neutrophils obtained from subjects with COPD.

Overnight urinary excretion of iPF $_{2\alpha}$ -III in the second group of subjects with COPD was significantly higher than that observed in the first group, averaging 766, 603–983 pg/mg creatinine (p < 0.05). This result well reflects the higher oxidative stress that characterizes this subgroup of subjects with COPD, all spe-



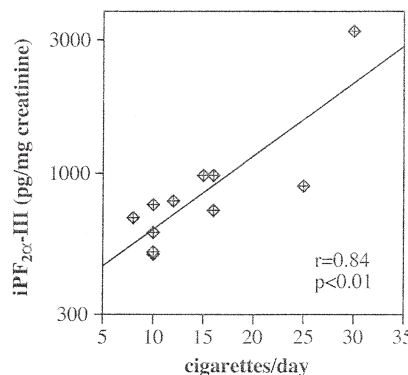
**Figure 2.** Production of leukotriene (LT) B $_4$  (LTB $_4$ ) and its  $\omega$ -oxidized metabolites by peripheral blood neutrophils in subjects with COPD (n = 6) and control subjects (n = 5). LTB $_4$  production was evaluated after granulocyte macrophage colony-stimulating factor (GM-CSF) priming (1 nM, 30 minutes) and formylmethionylleucylphenylalanine (FMLP) challenge ( $0.1 \mu\text{M}$ ). 5-Lipoxygenase (5-LO) metabolites were measured by reverse phase (RP)-HPLC coupled to diode-array ultraviolet detection. \*p < 0.05.



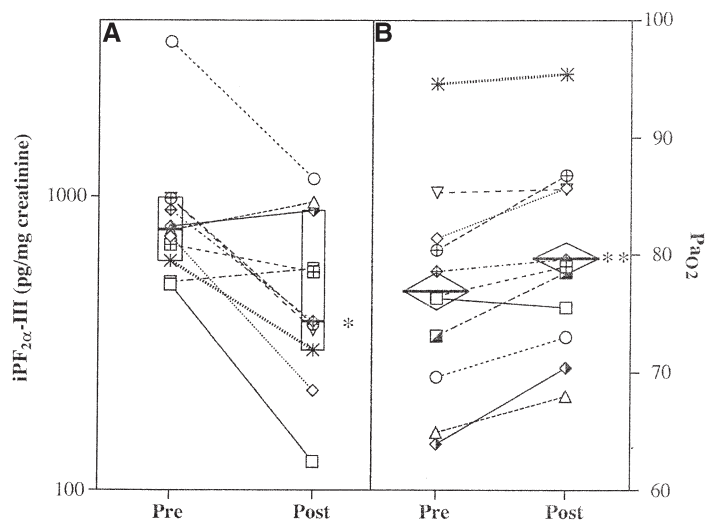
**Figure 3.** Production of LTB $_4$  and its  $\omega$ -oxidized metabolites by peripheral blood neutrophils obtained from control subjects (n = 5) and subjects with COPD (n = 6): effect of pretreatment with  $\alpha$ -tocopherol. Neutrophils were primed with GM-CSF (1 nM, 30 minutes) and challenged with fMLP ( $0.1 \mu\text{M}$ ) in the presence or absence of  $\alpha$ -tocopherol. 5-LO metabolites were measured by RP-HPLC coupled to diode-array UV detection. LTB production in untreated neutrophils was  $72 \pm 11$  and  $121 \pm 41$  pmol/ $5 \times 10^6$  neutrophils in control and COPD, respectively (mean  $\pm$  SD, p < 0.03). \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

cifically chosen because of being active smokers. Indeed, whereas urinary concentrations of iPF $_{2\alpha}$ -III did not correlate with any of the pulmonary functional parameters evaluated, they showed a statistically significant direct correlation either with the number of cigarettes currently smoked per day (r = 0.84, p < 0.01, Figure 4) or the number of packs/year (r = 0.6, p = 0.05).

Treatment with the standardized extract of *Vitis vinifera* was well tolerated by all subjects. After 40 days of treatment, a decrease of more than 50% in F $_2$ -isoprostane excretion was observed, with a mean value of 375, 300–896 pg/mg creatinine (Figure 5A; this change in F $_2$ -isoprostane excretion was accompanied by a small but statistically significant increase of PaO $_2$  values from  $76.8 \pm 2.7$  to  $79.8 \pm 2.4$  mm Hg (p < 0.01, Figure 5B). Furthermore, although FEV $_1$  values did not statistically improve throughout the group of treated subjects with COPD, it is interesting to note that changes in FEV $_1$  significantly correlated with changes in iPF $_{2\alpha}$ -III urinary excretion observed from enrollment to the end of treatment (p < 0.01) (Figure 6), suggesting that the urinary excretion of isoprostanes reflected well the efficacy (or lack of) of the antioxidant treatment on respiratory capacity.



**Figure 4.** Correlation between the urinary excretion of iPF $_{2\alpha}$ -III and the average number of cigarettes/day in a group of 10 active smokers with COPD. iPF $_{2\alpha}$ -III in urine was measured by specific EIA after double extraction on reverse- and normal-phase extraction cartridges, as described in METHODS.



**Figure 5.** Effects of a 6-week treatment with a dry extract from selected *Vitis vinifera* grapes standardized at 180 mg of polyphenols per tablet (three times a day) on urinary excretion of  $iPF_{2\alpha}$ -III (A) and  $PaO_2$  (B) in a group of 10 COPD, active smoker subjects.  $iPF_{2\alpha}$ -III in urine was measured by specific EIA after double extraction on reverse- and normal-phase extraction cartridges, as described in METHODS. \* $p < 0.05$ , \*\* $p < 0.01$ .

No statistically significant changes were observed in control subjects after 6 weeks of treatment with the standardized extract of *Vitis vinifera*, in either  $iPF_{2\alpha}$ -III urinary excretion (217, 191–299 and 234, 176–286 pg/mg creatinine, before and after treatment, respectively),  $FEV_1/FVC$  ( $75.2 \pm 3.8$  and  $75.4 \pm 3\%$ , respectively),  $FEV_1$  ( $3 \pm 0.4$  and  $3 \pm 0.4$  L, respectively), or  $PaO_2$  ( $89.8 \pm 1.2$  and  $90.7 \pm 1$  mm Hg, respectively).

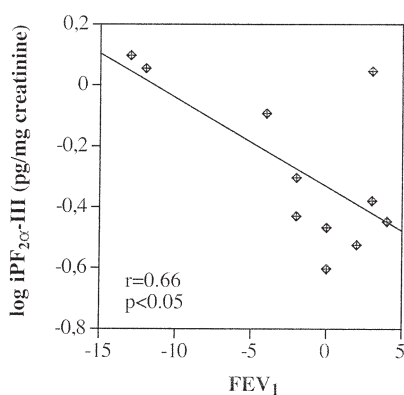
## DISCUSSION

In agreement with previously published data (13), determination of overnight urinary excretion of the  $F_2$ -isoprostane  $iPF_{2\alpha}$ -III using repeated collections over 3 consecutive days showed significantly increased concentrations in subjects with COPD. Over the last 10 years isoprostane excretion has been proposed as a reliable marker of oxidant stress *in vivo* (28), and increased urinary concentrations of  $iPF_{2\alpha}$ -III have been associated with a number of pathologic conditions where oxidative stress is known to occur (15). Given the strong association between cigarette smoking and COPD and the compelling evidence that smoke represents a significant source of oxidant species (29), oxidative stress (i.e., the unbalance of oxidant and antioxidant) within the lung has long been linked to COPD (26). All subjects with COPD studied were under treatment with inhaled corticosteroids, and it is possible that, given the potent antiinflammatory activity of these com-

pounds, production of oxidants by inflammatory cells might have been reduced. Indeed, inhaled corticosteroid treatment has been shown (30) to decrease ethane concentrations in exhaled breath condensate from subjects with COPD, suggesting a significant decrease of lipid peroxidation. Nevertheless, inhaled corticosteroid treatment in the same group of subjects did not change exhaled CO, a parameter possibly reflecting inflammation and oxidative stress. More recently it has been reported that despite their potent antiinflammatory activity, inhaled corticosteroids were not able to significantly decrease either  $H_2O_2$  or  $F_2$ -isoprostane concentrations in expired breath condensate from subjects with COPD (31). Even in the event of a decreased lipid peroxidation as a result of the inhaled corticosteroid treatment, the evidence that the subjects studied were still undergoing a significant oxidant stress, as shown by the  $F_2$ -isoprostane excretion, provides further support of a significant role of altered cellular redox state in the pathogenesis of COPD. Nevertheless, the relationship between oxidant stress and the characteristic pathologic features of COPD appears to be particularly complex. It is known that oxidant and cigarette smoke may increase the susceptibility to elastase and therefore facilitate structural damage and emphysema through the inactivation of  $\alpha_1$ -antitrypsin (32, 33). In addition, oxidants can increase mucus production (34) and impair cilia function (35), and can cause direct damage to lung structure through the alteration of DNA, lipids, and protein constituents (36). Oxidation by reactive oxygen species is a facile reaction in polyunsaturated fatty acids and may lead to the formation of lipid hydroperoxides, which are known to be extremely important for the activity of 5-LO in neutrophils (18).

Neutrophil infiltration is a major feature of COPD, and several factors, such as adhesion molecules (37), neurokinins (38), interleukin-8 (39), and tumor necrosis factor- $\alpha$  (40), may contribute to neutrophil migration into the airways of subjects with COPD.

$LTB_4$ , the final metabolite resulting from activation of 5-LO in neutrophils and alveolar macrophages, is among the most potent chemotactic and activating factors for neutrophils (41, 42). Increased  $LTB_4$  concentrations have been reported in the sputum as well as in breath condensate of patients with COPD (43), where it significantly contributes to the chemotactic activity present within the airways (44, 45). Mitsunobu and colleagues have recently published that enhanced LT generations from peripheral blood leukocytes could be observed in subjects with COPD on challenge with the calcium ionophore A23187 (46). In the present



**Figure 6.** Correlation between changes from the values observed at enrollment and at the end of the 6-week antioxidant treatment in urinary excretion of  $iPF_{2\alpha}$ -III (log-transformed data) and in  $FEV_1$  in a group of 10 COPD, active smoker subjects.  $iPF_{2\alpha}$ -III in urine was measured by specific EIA after double extraction on reverse- and normal-phase extraction cartridges, as described in METHODS.

study we provide evidence that a much more physiologically relevant stimulus, such as the formylated tripeptide formylmethionylleucylphenylalanine, causing receptor-mediated activation of peripheral blood neutrophils from subjects with COPD, resulted in a significantly higher production of LTB<sub>4</sub> when compared with that observed in age-matched, healthy control subjects. This evidence provides support to the potential involvement of LTB<sub>4</sub> in the recruitment and activation of neutrophils in subjects with COPD, and although no causative link can be implied from our data, it supports the hypothesis that oxidant stress *in vivo* may enhance the activity of 5-LO in neutrophils through the formation of lipid hydroperoxides. Increased production of LTB<sub>4</sub> from neutrophils in subjects with COPD may also arise from different factors: it is known that inflammatory cytokines are able to enhance the synthesis of 5-LO metabolites in humans (47). Nevertheless, lending further support to our hypothesis, we found that LTB<sub>4</sub> production by neutrophils from subjects with COPD was significantly less affected by the pretreatment with  $\alpha$ -tocopherol, suggesting that its antioxidant activity could not efficiently counterbalance the altered redox status of COPD neutrophils. On the contrary, in control neutrophils,  $\alpha$ -tocopherol was able to dose dependently inhibit the formation of LTB<sub>4</sub>, well in agreement with its role as a hydroperoxide scavenger (48).

The ability of *in vivo* antioxidant treatment to affect the urinary excretion of F<sub>2</sub>-isoprostanes (12) and the suggested hypothesis that urinary iPF<sub>2 $\alpha$</sub> -III may reflect the severity of the disease in subjects with COPD (13) led us to evaluate the effect of treatment with a dietary supplement, a standardized dry extract of *Vitis vinifera* skins (Zantox) with a high content of polyphenols (30%), on several pulmonary functional parameters, monitoring the effects on *in vivo* oxidant stress through the measurement of iPF<sub>2 $\alpha$</sub> -III urinary excretion.

Polyphenols, which belong to a heterogeneous class of compounds found in plants, are capable of interfering with the oxidative-antioxidative potential of cells or acting as free radical scavengers (49). Furthermore, they may inhibit phosphodiesterases and protein kinases, interfering with cell signaling (50), as well as the release of inflammatory cytokines from alveolar macrophages (51).

Evaluation of iPF<sub>2 $\alpha$</sub> -III in subjects with COPD selected according to the additional criterion of being currently active smokers but free of other diseases potentially associated with systemic oxidative stress further demonstrated that smoking represents a primary cause of oxidant stress in patients with COPD. Average urinary excretion of F<sub>2</sub>-isoprostanes was indeed significantly higher than that observed in the previous group of subjects with COPD and showed a statistically significant correlation with the number of cigarettes currently smoked per day. Although it has been reported that in exhaled breath condensates from current or exsmokers with COPD the concentrations of F<sub>2</sub>-isoprostanes was not different (52), our observation is well in agreement with previously published data on the effect of smoking cessation on urinary excretion of F<sub>2</sub>-isoprostanes (12), and further stresses the significant biological consequences directly arising from the burden of oxidant compounds present both in the tar and in the gas phase of cigarettes (29).

The treatment with natural antioxidant was able to significantly blunt the urinary excretion of iPF<sub>2 $\alpha$</sub> -III, and this effect was accompanied by a modest but statistically significant increase in the PaO<sub>2</sub>. This evidence provides additional support to the initial observation by Praticò and coworkers (13) suggesting a quite interesting correlation between the amount of oxidant stress as indicated by iPF<sub>2 $\alpha$</sub> -III and respiratory function. In fact, whereas FEV<sub>1</sub> on the average did not show a statistical improvement with treatment, it is remarkable that we have been able to observe a statistically significant correlation between the changes

(increase or decrease) in urinary excretion of iPF<sub>2 $\alpha$</sub> -III and the changes in FEV<sub>1</sub> between enrollment and the end of the treatment period. Many factors can certainly contribute to the observed link between oxidative stress and airways obstruction (26), but the combined results we have obtained support the hypothesis that enhanced 5-LO activity in cells such as neutrophils and macrophages, as a result of higher hydroperoxide tone in COPD, may play a role through the enhanced production of the neutrophil chemotactic and activating factor LTB<sub>4</sub>, resulting in a significantly increased airways inflammation.

In conclusion, the results of this study suggest that enhanced oxidative stress in subjects with COPD is paralleled by the increased ability of neutrophils to synthesize the chemotactic factor LTB<sub>4</sub>, and may ultimately contribute to the infiltration and the activation of neutrophils into the airways of subjects with COPD. Antioxidant treatment is effective in reducing oxidant stress, as indicated by the urinary excretions of iPF<sub>2 $\alpha$</sub> -III, and may lead to an improved control of airways inflammation in COPD. The results obtained provide the necessary basis for a larger, placebo-controlled study on the efficacy of grape-derived polyphenols in subjects with COPD.

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