EFFECTS OF INDOMETHACIN PRETREATMENT ON SHORT-TERM OZONE EXPOSURE IN ASTHMATICS

by

NEIL ALEXIS

A thesis submitted in conformity with the requirements for the
degree of Doctor of Philosophy
Graduate Department of Community Health
University of Toronto

Canada

EFFECTS OF INDOMETHACIN PRETREATMENT ON SHORT-TERM OZONE EXPOSURE IN ASTHMATICS, PH.D., 1998, NEIL EARL ALEXIS, DEPARTMENT OF COMMUNITY HEALTH, UNIVERSITY OF TORONTO

ABSTRACT

In a 2x2 randomized factorial design, 13 asthmatic volunteers were exposed to 400 ppb ozone or clean air for 2 hours while moderately exercising and pretreated with indomethacin or placebo. Baseline changes in pulmonary function, airway reactivity, symptom responses, sputum inflammatory cells and soluble markers of inflammation (PGF2-alpha, IL-8) were compared relative to air exposure to determine the ozone responses, and the ozone responses were compared between placebo and indomethacin to determine the interaction effect of indomethacin. Results demonstrated significant reductions in pulmonary function (FVC, FEV_1, V50, V25), significant increases in the reporting frequency and severity of respiratory symptoms and significant increases in total cells and percent neutrophils following ozone exposure. Only four subjects demonstrated airway hyperreactivity following ozone exposure. Indomethacin pretreatment did not significantly attenuate ozone-induced decreases in spirometry, increases in symptom reporting and severity, increases in airway reactivity, or increases in total inflammatory cells and percent neutrophils. Pre-exposure PGF2-alpha levels suggested appreciable cyclooxygenase (CO) inhibition with indomethacin prior to ozone exposure, while post exposure levels suggested no CO inhibition with indomethacin following ozone exposure. In a pilot study conducted by this

investigator, 9 non-asthmatics showed similar ozone-induced declines in FVC and FEV_1 to asthmatics, but indomethacin significantly attenuated these declines. Relative to baseline, PGF2-alpha levels were suppressed following ozone exposure in non-asthmatics. We conclude that lack of suppression of PGF2-alpha levels in asthmatics following ozone exposure, may have been *partially* responsible for the lack of pulmonary function protection we observed when asthmatics were pretreated with indomethacin. We hypothesize that due to higher baseline CO activity, asthmatics may require higher doses of indomethacin than non-asthmatics in order to achieve significant protection against ozone-induced declines in pulmonary function. However, despite higher baseline CO activity, asthmatics did not respond with substantially greater decrements in FVC and FEV_1 following ozone exposure than non-asthmatics. This suggests that non-cyclooxygenase mechanisms may be active in asthmatics exposed to ozone.

ACKNOWLEDGMENTS

I would like to acknowledge the following individuals for lending me their expertise in their respective fields of interest:

Thesis committee:

    Dr. Frances Silverman (supervisor)

    Dr. Susan Tarlo

    Dr. Paul Corey

    Dr. David Pengelly

    Dr. Paul O'Byrne

Mr. Bruce Urch

Dr. F. Hargreave

Dr. E. Diamindis

Dr. M. Vas

Dr. D. Banks

Ms. L. Grass

Dr. J. Edelson

Dr. Irv Broder

TABLE OF CONTENTS

TABLES

FIGURES

THESIS FORMAT

This thesis was written in the "journal article" format where chapters 4-7 were treated as a series of individual papers that logically followed one another. This format style dictated a separate introduction, methods, results, discussion and conclusion section for chapters 4-7. A comprehensive introduction section appears at the beginning of the thesis and covers the entire scope of the thesis. An overall summary and conclusion section appears at the end of the thesis in an attempt to tie chapters 4-7 together.

CHAPTER 1

OVERALL INTRODUCTION

INTRODUCTION*

Exposure to ozone (O_3), a principal component of photochemical smog, has been shown to have several negative effects on the respiratory system of humans. The health significance of these effects in the pathogenesis of respiratory disease, remains unclear. The human health effects of O_3 exposure is an area that has received much attention and dedicated research. In general, two main scientific approaches have generated much of the current data on O_3, namely toxicologic studies, and epidemiologic studies. Toxicologic studies have encompassed animal work, in vitro work and controlled human exposures. Exposure studies can be divided into acute and chronic protocols and then further subdivided into single or repeat exposure protocols. With respect to acute, single exposure studies, many areas are currently under investigation with respect to the health effects of ozone. Among these are lung function changes, exposure-response relationships, mechanisms of lung function changes, mechanisms of inflammation, increased airway responsiveness and lower and upper respiratory tract injury with inflammation and cell damage. Additional and more recent areas of investigation include immunologic effects of O_3, genotoxicity, susceptible populations and the interaction of O_3 with other pollutants. In general, epidemiologic studies have shown the following health effects to be associated with O_3 exposure: excess cardiorespiratory mortality, increased hospital visits, increased

* references for introduction appear in Chapter 2

physician visits, increased emergency room visits, asthma exacerbations, increased medication use, and increased respiratory illness. Over the last 15 years, toxicologic studies have analyzed several biologic markers of response associated with acute O_3 exposure. In general four types of lung response have been demonstrated: 1) symptoms, irritative cough and substernal chest pain on deep inspiration; 2) decrements in Forced Vital Capacity (FVC) and Forced Expiratory Effort in 1 second (FEV_1) due chiefly to decreased inspiratory capacity rather than airway narrowing; 3) neutrophilic inflammation of the airway submucosa accompanied by increased levels of mediators, proteins and cytokines as measured in Bronchoalveolar Lavage (BAL) fluid, nasal lavage fluid and recently in sputum samples, and 4) increased non-specific airway reactivity. Relatively homogeneous study populations of healthy subjects have shown a wide range of susceptibility to the above effects, however good intrasubject reproducibility has been demonstrated with symptoms and spirometric indices. An overview of the literature suggests that a small percentage of study subjects, perhaps less than 10%, are non-responsive to acute O_3 exposure as measured by spirometry. Recent evidence indicates that up to 50% of subjects can be non-responsive to O_3 as measured by methacholine challenge testing. The heterogeneity in response among different groups of individuals is unexplained at present and susceptibility remains an active area of research. Currently the weight of evidence suggests that paradoxically, individuals with compromised airways, i.e. asthmatics and chronic bronchitics, do not have

responses to O_3 compared to healthy subjects. These studies of asthmatic subjects typically involve mild asthmatics and have focussed on spirometric measures of disease. The comparable findings of pulmonary function decrement between asthmatics and normals must be considered in light of the fact that asthmatics have a lower spirometric baseline and higher level of baseline airway responsiveness than do normals. As a result, a comparable ozone response will have more clinical significance with possible respiratory sequelae in the asthmatic than the normal healthy subject. Several studies have examined the possible relationships among the four types of airway responses described above. In general the evidence indicates that there is no association between neutrophilic inflammation and spirometric responses, as measured by FVC or FEV_1; and neutrophilic inflammation and symptom responses. Furthermore, there do not appear to be strong correlations among the various cellular and biochemical markers of response following ozone exposure, i.e. neutrophils, proteins, interleukin-8 (IL-8), interleukin-6 (IL-6), prostaglandin E2 (PGE2) and fibronectin. One putative mechanism behind ozone-induced reductions in FVC and FEV_1 is that these reductions are neurally mediated events and do not involve changes in Forced Residual Capacity (FRC) and Residual Volume (RV). Studies have shown that decreased inspiratory capacity is not due to increased elastic recoil, pulmonary edema or a reduction in inspiratory muscle force, but rather from neurally mediated involuntary inhibition of inspiration via C- fiber stimulation. Airway C-fibers located in the intraepithelial layer

can release bronchoconstricting agents such as neuropeptides (substance P) and kinins (tachykinins) which can act locally to constrict airway smooth muscle. Anti-inflammatory pretreatment studies with cyclooxygenase inhibitors (indomethacin, ibuprofin) suggest that cyclooxygenase metabolites may contribute to the reduction in inspiratory capacity following acute ozone exposure. However, in acute ozone exposure studies, % decrement in FEV_1 is often larger than % decrement in FVC suggesting an additional airway affect along with the decreased inspiratory capacity. Other mechanisms behind ozone-induced changes in lung function have been investigated. Studies with atropine pretreatment have shown that efferent vagal mediation does not play a role in ozone-induced FVC decline, since atropine pretreatment or administration of beta-adrenergic agonists do not prevent the fall in FVC or symptom reporting. Atropine does however reverse increased Raw after ozone inhalation suggesting that airway caliber is mediated by parasympathetic mechanisms.

This thesis in total will examine whether cyclooxygenase metabolites, which are sensitive to indomethacin inhibition, play a role in several airway changes following acute ozone exposure, and whether cyclooxygenase metabolites significantly affect the associations among the various airway changes. Airway changes include changes in lung function, symptom responses, airway reactivity, inflammatory cells, inflammatory mediators, i.e. prostaglandin F2-alpha (PGF2-alpha), and cytokines, interleukin-8

(IL-8).

With respect to pulmonary function, the thesis will address changes in maximal expiratory flows and volumes, and which airway segment is most affected, since recent evidence has implicated the small airways as a preferential target site of action for ozone. With respect to symptom responses, this thesis will measure changes in symptom reporting and symptom severity following acute ozone exposure. Previous studies have shown that indomethacin did not attenuate increases in symptom severity following acute ozone exposure in normal subjects. With respect to airway reactivity, this thesis will examine the response to inhaled methacholine as measured by the PC_{20}, i.e. provocation concentration of methacholine aerosol that causes a 20% fall in FEV_1. Several studies both in animals and humans, have shown increased airway reactivity to inhaled methacholine following acute ozone exposure. One study in normal subjects using indomethacin showed no attenuation of increased airway reactivity following ozone, but animal evidence has suggested a role for cyclooxygenase metabolites in ozone-induced airway hyperreactivity in dogs. This thesis will attempt to determine whether cyclooxygenase metabolites play a role in ozone-induced airway hyperreactivity in asthmatics.

The airway inflammatory response in humans has been examined through use of such tools as bronchoalveolar lavage (BAL), mucosal biopsy, segmental airway lavage, and recently induced sputum. The

cellular response following acute ozone exposure is dominated by neutrophil influx, peaking 4-6 hours post exposure. Several classes of soluble compounds have been found in airway fluid samples, including proteins, cytokines, eicosanoids, fibronectin, albumin, and complement components. This thesis will examine the cellular and biochemical responses in induced sputum samples following ozone exposure, specifically measuring differential leukocytes, PGF2-alpha and IL-8. This thesis will attempt to determine whether cyclooxygenase metabolites which are sensitive to indomethacin inhibition, play a role in altering the cellular and biochemical response to acute ozone exposure.

Finally, this thesis will attempt to measure the associations between outcome measures and determine whether cyclooxygenase metabolites which are sensitive to indomethacin inhibition, play a role in the formation of any observed associations.

CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

Study Approach

Approaches to the study of the human health effects of ozone (O3) exposure, using a whole body model, can be divided into approximately two main categories: 1. toxicologic studies; and 2. epidemiological studies. Toxicologic studies include controlled laboratory exposures and field exposures. This thesis used approach number one employing a single acute exposure protocol in a controlled environmental chamber. The airway responses examined in this thesis therefore, will be acute responses generated from exposures to O_3 via the inhalation route. There exists an extensive animal O_3 data base, as well as an In Vitro data base. Studies from these data bases will be referenced only in the context of comparing data to the human model used in this thesis. In summary, this thesis has focused on a particular segment of the overall O_3 literature with respect to human health effects, namely, controlled whole body exposures to O_3 and examining the subsequent acute airway effects.

Ozone Uptake and General Site of Action

Before one discusses the specific respiratory effects of O_3 exposure, it is important to understand the anatomic distribution of O_3 and it's uptake in the lungs, and what factors like flow, affect both of these characteristics. Hu et al., (1992) developed a relatively non-invasive bolus inhalation apparatus which can

measure longitudinal distribution of O_3 uptake in the intact human lung. In this technique, the fraction of O3 that is absorbed from the inhaled bolus is determined in a series of single breaths at different bolus penetrations. The difference between the integrals under the inspiration and expiration concentration curves is computed to determine the uptake. Recently Hu et al., (1994) employed this technique in healthy subjects using different longitudinal penetration depths that corresponded to the upper airways (20-70 milliliter (ml) penetration), the lower conducting airways (70-180 ml), and the respiratory airspaces (>180 ml). Hu found that during quiet oral breathing (250 ml/s), uptake increased as penetration increased, with 50% of the inhaled O_3 absorbed into the upper airways and the remainder absorbed in the conducting airways, such that no O_3 reached the respiratory airspaces. These data compare favorably with Gerrity et al (1988) data which reported that 40% of continuously inhaled O_3 in healthy subjects, was removed by the extrathoracic airways during quiet breathing (250 ml/second (s)). During exercise however, Hu et al (1994) found that the uptake-penetration distribution shifted distally, such that significantly less O_3 was absorbed in the upper airways (10%), more was absorbed in the conducting airways (65%), and some reached the respiratory airspaces (25%). This is important since the acute spirometric response of the human airway to ozone is proportional to exercise level (Tilton, 1989). Therefore, as a result of exercise, there is an increased dose of O_3 delivered to the conducting airways and distal alveolar region, and this is due

primarily to increased minute ventilation. Also, during exercise, the changes in respiratory pattern may affect lung function by redistributing the O_3 dose to more sensitive tissue. Hu et al., (1992) calculated the O_3 absorption rate constant, which revealed that the efficiency of O_3 uptake in the lungs increased with longitudinal position throughout the conducting airway region. Efficiency however, began to fall off at the beginning of the proximal alveolar region. Biscardi et al., (1993) showed a similar location effect in healthy human subjects for O_3 uptake in the lungs. He measured the fraction of O_3 mass taken up, and found that uptake increased, as O_3 moved from the larynx (0.176), to the upper trachea (0.271), to the mainstream bronchus (0.355), and finally falling off at the bronchus intermedius (0.325).

Molecular Target Site of Ozone in the Lung

It is generally held that the plasma membrane of the pulmonary epithelium is a principle target site of O_3. Specifically, O_3 is likely to react with cysteine, methionine, tyrosine, tryptophan, and histidine residues in proteins, and carbon-carbon double bonds in unsaturated fatty acids in membrane phospholipid. Two pieces of evidence point to the apical membrane as a specific target site for O_3. The high solubility of O_3 in nonaqueous solvents, and the rapid ozonolysis of unsaturated compounds, support the hypothesis that unsaturated lipids above or in the apical membrane are preferred target sites for O_3 in the lung (Leikauf, 1993). O_3 therefore, may

influence cellular function by chemical modification of membrane lipid, a direct effect, or it may alter cell function by the action of one of its secondary products, an indirect effect.

Chemical Reaction of Ozone in the Lung

The chemical reaction of O_3 in the lungs can be described generally as oxidations of macromolecular constituents of respiratory cells and respiratory tract fluid. It is generally believed that O_3 forms secondary products, namely aldehyde and hydroxyhydroperoxide, when reacted with unsaturated fatty acids (Leikauf, 1993). This reaction is extremely rapid with an estimated first order rate constant of $10^6 s^{-1}$ (Hu, 1994). These secondary products are far less reactive than O_3 itself and can therefore be formed above or in the plasma membrane. As a result, secondary reaction products can penetrate into the cell membrane, or leave the cell membrane, to act subsequently at distal intracellular sites.

Variability of the Human Ozone Response

Study subjects, in whom the respiratory responses to inhaled O_3 are evaluated, fall into 2 major categories: "responders" and "nonresponders". "Responsiveness" traditionally refers to spirometric responsiveness, as measured by changes in FEV_1 and FVC. Investigators define responsiveness differently, such that different cutoff levels are imposed. Nonresponsive cutoff levels

range from a less than 5%-15% fall in FEV_1. The explanation of or mechanism behind why a certain percentage of individuals do not respond with a change in spirometry to O_3 is not clear. Non-responsiveness occurs in both healthy and asthmatic subjects and has been estimated to be approximately less than 10% of the population.

Intersubject Variability

Several studies have measured the changes in respiratory function in healthy subjects following an acute exposure to 400 ppb O_3 at rest or with exercise. They have reported decrements in spirometry (FEV_1) ranging from 2 to 48% of baseline, and changes in specific airway resistance (SRaw) from -20 to 80% (Horvath, 1981; McDonnell, 1983). McDonnell (1993) recently determined the individual characteristics that accounted for the majority of the observed intersubject variability in FEV_1 decrement in healthy subjects following a 2 hour, 400 ppb exposure to O_3. McDonnell initially considered the following predictor variables which he thought may have accounted for intersubject variability : O_3 concentration, baseline pulmonary function, cardiovascular fitness, ventilation and respiratory pattern, respiratory health history, environmental and occupational exposures, skin test status, age, and season of exposure. Variables were selected on the basis of potentially influencing the following factors: delivered dose; molecular and cell responses to exposure; and neural responses to exposure.

McDonnell reported that O_3 concentration significantly explained 31% of the variance in FEV_1 decline between subjects, with age accounting for an additional 4%. With respect to age, McDonnell's model predicted an age effect with a decreasing FEV_1 response of 1.07, and 0.47 liters for 18 and 30 years olds, respectively. Recently, Drechsler-Parks examined the age effect with O_3 exposure in healthy males of varying ages. In the older age categories (56-71 year old males), Drechsler-Parks (1994) could not induce a spirometric decrement following a 2 hour, 450 ppb O_3 exposure at varying minute ventilations. Drechsler-Parks concluded that the mechanism responsible for O_3-induced FVC decrement, i.e. a decreased Inspiratory Capacity (IC) due to pain on deep inspiration, is less active in older adults.

Intrasubject Variability

Intrasubject variability has been examined by a number of investigators. McDonnell (1985) reported that changes in FEV_1 are highly reproducible at all levels of O_3 exposure greater than or equal to 180 ppb for a period of 3 months. Reproducibility decreased at 120 ppb O_3 for exposures separated by longer intervals. This work supports earlier findings by Gliner (1983) who reported a significant correlation coefficient (R = 0.9) between the magnitude of FEV_1 response on 2 occasions separated by 7 weeks. However, as the separation period increased, the correlation coefficient decreased. These findings suggested that the individual

differences between subjects in response (i.e. intersubject variability) are likely due to specific factors such as age, which change uniformly over time.

Covariability of the Human Ozone Response

A number of studies have attempted to determine the influence of additional covariate factors on subject variability, such as gender, race, pre-existing pulmonary disease, and smoking history, on the acute O_3 response.

Gender

Early evidence for a gender effect has been inconsistent due partly to different approaches taken to achieve equivalent doses of O_3 when inequalities in lung size between males and females have existed (Lauritzen, 1985; Adams, 1987; Messineo, 1990). A recent study by Weinmann (1995) used a predetermined exercise workload to help standardize minute ventilation, and they normalized minute ventilation (Ve_{min}) to FVC, in order to adjust for gender based differences in lung size. Weinmann reported no gender based differences in functional or symptomatic responses. This evidence supports earlier work by Seal (1993) and Adams (1987), who maintained as long as Ve_{min} was kept proportional to lung size or body weight, gender was not a significant factor in the O_3 response. Gerbase (1993) further examined the effect of menstrual

to different approaches taken to achieve equivalent doses of O_3 when inequalities in lung size between males and females have existed (Lauritzen, 1985; Adams, 1987; Messineo, 1990). A recent study by Weinmann (1995) used a predetermined exercise workload to help standardize minute ventilation, and they normalized minute ventilation ($\dot{V}e_{min}$) to FVC, in order to adjust for gender based differences in lung size. Weinmann reported no gender based differences in functional or symptomatic responses. This evidence supports earlier work by Seal (1993) and Adams (1987), who maintained as long as $\dot{V}e_{min}$ was kept proportional to lung size or body weight, gender was not a significant factor in the O_3 response. Gerbase (1993) further examined the effect of menstrual cycle on O_3 spirometric responses in female adults. Gerbase concluded that there was no consistent effect of menstrual cycle phase on expiratory flows and volumes. This work was corroborated by Seal (1993) who reported no significant effect of menstrual cycle on specific airway resistance or cough in female adults.

Race

The effect of race on O_3 responses has not been extensively examined, but a few reports have looked at the question. Seal (1993) investigated the possibility that black adults would have a greater O_3 response than white adults. The premise being that black adults have smaller lungs than white adults for a given height, therefore it is likely that for equivalent inhaled volumes of O_3,

between healthy subjects. Pre-existing disease status may affect the overall O_3 response and influence the intersubject variability. Intuitively, one would suspect that individuals with pulmonary disease, would respond to inhaled O_3 in an exaggerated fashion compared to normal, healthy individuals. In fact, several studies have indicated that functional responsiveness to O_3, i.e. spirometric change, is no greater, and usually lower among cigarette smokers (Kagawa, 1984; Shepard, 1983), asthmatics (Koenig, 1987; Linn, 1980), patients with Chronic Obstructive Pulmonary Disease (COPD) (Linn, 1980; Solic, 1982); and patients with allergic rhinitis (McDonnell, 1987).

A recent study by Scannell (1995) compared the asthmatic and non-asthmatic response to a 4 hour, 200 ppb O_3 exposure with respect to spirometry and cellular and biochemical indices of inflammation. His evidence showed that asthmatics did not differ from normals in their spirometric or total protein response to O_3, but they did have a more intense neutrophil response in BAL. Jorres (1993) compared the effect of a 3 hour, 250 ppb O_3 exposure on lung function and airway responsiveness in healthy subjects, subjects with rhinitis and subjects with atopic asthma. Jorres reported large intersubject variability in the response to O_3 across all disease groups, yet found no significant differences in lung function and AR between groups. He concluded that interindividual variation in the O_3 response was much more pronounced than differences in response arising from asthma or rhinitis. Holtzman

(1979) in earlier work, examined the question of atopy as it affects the acute O_3 response. Holtzman examined atopic and nonatopic subjects and concluded that inducibility of airway hyperreactivity by exposure to 600 ppb O_3 for 2 hours, was not related to atopy. Several studies (Koenig, 1985; Linn, 1978; Linn, 1980; Silverman, 1979) have not demonstrated asthmatics to be more spirometrically responsive than normals following acute O_3 exposure. However, Kreit (1989) demonstrated an enhanced asthmatic response to O_3 in some pulmonary function variables. Following a 2 hour, 400 ppb O_3 exposure, asthmatics showed a significantly greater decrease in FEV_1, $FEV_1\%$ and Forced Expiratory Flow at 25-75% of vital capacity (FEF_{25-75}), suggesting greater airways obstruction in asthmatics versus normals. However, considering asthmatics begin with a lower spirometric baseline than normals, any comparable change in FVC or FEV_1 will have greater clinical significance in the asthmatic individual. In summary, the weight of evidence suggests that the asthmatic and rhinitic response to acute O_3 exposure, i.e. spirometry and airway responsiveness, does not significantly differ from the normal healthy response.

Smoking Status

Frampton (1993) examined whether smokers differ from non-smokers in their pulmonary function response to acute O_3 exposure. He reported that following a 220 ppb, 4 hour O_3 exposure, smokers had a significantly decreased response in FVC, FEV_1 and Specific Airway

Conductance (SGaw) compared to non-smokers. Recently, Torres (1995) examined the inflammatory response in smokers and non-smokers following a 4 hour, 200 ppb O_3 exposure. Torres reported similar responses for both cells (alveolar macrophages (AMs)) and cytokines (IL-8) in smokers and non-smokers. Voter (1993) examined BAL cells from smokers and non-smokers who were exposed to 220 ppb O_3 for 4 hours. He measured the ability of recovered cells to generate superoxide anion (O2-), AMs ability to generate hydrogen peroxide (H_2O_2), and the ability of AMs to express receptors for IgG (Fc). He reported that smokers showed higher O2- production and increased expression of Fc receptors versus non-smokers. It appears then, that the influence of cigarette smoke is measurement dependent, with functional variables not showing a difference in response, while inflammatory cells showing equivocal results.

Study Outcome Measures

Spirometry responses

Several spirometric responses have been examined in humans following short-term O_3 exposures at relatively low doses (<500 ppb). They can be grouped as maximal expiratory flows and volumes, plythysmographic derived lung volumes and resistances, ventilation characteristics, exercise parameters, static and dynamic pulmonary compliance and elastic work. Among these parameters FVC and FEV_1 are the most widely studied due to their high reproducibility among

study subjects, compared to other more variable measurements, such as lung flows and airway resistance. In experimental protocols involving exposures to levels as high as 400 ppb, strong effects were not observed at rest. However, effects were observed when subjects exercised by raising ventilation and possibly altering the pattern of ozone uptake along the respiratory tract. McDonnell (1983) reported in groups of healthy, non-smoking young men, that exposure to ozone concentrations ranging from 120 to 400 ppb for 2 hours while performing intermittent heavy exercise ($\dot{V}e_{min}$=35L/min/meter squared (m^2) body surface area) produced consistent reductions in mean FVC and FEV_1 with increasing ozone concentration. Ozone-induced reductions in FVC and FEV_1 typically begin to recover immediately post exposure and within the first 2 hours, 50% of lung function has returned to pre-exposure baseline levels (Hazucha, 1996). Complete recovery of lung function decrement usually occurs within 24 hours of exposure, however in some individuals recovery is still incomplete at 18 and 24 hours post exposure (Folinsbee, 1982). Recent evidence suggests that decreases in isovolume flow in the small airways persists at 24 hours in healthy subjects (Foster, 1993; Weinmann, 1993; Gerbase, 1993). Other labs have confirmed these findings, with somewhat lesser responses in the exposure range 120 - 160 ppb (Kulle, 1985; Linn, 1986). Studies of acute responses have also used protocols of 6 to 8 hour exposures with ozone concentrations of 80 - 120 ppb with intermittent exercise. Results indicated that statistically significant progressive decrements in mean FEV_1 were demonstrated

in healthy young men exposed to as little as 80 ppb for 6.6 hours at a minute ventilation ($\dot{V}e_{min}$) of 40 L/min. (Horstman, 1989; Horstman, 1990).

Exposure-Response Relationship

Data from many labs over the years (Folinsbee, 1975; Hackney, 1975; Silverman, 1977; McDonnell, 1983; Gibbons, 1984; Kulle, 1985; Linn, 1988; Folinsbee, 1988; Kreit, 1989) have confirmed the observation that a fall in FVC and FEV_1 is a consistent response among subjects who respond to O_3 exposure. The magnitude of the FVC and FEV_1 decline shows considerable variation between individuals (5-25 %), and is reproducible in repeat testing. The fall in FVC and FEV_1 are related to the minute ventilation, the concentration of O_3 and the duration of exposure.

The curvilinear nature of the exposure-response (FEV_1) curve at low O_3 concentrations is well documented (Adams, 1981; Avol, 1984; Folinsbee, 1988; Horstman, 1990). It is generally agreed upon that at higher concentrations, a plateau in response occurs (Hazucha, 1992). Further, Gliner (1983) observed that 70% of the response following 420 and 500 ppb O_3 occurs in the first hour of exposure. Work by Hazucha (1987) synthesized the results from over 75 acute exposure chamber studies with normal subjects at varying O_3 concentrations. Hazucha fitted a quadratic equation for each pulmonary function variable and ventilation level. Hazucha showed

that the slope for each variable within a group, and almost all variables between groups, were significantly different from zero and from each other. Hazucha also determined that 100 ppb was the threshold exposure concentration (C) at high $\dot{V}e_{min}$ (>50 1\min) at which pulmonary function slopes became significant. McDonnell (1994) described the acute O_3 response as a function of exposure rate (C x $\dot{V}e_{min}$) and total inhaled dose [C x $\dot{V}e$ x Time (T)]. McDonnell demonstrated rather clearly that for low level (<500 ppb), short-term exposures (< 3 hours), the exposure-response relationship was non-linear. In fact, McDonnell described a general sigmoid-shaped model that fitted the mean responses in terms of exposure rate and total inhaled dose over a wide range of concentrations and times. Among the three variables, C, $\dot{V}e_{min}$ and T, McDonnell concluded that concentration was the strongest predictor of O_3-induced FEV_1 decrement. This was consistent with McDonnell's (1993) earlier report where he showed that concentration explained the majority of the variance in FEV_1 decline. McDonnell's (1994) sigmoid model supported previous work by Larsen (1991) who described a sigmoid function of CxT for a constant ventilation pattern. It must be realized that in the above studies, group mean data were used to determine the shape of the dose response curve for FEV_1 following ozone exposure. Due to high intersubject variability, only a small shift in group mean value may occur with ozone, but a much greater shift (and individually significant) will have occurred in a small number of more affected subjects. Therefore, in studies that have examined the shape of the dose

response curve, a small change in sample means reflect some individuals with large reductions in FEV_1, as well as a larger group with smaller or no decrements.

Mechanism Of Response In Inspiratory Mechanism

Hazucha (1989) determined that the O_3-induced fall in FVC was not due to a change in FRC or an increase in RV, but was the result of a fall in Inspiratory Capacity (IC). Various mechanisms have been postulated to explain the fall in IC contributing to O_3-induced decreases in FVC, FEV_1 and changes in ventilatory pattern. It had been suggested by Mills (1970) and many others since, that stimulated irritant receptors, located in the upper airways, caused tachypnea, which resulted in hyperventilation, and reflex bronchoconstriction. Taken together, these responses will lead to decreased FVC and FEV_1, and increased breathing frequency. Further, it was noted by Mills that irritant receptors respond primarily at the height of IC. This is consistent with the often reported symptom of pain on deep inspiration following acute exposure to O_3. The fall in IC was also responsible for observed decreases in VC and Total Lung Capacity (TLC), which contributed to decreased FVC and FEV_1. This has been confirmed by other investigators, most recently by Weinmann (1995). Hazucha (1989) further concluded that increases in both mean airway resistance (Raw) and SRaw were not due to changes in dynamic or static pulmonary compliance or elastic properties of the lungs. This is consistent with earlier work by

Bates (1972) and Kerr (1975) who also reported no change in the elastic properties of the lungs following acute O_3 exposure. Hazucha (1989), like Mills (1970), also noted a significantly increased respiratory frequency (Rf) and a decreased tidal volume (Vt) following acute O_3 exposure. These findings were also demonstrated in earlier work (Folinsbee, 1975; Folinsbee, 1977). Recent evidence regarding the inability to inspire fully following ozone exposure, points to neural mediation of irritant C-fibers located in the intraepithelial layer of the airway, as well as involvement of cyclooxygenase metabolites. Animal evidence showing airway C-fiber stimulation has been found in ozone exposed guinea pigs (Tepper, 1993) and in ozone exposed dogs using single fiber vagal preparations (Coleridge, 1993). Evidence of C-fiber stimulation in human subjects has been reported by Hazburn (1993), who showed increased levels of substance P following ozone exposure. Substance P is a neuropeptide found in C-fiber nerve endings and has bronchoconstricting properties. Pretreatment studies with indomethacin in healthy subjects has shown inhibition of the spirometric response (FVC, FEV_1) following an acute ozone exposure, suggesting that the ozone effect on IC is mediated in part by cyclooxygenase metabolites (Schelegle, 1987).

Small Airways Effects

Recently, Weinmann (1995) examined the effect of a 130 minute, 350 ppb O_3 exposure on small airway function using isovolumetric flow

measurements to adjust for O_3-induced decrements in VC. Weinmann reported approximately 10-15% reductions in isoflow forced expiratory flow rate at 50% VC (iso$\dot{V}_{50}$) and isoflow flow rate at 25-75% VC (iso$\dot{V}_{25-75}$) following O_3 versus filtered air exposures. Weinmann also reported that recovery of isoflow $\dot{V}_{25-75}$ to baseline, was not apparent at 24 hours post exposure, but partial recovery of FVC and FEV was apparent. This provided evidence that small airway effects persisted beyond large airway recovery.

Summary

Acute exposures (2-3 hours) to relatively low doses of O_3 (<500 ppb) have produced the following spirometric results: decreases have been demonstrated in FVC, FEV_1, $\dot{V}_{50}$, iso$\dot{V}_{50}$, $\dot{V}_{25-75}$, iso$\dot{V}_{25-75}$, Vt, IC, VC, FVC, TLC and Sgaw; increases have been demonstrated in Raw, SRaw, Heart Rate (HR) and Rf; no changes have been demonstrated in FRC, with slight increases in RV, and no changes in static/dynamic pulmonary compliance or elastic properties of the lungs.

Symptom Response

Symptoms such as cough, substernal pain, wheezing, shortness of breath, and pain on deep inspiration have been well documented following acute O_3 exposures in both healthy subjects and those with pre-existing pulmonary disease. (Hackney, 1975; Linn, 1980;

Koenig, 1985; Folinsbee, 1988; Horstman, 1990). Many of these studies also reported close associations between symptoms and mean pulmonary function decrement induced by O_3 exposure (Linn, 1980; Folinsbee, 1988; Schelegle, 1987; Horstman, 1990). For example, Schelegle (1987) pretreated healthy subjects with indomethacin and exposed them for 1 hour to 350 ppb O_3. Schelegle reported, reduced overall symptom severity, and inhibition of pulmonary function decrement. Lippman (1989) graded O_3-induced symptom responses in study subjects as mild, moderate and severe and placed cough, shortness of breath and severe pain on deep inspiration in these respective categories.

Inflammatory Response

It is well documented that acute O_3 exposure in humans causes increased concentrations of cellular and biochemical indices of airway inflammation. This occurs in both the lower airways, as measured by bronchoalveolar lavage (BAL) fluid samples (Seltzer, 1986; Koren, 1989; McDonnell, 1990; Devlin, 1991), and upper airways using nasal lavage samples (Graham, 1988; Graham,1990; Bascom,1990; McBride, 1994). Other sampling techniques such as isolated proximal airway lavage (PAL) (Aris, 1993), collection of the bronchial fraction (first 60 ml aliquot) of the BAL sample (Schelegle, 1991), induced sputum (IS) (Fahy, 1995) and bronchial biopsy (Aris, 1993) have been employed to compare the inflammatory

response in the proximal airway region to the distal airway region and alveolar surfaces. Early studies as well as a few recent ones also examined indicators of inflammation in plasma and blood following O_3 exposure (Schelegle, 1989; Hazbun, 1993).

Many investigators include the use of segmental PAL and bronchial fraction BAL when assessing lower airway inflammation. This is due to the fact that O_3 exerts a large part of its effects in the conducting proximal airway region, and BAL is primarily influenced by events occurring in the terminal bronchioles and alveoli. Schelegle (1991) examined the differences between pooled BAL samples and bronchial fraction samples. Schelegle compared data obtained from the first 60 ml aliquot BAL sample (bronchial fraction) versus the data obtained from pooling sequential BAL washes. He reported that pooling BAL washes acts to obscure the intensity of the bronchiolitis induced by acute O_3 exposure. In fact Duddridge (1988) observed that on average 24% of the return of the second 60 ml aliquot lavage was residual from the first lavage, and that 8% of the return of the third lavage was residual from the first. Other studies using sequential BAL washes have shown that variations in cell yield and in differential cell count depend on the volume of lavagate, which is not always uniform (Davis, 1982; Pingleton, 1983). Kelly (1987) demonstrated that the first 60 ml lavage filled the proximal airways next to the bronchoscope, while only the second lavage filled the segment of the more distal right middle lobe. These data support the contention of several

investigators (Davis, 1982; Robinson, 1988; Schelegle, 1991) that the first BAL wash samples are from the more proximal conducting airways of the wedged subsegment. Therefore, by separating proximal airway samples from pooled samples, one can infer that acute inflammation is occurring in the proximal airway (bronchiolitis), and that the distal airway is affected to a lesser degree.

In addition to analyzing bronchial fraction samples for the purpose of examining the proximal airway region, some investigators have elected to perform segmental airway lavages, usually in the right or left mainstem bronchus (Hazbun, 1993; Aris, 1993). Segmental PAL is a technique that appears to have a role in assessing the response of the airway epithelium, the chief molecular target site of O_3, to inhaled pollutants (Aris, 1993). This further supports evidence of inflammation in the proximal airways.

The additional measurement technique of tissue biopsy has also been used. Several investigators have used forceps biopsy of the bronchial mucosa of the upper lobes to directly assess proximal airway inflammation and tissue injury. (Aris, 1993; Scannell, 1995; Aris, 1995). Biopsy histology can then be used to identify evidence of inflammation (presence of neutrophils) and morphometry employed to quantitate inflammation (number of neutrophils).

The above bronchoscopic methods are all invasive, require the use of anaesthetic and have a significant recovery period for the study

subjects. For these reasons, these procedures cannot by used repeatedly on the same subject within a short period of time, and are not tolerated well by people who have severe pulmonary disease. Recently, a non-invasive method of collecting fluid samples from the lower airways has become available. It is induced sputum (IS) with hypertonic saline. This procedure analyzes cellular and biochemical constituents from sputum samples originating from the lower airways. Portions of the sample deemed originating from the upper airways region (> 20% squamous cells) are excluded from analysis. IS has the advantage of being much less invasive than bronchoscopic procedures and can be used repeatedly over relatively short time periods, and safely in subjects with moderate to severe pulmonary disease. This procedure is now being applied in controlled human exposure studies with O_3 (Wong, 1995; Alexis, 1995).

All the above methods, excluding plasma and blood analysis, have been used to measure the cellular response in humans following acute O_3 exposures. The total cell count and differential leukocyte count are two measures commonly reported in the O_3 literature. The differential cell counts are expressed as a percentage of the total cell count which usually includes only nucleated cells (i.e. no squamous epithelial cells) and are generally recorded as the percentage of polymorphonuclear neutrophils (PMN), eosinophils, macrophages, lymphocytes and bronchial epithelial cells.

Following an acute O_3 exposure, total cells, as measured in the lower and upper airways, are reported to increase in healthy subjects, compared to filtered air exposures (Aris, 1993; Wong, 1995). Many studies, both in animals, and humans, have reported that the specific cellular response following an acute O_3 exposure is dominated by PMN influx into the lower and upper airways. Holtzman (1983) and later Fabbri (1984) reported PMN influx into the epithelial layer of the airway wall in dogs. Human studies have reported PMN influx in the lower airways, as measured by BAL (Schelegle, 1991; Koren, 1989; Aris, 1993; Devlin, 1991; Gwizdala, 1993; Torres, 1995; Morrison, 1995; Scannell, 1994), and induced sputum (Alexis, 1996; Wong, 1996; Vagaggini, 1996; Fahy, 1995), and upper airways, as measured by nasal lavage samples (Graham, 1990; Graham, 1988). In addition to the neutrophil influx following ozone exposure, some studies have observed changes in other cell types including macrophages, eosinophils, and epithelial cells. Although these cell types are recovered in far fewer numbers compared to neutrophils following ozone, changes in their absolute numbers can have important physiological implications in how the airways respond to environmental insults. In a small number of studies, significant decrease in the absolute number of macrophages have been observed (Weinmann, 1993; Koren, 1989; Torres, 1995; Alexis, 1995).

With respect to eosinophils, one study exposed allergic asthmatics for 2 hours to 400 ppb O_3 or filtered air and reported a

significant eosinophil influx in nasal lavage fluid samples following O₃ exposure (Michelson, 1995). Bascom (1990) similarly examined upper airways responses. She exposed asymptomatic allergic rhinitics to 500 ppb O_3 for 2 hours, and found increased eosinophils in nasal lavage fluid samples compared to filtered air exposure. Only one study to date has reported an eosinophil response in the lower airways. Torres (1995) measured BAL fluid samples in healthy and smoking subjects, and reported an increased eosinophil response in both subject types. BAL was measured 18 hours following a 2 hour, 220 ppb O_3 exposure.

With respect to epithelial cells, only two human studies to date (McBride, 1994; Aris, 1993) have reported an increase following an acute O_3 exposure versus FA exposure. McBride (1994), examined nasal lavage samples in asthmatics exposed for 90 minutes to 240 ppb O_3, while Aris (1993) examined PAL samples in healthy subjects exposed for 4 hours to 200 ppb O_3. Both of these studies are supported by animal toxicology studies where increased epithelial cell injury was observed following acute O_3 exposure (Wilson, 1984; Schwartz, 1976).

Apart from determining the total and differential cell counts in BAL, PAL, bronchial fraction washes, Nasal Lavage (NL), IS and bronchial biopsy, some recent studies have examined other cellular characteristics, such as markers of cell activity, or adhesion molecule expression, following acute O_3 exposure (Voter, 1995;

Frampton, 1994; Morrison, 1995; Devlin, 1991; Aris, 1995).

Several biochemical markers of inflammation have been measured from the array of sampling techniques described above. These include BAL fluid eicosanoids such as prostaglandins, chemical indicators of cell damage such as total protein, albumin, and Immunoglobulin G (IgG), fibrolytic and inflammatory proteins, cytokines such as IL-8, and enzymes. With respect to eicosanoids, Seltzer (1986) reported increased levels of PGF2-alpha, which is a potent bronchoconstrictor of airways smooth muscle (Hamberg, 1975). PGF2-alpha also causes increased airway responsiveness in normals, (Mathe, 1975) and can heighten reflex bronchoconstriction by sensitizing airway nerve endings (O'Byrne, 1984). Seltzer (1986) also reported increased levels of Thromboxane B2 (TXB2) which is a bronchoconstrictor formed from unstable Thromboxane A2 (TXA2) (Sladek, 1990), PGE2 has been reported in several studies to increase following O_3 exposure (Devlin, 1991; McDonnell, 1990; Koren, 1989). PGE2 has a bronchoconstricting and bronchodilating effect on airway smooth muscle and is a down regulator of functional responses in macrophages (phagocytosis) and neutrophils (Downey, 1988; Kuehl, 1980; Davies, 1984).

With respect to cytokines, IL-8 has been reported to increase in BAL fluid following O_3 exposure (Gwizdala, 1993; Torres, 1995). IL-8 is a chemoattractant for neutrophils and activates neutrophils to release lysosomal enzymes (Oppenheim, 1991). Wong (1995) recently

reported increased levels of IL-6 and IL-8 in sputum samples following acute O_3 exposure. Scannell (1994) observed increased levels of Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF), which acts to promote all cells in the granulocyte, macrophage and eosinophil lineage (Burgess, 1990).

Airway Reactivity

Many investigators have demonstrated increased nonspecific airway reactivity to methacholine or histamine following acute, O_3 exposure. This has been demonstrated in both animal (Lee, 1977; Holtzman, 1983) and human studies (Aris, 1993; Ying, 1990; Folinsbee, 1989; Eschenbacher, 1989; Gong, 1988; McDonnell, 1987; Seltzer, 1986; Dimeo, 1981; Holtzman, 1979). But it was Golden (1978) who first demonstrated increased nonspecific airway reactivity to methacholine in healthy subjects following a 2 hour, 600 ppb O_3 exposure. Holtzman (1979) shortly thereafter induced airway hyperreactivity in both atopic and non-atopic subjects to the same degree following a 2 hour, 600 ppb O_3 exposure. Horstman (1989) later determined that the minimal dose capable of eliciting increased nonspecific airway reactivity to methacholine in healthy subjects, was 80 ppb, but this was in a repeated 6.6 hour exposure protocol with exercise. Kreit (1989) further demonstrated that following a 2 hour, 400 ppb O_3 exposure, asthmatics and healthy subjects did not differ in their increased reactivity to methacholine challenge. Reactivity to other bronchoconstrictive

agents have been recorded, mainly with histamine, with similar results to methacholine data (Holtzman, 1979; Seltzer, 1986). Not all studies however, have been 100% successful in inducing increased nonspecific airway reactivity following acute O_3 exposures. Ying (1990) for example, was only able to demonstrate increased nonspecific airway reactivity in 7 of 13 healthy male subjects following a 2 hour, 400 ppb O_3 exposure.

Associations Between Outcome Measures

Several investigators have attempted to determine whether O_3 response variables are associated with one another, i.e. do relationships exist among indices of inflammation, pulmonary function changes, symptom reporting and changes in non-specific airway reactivity.

The association between airway hyperreactivity and inflammation following O_3 exposure has received much attention. Respiratory exposures to acute O_3 can cause non-specific hyperreactivity in healthy humans (Golden, 1978; Holtzman, 1979) and animals (Lee, 1977; Holtzman, 1983). The hypothesis that an association exists between O_3-induced non-specific hyperreactivity and airway inflammation is suggested by the fact that stimuli, such as O_3, can cause both reactions to occur concomitantly. The literature however, has revealed inconsistent results supporting this association, but this may due to species differences and exposure

levels (Ying, 1990; Tepper, 1993; Seltzer, 1986; Janssen, 1991; Yeadon, 1992). The association was first demonstrated in dogs where Holtzman (1983) and later Fabbri (1984), showed that dogs who became hyperresponsive following a 2 hour exposure to 2100 ppb O_3, also developed epithelial cell desquamation from the airways, and a marked neutrophil influx into the epithelial layer of the airway wall. O'Byrne (1984) depleted dogs of their neutrophils in an effort to determine whether the development of non-specific hyperreactivity depended on the neutrophil influx into the epithelial layer. He found that neutrophil depletion prevented the neutrophil influx to occur, and it also prevented the development of O_3-induced airway hyperreactivity. However, O'Byrne (1984) also demonstrated that indomethacin pretreatment significantly inhibited airway hyperreactivity in dogs without affecting neutrophil influx. O'Byrne's studies suggest that both neutrophils and cyclooxygenase metabolites are important singular factors that can individually mediate airway responsiveness. Seltzer (1986) first examined the relationship between increased non-specific airway reactivity and airway inflammation in humans, by examining BAL samples following 400 or 600 ppb O_3 exposure. Seltzer found significant increases in the concentration of neutrophils, and several arachidonic acid metabolites, such as PGF2-alpha, PGE2 and TXB2 in BAL fluid. Seltzer concluded that an association existed between changes in non-specific airway reactivity and PMNs in BAL fluid. Other studies by Murlas (1991) and Li (1992) have shown that the presence of neutrophils is not essential for ozone-induced airway

hyperreactivity to occur.

Early animal studies had suggested the possibility that lung lipids may be involved in the development of O_3-induced hyperreactivity. In a rat model, Shimasaki (1976) showed that O_3 exposure resulted in an increase in arachidonic acid levels in BAL samples. This led O'Byrne (1984) to investigate whether oxygenation products of arachidonic acid were important in the development of O_3-induced airway hyperreactivity and neutrophil recruitment into the airway of dogs. He used indomethacin, a potent inhibitor of prostaglandin synthetase, to effectively block cyclooxygenase metabolite generation. He found indomethacin inhibited airway hyperreactivity in dogs exposed for 2 hours to 3000 ppb O_3, but neutrophil influx was unaffected. This study suggested that a role existed for cyclooxygenase products sensitive to indomethacin block, in the development of O_3-induced airway hyperreactivity. McDonnell (1990) determined correlation coefficients between airway reactivity as measured by the dose of methacholine required to increase specific airway resistance by 100% (PD100,Sraw) and BAL measures which changed significantly, in healthy subjects exposed to a 6.7 hour, 100 ppb O_3 exposure. Significant correlations were reported between PGE2 and PD100,Sraw (R=-0.86), but not between %PMN and PD100,Sraw, %PMN and protein or between sulfidopeptide leukotrienes (LT) (LTC4, LTD4, LTE4) and PD100.

Aris (1993) examined the hypothesis that methacholine

responsiveness (PC100,Sraw) might be an important determinant of susceptibility to O_3 as measured by FEV_1 response. Aris exposed 66 healthy subjects to a 4 hour exposure of 200 ppb O_3. He reported that baseline PC100,Sraw was significantly associated with increases in O_3-induced PC100,Sraw, but not FEV_1 or FVC decline.

Animal evidence (Coleridge, 1976; Roberts, 1985) has shown that PGE2 and PGF2-alpha can stimulate pulmonary neural afferents, which can initiate several responses characteristic of acute O_3 exposure. This evidence along with Seltzer's (1986) findings of increased prostaglandins (PGF2-alpha, PGE2) in the BAL fluid of subjects exposed to acute O_3 exposure, had led other investigators to speculate whether associations existed between inflammatory markers, pulmonary function decline, ventilatory changes and symptom reporting.

McDonnell (1990) performed BAL measurements in healthy subjects, and found a significant association between a biochemical marker of inflammation and spirometry. McDonnell observed a significant association between the eicosanoid PGE2 and FEV_1 decline (R=0.7), and between the reported symptom of pain on deep inspiration and protein level (R=0.8).

Recently Morrison (1995) examined the relationship between pulmonary function changes, inflammation, epithelial permeability and oxidant status in the airspaces of non-smokers exposed for 1

hour to 400 ppb O_3. He reported that O_3-induced bronchoconstriction (9% fall in FEV_1) was associated with PMN influx into the airspaces, as well as with reduced superoxide production by BAL leukocytes.

Scannell (1994) recently examined distal and proximal airway lavage samples in healthy subjects exposed to 200 ppb O_3 for 4 hours and determined correlations of O_3-induced decreases in FEV_1 and FVC with cellular and biochemical markers of inflammation (i.e. Total cells, differential cell counts, protein, fibronectin, IL-8, GM-CSF). By defining subjects as spirometrically sensitive (based on FEV_1 response to O_3) or insensitive, Scannell reported that the magnitude of the cellular and biochemical response did not differ between groups or airway regions sampled. This supports earlier work by Schelegle (1989) who defined his subjects as spirometrically sensitive (>24% fall in FEV_1) and insensitive (<11% fall in FEV_1) and exposed them to 350 ppb O3 for 80 minutes. He found no correlation between the eicosanoid PGF2-alpha, measured in plasma, and changes in FEV_1 and FVC. Framptom (1994), like Scannell (1994) and Schelegle (1989), similarly defined healthy subjects as sensitive (responders) (>15% fall in FEV_1) or insensitive (non-responders) (<5% fall in FEV_1) and exposed them to 220 ppb O_3 for 4 hours. In contrast to Scannell (1994) and Morrison (1995), Framptom showed that non-responders were associated with greater PMN influx 18 hours post exposure, while other investigators showed that subjects with the greatest FEV_1 decline were not necessarily

NOTE TO USERS

Page(s) not included in the original manuscript are unavailable from the author or university. The manuscript was microfilmed as received.

39

UMI

Hazbun (1993) examined the relationship between Substance P and 8 epi-PGF2-alpha (a non-cyclooxygenase derived prostaglandin) and changes in spirometry following acute ozone exposure. He failed to show a significant correlation between 8epi-PGF2-alpha and decreases in FEV_1. Hazbun did identify however, a significant correlation (R=0.89) between O_3-induced increases in Substance P and 8-epiPGF2-alpha.

McBride (1994) examined the correlation between upper airways inflammation in asthmatics exposed for 90 minutes to 240 ppb O_3. Although a significant increase in IL-8 levels was not observed, McBride found a significant correlation between IL-8 levels and Total Cell Counts (TCC)/ml (R=0.8) in nasal lavage (NL) samples taken 10 minutes post exposure.

As a consequence of airway inflammation, morphologic alterations may occur in the airways and persist beyond spirometric recovery. Animal evidence indicates that acute morphologic response to ozone involves epithelial cell injury along the entire respiratory tract, resulting in cell loss and replacement. In the lower airways of rats, the proximal acinar regions are most affected, with ciliated cells more sensitive to injury than Clara cells or mucus secreting cells (Evans, 1976). In the alveolus, Type 1 cells are highly sensitive to ozone whereas Type 2 cells are resistant and appear to serve as a stem cell for Type 1 cell replacement following ozone exposure (Evans, 1976).

Mechanisms of Ozone-Induced Responses

The mechanisms behind airway changes following ozone exposure are not fully understood, but it is clear that with respect to nonspecific airway reactivity and some spirometric indices, some abnormality exists in the parasympathetic nervous system.

Parasympathetic involvement has been largely implicated from studies that have used atropine pretreatment to block muscarinic receptors, including those located on airway smooth muscle, and from studies that have interrupted the vagal nerve reflex arc. Beckett (1985) pretreated subjects with aerosolized atropine and exposed them for 2.5 hours to 400 ppb O_3. Beckett reported that atropine prevented the significant increase in airway resistance, and partially blocked the decrease in expiratory flows. Atropine did not affect FVC, Rf, Vt or symptom reporting. Early work by Golden (1978) reported that atropine pretreatment prevented the increase in nonspecific airway reactivity to histamine after healthy subjects were exposed to 400 ppb O_3 for 2 hours. This indicated the involvement of the muscarinic cholinergic receptors. These results were later supported by Holtzman (1979) who exposed healthy subjects pretreated with atropine, to 600 ppb O_3 for 2 hours. Holtzman prevented increased nonspecific airway reactivity to histamine. In vitro studies also support a role for parasympathetic nervous system involvement in the acute O_3 response. Studies on isolated canine lung segments exposed to 100

and 1000 ppb O_3 have demonstrated a rapid increase in collateral resistance (Gertner, 1983; Gertner, 1983). At both of these concentrations of O3, the early increase in Raw was prevented by atropine pretreatment, yet at the higher concentration, a later increase in collateral resistance was not blocked by atropine.

Studies which have also implicated parasympathetic involvement in animals (Lee, 1977; Yeadon, 1992) and humans (Golden, 1978; Holtzman, 1979), have also demonstrated that either vagal cooling or vagotomy attenuated the O_3-induced responsiveness to histamine.

Holtzman (1979) postulated a mechanism whereby damage to the airway epithelial lumen, decreased the threshold of the vagal sensory nerve endings, thus allowing histamine to stimulate greater activity in both afferent and efferent pathways. This would lead to subsequent release of acetylcholine resulting in more severe airway smooth muscle bronchoconstriction. Several animal studies support Holtzman's theory of increased epithelial permeability following acute O_3 exposure.

Miller (1986) showed that O_3 increased airway epithelial permeability following acute exposure in guinea pigs. Bhalla (1986) also demonstrated increased bronchoalveolar permeability in rats exposed to O_3. Several human studies have examined changes in epithelial permeability following acute O_3 exposure, by measuring the airway clearance rate of gamma emitting ^{99m}Tc-Diethylene-

diaminepentaacetate (DTPA). Kehrl (1987) measured airway clearance of ^{99m}TC-DTPA in healthy subjects exposed to 400 ppb O_3 for 2 hours. Kehrl reported increased clearance following O_3 versus filtered air (FA) exposure, which is indicative of increased epithelial permeability. Recently, two studies have examined O_3-induced epithelial permeability changes at different post exposure time points. Foster (1995) reported increased clearance of 99mTC-DTPA in healthy subjects exposed for 130 minutes to 350 ppb O_3, 18-24 hours following exposure. In contrast, Morrison (1995) could find no alteration in ^{99m}TC-DTPA clearance rate 1 hour following a 1 hour, 400 ppb O_3 exposure. Holtzman (1979) suspected that O_3 caused either the number of Acetylcholine (Ach) receptors to increase, or their affinity for Ach to increase. Recent evidence from a guinea pig model (Schultheis, 1994) demonstrated that following high dose O_3 exposure (2000 ppb), inhibitory M2 muscarinic receptors became dysfunctional, thereby potentiating the release of Ach from vagal nerves, and increasing vagally mediated bronchoconstriction.

Several investigators have also studied the contribution of the sympathetic nervous system to O_3-induced increases in nonspecific airway reactivity, symptom reporting, and pulmonary function decrements. Gong (1988) pretreated healthy athletes with inhaled albuterol (beta-2 adrenergic bronchodilator) and found no improvement in O_3-induced pulmonary function decrement (FVC, FEV_1, V_{25-75}), symptoms and increased nonspecific airway reactivity. These results were consistent with an earlier report by Mckenzie (1987)

who used salbutamol (beta-2 adrenergic bronchodilator) pretreatment with no success on FVC, FEV_1 and symptom reporting. Beckett (1985) did report however, that post exposure treatment with metaproterenol (inhaled beta-2 agonist) quickly reversed O_3-induced increased specific airway resistance. Together these findings suggest that the contribution of the sympathetic nervous system, i.e. beta adrenergic mechanism, is minimal with respect to causing pulmonary function decrement, symptom reporting and increased nonspecific airway responsiveness.

On the other hand, these studies demonstrate the importance of smooth muscle contraction and do not exclude the importance of the parasympathetic nervous system as a principal mechanism in ozone-induced airway hyperreactivity.

The significance of the cyclooxygenase (COX) pathway in both lung disease (Marsh, 1985; Diaz, 1989) and in O_3-induced lung injury (Holtzman, 1983; O'Byrne, 1984; Schelegle, 1987), is demonstrated in studies which correlate abnormalities in airway function with the production of arachidonic acid (AA) products. The major focus of cell types generating arachidonic acid metabolites are those conspicuously involved in pulmonary inflammation, namely leukocytes and epithelial cells.

Linoleic acid is the w-6 essential fatty acid precursor of AA. The end products of this metabolic pathway via COX metabolism are

prostaglandins (D2, E2, I2), and thromboxane A2. This pathway has recently been referred to as cyclooxygenase 1 (COX1) since a second enzyme (COX2) has been reported to regulate AA derived prostaglandin production (Smith, 1993); via 5-lipoxygenase metabolism, the end products are leukotrienes (B4), and sulfidopeptide leukotrienes C4, D4, and E4 (Henderson, 1987). Alpha-linolenic acid is the w-3 fatty acid precursor of eicosapentaenoic acid (EPA). The end products of this metabolic pathway via cyclooxygenase are prostaglandins (D3, E3, I3) and thromboxane (A3); via the 5-lipoxygenase pathway the end products are leukotrienes (A5, B5, C5, D5) (Henderson, 1987). The end products of importance for this thesis are the w-6 derived oxygenated derivatives of AA, called eicosanoids.

The cyclooxygenase pathway gives rise to a distinct array of products. The availability of AA to oxygenation pathways is tightly controlled in most cells by the activity of phospholipases. Phospholipase A2 acts to release fatty acids from structural pools of glycerophospholipid in cell membranes. Upon activation by either cell surface receptor activation, mechanical perturbations of the cell membrane, or reaction with phospholipase activating protein (PLAP), PLA2 translocates from the cytosol to the cell membrane and in response to submicromolar calcium (Ca^{2+}) concentrations, preferentially cleaves membrane lipids to form free AA (Henderson, 1987). Free AA is then available for metabolism (Sladek, 1990). The enzyme cyclooxygenase (prostaglandin synthase) is found in the

endoplasmic reticulum and nuclear membrane of certain cells, and catalyzes the conversion of AA to prostaglandin endoperoxides, which serve as the precursors to a series of biologically active compounds called thromboxane, prostacyclin and prostaglandins (Holtzman, 1991). The cyclooxygenase activity of the enzyme (cyclooxygenase) (step 1) inserts two molecules of oxygen into arachidonic acid to yield prostaglandin (PG) G2, and the peroxidase activity of the enzyme (step 2), reduces PGG2 to its 15-hydroxy analogue, PGH2, which is the precursor for other PGs, prostacyclin, and thromboxane. In the absence of endoperoxidation (step 2), the initial step is followed by radical migration and oxygenation to yield 11- and 15-hydroxy-eicosatetraenoic acid (11-and 15-HETE). Non-steroidal anti-inflammatory drugs, like indomethacin, inhibit cyclooxygenase (step 1), but not the hydroperoxidase activity (step 2) of the cyclooxygenase enzyme (Holtzman, 1991).

Two other major enzymatic pathways involved in oxygenating fatty acids are lipoxygenase, and monooxygenase, from which identical compounds to the cyclooxygenase pathway can be formed (i.e. HETEs). Therefore, findings HETEs for example in biological fluids (i.e. BAL) is not sufficient to specify the cyclooxygenase mechanism of formation. However, unique to the cyclooxygenase pathway are prostaglandins, and thus they can be used as markers to specify the enzymatic mechanism of formation.

The cyclooxygenase pathway frequently generates oxygen radicals.

The conversion of PGG2 to PGH2 involves a peroxidase and liberates O⁻ which can then be used by other oxidative systems. Prostaglandin synthesis and the accompanying peroxidation occurring in the presence of NADH or NADPH, may also lead to the formation of O_2-, which may cause epithelial tissue injury after trauma in the lung (Kontos, 1987). Oxygen radicals also function as regulatory controls on the cyclooxygenase pathway. Lipid hydroperoxides derived from lipid interaction with activated oxygen species, such as those from O_3-epithelial lining fluid interactions, are activators of cyclooxygenase at low concentrations (< 100 nm), and inhibitors at higher concentrations (Holtzman, 1991). Free radical-generated systems therefore have the capacity to regulate eicosanoid effects by their ability to inactivate cyclooxygenase products or directly activate phospholipase. The net effect of oxidants or oxidant-induced peroxides however, depends on the concentration of reactive species and on the capacity for oxidant removal (free radical scavengers).

Each of the cells involved in mediation of upper and lower airway inflammation, release a distinct profile of mediators after cell stimulation. The following are cells which release mediators from cyclooxygenase metabolism: epithelial cells, eosinophils, lung mast cells, alveolar macrophages, monocytes, endothelial cells and platelets. Neutrophils do not release any products of cyclooxygenase metabolism, but since they are the predominant cell type in the O_3 response, they will be discussed as well.

Neutrophils release products from 5-lipoxygenase metabolism. All inflammatory cells mentioned release platelet activating factor (PAF), an end product of lyso-phospholipid metabolism.

Airway epithelial cells produce the most cyclooxygenase activity compared to other inflammatory leukocytes. Airway epithelial cyclooxygenase products have established roles in regulation of ion transport (Al-Bazzaz, 1981) and smooth muscle tone (Flavahan, 1985; Stuart-Smith, 1988). Human epithelial cells generate nearly equivalent amounts of PGF2-alpha, and PGE2 (Holtzman, 1980). Cultured human tracheal epithelial cells release a profile of cyclooxygenase products similar to freshly isolated cells (Churchill, 1989), namely, increased levels of PGE2.

Human eosinophils incubated with AA produce predominantly 15-HETE (Holtzman, 1989). If stimulated however, by agents such as calcium ionophore, eosinophils will produce LTC4 (Holtzman, 1991). Lung mast cells release PGD2, a bronchoconstrictor and pulmonary vasoconstrictor (Henderson, 1987). Pulmonary macrophages contain a 5-lipoxygenase pathway that produces high concentrations of LTB4. Data in humans indicate that LTB4 is the predominant neutrophil chemotaxin released by normal resident lung macrophages (Martin, 1987). Mononuclear phagocytes release large quantities of TXA2 and alveolar macrophages release large quantities of PGE2 (Holtzman, 1991).

Monocytes release products from both cyclooxygenase and 5-lipoxygenase pathways. They release LTB4, the potent neutrophil chemotaxin, and the sulfidopeptide LTC4. They also release TXA2 and PAF (Henderson, 1987). Endothelial cells release the bronchodilator PGI2 (prostacyclin) and platelets release TXA2 and PAF (Henderson, 1987).

Neutrophils, the predominant cell type in the O_2 response do not have cyclooxygenase activity. Neutrophils contain the 5-lipoxygenase pathway and produces mainly LTB4 when stimulated with calcium ionophore or cell surface receptor stimuli (Holtzman, 1991). LTB4 may also augment the release of additional inflammatory mediators (Tessner, 1989). The neutrophil also contains a P450-monooxygenase capable of rapidly oxidizing LTB4 to its hydroxylase metabolite, which shares similar functional activity with LTB4 (Clancy, 1984).

Cyclooxygenase activity is stimulated by a number of different growth factors, such as transforming growth factors (TGF-alpha/beta) (Diaz, 1989), epidermal growth factor (Yokota, 1986) and platelet-derived growth factor (Goerig, 1987). Tumor Necrosis Factor (TNF) indirectly influences cyclooxygenase by stimulating Phospholipase Activating Peptide (PLAP), the enzyme needed to stimulate cyclooxygenase activity (Clark, 1987). Actinomycin D inhibits cyclooxygenase activity (Yokota, 1986).

IL-1, IL-2, interferon gamma and GM-CSF stimulate cyclooxygenase and 5-lipoxygenase activity (Holtzman, 1991). Like TNF, IL-1 indirectly stimulates cyclooxygenase by stimulating PLAP (Clark, 1987). Communication between cytokines also serves to regulate cyclooxygenase activity.

Indomethacin

The ability of applying a specific pharmacologic block on the cyclooxygenase pathway involves affecting one of the four critical components of the oxygenation reaction: 1. the enzyme (cyclooxygenase); 2. the substrate arachidonic acid; 3. the substrate phospholipid; 4. the activator phospholipase A2 (PLA2). Interruption of the availability or function of any of these steps will block the formation of cyclooxygenase end products. Indomethacin alters the enzyme by specifically blocking the first step of the enzyme's action, that is the cyclooxygenase. It does not affect the second step, peroxidation.

Several studies in animals and humans have used indomethacin to implicate the involvement of the AA pathway as a mechanism of response in allergen induced asthma, Toluene Diisocyanate (TDI) induced asthma and O_3 induced airway responses. O'Byrne (1984) prevented airway hyperreactivity in dogs with Indomethacin pretreatment following acute O_3 exposure. Lanes (1988) also prevented specific antigen-induced hyperreactivity with

indomethacin pretreatment in sensitized sheep, but failed to inhibit the asthmatic responses. In contrast, Lee (1985) was unable to block the increase in airway resistance or airway responsiveness in guinea pigs following acute O_3 exposure, suggesting species differences.

Irritant exposure studies with humans pretreated with indomethacin have shown variable results with respect to preventing pulmonary function decline, and airway hyperreactivity. Both Fairfax (1983) and Shephard (1985) demonstrated that indomethacin inhibits the late asthmatic response after allergen inhalation. Shepard (1985) also showed that indomethacin prevented the increase in TXB2 following allergen inhalation. Neither study measured the changes in airway reactivity following allergen inhalation therefore the effect of indomethacin on airway reactivity was not evaluated. Fabbri (1985) examined spirometry and airway reactivity in TDI sensitive asthmatics pretreated with indomethacin following TDI exposure. Fabbri was unable to demonstrate any protective effect of indomethacin on airway hyperreactivity, and did not demonstrate an inhibitory affect on the spirometric response. Walters (1983) however, demonstrated that indomethacin pretreatment inhibited the development of airway hyperreactivity in normal subjects following upper respiratory viral infection. Kirby (1989) pretreated atopic subjects with indomethacin prior to allergen exposure. Kirby measured airway reactivity and the early and late asthmatic spirometry response. Kirby reported that indomethacin inhibited the

increase in airway reactivity. O'Byrne (1986) further examined the role of contractile prostaglandins in the initial broncho-constriction after exercise in asthmatics, and the role of inhibitory (bronchodilator) prostaglandins in the refractoriness following exercise. O'Byrne reported that indomethacin pretreatment did not alter initial bronchoconstriction, but did inhibit the exercise-induced refractoriness.

Schelegle (1987) was the first investigator to determine whether indomethacin pretreatment protected against pulmonary function decrement following a controlled exposure to acute O_3 in human subjects. He reported that indomethacin pretreatment significantly attenuated the fall in FEV_1 and FVC following a 1 hour exposure to 350 ppb O_3. Eschenbacher (1989) reported similar results at 400 ppb for 2 hours in healthy subjects. Ying (1990) attempted to replicate Schelegle's (1987) results with respect to pulmonary function protection, and also tried to demonstrate in healthy humans that indomethacin prevented ozone-induced airway hyperreactivity. Ying (1990) found that indomethacin pretreatment did not attenuate the increase in airway reactivity following a 2 hour exposure to 400 ppb O_3 in healthy subjects. He did confirm however, Schelegle's (1987) results that indomethacin will prevent the decline in FEV_1 and FVC following acute O_3 exposure.

REFERENCES

Adams, WC., Savin, WM., Christo, AE. Detection of ozone toxicity during continous exercise via the effective dose concept J Appl Physiol 51: 415-22, 1981

Adams, WC., Brooks, KA., Schelegle, ES. Effects of NO2 alone and in combination with O3 on young men and women. J Appl Physiol 62: 1698-704, 1987

Al-Bazzaz, F., Yadava, VP, VP., Westonfelder, C. Modification of Na and Cl transport in canine tracheal mucosa by prostaglandins. Am J Physiol; 240; F101-5, 1981

Alexis, N,. Silverman, F., Tarlo, S., Corey, P. Effects of indomethacin on acute ozone exposure in asthmatics (Abst) Am J Resp Crit Care Med Vol 151, No 4 April, 1995

Aris, R., Erle, D., Chen, LL., Christian, D., Heame, PQ. Ferrando, RE., Balmes, JR. Increased expression of the beta-2 integrins CD11b and CD11c on human lung neutrophils in the ozone model of acute lung injury. (Abst) Am J Respir Crit Care Med vol 149 No 4 April 1994

Aris, R., Ferrando, R., Chen, L., Christian, D., Balmes, JR. Increased ICAM expression on human bronchial endothelium after acute exposure to ozone (Abst) Am J Respir Crit Care Med vol 151, No 4, April, 1995

Aris, RM., Christian, D., Hearne, PQ., Finkbeiner, WE., Balmes, JR. Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. Am Rev Respir Dis 148: 1363-1372, 1993

Avol, EL., Linn, WS., Venet, TG., Shamoo, DA., Hackney, JF. Comparative respiratory effects of ozone and ambient oxidant pollution exposure during heavy exercise JAPCA 34: 804-809, 1984

Bascom, R., Naclerio, RM., Fitzgerald, TK., Kagy-Sobotka, A., Proud, D. Effect of ozone inhalation on the response to nasal challenge with antigen of allergic subjects Am Rev Respir Dis 142: 594-601, 1990

Bates, DV., Bell. GM., Burnham, CH., Hazucha, MJ., Mantha, J., Pengelly, LD., Silverman, F. Short-term effects of ozone on the lung. J Appl Physiol 32: 176-181, 1972

Beckett, WS., McDonald, WF., Horstman, DH., House, DE. Role of the parasympathetic nervous system in acute lung response to ozone. J Appl Physiol 59: 1879-1885, 1985

Bhalla, DK., Mannix, RC., Kleinman, MT., Crocker, TT. Relative

permeability of nasal, tracheal and bronchoalveolar mucusa to macromolecules in rats exposed to ozone. J Toxicol Envriron Health 17: 269-83, 1986

Biscardi, F., Gerrity, T., Strong, A., Garlington, R., Bromberg, P. Ozone uptake in human airways. Am Rev Resp and Crit Care Med. (Abst) Vol 147 No 4 April 1993.

Bitterman, PB., Rennard, SI., Adelberg, S., Crystal, RG. Role of fibronectin as a growth factor for fibroblasts. J Cell Biology 97: 1925-1932, 1983

Boven, RM., Hoidal, JR., Estensen, RD. Urokinase-type plasminogen activator in alveolar macrophage and bronchoalveolar lavage fluid from normal and smoke exposed hamsters and humans J Lab Clin Med 106: 667-673, 1985

Burgess, AW. Granulocyte Macrophage Colony-Stimulating Factor in peptide growth factors and their receptors 1, Sporn, MB and Roberts, AB, eds, Springer Verlog, New York, p 723, 1990

Churchill, L,. Chilton, PH., Resau, JH et al. Cyclooxygenase metabolism of endogenous arachidonic acid by cultured human tracheal epithelial cells. Am Rev Respir Dis; 140; 449-59; 1989

Clancy, RM., Dahinden, CA., Hugli, TE. Oxidation of leukotrienes at the w end: demonstration of a receptor for the 20 hydroxy derivative of LTB4 on human neutrophils and implications for the analysis of leukotriene receptors. Proc Natl Acad Sci USA 81: 5729-33, 1984

Clark, MA., Conway, TM., Shorr, RGL., Crook ST. Identification and isolation of a mamalian protein which is antigenically and functionally related to the phospholipase A2 stimulatory peptide melittin J Biol Chem; 262: 4402-6; 1987

Coleridge, HM., Coleridge, JC., Ginzel, KH., Baker, DG., Banzett, RB., Morrison, MA. Stimulation of "irritant" receptors and afferent C-fibers in the lungs of dogs. Nature (London) 264, 451, 1976

Coleridge, JCG., Coleridge, HM. Schelegle, ES., Green, JF. Acute inhalation of ozone stimulates bronchial C-fibers and rapidly adapting receptors in dogs J Appl Physiol 74: 2345-2352, 1993

Daughaday, CC., Douglas, SD. Membrane receptor on rabbit and human pulmonary alveolar macrophages. J Reticuloendothel Soc 19: 37-45, 1976

Davies, P., Bailey, PJ., Goldenberg, MM. The role of arachidonic acid oxygenation products in pain and inflammation Annu Rev Immunol 2:335-337, 1984

Davis, GS., Giancola, MS., Crotanza, MC., Low, RB. Analysis of sequential bronchoalveolar lavage samples from healthy human volunteers Am Rev Respir Dis 126: 611-6, 1982

Devlin, RB., McDonnell, WF., Mann, R., Becker, J., House, DE., Schreinemachers, D., Koren, HS. Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. Am J Respir Cell Mol Biol 4: 72-81, 1991

Diaz, P., Gonzalez, MC., Galleguillos, FR et al. Leukocytes and mediators in bronchoalveolar lavage during allergen-induced late phase asthmatic reactions. Am Rev Respir Dis 139: 13839, 1989

Dimeo, MJ., Glenn, MG., Holtzman, MJ., Sheller, JR., Nadel, JA., Boushey, HA. Threshold concentration of ozone causing an increase in bronchial reactivity in humans and adaptation with repeated exposures Am Rev Respir Dis; 124: 245-8, 1981

Dinarello, CA. Blood 77: 1627, 1991

Downey, GP., Gumbay, RS., Doherty, DE et al. Enhancement of pulmonary inflammation by PGE2: evidence for a vasodilator effect. J Appl Physiol 64: 728-741, 1988

Drechsler-Parks, D.M. The dose/response relationship in older men exposed to ozone. (Abst) J Resp Crit Care Med Vol 149 No 4 April 1994

Duddridge, M., Kelly, CA., Fenwick, J., Hendrick, DJ., Walters, EH. Assessment of mixing between sequential aliquots during bronchoalveolar lavage (Abst) Thorax, 43: 822-3, 1988

Eschenbacher, WC., Ying, RC., Kreit, JW., Gross, KB. Ozone-induced lung function changes in normal and asthmatic subjects and the effect of indomethacin. In T. Schneider, SD, Lee, GJR, Wolters, and LD Grant (Eds), Atmospheric Ozone Research and Its Policy Implications. Elsevier, Amsterdam, P493, 1989

Evans, MJ., Johnson, LV., Stephens, RJ., Freeman, G. Cell renewal in the lungs of rats exposed to low levelse of ozone. Exp Mol Pathol 24: 70-83, 1976

Fabbri, LM., Aizawa, H., Alpert, SE, et al. Airway hyperresponsiveness and changes in cell counts in bronchoalveolar lavage after ozone exposure in dogs. Am Rev Respir Dis 129: 288-91, 1984

Fabbri, LM., Di Giacomo, R., Del Vecchio, L., Zocca, E., De Marzo, N., Maestrelli, P., Mapp, CE. Prednisolone, indomethacin and airway responsiveness in toluene diisocyanate sesitized subjects. Clin Respir Physiol 21: 421-426, 1985

Fahy, JV., Liu, L., Boushey, HA. Reproducibility of measurements made in induced sputum from asthmatic subjects (Abst) Am J Respir Crit Care Med Vol 151, No 4, April 1995

Fairfax, AJ., Hanson, JM., Morley, J. The late reaction following bronchial provocation with house dust mite allergen. Dependence on arachidonic acid metabolism. Clin Exp Immunol 52: 393-398, 1983

Flavahan, NA., Aarhus, LL., Rimele, TJ., Vanhouttem, PM. Respiratory epithelium inhibits bronchial smooth muscle tone. J Appl Physiol 58: 834-8; 1985

Folinsbee, LJ., Hazucha, MJ. Persistence of ozone-induced changes in lung function and airway responsiveness. In T. Schneider, S.D., Lee, GJR, Wolterss, and LD Grant (Eds) Atomospheric Ozone Research and Its Policy Implications Elsevier, Amsterdam, P483, 1989

Folinsbee, LJ., Silverman, F., Shephard, RJ. Decrease of maximum work performance following ozone exposure. J Appl Physiol: Respirat Environ Exercise Physiol 42 (4) 531-536, 1977

Folinsbee, LJ., Silverman, F., Shephard, RJ. Exercise responses following ozone exposure. J Appl Physiol 38, 996, 1975

Folinsbee, LJ., McDonnell, WF., Horstman, DH. Pulmonary function and symptom response after 6.6 hour exposure to 0.12 ppm ozone with moderate exercise JAPCA 38, 28, 1988

Foster, WM., Stetkiewicz, P. Permeability of respiratory epithelia of humans following exposure to ozone. (Abst) Am J Respir Crit Care Med Vol 151, No 4, April, 1995

Frampton, M., Morrow, PE., gibb, FR., Speers, DM, Gavros, JB., Utell, MJ. Airways responsivness to ozone in smokers and nonsmokers. (Abst) Am Rev Respir Dis vol 147 No 4 1993

Frampton, MW., Voter, KZ., Fogarty, JP., Morrow, PE., Gibb, FR., Speers, DM., Tsai, Y., Utell, MJ. Airway inflammation and functional responsiveness to ozone in humans. (Abst) Am J Respir Crit Care Med vol 149 No 4 April 1994

Gerbase, MN., Foster, WM., Thomas, K., Zacur, H., Weinmann, GG. Effect of menstrual cycle on ozone responsiveness. (Abst) vol 147 No 4 April 1993

Gerrity, T.r., Weaver, R.A., Bernstein, J., House, D.E., O'Neil, J.J. Extrathoracic and intrathoracic removal of ozone in tidal breathing humans. J. Appl Physiol 65:393-400, 1988.

Gertner, A., Bromberger-Barnea, B., Traystman, R., Berzon, D., Menkes, H. Responses of the lung periphery to ozone and histamine J Appl Physiol 54: 640-646, 1983

Gertner, A., Bromberger-Barnea, B., Connenberg, AM, Jr., Traystman, R., Menkes, H. Responses of the lung periphery to 1 ppm ozone J Appl Physiol 55: 770-776, 1983

Gibbons, SI., Adams, WC. Combined effects of ozone exposure and ambient heat on exercising females J Appl Physiol 57, 450, 1984

Gliner, JA., Horvath, SM., Folinsbee, LJ. Pre-exposure to low ozone concentrations does not diminish the pulmonary function response on exposure to higher ozone concentration. Am Rev Respir Dis 127: 51-55, 1983

Goerig, M., Habenicht, AJR., Heitz, R et al. J Clin Invest 79: 90-3; 1987

Golden, JA., Nadel, JA., Boushey, HA. Bronchial hyperirritability in healthy subjects after exposure to ozone. Am Rev Respir Dis 118, 287, 1978

Gong, H., Bedi, JF., Horvath, SM. Inhaled albuterol does not protect against ozone toxicity in nonasthmatic athletes Arch Environ Health 43, 46, 1988

Graham, DE., Henderson, F., House, DE. Neutrophil influx measured in nasal lavage of humans exposed to ozone. Arch Environ Health 43: 229-233, 1988

Graham, DE., Koren, HS. Biomarkers of inflammation in ozone-exposed humans. Am Rev Respir Dis 142: 152-156, 1990

Gwizdala, CJ., Basha, MA., Gross, KB., Haider, AU., Popvich, J. Airway inflammation in asthmatic and healthy volunteers after controlled exposure to ozone and filtered air. (Abst) Am Rev Respir Dis Vol 147, No 4, April 1993

Hackney, JD., Linn, WS., Mohler, JG., Perderson, EE., Breisacher, P., Russo, A. Experimental studies on human health effects of air pollution. Arch Environ Health, 30, 359, 1975

Hamberg, M. Inhibition of prostaglandin systhesis in man. Biochem Biophys Res Commun 49: 720-6, 1972

Hazbun, ME. Hamilton, R., Holian, A., Eschenbacher, WL. Ozone induced increases in substance P and 8-epi-prostaglandin F2-alpha in the airways of human subjects. Am J Respir Cell Mol Biol vol 9 pp 568-572, 1993

Hazucha, MJ., Bates, DV., Bromberg, P. Mechanism of action of ozone on the human lung. J Appl Physiol 67(4) 1535-1541, 1989

Hazucha, MJ., Folinsbee, LJ., Seal, E. Jr. Effects of steady state and variable ozone concentration profiles on pulmonary function Am

Rev Respir dis 146: 1487-1493, 1992

Hazucha, MJ. Relationship between ozone exposure and pulmonary function changes J Appl Physiol 62: 1671-1680, 1987

Henderson, WR., Jr. The role of leukotrienes in inflammation Ann Intern Med 121: 684-697, 1994

Henderson, Wr. Jr. Eicosanoids and lung inflammation. Am Rev Respir dis; 135; 1176-1185; 1989

Holtzman, MJ., Cunnigham, JH., Sheller, JR., Irsigler, GB., Nadel, JA., Boushey, H. Effect of ozone on bronchial reactivity in atopic and nonatopic subjects. Am Rev Respir Dis 120, 1059, 1979

Holtzman, MJ., Aizawa, H., Nadel, J., Goetz, EJ. Selective generation of leukotriene B4 by tracheal epithelial cells from dogs. Biochem Biophys Res Commun 114: 1071-6, 1983

Horstman, DH., McDonnell, WF., Folinsbee, LJ., Salaam, A., Ives, P. Changes in pulmonary function and airway reactivity due to prolonged exposure to typical ambient O3 levels In: Schneider, SD Lee, GJR Wolters and LD Grant (Eds), Atmospheric ozone research and its policy implications. Elsevier, Amsterdam, p 75, 1989

Horstman, DH., Folinsbee, LJ., Ives, PJ., Abdul, S., McDonnell, WF. Ozone concentration and pulmonary response relationships for 6.6 hours with 5 hours of moderate exercise to 0.08, 0.10 and 0.12 ppm. Am Rev Resp Dis 142 1158, 1990

Horvath, S.M., Gliner, J.A., Folinsbee, L.J. Adaptation to ozone: Duration of effect. Am Rev Resp Dis, 123, 496, 1981.

Hu, S.C., Ben-Jebria, A., Ultman, J.S. Longitudinal distribution of ozone absorption in the lung: effects of respiratory flow. J Appl Physiol 77 (2): 574-583, 1994.

Hu, S.C., Ben-Jebria, A., Ultman, J.S. Longitudinal distribution of ozone absorption in the lung: quiet respiration in healthy subjects. J Appl Physiol 73 (4): 1665-1661, 1992

Jorres, R., Nowak, D., Magnussen, H. Coparison of the effect of ozone on lung function and airway responsiveness in healthy subjects and subjects with rhinitis or bronchial asthma. (Abst) Am Rev Respir Dis Vol 147 No 4, 1993

Kagawa, J. Exposure-effect relationship of selected pulmonary function measurements in subjects exposed to ozone. Int Arch Occup Environ Health, 53, 345, 1984

Kehrl, HR., Vincent, LM., Kowalsky, RJ., Horstman, DH., O'Neil, J., McCartney, WA., Bromberg, PH. Ozone exposure increases respiratory

epithelial permeability in humans. Am Rev Respir Dis 135, 1174, 1987

Kelly, CA., Kotre, CJ., Ward, C., Hendrick, DJ., Walters, EH. Anatomical distribution of bronchalveolar lavage fluid as assessed by digital subtraction radiography. Thorax 42: 624-8, 1987

Kerr, HD., Kulle, TJ., McIlhany, Seidersky, P. Effects of ozone on pulmonary function in normal subjects Am Rev Respir Dis 111: 763-773, 1975

Kirby, JG., Hargreave, FE., Cockcroft, DW. O'Byrne, PM. Effect of indomethacin on allergen-induced asthmatic responses. J Appl Physiol 66(2): 578-583, 1989

Koenig, JQ., Covert, DS., Marshall, SG., Van Belle, G., Pierson, WE. The effects of ozone and nitrogen dioxide on pulmonary function in healthy and in asthmatic adolescents. Am Rev Respir Dis, 136, 1152, 1987

Koenig, JQ., Covert, DS., Morgan, MS., Horike, M., Marshall, SG., Pierson, WE. Acute effects of 0.12 ppm ozone or 0.12 ppm nitrogen dioxide on pulmonary function in healthy and asthmatic adolescents Am Rev Respir Dis 132, 648, 1985

Kontos, HA. Oxygen radicals from arachidonate metabolism in abnormal vascular responses. Am Rev Respir Dis; 136; 474-7; 1987

Koren, H., Devlin, RB., Graham, D., Mann, R., McGee, M., Horstman, DH., Kozumbo, WJ., Becher, S., House, DE., McDonnell, WF., Bromberg, P. Ozone-induced inflammation in the lower airways to human subjects Am Rev Respir Dis 139, 407, 1989

Kreit, JW. Gross, KB., Moore, TB., Lorenzen, TJ., D'Arcy, J., Eschenbacher, WL. Ozone induced changes in pulmonary function and bronchial responsiveness in asthmatics. J Appl Physiol 60, 217, 1989

Kuehl, FA., Egan, RW. Prostaglandins, arachidonic acid, and inflammation. Science 210: 978-984, 1980

Kulle, TJ., Sauder, LR., Hebel, JR., Chatham, MD. Ozone response relationships in healthy nonsmokers. Am Rev Respir Dis 132, 36, 1985

Lanes, S. Stevenson, JS., Codias, E., Hernandex, A., Sialczak, MN., Wanner, H., Abraham, WM. Indomethacin and FPL 55321 inhibit antigen-induced airway hyperresponsiveness in sheep. J Appl Physiol 61: 864-872, 1988

Larsen, RI., McDonnell, WF., Horstman, DH., Folinsbee, LJ. An air quality data analysis system for interrelating effects, standards,

and needed source reductions. Part 11. A log normal model relating human lung function decreases to ozone exposures J Air Waste Manage Assoc 41: 455-459, 1991

Lauritzen, SK., Adams, WC. Ozone inhalation effects consequent to continuous exercise in females: comparison to males. J Appl Physiol 59, 1601-1606, 1985

Lee, HK. Murlas, C. Ozone-induced bronchial hyperreactivity in guinea pigs is abolished by BW755C or FPL55712 but not by indomethacin. Am Rev Respir Dis 132: 1005-9, 1985

Lee, LY., Bleeker, ER., Nadel, JA. Effect of ozone on the bronchomotor response to inhaled histamine aerosol in dogs J Appl Physiol; 43: 626-31, 1977

Leikauf, G.D., Zhao, Q., Zhao, S., Santrock, J. Ozonolysis products of membrane fatty acids activate eicosanoid metabolism in human airway eipithelial cells. Am J Respir Cell Mol Biol Vol 9, 594-602, 1993

Li, Z., Daniel, EE., Lane, CG., Arnout, MA., O'Byrne, PM. Effect of an anti-Mol MAb on ozone-induced airway inflammation and airway hyperresponsiveness in dogs. Am J Physiol (Lung Cell Mol Physiol) 263: L723-L726, 1992

Linn, WS., Avol, EL., Shamoo, DA., Peng, RC,. Valencia, LM., Little, DE, Hackney, JD. Repeated laboratory ozone exposure of volunteers Los Angeles residents: An apparent seasonal variation variation in response Toxicol Ind Health 4, 504, 1988

Linn, WS., Avol, EL., Shamoo, DA., Spier, CE., Valencia, LM., Venet, TG., Fischer, DA., Hackney, JD. A dose-response study of healthy heavily exercising men exposed to ozone at concentrations near the ambient air quality standard. Toxicol Ind Health 2:99-112, 1986

Linn, WS., Jones, MP., Bachmyer, EA., Spier, CE,. Mazur, SF., Avol, EL., Hackney, JD. Short term respiratory effects of polluted air: A laboratory study of volunteers in a high oxidant community. Am Rev Respir Dis 121, 243, 1980

Linn, WS., Buckley, RD., Spier, CE, Blessey, RL., Jones, MP., Fisher, DA., Hackney, JD. Health effects of ozone exposure in asthmatics. Am Rev Respir Dis 117: 835, 1978

Linn, WS., Jones, MP., Bachmyer, EA., Spier, CE., Mazur, SF., Avol, EL., Hackney, JD. Short term respiratory effects of polluted air: A laboratory study of volunteers in a high oxidant community. Am Rev Respir Dis 121, 243, 1980

Lippman, M. Health effects of ozone. A critical review. JAPCA 39,

672, 1989

Lundberg, J., Hokfelt, T., Martling, C., Saria, A., Cuello, C. Substance-P immunoreactive sensory nerves in the lower respiratory tract of various mamals including man. Cell Tissue Res 235: 251-261, 1984

Malek, M., Utell, MJ., Looney, RJ., Morrow, PE, Levy, PL., Tsai, Y., Barth, R., Frampton, MW. Does exposure to ozone induce systemic acute phase response in humans (Abst) Am J Respir Crit Care Med vol 151 No 4, April 1995

Marsh, WR., Irvin, CG., Murphy, KR., Behrens, BL., Larsen, GL. Increases in airway reactivity to histamine and inflammatory cells in bronchoalveolar lavage after the late asthmatic response in an animal model. Am Rev Respir Dis 131: 875-879, 1985

Martin, TR., Raugi,G., Menritt, TL., Henderson, Wr. Jr. Relative contribution of leukotrience B4 to the neutrophil chemotactic activity produced by the resident human alveolar macrophage. J Clin Invest 80; 1114-24; 1987

Mathe, A., Hedqvist, P. Effect of prostaglandin F2-alpha and E2 on airway conductance in healthy subjects and asthmatic patients. Am Rev Respir dis 11: 313, 1975

McBride, DE., Koenig, JQ., Luchtel, DL., Williams, PV., Henderson, WR., Jr. Inflammatory effecgs of ozone in the upper airways of subjects with asthma. Am J Respir Crit Care Med 149: 1192-7, 1994

Mcdonnell, WF., Smith, MV. Description of acute ozone responses as a function of exposure rate and total inhaled dose J Appl Physiol 76(6): 2776-2784, 1994

McDonnell, W., Koren, H., Devlin, R., Salaam, A., Ives, P., O'Neil, J. Biochemical and cellular correlates of changes in pulmonary function and symptoms in humans exposed to ozone. Am Rev Respir Dis 141: A72, 1990

McDonnell, W.F., Muller, K.E., Bromberg, P., Shy, C.M. Predictors of individual differencers in acute response to ozone exposure. Am Rev Respir Dis 147: 818-825, 1993

McDonnell, WF., Horstman, DH., Abdul, S., Ragio, LS. Green, JA. The respiratory responses of subjects with allergic rhinitis to ozone exposure and the relationship to nonspecific airway reactivity. Toxicol Ind Health, 3, 507, 1987

McDonnell, W.F., Horstman, D.H., Hazucha, M., Seal, E., Hack, D., Salaam, D., House, D.E. Pulmonary effects of ozone exposure during exercise: Dose-response characteristics. J Appl Physiol 54, 1345, 1983.

McDonnell, WF., Horstman, DA., Abdul-Salaam, S., House, DE. Reproducibility of individual responses to ozone exposure. Am Rev Respir Dis, 131: 36-40, 1985

McKenzie, DC., Stirling, DR., Fadl, S., Allen, M. The effects of salbutamol on pulmonary function in cyclists exposed to ozone: A pilot study. Can J Spt Sci 12: 146-48, 1987

Messineo, TD., Adam, WC. Ozone inhalation effects in females varying widely in lung size: comparison with males. J Appl Physiol 69: 96-103, 1990

Michelson, P., Dailey, L., Carter, J., Devlin, R., Pedan, D. Characterization of the nasal inflammatory response of extrinsic asthmatics following ozone exposure. (Abst) Am Rev Respir Dis Vol 151 No 4 April 1995

Miller, PD., Gorden, T., Warrick, M., Ander, MO. Effect of ozone and histamine on airway permeability to horseradish peroxidase in guinea pigs J Tox Environ Health 18: 121-32, 1986

Mills, J., Sellic, H., Widdicombe, W. Breathing. London, Churchill, P 77, 1970

Morrison, D., Langridge, F., Rahman, I., MacNee, W. Epithelial permeability, inflammation and oxidant status in the airspaces of non-smokers exposed to ozone. (Abst) Am J Respir Crit Care Med Vol 151, No 4 April 1995

Morrow, J., Roberts, J. Quantification of noncyclooxygenase oxidative formation of a series of novel prostaglandins: analytical ramification for measurement of eicosanoids Anal Biochem 184: 1-10, 1991

Murlas, CG. Enviornmental airway injury: mucosoal changes and airway hyperreactivity. In SG Farmer and DNP Hay, eds. The airway epithelium. Marcel Dekker, New York 213-253

O'Byrne, PM., Walters, EH., Fabbri, LM., Holtzman, MJ., Nadel, JA. Indomethacin inhibits the airway hyperresponsiveness but not the neutrophil influx induced by ozone in dogs. Am Rev Respir Dis 130: 220-224, 1984

O'Byrne, PM., Junes, GL. The effect of indomethacin on exercise-induced bronchoconstriction and refractoriness after exercise. Am Rev Respir Dis 134: 69-72, 1986

O'Byrne, PM., Walters, EH., Gold, BD., Aizawa, HA., Fabbri, LM., Alpert, SE., Nadel, JA., Holtzman, MJ. Neutrophil depletion inhibits airway hyperresponsiveness induced by ozone exposure. Am Rev Respir Dis 130: 214-9, 1984

O'Byrne, P., Aizawa, H., Bethel, R et al.: Prostaglandin F2-alpha increases responsiveness in pulmonary airways of dogs. Prostaglandins 28: 537, 1984

Oppenheimer, JJ et al. Annu Rev Immunol, 9:617-648, 1991

Pingleton, SK., Harrison, GF., Stecschalte, DJ., Wesseluis, LJ., Kerby, GR., Ruth, WE. Effect of location, PH, and temperature of installate in bronchoalveolar lavage in normal volunteers Am Rev Resir Dis 128: 1035-7, 1983

Rennard, SI., Hunninghake, GW., Bitterman, PB., Crystal, RB. Production of fibronectin by human alveolar macrophages: mechanism for the recruitment of fibroblasts to sites of tissue injury in interstitial lung diseases. Proc Natl Acad Sci USA, 78: 7147-51, 1981

Reynolds, HY., Atkinson, JP., Newball, HH., Frank, MM. Receptors for immunoglobulin and complement on human alveolar macrophages. J immunol 114: 1813-1819, 1975

Roberts, AM., Schultz, HD., Green, JF., Armstrong, DJ., Kaufman, MP., Coleridge, HM., Coleridge, JCG. Reflex tracheal contraction evoked in dogs by bronchodilator prostaglandins E2 and I2. J Appl Physiol 58, 1823, 1985

Robinson, BW., James, A., Rose, AH., Sterett, GF., Musk, AW. Bronchoalveolar lavage sampling of airway and alveolar cells Br J Dis Chest, 82: 45-55, 1988

Scannell, CH., Aris, R., Christian, D., Hearne, P., Tager, I., Ferrando, R., Chen, L., Balmes, JR. Ozone-induced airway inflammation is not correlated with physiological responses (Abst) Am J Respir Crit Care Med vol 149 No 4 April, 1994

Scannell, CH., Chen, LL, Tager, I., Christian, D., Walsh, B., Kelly, T., Ferrando, R., Boylen, K., Balmes, JR. Ozone-induced pulmonary function changes and airway inflammation in asthmatic and non-asthmatic subjects (Abst) Am J Resir Crit Care Med vol 151, No 4 April, 1995

Schelegle, ES., Adam, WC., Siefkin, AD. Indomethacin pretreatment reduces ozone-induced pulmonary function decrements in human subjects Am Rev Respir Dis, 136, 1350-1354, 1987

Schelegle, ES., Siefkin, AD., McDonald, RJ. Time course of ozone-induced neutrophilia in normal humans. Am Rev Respir Dis 143: 1353-1358, 1991

Schelegle, ES., Adams, WC., Giri, SN., Siefkin, AD. Acute ozone exposure increases plasma prostaglandin F2 in ozone sensitive human subjects Am Rev Respir Dis 140: 211-216, 1989

Schulteiss, AH., Bassett, D., Fryer, A. Ozone-induced airway hyperresponsiveness and loss of neuronal M2 muscarinic receptor function J Appl Physiol 76(3): 1088-1097, 1994

Schwartz, WL., Dungworth, DL., Mustafa, MG., Tarkington, BK., Tyler, WS. Pulmonary response of rats to ambient levels of ozone: Effects of 7 day intermittent or continuous exposure. Lab Invest: 34, 565-578, 1976

Seal, E. Jr., McDonnell, WF., House, D., Salaam, SA., Dewitt, PJ., Butler, SO., Green, J., Raggio, L. The pulmonary response of white and black adults to six concentrations of ozone. Am Rev Resp Dis Vol 147, 804-810, 1993

Seltzer, J., Bigby, BG., Stulburg, M., Holtzman, J., Wadel, JA. Ozone-induced change in bronchial reactivity to methacholine and airway inflammation in humans J Appl Physiol 60, 1321, 1986

Shephard, EG., Malan, L., MacFarlane, CM., Mouton, W. Joubert, JR. Lung function and plasma levels of thromboxane B2, 6-keto-prostaglandin F1a and B-Thromboglobulin in antigen-induced asthma before and after indomethacin pretreatment. Br J Clin Pharmacol 19: 459-470, 1985

Shephard, RJ., Urch, B., Silverman, F., Corey, P. Interaction of ozone and cigarette smoke exposure. Environ Res 31, 125, 1983

Shimasaki, H., Takatori, T., Anderson, WR., Horten, HL., Privett, OS. Alteration of lung lipids in ozone exposed rats. Biochem Biophys Res Commun 68: 1256-62, 1976

Shurin, SB., Stossel, TP. Complement (C3)-activated phagocytosis by lung macrophages. J immunol 120: 1305-1312, 1978

Silverman, F. Asthma and respiratory irritants (ozone). Environ Health Persp Vol 29, 131-136, 1979

Silverman, F., Shephard, RJ., Folinsbee, L. Effects of physical activity on lung responses to acute ozone exposure. Procedings of the fourth international clean air congress, 15-9, 1977

Sladek, K., Sheller JR. Cyclooxygenase mediators. Allergic inflammatory mediators and bronchial hyperresponsiveness I. Immunol and Allergy Clinics of North America 10:2, May, 1990

Slovik, L., Bromberg, P., Rodgers, J., McDonnell, W., Hazucha, M. Genetic influence in the lung function response to ozone (Abst) J Respir & Crit Care Med Vol 149 No 4 April 1994

Smith, C., Johnson, DA. Human bronchial leukocyte4s proteinase inhibitor Biochem J 225: 463-472, 1985

Solic, JJ., Hazucha, M., Bromberg, P. The acute effects of 0.2 ppm ozone in patients with chronic obstructive pulmonary disease. Am Rev Respir Dis 125, 664, 1982

Stuart-Smith, K., Vanhoutte, PM. Arachidonic acid evokes epithelium-dependent relaxation in canine airways. J Appl Physiol 65; 2170-80; 1988

Tepper, JS., Costa, DL., Lehmann, JR. Extrapolation of animal data to humans: homology of pulmonary physiological responses with ozone exposure. In DE Gardner, editor. Toxicology of the lung. Raven Press, New York, 217-251

Tessner, TG., O'Flaherty, JT., Wykle, RL. Stimulation of platelet-activating factor synthesis by a nonmetabolizable bioactive analog of platelet activating factor and influence of arachidonic acid metabolism. J Biol Chem; 284; 4794-9, 1989

Tilton, B.E. Health effects of tropospheric ozone. Environ Sci Technol 23: 257-263, 1989.

Torres, A., Utell, MJ., Morrow, PE., Voter, KZ., Tsai, Y., Barth, R., Frampton, MW. Airway inflammatory responses to ozone: relationship to lung function changes in smokers and nonsmokers. (Abst) Am J Respir Crit Care Med Vol 151 No 4 April, 1995

Vagaggini, B., Taccola, M., Giannini, D., Di Franco, H., Cannevali, S. Gianchetti, S., Bacci, E., Bancalari, L., Bartoli, ML., Dente, FL., Pagiaro, PL., Giantini, C. The effects of ozone exposure in the inflammatory cell percentages in the induced sputum of asthmatic subjects (Abst). Am J Respir Crit Care Med Vol 153 No 4 April 1996

Van Snick, J. Annu Rev Immunol 8:213, 1990

Voter, KZ., Frampton, MW., Whitin, JC., Cox, C., Tsai, Y., Morrow, P., Utell, MJ. Immediate and delayed effects of ozone exposure on bronchoalveolar lavage cells in smokers and nonsmokers. (Abst) Am Rev Respir Dis vol `47 No 4 April, 1993

Voter, KZ., Whitin, JC., Utell, MJ., Tsai, Y., Morrow, PE., Frampton, MW. Superoxide anion generation by bronchoalveolar lavage cells from smokers and non-smokers exposed to ozone. (Abst) Am Rev Respir Dis vol 151 No 4 April 1995

Walters, EH. Effects of inhibition of prostagland synthesis on induced bronchial hyperresponsiveness. Thorax 38: 195-199, 1983

Weinmann, G., Liu, MC., Proud, D., Hubbard, W., Frank, R. Repeated ozone exposure in humans: Inflammatory, small and peripheral airway responses (Abst) Am J Respir Crit Care Med Vol 151 No 4 April, 1995

Weinmann, GG., Lin, M., Gerbase, MW., Foster, WM., Frank, R. Baseline peripheral airways resistance is significantly associated with spirometric responsiveness to ozone in humans. (Abst) Am Rev Respir Dis Vol 147, No 4, 1993

Wilson, DW., Plopper, CG., Gungworth, DL. The response of the macaque tracheobronchial epithelium to acute ozone injury. Am J Path 116: 193-206, 1984

Wong, HH., Liu, J., Bouchey, HA., Fahy, JV. The effect of ozone exposure on markers of inflammation in induced sputum. (Abst) Am J Respir Crit Care Med vol 151, No 4 April, 1995

Yeadon, M., Wilkinson, D., Darley-Usmar, V., O'Leary, VJ., Payne, AN. Mechanisms contributing to ozone-induced bronchial hyperreactivity in guinea pigs. Pulm Pharmacol 5: 39-50, 1992

Ying, RL., Gross, KB., Terzo, TS., Eschenbacher, WC. Indomethacin does not inhibit the ozone-induced increase in bronchial responsiveness in human subjects. Am Rev Respir Dis 142, 817, 1990

Yokota, K., Kusaka, M., Ohshima, T et al. Stimulation of PGE2 synthesis in cloned osteoblastic cells of mouse by epidermal growth factor. J Biol Chem; 261; 15410-5, 1986

CHAPTER 3

THESIS PREVEIW

The following three chapters comprise the main thesis study which address a number of end points with respect to the asthmatic airway response to ozone. Chapter 4 (study 1) will address the effect of cyclooxygenase inhibition on ozone-induced changes in spirometry, symptom responses, and airway reactivity in asthmatics. Chapter 5 (study 2) will examine the ozone-induced inflammatory response in asthmatics using the method of sputum induction with hypertonic saline, and determine the effect of cyclooxygenase inhibition. Chapter 6 (study 3) will address whether cyclooxygenase inhibition affects the relationships between various outcome measures following ozone exposure.

Chapter 4 will attempt to first confirm the work of Eschenbacher (1989) on the effect of ozone on FEV_1 in asthmatics, and second, extend Eschenbacher's (1989) work in asthmatics by examining the effect of cyclooxygenase inhibition on pulmonary function variables other than FVC and FEV_1. With respect to ozone-induced airway hyperreactivity, this study will advance the work of Eschenbacher (1989) who did not examine the effect of cyclooxygenase inhibition on non-specific airway reactivity to ozone in asthmatics. This study will depart from the work by Ying (1990) who examined healthy subjects, by addressing whether cyclooxygenase inhibition can prevent ozone-induced airway hyperreactivity in asthmatics. Finally, with respect to symptom responses, this study will extend the work of Schelegle (1987) and Eschenbacher (1989) by describing

the effect of cyclooxygenase inhibition on both symptom reporting and symptom severity in asthmatics following ozone exposure.

Chapter 5 will examine cellular and specific biochemical aspects of the inflammatory response following acute ozone exposure in asthmatics. It will differ from recent work by Hazucha (1996) who also examined the effect of cyclooxygenase inhibition on the inflammatory response following ozone exposure, but measured inflammation in the BAL fluid rather than sputum, used healthy subjects versus asthmatics, and pretreated with Ibuprofen instead of indomethacin. Our study will examine the effect of cyclooxygenase inhibition on the inflammatory response in the induced sputum of asthmatic subjects. The use of induced sputum has several advantages over BAL with respect to ozone studies, one of which is that it samples lower conducting airways (excluding alveolar regions), the area where ozone is known to exert the majority of it's effects. Also, our use of asthmatics subjects promotes the opportunity to compare the results of cyclooxygenase inhibition on ozone-induced inflammation between asthmatics and previous reports on healthy subjects.

Chapter 6 will explore potential relationships among several outcome measures in asthmatics following acute ozone exposure, including pulmonary function, symptoms, airway reactivity and inflammatory indices. This chapter will also examine the effect of cyclooxygenase inhibition on the associations between outcome

measures. This study will advance recent work by Balmes (1996) and Hazucha (1996) who examined whether ozone-induced changes in FEV_1 or FVC were related to changes in BAL cells and cytokines. This study will examine relationships between expiratory volumes and flows, inflammatory cells, and cytokines.

Chapter 7 is based on a pilot study examining the effects of cyclooxygenase pretreatment on non-asthmatic subjects following an acute exposure to ozone. It will address aspects of the non-asthmatic airway response to ozone exposure and compare these with asthmatic responses found in the main thesis study. In this chapter outcome measures will include pulmonary function and cellular and biochemical indices of inflammation.

Chapter 8 will provide a summary of each chapter's critical findings, while Chapter 9 will summarize the overall conclusions of the study. Chapter 10 will discuss limitations of the thesis, and Chapter 11 will provide a perspective on future research directions.

OVERALL STUDY DESIGN

Fourteen asthmatic volunteers were recruited to participate in a 2x2 randomized factorial design where each subject underwent a controlled 2 hour exposure to 400 ppb ozone or clean air while pretreated with indomethacin or placebo. One asthmatic subject did

not complete the entire 2x2 design but did complete a 1x2 design where he received only one exposure condition, namely ozone, with both pretreatment conditions. Prior to beginning this study, a small scale pilot study was conducted by this investigator on the airway effects of ozone exposure using 9 non-asthmatic subjects. This pilot study examined several of the same outcome measures as the larger subsequent thesis study, however in 5 subjects clean air exposures were not included as part of the study protocol. In all cases, regardless of whether subjects received the full 2x2 study protocol, or a 1x2 protocol, identical experimental conditions existed to the thesis study, including exposure methods and assessment techniques. Although the data generated from the pilot study was not intended to be part of the thesis, its results have been determined to be of some importance in shedding light on some of the results of the thesis. Consequently specific results from the pilot study on non-asthmatics will be presented in Chapter 7 as they pertain to asthmatic results demonstrated in the thesis study.

OVERALL STATISTICAL METHODS

The Statistical Analysis System (SAS) software package was used for data analysis. Arithmetic means and standard deviations were employed for the statistical summaries of the variables. Paired T-Test analyses were performed comparing percent change and absolute differences from pre exposure baseline between exposures to determine the ozone response. The ozone response was compared

between treatments to determine the indomethacin interaction effect. Percent changes from baseline were calculated as pre minus post exposure/pre-exposure x 100%. The overall statistical result was testing the difference of a difference. Multiple linear regression analyses were used to confirm paired T-Test results. Significance was accepted at the 0.05 level for all statistical analyses.

Since this thesis involved several outcome measures (i.e. pulmonary function, airway reactivity, inflammatory cells) each with different variances and effect sizes, we calculated several sample size estimates and based the respective standard deviations and effect sizes for each on studies which best resembled the protocol for this study.

With respect to pulmonary function outcome measures, all of our subjects (N=14) had complete data to examine the ozone-induced changes in maximal expiratory flows and volumes with indomethacin. Two sample size estimates were used to assess two deltas. First, a sample size estimate was determined to evaluate the percent change difference from baseline in FEV_1 following ozone exposure with indomethacin. Our estimate was based on data from Ying (1990) who showed a significant mean (+/-SE) ozone-induced change from baseline in FEV_1 of 4.8% (3.7) in 7 healthy subjects. Since Ying was able to show small, significant changes with as few as 7 subjects, and we anticipated somewhat larger declines in FEV_1 with

asthmatics, we felt quite comfortable estimating an effect size in the range of 5-8 %. We determined that 13 asthmatic subjects would detect a change from baseline in FEV_1 of 7.6% with indomethacin. Our second sample size estimate examined the percent change from baseline in FEV_1 between placebo and indomethacin. Our estimate made use of a recent study by Hazucha who showed a significant mean (+/-SE) difference in FEV_1 decline between placebo and ibuprofen (a similar acting non-steroidal anti-inflammatory drug to indomethacin) of 10 % points (3%) in 10 healthy subjects. We determined that 13 subjects would be sufficient to detect a difference of 7.3 % points between placebo and indomethacin for FEV_1 decline following ozone exposure. Since our study was also interested in examining the effects of ozone on small airways flow variables in asthmatics, we needed a sample size estimate to detect changes in $\dot{V}25$ following acute ozone exposure. From Folinsbee's (1977) data we learned that a mean (+/- SD) decline of 21% (28%) in $\dot{V}25$ could be expected following an acute ozone exposure in normal subjects. In our asthmatics, we expected the decline in $\dot{V}25$ to be somewhat higher than 21% due to their preexisting asthmatic condition. Based on Folinsbee (1977) we determined that a sample size of 13 would detect a 22% fall in $\dot{V}25$ following ozone exposure versus filtered air exposure. We could not determine an estimate of the magnitude of decline in $\dot{V}25$ with indomethacin since this data has not been published although we expect it to be lower than the placebo condition.

For the airway reactivity outcome measure, we used the concentration of methacholine that causes a 20% drop in FEV_1 (PC_{20}) from pre exposure baseline in order to determine a sample size estimate. For each subject the PC_{20} on the ozone exposure day relative to the previous day (where no exposure was given) is a ratio measure of the effect of ozone on airway reactivity. An estimate of the standard deviation of this ratio can be obtained from the paper by Molfino (1991) in which asthmatics were given methacholine challenge on two days. The two days were not consecutive as in this study, they were separated by an intervening day where an exposure was given. An estimate of the variance for the PC_{20} ratio in the Molfino study is done by pooling the results across the four exposure conditions of the study. The variance was 0.5436, which is a standard deviation of 0.74. The variance of 0.5436 was not used in the sample size calculation for this study since we are comparing the PC_{20} ratio across only two conditions, namely the day with indomethacin and the day with placebo. The variance 0.5436 is adjusted by multiplying it by a factor less than two since each subject in this study is operating as his/her own control and therefore the multiplier is reduced by a factor (1-r) where r is the correlation coefficient between repeated ratio measures on the same subject. From Molfino the 6 pairwise correlation coefficients range from -0.27 to 0.58.

To complete our sample size calculation we need an estimate of the ozone effect that we can anticipate. Results from an abstract by

Jorres (1993) show that for a group of 26 asthmatics exposed to 250 ppb ozone for 3 hours while intermittently exercising, the change in PC_{20} from the ozone day to the air day is equivalent to 0.95 of a doubling concentration, or a PC_{20} ratio of 0.52. What we could expect with indomethacin was ascertained from a study by Ying (1990) who pretreated healthy ozone sensitive subjects with indomethacin and exposed them to 400 ppb ozone for 2 hours. In this study the concentration of methacholine that would have resulted in a 100% increase in the baseline specific airway resistance (PC_{100}, SRaw) was used and not the PC_{20}, but the PC100,SRaw ratio of ozone to air exposure with indomethacin was 0.35 which was approximately a 2 doubling dose concentration shift in methacholine.

Using an alpha level = 0.05 and a beta = 0.20, a sample size estimate was calculated using an effect size PC_{20} ratio of 0.40 (65% drop in PC_{20} or slightly greater than 1 doubling dose fall in PC_{20}, methacholine) and a standard deviation of 0.37 (correlation coefficient (r) for repeated measurements of 0.5 was used). A sample size of 12 subjects was determined to be adequate.

For the inflammatory cell response, we were unable to base our sample size calculation on the expected attenuation of the ozone-induced sputum neutrophilia by indomethacin since this experiment had not been conducted in human subjects, and therefore a standard deviation and effect size could not be derived. In addition, two similar human studies using ibuprofin as the cyclooxygenase

blocking agent (Hazucha, 1991; Madden, 1991) and one study in dogs using indomethacin (O'Byrne, 1984) found no attenuation of the ozone-induced neutrophil response when compared to placebo pretreatment; this meant an estimated effect size of zero. The sample size calculation for the inflammatory cell response was based on detecting a mean change of 10 percentage points in sputum neutrophil cell counts following ozone exposure compared to filtered air exposure. A standard deviation of 15 percentage points was used in the sample size calculation and this was based on six studies (Scannell, 1996; Balmes, 1996; Wong, 1995; Aris, 1993; Schelegle, 1991; Koren, 1989) where in five out of six studies, bronchoalveolar lavage (BAL) inflammatory cells were examined and in one study sputum inflammatory cells were examined (Wong, 1995). Since the number of controlled human ozone exposure studies using sputum induction were limited, studies using bronchoalveolar lavage (BAL) neutrophils were included in the pooled standard deviation as the next best alternative in arriving at a standard deviation for our sample size calculation. Of the six studies, four examined healthy subjects and two examined asthmatic subjects. All six studies used similar ozone exposure protocols to our study (400 ppb ozone, 2 hours) and recorded mean changes and standard deviations for percent neutrophils following ozone exposure. From these six studies we determined that a mean increase in neutrophils of 19.5 percentage points (range = 8 - 29 % points) could be expected following a 2 hour, 400 ppb ozone exposure (minus filtered air exposure). The pooled SD for the six studies was 15.2 percentage

points for the ozone exposures. Our sample size calculation was based on a more conservative effect size of 12 percentage points rather than 19.5 since our asthmatic subjects were mild and probably would have ozone-induced neutrophil responses slightly higher than healthy individuals compared to moderate and severe asthmatics. For example Hazucha's (1991) healthy subjects demonstrated an increase in neutrophils of 9.6% following a 2 hour exposure to 400 ppb ozone. A power of 80% (Z1-beta = 0.8416) and an alpha level of 0.05 (Z1-alpha = 1.96) were used to determine that 13 subjects would provide the appropriate sample size to detect a change in neutrophils of 12 % points.

With respect to the biochemical markers of inflammation measured in this study (PGF2-alpha and IL-8), standard deviations for sample size estimates were based on two studies which closely resembled this study as far as ozone concentration, exposure duration and use of a cyclooxygenase inhibitor, but analyzed different biochemical markers. Hazucha (1991) and Madden (1991) measured the response of prostaglandin E2 (PGE2) and interleukin-6 (IL-6) following acute exposure to 400 ppb ozone for 2 hours in healthy subjects pretreated with ibuprofin. Ibuprofin, like indomethacin is a non-steroidal anti-inflammatory drug which acts on prostaglandin synthase (cyclooxygenase). PGE2 like PGF2-alpha, is a direct metabolite of prostaglandin G2/H2, while Il-6 is somewhat representative of the IL-8 response to ozone, since it has also been found in increased concentrations in BAL fluid after ozone

exposure (Devlin, 1993). Hazucha (1991) reported a mean (+/-SEM) decrease in PGE2 levels following ozone of 60% (12%) with ibuprofen, while Madden (1991) reported a mean (+/- SEM) decrease in IL-6 levels following ozone of 45% (13%) with ibuprofen. Based on these data standard deviations were derived for sample size estimates for PGF2-alpha and IL-8. Of our 13 asthmatic subjects, 7 had complete data on IL-8 and PGF2-alpha, therefore a sample size of 7 was estimated to observe an effect size of 41% for PGF2-alpha, and 44% for IL-8. In other words, we have the power to detect nothing lower than a 41% and 44% decrease in PGF2-alpha and IL-8 responses respectively, with indomethacin following ozone exposure. Due to our having only 7 subjects with available biochemical data, this aspect of the study is somewhat underpowered.

The sample size equation is given below.

$$N = \left(SD \; \frac{(Z1_{beta} + Z1_{alpha})}{effect \; size} \right)^2$$

$Z1_{beta}$ = 0.8416 for 80% power

$Z1_{alpha}$ = 1.96 for significance level of 0.05

Since the time our initial sample size estimate for neutrophils was calculated, several new studies on sputum induction have been reported in the literature (Keatings, 1996; Gershman, 1996; Veen, 1996; Turner, 1995; Pizzichini, 1995; Popov, 1995; Maestrelli, 1995; Pizzichini, 1996). We conducted post hoc sample size estimates using the pooled variance from these recent studies to

determine whether our inclusion of BAL studies in our initial sample size calculation underestimated our sample N. We also compared the actual variance we observed in our study with the original variance estimate to determine whether our estimate served to underestimate our sample N. Since the method of BAL is a routinely used, standardized procedure compared to induced sputum, we suspected it has less variability associated with it with respect to cell recovery versus the new technique of induced sputum. As a result our pooled variance estimate may have been an underestimate and produced a lower sample size estimate. Depending on the size of the difference between the variance estimate that included BAL studies (i.e. actual one used) versus one that uses only sputum studies (i.e. post hoc calculated) versus the actual variance we observed in the study, the effect of including BAL studies in the sample size estimate may have some bearing on some of the negative findings of the study, particularly where the number of subjects examined was limited, i.e. fluid phase components PGF2-alpha, IL-8. From eight studies that used a total of 11 sputum induction measurements of neutrophil responses in asthmatics, the pooled standard deviation (SD) was 16.5 versus 15.2 that was actually used. As expected, the SD was somewhat higher using only sputum induction studies. Using 16.5 as the SD, we determined that instead of 13 subjects, 15 would be needed to detect an effect size of 12 percentage points. Our inclusion of BAL studies in our sample size calculation served to underestimate our N by only 2 subjects. It appears that our estimate which was based

on mainly BAL studies, did not underestimate our sample size to a great degree. The SD we observed in our study for percent neutrophils following ozone exposure was 33 compared to 15.2. This is 2.1 times higher than the actual SD estimate, and has a substantial effect on the effect size increasing it to 26% points from 12 using N=13 at the 0.05 level of significance. It appears then that findings that did not achieve statistical significance (with respect to neutrophil responses), were likely due to an insufficient number of subjects studied.

REFERENCES

Aris, RM., Christian, D., Hearne, PQ., Finkbeiner, WE., Balmes, JR. Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. Am Rev Respir Dis 148: 1363-1372, 1993

Balmes, J., Chen, L., Scannell, C., Tager, I., Christian, D., Hearne, P., Kelly, T., Aris, R. Ozone-induced decrements in FEV1 and FVC do not correlate with measures of inflammation. Am J Respir Crit Care Med, 153: 904-9, 1996

Eschenbacher, WC., Ying, RC., Kreit, JW., Gross, KB. Ozone-induced lung function changes in normal and asthmatic subjects and the effect of indomethacin. In T. Schneider, SD, Lee, GJR, Wolters, and LD Grant (Eds), Atmospheric Ozone Research and Its Policy Implications. Elsevier, Amsterdam, P493, 1989

Folinsbee, LJ., Silverman, F., Shephard, RJ. Decrease of maximum work performance following ozone exposure. J Appl Physiol: Respirat Environ Exercise Physiol 42 (4) 531-536, 1977

Gershman, NH., Wong, HH., Liu, JT., Manimeister, MJ., Fahy, JV. Comparison of two methods of collecting induced sputum in asthmatic subjects. Eur Respir J, vol 9 2448-2453, 1996

Hazucha, M., Madden, M., Pape, G., Becker, S., Devlin, R., Koren, H., Kehrl, H., Bromberg, P. Effects of cyclo-oxygenase inhibition on ozone-induced respiratory inflammation and lung function changes. Eur J Appl Physiol, 73: 17-27, 1996

Hazucha, MJ., Pape, G., Madden, M., Koren, H., Kehrl, H., Bromberg, P. Effects of cyclooxygenase inhibition on ozone-induced respiratory inflammation and lung function changes (Abst). Am Rev Respir Dis 143: A701, 1991

Jorres, R., Nowak, D., Magnussen, H. Comparison of the effect of ozone on lung function and airway responsiveness in healthy subjects and subjects with rhinitis or bronchial asthma. (Abst) Am Rev Respir Dis Vol 147 No 4, 1993

Keatings, VM., Collins, PD., Scott, DM., Barnes, PJ. Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. Am J Respir Crit Care Med; 153: 530-4, 1996

Koren, H., Devlin, RB., Graham, D., Mann, R., McGee, M., Horstman, DH., Kozumbo, WJ., Becher, S., House, DE., McDonnell, WF., Bromberg, P. Ozone-induced inflammation in the lower airways to human subjects Am Rev Respir Dis 139, 407, 1989

Madden, M., Pape, G., Hazucha, M., Devlin, R., Harder, S., Cole, L., Becker, S., Koren, H. Effects of ibuprofin on lung lavage

composition in ozone-exposed subjects. Am Rev Respir Dis (Abst), Vol 143, No 4., 1991

Maestrelli, P., Saetta, M., Di Stefano, A., Calcagni, PG., Turato, G., Ruggieri, MP., Roggeri, A., Mapp, CE., Fabbri, L. Comparison of leukocyte counts in sputum, bronchial biopsies, and bronchoalveolar lavage. Am J Respir Crit Care Med, 152: 1926-31, 1996

Molfino, N., Wright, S., Katz, I., Tarlo, S., Silverman, F., McClean, P., Szalai, J., Raizenne., Slutsky, A., Zamel., N. Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. Lancet 338: 199-220, 1991

O'Byrne, PM., Walters, EH., Fabbri, LM., Holtzman, MJ., Nadel, JA. Indomethacin inhibits the airway hyperresponsiveness but not the neutrophil influx induced by ozone in dogs. Am Rev Respir Dis 130: 220-224, 1984

Pizzichini, MM., Popov, T., Efthimiadis, A., Hussack, P., Evans, S., Pizzichini, E., Dolovich, J., Hargreave, FE. Spontaneous and induced sputum to measure indices fo airway inflammation in asthma. Am J Respir Crit Care Med, 154: 866-9, 1996

Pizzichini, MM., Pizzichini, EM., Efthimiadis, A., Evans, S., Dolovich, J., Hargreave, FE. Repeatability of induced sputum (IS) cell counts on cytospins and fluid phase indices of inflammation. Clin and Invest Med, B137, 1995

Popov, T., Pizzichini, MM., Pizzichini, EM., Kolendowicz, R., St. Pierre, M., Hargreave, FE. Spontaneous and induced sputu: Are they the same ? Pg 78S, 1994

Scannell, C., Chen, L., Aris, R,. Tager, I., Christian, D., Ferrando, R., Welch, B., Kelly, T., Balmes, J. Greater ozone-induced inflammatory responses in subjects with asthma. Am J Respir Crit Care Med 154: 24-9, 1996

Schelegle, ES., Siefkin, AD., McDonald, RJ. Time course of ozone-induced neutrophilia in normal humans. Am Rev Respir Dis 143: 1353-1358, 1991

Turner, MO., Hussack, P., Sears, MR., Dolovich, J., Hargreave, FE. Exacerbations of asthma without sputum eosinophilia. Thorax; 50: 1057-1061, 1995

`t Veen, JCCM., de Gouw, HWFM., Smits, HH., Sont, JK., Hiemstra, PS., Sterk, PJ., Bel, EH. Repeatability of cellular and soluble markers of inflammation in induced sputum from patients with asthma. Eur Respir J, vol 9 2441-2447, 1996

Wong, HH., Liu, J., Bouchey, HA., Fahy, JV. The effect of ozone exposure on markers of inflammation in induced sputum. (Abst) Am J

Respir Crit Care Med vol 151, No 4 April, 1995

Ying, RL., Gross, KB., Terzo, TS., Eschenbacher, WC. Indomethacin does not inhibit the ozone-induced increase in bronchial responsiveness in human subjects. Am Rev Respir Dis 142, 817, 1990

CHAPTER 4

PULMONARY FUNCTION, SYMPTOMS AND AIRWAY REACTIVITY: EFFECTS OF INDOMETHACIN PRETREATMENT

INTRODUCTION

Acute ozone exposure in humans can produce transient and reversible changes in spirometry, which include a decrease in lung volumes as measured by FVC and FEV_1 and a modest increase in specific airways resistance (SRaw) (Folinsbee, 1975; Hackney, 1975; Silverman, 1977; Linn, 1988; Kreit, 1989). The severity of the spirometric decline depends most heavily on the concentration of ozone inhaled, and progresses more rapidly with exercise throughout the exposure period (McDonnell, 1994). Recovery from pulmonary function decline begins almost immediately once exposure has ceased and is generally 50% complete after 2 hours post exposure, resolving completely by 24 hours (Hazucha, 1996). The ozone-induced changes in vital capacity (VC) are almost entirely due to a reduction in inspiratory capacity (IC) and not from changes in Functional Residual Capacity (FRC) or Residual Volume (RV) (Hazucha, 1989; Beckett, 1985). Evidence has also shown that decreased IC is not due to increased elastic recoil, pulmonary edema, or a reduction in inspiratory muscle force, but rather seems attributable to neural mediation of C-fibres located in the intraepithelial layer of the airways (Hazucha, 1989; Tepper, 1993; Coleridge, 1993). In addition to effects on IC, exposure to ozone often shows a disproportionate decrease in FEV_1 versus FVC, indicative that airway effects may also play a role. A recent study by Weinmann (1995) highlighted the small airways region as particularly sensitive to ozone exposure in normal subjects. In her study the ozone-induced isovolume flow decrement persisted post exposure while FVC and FEV_1 recovered.

Recent evidence by Salmon (1996) in rats also suggested that the small airways are more susceptible to ozone exposure. The mechanism behind ozone's effects on the airways remains unclear.

Studies in humans have demonstrated indomethacin's protective function against different stimulus-induced decreases in spirometry which include exposures to allergen (Fairfax, 1983; Shephard), and viral infection (Walters, 1983). Schelegle (1987) first demonstrated cyclooxygenase metabolite involvement in ozone-induced spirometric decrement in normal subjects. In healthy subjects pretreated with indomethacin or placebo and exposed for 1 hour to 350 ppb ozone, there was a significant attenuation of the ozone-induced declines in FVC and FEV_1. Since indomethacin acts on the cyclooxygenase pathway of arachidonic acid, this suggested that the ozone effect on IC may be mediated in part by release of cyclooxygenase products. Eschenbacher (1989) and Ying (1990) also pretreated healthy subjects with indomethacin. They both showed attenuation of ozone-induced decreases in FVC and FEV_1 following a two hour, 400 ppb ozone exposure. This evidence suggests that in normals, products of the cyclooxygenase pathway sensitive to indomethacin inhibition, play an important role in the pulmonary function changes following acute ozone exposure. Since the weight of evidence suggests that asthmatics do not differ substantially from healthy subjects in the magnitude of their respiratory response to acute ozone exposure (Holtzman, 1979; McDonnell, 1987; Linn, 1978; Koenig, 1985; Koenig, 1987), we hypothesized that with

respect to pulmonary function changes following ozone exposure, the cyclooxygenase pathway plays an equally important role in asthmatics as it does in normal subjects.

Respiratory symptoms associated with acute ozone exposure include pain on deep inspiration, cough, wheeze, shortness of breath, nasal and eye irritation and sore throat. These symptoms have been documented in healthy individuals and in people with preexisting pulmonary disease (Hackney, 1975; Linn, 1980; Koenig, 1985; Folinsbee, 1988; Horstman, 1990). Several studies have suggested that respiratory symptoms are associated with spirometric decrements following ozone exposure (Linn, 1980; Folinsbee, 1988; Schelegle, 1987; Horstman, 1990). Since cyclooxygenase metabolites were purported to be involved in the spirometric response to ozone, Schelegle (1987) attempted to determine whether cyclooxygenase metabolites sensitive to indomethacin inhibition, played a role in symptom reporting and severity. He pretreated healthy subjects with indomethacin and exposed them to 350 ppb ozone for one hour. He reported a reduced symptom reporting along with an inhibition of pulmonary function decrement. Schelegle's (1987) data supports a role for cyclooxygenase metabolites as a mechanism associated with symptom reporting in healthy individuals, but its role in the symptom response in asthmatics remains unclear.

Acute exposure to ozone can produce increased airway reactivity to non-specific stimuli, such as methacholine or histamine (Aris,

1993; Ying, 1990; Kreit, 1989; Dimeo, 1981; Golden, 1978; Holtzman, 1979; Seltzer, 1986). This has been demonstrated in both healthy and asthmatic subjects. Animal models have also shown increased airway reactivity following ozone exposure (O'Byrne, 1984; Lee, 1977; Holtzman, 1983). In studies that have attempted to determine the mechanism behind ozone-induced increased airway reactivity, two different airway responses have been excluded as potential mechanisms. The data of McDonnell (1989) have suggested that the degree of increased reactivity is not associated with spirometric decline, while other studies have suggested that the presence of neutrophils are not essential for hyperreactivity to occur (Murlas, 1991; Li, 1992), although earlier work by Seltzer (1986) supported a role for neutrophilic inflammation.

With respect to a cyclooxygenase product of arachidonate metabolism being involved in the pathogenesis of airway hyperreactivity, several studies examining different stimuli have demonstrated evidence to support the involvement of a cyclooxygenase metabolite. Indomethacin has been shown to inhibit airway hyperreactivity following viral infection in humans (Walters, 1983), following allergen inhalation in atopic subjects (Kirby, 1989), and following ozone inhalation in dogs (O'Byrne, 1984). With respect to ozone exposure, Aizawa (1985) suggested that thromboxane may be more directly involved in airway reactivity since he successfully attenuated increased airway reactivity in dogs with a specific thromboxane synthetase inhibitor. Two studies on healthy subjects

examined the role of cyclooxygenase products of arachidonate metabolism in ozone-induced airway hyperreactivity. Both studies were unable to attenuate airway hyperreactivity when subjects were pretreated with indomethacin (Eschenbacher, 1989; Ying, 1990). Both these studies concluded that cyclooxygenase metabolites of arachidonic acid were not important factors in ozone-induced increased airway reactivity.

PURPOSE

To determine whether cyclooxygenase metabolites sensitive to indomethacin inhibition, play a role in the pulmonary function responses, symptom responses and airway reactivity responses of asthmatics exposed to acute ozone.

HYPOTHESIS

Indomethacin will significantly inhibit decreases in pulmonary function, increases in symptom reporting and severity scores, and increases in airway reactivity, following a 2 hour exposure to 400 ppb ozone.

OBJECTIVES

The first objective is to demonstrate significant changes in the following outcome measures: maximum expiratory flows and volumes,

the number of symptoms reported, the mean symptom severity score, and the PC_{20}, methacholine following a 2 hour exposure to 400 ppb ozone. Second, to determine whether indomethacin pretreatment will inhibit the above changes.

METHODS

Subject Selection

Study approval was obtained from the University of Toronto Review Committee on the Use of Human Subjects. Informed consent was obtained prior to entry into the study. Fourteen asthmatic volunteers between the ages of 18 and 40 were recruited into the study. Thirteen asthmatics completed the entire 2x2 factorial randomized study protocol (ozone/placebo; ozone/indomethacin; air/placebo; air/indomethacin). One asthmatic subject did not complete the entire 2x2 study protocol due to time constraints, but completed a 1x2 study protocol (ozone/placebo; ozone/indomethacin). Mild atopic and non-atopic asthmatic volunteers were chosen for study so as to have as representative a group of asthmatics as possible. Seasonal (i.e. ragweed, grass, tree pollen or fungal spore sensitive) asthmatics whose allergy season would have likely overlapped with their period of study were excluded. Asthmatic subjects had to meet as a minimum, the first criteria item for the study definition of asthma to be allowed entry into the study: Criteria item 1. positive history of physician diagnosed asthma (as

confirmed by family physician). Criteria item 2. methacholine challenge PC_{20} test result of less than 8 mg/ml; and criteria item 3. a post FEV_1 bronchodilator response of greater than 15%. For asthmatics, criteria items 2 and 3 did not have to be met during the first baseline week, but did have to be met on all subsequent pre-exposure baseline days during each of the study visits. This inclusion/ exclusion system for asthma allowed for subjects who did not present with hyperactive airways during the first baseline week (i.e. out of season atopic subject) to still be entered into the study.

Subjects underwent a baseline week of pulmonary function testing, bronchoprovocation testing with methacholine aerosol, sputum induction with hypertonic saline, a medical examination by the study physician, exercise testing on a stationary bicycle, and evaluation of spirometric and symptomatic tolerance to indomethacin.

Inclusion criteria included the following: 1) age 18-40; 2) no adverse response to ingestion of Acetylsalicylic Acid (ASA). Subjects were only included for study if they had ingested ASA within the previous 6 months and had not experienced an exacerbation of their asthma associated with this; 3) no requirement for oral or inhaled steroids; 5) able to discontinue methylxanthines for 36 hours and beta 2 adrenergic agonists for 12 hours before each study period and then only with the permission of

their family physician. No subject was currently using nor had used inhaled or oral steroids for at least one year. No subject was currently using nor had used methylxanthines. All subjects were on beta 2 adrenergic agonists (Ventolin) on an as needed basis and all were able to discontinue their use for 12 hours prior to any study period. All subjects met the inclusion criteria.

Exclusion criteria included the presence of any of the following: 1) ages <18 or >40; 2) nasal polyps; 3) history of ASA sensitivity; 4) significant cardiac/renal/hepatic disease; 5) history of peptic ulcer; 6) pregnancy during the course of the study; and 7) current or past smoker (none in last year and no more than 1 per day prior to that). No subjects were excluded from the study based on the above exclusion criteria.

All subjects had no recent history of viral infection upon entry into the study. Three subjects who were seasonal atopic (grass, ragweed) asthmatics, as defined by medical history and allergen skin test results, were studied during the winter months out of season. Evidence of upper respiratory tract infection at any time during the study were grounds for a six week postponement in a subjects participation. This occurred in two subjects.

All subjects demonstrated airway hyperreactivity (<8mg/ml) when challenged with methacholine on pre exposure baseline study days. The group mean (+/- SEM) pre exposure PC_{20} (mg/ml) with placebo was

0.62mg/ml (0.14) and 1.8 mg/ml (0.12) with indomethacin (Table 9). The difference between placebo and indomethacin was not statistically significant (p=0.07).

Environmental Chamber

The controlled environmental chamber has an inside space measuring 2.44 x 2.44 x 2.08 meters (m) [(12.4 cubic meters (m^3)], and is constructed from a channel iron frame covered internally with 6.35 millimeters (mm) plexi-glass sheets. Air is supplied to the chamber from the outside atmosphere at a flow rate of 3.29 m^3/min. or 15.9 air changes per hour, and filtered through activated charcoal plus a fabric after filter before entering the chamber. Air mixing is provided both by the movement of air through the supply inlet, located at ceiling height, to the exhaust outlet, located diagonally opposite on the floor; as well as by an oscillating fan (38 cm blade), adjusted to medium speed, and positioned at a height of 1.3 m that faced into the corner toward the air inlet.

The relative humidity (RH) inside the.chamber was monitored by a Vaisala (model 03334-20) RH transmitter with ± 2% accuracy from 0-90% RH; ± 3% accuracy from 90-100%. The Vaisala had dimensions 4 3/8" L x 3/8" W x 1 3/8" D and was wall mounted. It had a response time of 15 sec. with the filter. A Honeywell humidifier system was built into the ventilation supply and controlled from the outside

of the chamber. Both relative humidity and temperature inside the chamber were recorded on a Honeywell chart recorder against time. Temperature and relative humidity could be maintained at a predetermined constant level within the range 13 to 24° Celsius (C), and 5 to 95% RH, respectively. Past experience has shown that 21°C and 40% RH is comfortable for subjects, and thus all exposures were carried out under these conditions.

Ozone Generation and Monitoring

Ozone was produced by passing 100% oxygen through a high intensity electric field. The use of pure medical grade oxygen to generate ozone eliminated the possibility of generating other compounds, such as oxides of nitrogen which might be produced if air were used. Ozone concentration in chamber studies is not without variation. The literature reports ranges of 1.4% to 16.7% variability (Gong, 1986; Molfino, 1991). Trials with our design have produced just over 1% variability at the 400 ppb level of ozone. Ozone concentration was monitored continuously using an ultraviolet photometric ozone analyzer (Dasibi, range 0-1.0 parts per million (ppm); precision: 0.001-0.002 ppm; LDL = 0.002 ppm; stability: =/- 0.002 ppm @ 0.5 ppm). The Dasibi was specific for ozone and was not influenced by other gases. The analyzer was calibrated every 6-8 weeks by the Ontario Ministry of Environment. They performed dynamic calibration and used an ozone source as analyzed against an ultra-violet photometer traceable to the

National Bureau of Standards primary standards.

Four hundred ppb ozone was selected as the exposure concentration for several reasons. Our laboratory has had extensive experience working with this concentration in similar exposure protocols. It has proven to be a safe and effective concentration at which one can measure reversible spirometric changes, symptoms and airway hyperreactivity in asthmatics. In addition, other investigators have successfully used this concentration in similar studies using indomethacin (Schelegle, '87; Ying, '90) without harmful effects to subjects.

Lung Function Measurements

All pulmonary function measurements, pre, during and post exposure, were corrected to Body Temperature Pressure Saturated (BTPS), i.e. 37 degrees C, and 47 mm mercury water vapour pressure. Forced expiratory volumes (measured in litres) and flows (measured in litres/second) were measured using a Wedge Spirometer (Medscience Electronics, St. Louis, Mi USA), traced on an X-Y recorder (Hewlett Packard, model 7045A). The spirometer was calibrated monthly for volume (using a 4.0 litre calibration syringe) and every 3 months for flow (using a calibrated 10 l/sec^{-1} rotameter). This tested the full range of the spirometer (0-10 l/sec); results were considered acceptable if they varied by less than 5% between calibrations. The flow and volume were corrected, if it was necessary, to ensure

that they were within 100 ± 2% of the actual (physical calibration) value, after every calibration. The wedge spirometer was checked for leaks before each testing series, and for volume at the mid-range of the wedge spirometer (3-7 litres). The X-Y recorder had an accuracy of ± 0.2% of full scale. Subjects completed two partial expiratory flow-volume (PEFV) curves and one maximum expiratory flow volume (MEFV) curve as one set. PEFV curves were generated in the following manner: the subject performed several slow vital capacity (VC) maneuvers, the largest of which was recorded. A compass point was marked at 40% of VC (measured from TLC) and locked in place. The subject then inspired to total lung capacity (TLC) and then returned to normal breaths for 15 seconds. After 15 seconds had elapsed, the subject inspired to exactly the 40% mark and exhaled forcefully to RV, thus completing the first PEFV curve. With no pause, a second PEFV was produced in exactly the same manner. Again with no pause the subject inspired fully to TLC and exhaled to RV thus producing a MEFV curve, for a total of 3 curves. The subjects repeated this procedure at least once more until the largest FVCs were reproducible to within 5% and it was judged that a maximum effort had been obtained (sharp peaks, reproducible curves). Standard spirometric values were measured from the MEFV curves and flow at 40% VC ($\dot{V}40P$) was measured from the average of two PEFV curves before exposure, and every 30 minutes during exposure for 2 hours. Flow rates were measured as a percentage of vital capacity starting from the residual volume (RV) end of the MEFV curve. Flow rates are expressed using the "$\dot{V}$" terminology

followed by a number (i.e. 25) expressing the percentage of vital capacity, i.e. the point along the x-axis of the MEFV curve. This number corresponds to the point where a perpendicular line is used to intersect the MEFV curve and the distance between the curve and the x-axis is recorded. A standard calibration box is used to convert the measurement (millimeters) to a flow rate in litres per second. The following are measured from the flow volume curve: Forced Vital Capacity (FVC), Forced Expiratory Volume in 1 second (FEV$_1$), Forced Expiratory Flow Rate at 50% vital capacity ($\dot{V}$50), Forced Expiratory Flow Rate at 25% vital capacity ($\dot{V}$25), Forced Expiratory Flow Rate (from MEFV) at 40% vital capacity ($\dot{V}$40F), Forced Expiratory Flow Rate (from PEFV) at 40% vital capacity ($\dot{V}$40P), Forced Expiratory Flow Rate at 75% vital capacity ($\dot{V}$75) and Peak Expiratory Flow Rate (PEFR). Partial flow rates were produced from the average of two partial flow volume curves measured at 40% of the largest FVC. Partial flow volume curves may provide a more sensitive indicator of bronchoconstriction in the small airways and show reductions in airflow at lower lung volumes than do full curves at 40% vital capacity ($\dot{V}$40F) (Zamel, 1984; Habib, 1979; Bouhnys, 1969). The largest FVC and FEV$_1$ at each time point (pre, 30, 60, 90, 120, 90 minutes post exposure) were reported. Flows were measured from the MEFV curve yielding the largest FVC and FEV$_1$ within 5%. The largest and most reproducible flows were reported. The investigator calculated all flow/volume curves by hand and was blind to the subject name and study conditions during the calculations to avoid measurement bias.

Exercise Testing

Subjects exercised intermittently using alternating bouts of 15 minutes exercise, 15 minutes rest, over the 2 hour exposure periods. Exercise was carried out on a Monark stationary bicycle ergometer at a workrate that generated a target inspiratory exercise minute ventilation ($\dot{V}i_{min}$) of 30 litres/minute (1/min), at a cadence of 60 revolutions per minute. The cadence was set by a metronome placed in the chamber. For each subject, the workrate was established during a preliminary test in the baseline week, in which subjects performed a "three workrate" test. That is, each subject achieved 5-6 minutes of steady state, sub-maximum cycling, at inspired minute ventilations of approximately 20, 30, and 40 1/min. A plot of minute ventilation (1/min) versus workload (kg) was produced from the three testing conditions, and simple linear regression was applied to determine an equation for the line. A perpendicular line was dropped from the curve corresponding to the y-axis mark of 30 1/min. The workload weight was then read off the x-axis. Subjects were prompted when they fell outside a critical zone of 57-63 revolutions/min. This was picked up from the trachometer output. The cycle ergometer was calibrated using standardized known weights before each test.

Symptom Reporting

During the exposure, subjects were asked to record any symptoms and the time they occurred. Immediately following the exposure, these symptoms were transferred to a formatted questionnaire used in previous studies at the GAGE Research Institute. The questionnaire was completed after the exposure to minimize possible under or over-reporting bias. Under-reporting bias may occur if subjects given the questionnaire during the exposure period feel limited to record only the symptoms that appeared on the questionnaire, and thus underreport the symptoms they actually experienced during an exposure period. Over-reporting may occur if the questionnaire is given during the exposure and becomes suggestive of symptoms that otherwise would not be reported. The symptom questionnaire covered the pre, during and post-exposure time periods. The number of symptoms reported was the difference between pre-exposure baseline and the total number of symptoms reported by the subject at the end of two hours of exposure. Symptoms were classified as respiratory symptoms (SYMR) or general symptoms (SYMG). SYMR included symptoms pertaining to the upper and lower respiratory tract over the entire 2 hour exposure period and included such symptoms as chest pain, difficulty breathing, wheezing and cough. SYMG included all other complaints such as fatigue, headache and sore muscles over the two hour exposure period.

Symptom Severity

Symptom severity was scored using a categorical Borg scale, 0

representing no complaint, 1-minimal, 2-mild, 3-moderate, 4-severe and 5-incapacitating. Severity scores were reported for the pre, during and post-exposure time points and were mean (+/-SEM) values. For example, respiratory symptom severity scores of 2 for cough in hour one, 3 for chest pain in hour 2, and 1 for wheeze in hour 2 would result in a SYMR score of 2+3+1/3 = 2 (+/-0.58).

Methacholine Challenge Testing

Methacholine challenges were given one day prior to all exposure days and 90 minutes following each exposure. A period of 90 minutes post exposure allowed for the return of pulmonary function (FEV_1) to pre-exposure baseline levels prior to starting a methacholine challenge. This was done as a safety precaution and to avoid subject discomfort. The methacholine procedure is designed to test airway responsiveness to an inhaled bronchoconstricting agent given in increasing doubling dosages from 0.06 to 256 mg/ml. The first dose delivered was a diluent solution (0.9% NaCl) to establish a baseline FEV_1 response. Each dose was inhaled for exactly 2 minutes after which bronchoconstriction was determined by measuring the FEV_1 from the maximum expiratory flow-volume curve. When the FEV_1 dropped by more than 20% from the subject's measured mean baseline or below 1 litre, the test was stopped. The provocation concentration of methacholine which caused a 20% drop in FEV_1 (PC_{20}) was determined by interpolation of the last two doses using a plot which plotted the percent drop in FEV_1 (Y-axis) versus the log

methacholine concentration (mg/ml) (X-axis). The PC_{20} was calculated by dropping a perpendicular line from the curve at the point which corresponded to a 20% fall in FEV_1 on the Y-axis. The PC_{20} was then read off the X-axis.

Measurement of FEV_1 after each dose of methacholine was only performed once if the curves generated were technically correct and there was less than 5% variability compared to baseline. If the variability was greater than 5% or the curves were not technically acceptable, a second measurement was required at the 90 second mark. A final third measurement at the 3 minute mark was performed if the first two measurements were unsatisfactory. At five minutes the next doubling dose of methacholine was given provided satisfactory curves were obtained before this point.

The methacholine aerosol was generated with a Wright nebulizer (Roxon Medi-Tech Ltd., Mtl. PQ.) and inhaled with normal tidal breaths through the mouth with the nose clipped. The aerosol delivered to the lung might be altered by nebulizer output, the duration of inhalation and the particle size. The influence of each of these factors has been investigated by Hargreave (1984) who showed that nebulizer output and the duration of inhalation need to be regulated. The nebulizer is regulated at 0.13 ml/min and the duration of inhalation is 2 minutes i.e. the total dose delivered to the mouth during the period of tidal breathing is 0.26 ml. The reproducibility of the dose delivered by the Wright nebulizer to

the lung and of the measured response is equal to that obtained by delivery of a bolus aerosol by a dose-measuring device (dosimeter) during a number of inspiratory breaths (Ryan, 1981; Juniper, 1978; Dehaut, 1983). Each Wright nebulizer is calibrated with the flow meter used to operate it. The Wright nebulizer has been found to remain stable with respect to desired output, for a period of up to 1.5 years (Hargreave, 1984).

PC_{20} is defined as the concentration of methacholine that produces a 20% fall in FEV_1. The lower the PC_{20} value, the greater the airway reactivity. Following ozone exposure, a decrease in PC_{20} from baseline would reflect an increase in airway reactivity. Pre exposure $PC_{20}s$ were measured 1 day prior to exposure, and post exposure $PC_{20}s$ were measured 90 minutes following exposure. Table 9 (pg 134) shows the pre minus post exposure differences and the difference between the pre minus post exposure differences between the placebo and indomethacin condition. Positive values for the difference between pre minus post exposure are declines in PC_{20} and therefore increases in airway reactivity.

Study Protocol

The study protocol was comprised of five weeks per subject (one baseline testing week plus four study weeks). The baseline week consisted of 5 consecutive days and a study week comprised three consecutive days. The last day of each week (baseline and study)

was the exposure day where, in the case of a baseline week, subjects were exposed in a controlled environmental chamber to clean filtered air (FA) for 2 hours, while intermittently exercising on a stationary bicycle every 15 minutes. The study week exposure days were identical to baseline, except subjects received ozone at a concentration of 400 ppb or FA in a randomized, single blind manner. Each study week was separated by a minimum of 2 weeks to allow for the possibility of ozone washout. The two week washout period was implemented whether or not a subject was exposed to ozone.

Baseline Week

On the first day of baseline week, subjects completed a health questionnaire and received a medical exam to ensure all medical inclusion and exclusion criteria were met. If all criteria had been satisfied, subjects were tested as follows: spirometry (% of predicted values for normal flow-volume curves according to sex, height, weight and age of subject), exercise level (establishing cycle-ergometer work load to achieve a target inspiratory $\dot{V}i_{min}$ of 30 L/min.), responsiveness to a methacholine challenge and allergic reaction to common allergens (skin test). On day 2 subjects provided sputum samples via the induced sputum procedure (Pin, 1992) for later analysis. On day 3 subjects took a one quarter dose capsule of indomethacin (6.25mg) and spirometry (FEV_1) was monitored every 30 minutes for 2 hours. A subject whose FEV_1 fell

by more than 15% was considered to be aspirin sensitive and at risk of developing a bronchospasm to higher doses of indomethacin and would become ineligible for the study. If the drop was less than 15%, the subject took 1 full dose capsule (25mg) and spirometry was again monitored every 30 minutes for 2 hours. If the FEV_1 drop was again less than 15%, the subject took home six 25 mg capsules and ingested them with food on a three times a day schedule (t.i.d.). for 2 days. On day 4 while continuing on the indomethacin regimen (25mg t.i.d.) the subjects received a methacholine challenge. Two hours following the methacholine challenge, induced sputum samples were collected. On day 5 (the last day of the baseline week), the subject took the last indomethacin capsule (25mg) approximately one hour before exposure, performed pre-exposure spirometry, and then underwent a two hour FA exposure with exercise. Approximately 90 minutes post exposure, a methacholine challenge was given, and two hours subsequent to the methacholine challenge, induced sputum samples were collected and analyzed.

Study Weeks

At least 2 weeks later subjects began study week 1 as follows: day 1 they randomly received a double-blind ingestion of either one 25 mg indomethacin capsule or an identical looking placebo capsule. Spirometry was then monitored over the next 2 hours. If results were satisfactory (< 15% drop in FEV_1) subjects ingested 2 more

capsules that day (day 1) to total 3 capsules/day. Three 25mg capsules were ingested the following day (day 2), and one 25mg capsule was ingested the morning of the exposure (day 3) approximately one hour prior to exposure. Subjects therefore ingested a total of 7 capsules over 2 days (plus a morning). Capsules were ingested with food, i.e. after breakfast, lunch and dinner. On day 2 a methacholine challenge was performed followed two hours later by induced sputum. On day 3, subjects were exposed for 2 hours to ozone (400 ppb) or FA (single blind fashion) with alternating 15 minute exercise and rest periods. One MEFV curve, and two PEFV curves were performed as one manoeuvre (3 curves), immediately pre exposure, every 30 minutes during exposure and 90 minutes post exposure for a total of 6 times. Subjects remained inside the chamber for all spirometry testing procedures. Inspiratory minute ventilation ($\dot{V}i_{min}$) was recorded while subjects breathed through a mouthpiece with clipped nose for at least two minutes. Minute ventilation was monitored once during each exercise and rest period, and heart rate was recorded every five minutes throughout the exposure period. A symptom questionnaire (described in chapter 4) was administered at the end of the exposure. To avoid ozone odor bias, a short spike of ozone (< 50 ppb) for 2-5 minutes was introduced on FA days at the beginning of the exposure. Temperature and relative humidity were kept at 21 degrees C and 40%, respectively, for subject comfort. Bronchodilator therapy (ventolin) was always on hand, if needed, to relieve respiratory distress, and all exposures, whether FA or with ozone, were

conducted with a physician's knowledge who was in the building. The plexi-glass walls of the chamber were transparent such that visual contact with the subject was maintained at all times. Ninety minutes after exiting the chamber a methacholine challenge was performed. The 90 minute period allowed for resolution of the ozone-induced changes in lung function before beginning the post exposure methacholine challenge. Two hours later, induced sputum samples were collected. This time coincided with the reported peak neutrophil response time in humans of 4-6 hours post ozone exposure (Schelegle, 1991). The combination of treatment and exposure were randomized such that a subject did not receive the same combination of treatment and exposure twice.

Study weeks three through five were identical to study week two, except a different treatment-exposure combination condition was randomly assigned in each case. Subjects served as their own controls and comparison of dependent variables were made between treatment (placebo and indomethacin) and exposure (ozone and filtered air) conditions.

RESULTS

Anthropometric data and all baseline data on pulmonary function tests appear in Table A1 (Appendix, pg A145) at the end of this chapter. Also included in the appendix are results on the effects of indomethacin on baseline FVC and FEV$_1$ (Table A2, pg A146), mean

subject minute ventilation and respiratory frequency values (Table A3, pg A147), and the effect of exercise on pulmonary function variables with placebo (Table A4A, pg A148) and indomethacin (Table A4B, pg A148).

Response with Placebo and Indomethacin

Pulmonary Function

Table 1 (pg128) shows individual subject FVC values for all pre and 2 hour post exposure conditions, and all pretreatment conditions for 14 asthmatic subjects. Also shown are the percent change differences between pre and post exposure, and the difference between the percent change differences with placebo and indomethacin. Percent change differences are calculated as (pre exposure - post exposure)/pre-exposure x 100, thus in the table a positive percent change difference indicated a decrease in pulmonary function from baseline, and a negative percent change difference indicated an increase in pulmonary function. The difference between the percent change differences was calculated as follows: {percent change difference$_{(placebo)}$} - {percent change difference$_{(indomethacin)}$}, thus in the table a positive value indicated an overall greater decrease in pulmonary function with placebo versus indomethacin, and a negative value indicated an overall smaller decrease in pulmonary function with placebo versus indomethacin.

Table 2 (pg129) shows the group mean (+/-SEM) pulmonary function data in all exposure (pre and 2 hours post) and pretreatment conditions for all pulmonary function variables in 13 asthmatic subjects. One subject (number 4) did not complete the air exposure study condition, thus N=13.

Table 3 below shows the difference (+/-SEM) in baseline changes (%) between ozone and air exposure (2 hours) with placebo and indomethacin for all maximum expiratory flows and volumes in 13 asthmatic subjects.

TABLE 3

MEAN (+/-SEM) DIFFERENCE IN BASELINE CHANGES (%) BETWEEN OZONE AND AIR EXPOSURE (N=13)

	PLACEBO	P VALUE	INDOMETHACIN	P VALUE
FVC	-12 (3)	0.001	-11 (4)	0.02
FEV1	-13 (2)	0.0001	-10 (3)	0.01
V50	-21 (6)	0.004	-12 (4)	0.02
V25*	-25 (10)	0.02	-9 (6)	0.14
V40F	-15 (8)	0.07	-10 (6)	0.12
V40P	-26 (13)	0.09	-16 (9)	0.11
V75	-19 (7)	0.02	-15 (4)	0.006
PEFR	-13 (4)	0.005	-14 (4)	0.004

* difference between placebo and indomethacin approached statistical significance (p=0.07);SEM = standard error of the mean

Data from Table 3 is displayed in graphical form in Figure 1A (pg136). Figure 1A shows the mean (+/-SEM) difference in baseline changes (%) between ozone and air exposure with placebo (clear bar) and indomethacin (shaded bar) in 13 asthmatic subjects. The ozone response is defined as: (pre ozone exposure - ozone exposure at 2 hours) - (pre air exposure - air exposure at 2 hours). An * indicates a statistically significant difference between ozone and air exposure at the 0.05 level. The "indomethacin" or "drug interaction effect" is defined as: (ozone response with placebo) - (ozone response with indomethacin). No significant mean (p>0.05) differences between the placebo and indomethacin condition, i.e. no significant interaction effects, were found for any of the following pulmonary function variables following 2 hours of ozone exposure (although statistical significance was approached for $\dot{V}25$): FVC (p=0.84), FEV_1 (p=0.27), $\dot{V}50$ (p=0.19), $\dot{V}25$ (p=0.07), $\dot{V}40f$ (p=0.93), $\dot{V}40p$ (p=0.96), $\dot{V}75$ (p=0.59), and PEFR (p=0.86). Figure 1A also shows that the greatest ozone responses with placebo occurred with $\dot{V}25$ (25% decrease) and $\dot{V}40P$ (26% decrease), compared to a mean decline of 12% and 13% for FVC and FEV_1, respectively. Among all variables, $\dot{V}25$ showed the greatest response to indomethacin, i.e. a 57% reduction in decline (p=0.07), therefore was more sensitive to the effects of indomethacin than the other variables.

Table 4 below (pg110) shows the mean (+/-SEM) difference in baseline changes (%) between ozone and air exposure with placebo and indomethacin following 1 hour of exposure in 13 asthmatics.

TABLE 4

MEAN (+/-SEM) DIFFERENCE IN BASELINE CHANGES (%) BETWEEN OZONE AND AIR EXPOSURE AFTER 1 HOUR EXPOSURE IN 13 ASTHMATIC SUBJECTS

	PLACEBO	P VALUE	INDOMETHACIN	P VALUE
FVC*	-7 (2)	0.01	-5 (2)	0.04
FEV1*	-9 (3)	0.01	-4 (2)	0.08
$\dot{V}50$	-16 (7)	0.04	-3 (4)	0.46
$\dot{V}25$	-18 (8)	0.06	-6 (6)	0.35
$\dot{V}40F$	-12 (7)	0.11	-9 (5)	0.08
$\dot{V}40P$	-16 (13)	0.25	-16 (8)	0.09
$\dot{V}75$	-12 (6)	0.06	-2 (4)	0.53
PEFR	-11 (4)	0.03	-5 (2)	0.08

---

* difference between placebo and indomethacin approached statistical significance (p=0.08); SEM = standard error of the mean

Data from Table 4 is displayed in graphical form in Figure 1B (pg136). Figure 1B shows the mean (+/-SEM) difference in baseline changes (%) between ozone and air exposure with placebo (clear bar) and indomethacin (shaded bar) following 1 hour of exposure in 13 asthmatic subjects. An * indicates a significant difference between ozone and air at the 0.05 level. No significant differences (p>0.05) between placebo and indomethacin were found, although FVC (p=0.08) and FEV_1 (p=0.09)approached statistical significance.

Individual FEV_1 responses at 30, 60, 90 and 120 minutes of exposure with placebo and indomethacin are shown in Figures 2A-2M (pg138-

139). Eleven of 13 subjects showed maximum FEV_1 decrement after the first hour of exposure. Of these 11 subjects, 9 subjects showed the greatest decline in FEV_1 at 120 minutes of exposure; 2 subjects showed maximum decrement at 90 minutes, and 2 subjects had a peak decrement at 60 minutes. In each subject, the difference between placebo and indomethacin at 2 hours of exposure was not statistically significant ($p>0.05$). Figures 3A, 3B, 3C and 3D (pg 140) show scatter plots of the relationship between the ozone response, i.e. the ozone-induced baseline change in FVC, FEV_1, $\overset{\bullet}{V}50$ and $\overset{\bullet}{V}25$ with placebo, versus % protection with indomethacin after 2 hours of exposure. Protection is measured as a percent change between the ozone-induced baseline changes with placebo and indomethacin, i.e. % protection=(baseline change in $FVC_{placebo}$) - (baseline change in $FVC_{indomethacin}$)/baseline change in $FVC_{placebo}$ x 100. An arbitrary cut-off point of a 15% decline from baseline was selected to define FVC and FEV_1 responsive subjects, although this cut-off point for FEV_1 responsiveness has been used by others (Torres, 1995). A 20% cut-off point was selected for $\overset{\bullet}{V}50$ and $\overset{\bullet}{V}25$ to define ozone-responsive individuals. Protection was either present (yes > zero) or not present (no < zero). Using these limits, spirometrically responsive subjects were divided into two quadrants, protection = "yes" or protection = "no". For FVC and FEV_1, 6 and 7 subjects respectively were classified as responsive. In Figure 3A (FVC), equal numbers of subjects demonstrated protection ("yes") and no protection ("no") against ozone-induced FVC decline. For ozone-induced FEV_1 decline the majority of subjects (6 out of

7) demonstrated no protection. In both scatter plots, there does
not appear to be a discernable linear relationship among the data
points in either quadrant. For $\dot{V}50$ and $\dot{V}25$, 9 subjects were
classified as responsive, and for both variables, twice as many
subjects (6) were represented in the no protection quadrant. The
relationship of the data points in either quadrant did not appear
linear.

Symptom Reporting

Tables 5 (pg130) and 6 (pg131) report the individual and group mean
(+/- SEM) pre and post exposure symptom reporting values across all
exposure and treatment conditions for general (Table 5) and
respiratory symptoms (Table 6) in asthmatic subjects. Also shown
are the pre minus post exposure differences and the difference
between the pre minus post exposure differences in the placebo and
indomethacin condition. Table 6 shows that following ozone
exposure, the mean number of respiratory symptoms reported was just
under 2 (i.e. 1.85) with placebo.

Figure 4 (pg141) shows the mean (+/-SEM) difference in absolute
baseline changes for symptom reporting between ozone and air
exposure with placebo (clear bar) and indomethacin (shaded bar)
following 2 hour exposures. Symptom reporting was classified into
general type symptoms (SYMG), i.e. General fatigue, muscle soreness
and respiratory only (SYMR), i.e. Cough, chest pain, dyspnea. An *
indicates a significant difference (i.e. Ozone response) between

ozone and air exposure at the 0.05 level. Significant ozone responses with placebo were found for SYMG (p=0.04) and SYMR (p=0.001). Significant ozone responses with indomethacin were found for SYMG (p=0.01) and SYMR (p=0.03), but the magnitude of response was smaller with indomethacin compared to placebo. There were no significant differences between placebo and indomethacin (i.e. indomethacin interaction effects) for either general or respiratory symptom reporting (p>0.21).

Symptom Severity

Tables 7 (pg132) and 8 (pg133) show the difference in absolute baseline changes for symptom severity scores between ozone and air exposure with placebo and indomethacin. Also shown are the individual and group mean (+/- SEM) pre and post exposure symptom severity scores for all exposure and treatment conditions for general and respiratory symptoms, respectively. From table 7 the mean respiratory severity score following ozone exposure with placebo was 2.14 which is equal to a descriptive rating of "mild".

Figure 5 (pg142) shows the mean (+/-SEM) difference in absolute baseline changes between ozone and air exposure for symptom severity scores for general and respiratory symptoms with placebo (clear bar) and indomethacin (shaded bar). Significant differences between ozone and air, i.e. the ozone response (* in the Figure), were found with placebo for SYMG (p<0.02) and SYMR (p<0.001).

Significant ozone responses were also found with indomethacin for SYMG (p<0.05) and SYMR (p<0.01). There were no significant differences between placebo and indomethacin i.e. indomethacin interaction effects, for SYMG and SYMR (p>0.05).

Airway Reactivity

Table 9 (pg134) shows individual and group mean (+/- SEM) pre and post exposure PC_{20} values across all exposure and treatment conditions in 13 asthmatic subjects.

Figure 6 (pg143) shows the difference in absolute baseline changes for PC_{20}, methacholine between ozone and air exposure, i.e. the ozone response, with placebo (clear bar) and indomethacin (shaded bar) in 13 asthmatic subjects. Figure 6 also shows the difference in baseline changes for PC_{20} between ozone and air exposure for 4 asthmatic subjects who had a greater than two doubling dose fall in methacholine following ozone exposure, i.e. ozone responsive. No significant difference (p=0.66) between ozone and air with placebo were found for the entire asthmatic subject group (N=13). The indomethacin interaction effect for PC_{20} is defined as the difference in ozone response between placebo and indomethacin. No significant difference in ozone response between placebo and indomethacin was found for the 4 ozone responsive subjects. Since a significant ozone response was not observed for the entire asthmatic subject group, a test to determine the difference between

placebo and indomethacin was not performed. In PC_{20} responsive subjects, indomethacin did not significantly reduce the reduction in PC_{20} following ozone exposure (p=0.89).

DISCUSSION

Results from this study showed that when compared to air exposure, asthmatic subjects demonstrated significant declines in several maximum expiratory flows and volumes following a two hour exposure to 400 ppb ozone. The maximum decrement occurred in variables which represent the status of the small airways ($\dot{V}40P$, $\dot{V}25$, $\dot{V}50$).

Our data support an earlier study by Eschenbacher (1989) who reported that asthmatics experienced reductions in FVC and FEV_1 following a 2 hour exposure to 400 ppb ozone. Our data demonstrated that pretreatment with indomethacin did not significantly reduce decrements in pulmonary function, i.e. maximum expiratory flows and volumes, following a 2 hour exposure to ozone. These results are in contrast to Eschenbacher's (1989) study which reported significant, but partial inhibition of FVC and FEV_1 decrements with indomethacin following a 2 hour exposure to 400 ppb ozone.

Several possible reasons may explain why we were unable to demonstrate significant attenuation of the ozone-induced declines in FVC and FEV_1 with indomethacin. These include too small a sample size, failure to achieve enough cyclooxygenase (COX1) inhibition

due to insufficient dose of indomethacin, or alternatively failure to achieve COX2 inhibition due to indomethacin's preference to inhibit COX1 versus COX2 inhibition, or finally that non-cyclooxygenase mechanism(s) produced the observed decrements in pulmonary function in asthmatics. Each of these possibilities will be discussed in turn.

With respect to sample size, the majority of studies demonstrating ozone-induced pulmonary function decrements as well as ones that successfully used cyclooxygenase blockers to prevent these declines, (Hazucha, 1996; Ying, 1990; Eschenbacher, 1989; Schelegle, 1987) all had samples sizes very similar to ours. In fact, our sample of 13 asthmatic subjects is on the high end compared to Ying (7 subjects), Hazucha (10 subjects) and Eschenbacher (10 subjects). What may be more relevant however, than just the total number of subjects is the magnitude of response. Compared to these previous studies, our subjects showed a lower mean decline in FEV_1 (13%) with placebo versus Schelegle (25%), Eschenbacher (24%) and Hazucha (18%) following ozone exposure. This was likely due to higher effective doses of ozone used in these latter studies. For example, Eschenbacher used 400 ppb for 2 hours at 30 $L/min/m^2$ body surface area with intermittent exercise (approximately 60 L/min, using a body surface area of 2.0 m^2 for a 180 cm, 75 kg male). Since indomethacin was not expected to reduce FEV_1 decrement back to complete baseline (i.e. 100%), but previous evidence suggested 50% - 60% was reasonable, a reduction in

this range applied to our data would have produced a relatively smaller absolute effect size, i.e. a small change from pre-exposure baseline. For example, a 50% reduction of a 26% drop in FEV_1 is a decline of 13% points, versus a 50% reduction of a 12% drop, which is a decline of 6% points. Given that normal baseline variability can be as high as 5% for FVC (Goldman, 1959) and FEV_1 (Morris, 1973), more statistical power (and more subjects) is required to demonstrate significance for a smaller effect size of 6 % points versus 13 % points at the 0.05 level. Since we in fact used more subjects than the other studies previously mentioned, we considered our study to have sufficient statistical power to demonstrate a relatively small difference between placebo and indomethacin (7% points, Chapter 3, pg. 73). In addition, results from our non-asthmatic subjects (N=9) (see Chapter 7) showed statistically significant inhibition of FEV_1 decline where the magnitude of decline and variance with placebo (10% +/- 2%) was very similar to that observed in our asthmatic subjects (12% +/- 3%). Therefore, other reasons such as dose of indomethacin need to be considered to explain our inability to demonstrate a significant indomethacin interaction effect.

Eschenbacher (1989) successfully inhibited ozone-induced FEV_1 decline with indomethacin but used higher doses (150 mg/day for 4-5 days) compared to our study (75 mg/day/3 days). Our results at one hour of exposure revealed that indomethacin protected against decrements in FVC and FEV_1 that approached statistical significance

Since protection against pulmonary function decline began to appear after one hour of exposure, but disappeared after two hours, suggests the possibility that an insufficient concentration of indomethacin was in the airways to sustain protection against pulmonary function decrement throughout the entire two hour exposure period. The absence of protection at 2 hours was not due to the presence of a minimal ozone response at 2 hours, since the fall in FVC and FEV_1 was greater at 2 hours versus one hour. Further we noted that indomethacin's protective effects became progressively weaker as ozone duration increased. Our data demonstrated the following inequality for mean FEV_1 protection (%) over 4 exposure time points: 120 min. < 90 min. < 60 min. < 30 min. If what our data suggest is true, several cyclooxygenase metabolites (i.e. PGE2, TXA2, PGD2) including PGF2-alpha may have increased over the exposure period as protection decreased. As a result, these eicosanoids may have contributed directly to airway bronchoconstriction, or indirectly by stimulating nociceptive airway nerve endings which are known to cause reduced inspiratory capacity. Both these actions may account for the lack of protection we observed against ozone-induced reductions in FVC and FEV_1 in asthmatic subjects pretreated with indomethacin.

A third potential explanation is that ozone-induced pulmonary function decline was mediated in part by eicosanoids derived from activation of the COX2 enzyme, not COX1. Indomethacin is 20 times

more potent at inhibiting COX1 versus COX2 in the mouse (Smith, 1993), therefore indomethacin may not have the pharmacologic specificity to inhibit all the appropriate metabolic pathways driving the ozone response in asthmatics. In addition, it's possible that a non-cyclooxygenase pathway completely immune to indomethacin inhibition, may have been activated in the airways and caused reductions in inspiratory capacity.

According to our data, the small airways region demonstrated the greatest magnitude of response with ozone and indomethacin. Our data also showed that when compared to the placebo condition, subjects demonstrated appreciable FVC decline with indomethacin, whereas the percent decreases in $\dot{V}25$ and $\dot{V}40$ were appreciably attenuated with indomethacin. This demonstrates that the effect of indomethacin on small airways variables was not related to its effect on FVC. This suggested to us that the changes we observed in small airways variables in asthmatics were due to small airways dysfunction, and not from changes in FVC which is a typical concern when flow rates are not determined iso-volumetrically.

Histological evidence supports the view that the small airways may be a preferential target site for ozone. The lung lining fluid layer of the airways is thought to provide some protection against inhaled ozone, but the small airways may be more vulnerable to ozone due to the "patchy" nature of the fluid layer found there.

Evidence in exposed animals also showed that ozone had an initial effect on the small airways (Gertner, 1983; Morgan, 1986). Recent studies on ozone uptake in humans have implicated the small airways as a target site of action. Hu (1992) used an inhalation bolus technique in healthy subjects, and reported that ozone penetration and uptake increased longitudinally toward the small airways as ventilation increased. This evidence along with our data support earlier predictive models which showed that the small conducting airways were subjected to higher doses of inhaled ozone per unit of tissue versus the larger upper airways (Miller, 1985).

Our data demonstrated that subjects reported more general and respiratory type symptoms relative to baseline following ozone exposure compared to air, i.e. General Symptoms (baseline = 0.23 vs post ozone exposure = 1.77), and Respiratory Symptoms (baseline = 0.23 vs post exposure 1.85). A significant increase in general type symptoms may indicate that ozone has health effects on other body systems, i.e. cardiovascular, other than respiratory. These results support a previous study in normal subjects (Schelegle, 1987) where a 2 hour exposure to 400 ppb ozone caused significant increases in cough, pain on deep inspiration and throat tickle. Our results showed that pretreatment with indomethacin did not significantly inhibit the increase in general or respiratory symptom reporting, although increased respiratory symptom reporting was inhibited to a small degree. These results are in contrast to a previous report by Schelegle (1987) where indomethacin significantly inhibited

increased reporting of cough and throat tickle. Our symptom data appeared to track our pulmonary function results with respect to exposure (ozone) and pretreatment (indomethacin), i.e. increased symptom reporting following ozone, and the partial but non-significant attenuation of the increased symptom responses with indomethacin. The partial attenuation was mainly on cough and chest tightness. The apparent relationship we observed between pulmonary function decrement and increased symptom reporting has been previously demonstrated (Linn, 1980; Folinsbee, 1988; Schelegle, 1987; Horstman, 1990).

Our data showed that ozone compared to air, caused significant increases from baseline in symptom severity scores for both general (baseline = 0.08 vs post exposure = 2.14) and respiratory (baseline = 0.08 vs post exposure = 2.37) symptoms. Ratings of 2.1 and 2.4 have descriptive Borg scale ratings of "mild". Our severity ratings following ozone exposure were very similar to Schelegle's (1987) results in healthy subjects. Schelegle used a categorical Borg scale and reported "mild" severity following ozone (with placebo) exposure. Like our symptom reporting results, our data showed that indomethacin provided partial but non-significant inhibition of increases in severity scores for general and respiratory symptoms. These data support Schelegle's (1987) results who found no significant inhibition of symptom severity with indomethacin.

With respect to airway reactivity, ozone exposure did not produce

a significant mean increase in airway reactivity in our subjects. These data do not support previous findings where under similar exposure conditions, ozone was able to produce increased airway reactivity in asthmatic subjects (Ying, 1990; Kreit, 1989; Holtzman, 1979). To help explain these results, we measured the individual PC_{20} for each subject following ozone exposure. We discovered that only 4 of 13, or 30% of subjects, experienced a fall in PC_{20} of greater than two doubling doses of methacholine, i.e. PC_{20} sensitive to ozone. When these subjects were analyzed as a separate group (N=4), a significant increase in airway reactivity (decline in PC_{20}) was found in the placebo condition following ozone exposure (p=0.05) (Figure 6). Our difficulty in observing a mean increase in airway reactivity for all asthmatic subjects was no doubt due to the influence of subjects who were PC_{20} insensitive to ozone. With respect to individual sensitivity, our data are consistent with a recent report by Stolk (1996) and an earlier report by Ying (1990). Ying (1990) used a similar ozone sensitivity cut-off point to ours in normal subjects (>50% decline in PC100 Sraw), while Stolk (1996) defined non-responders as demonstrating a less than 0.5 doubling dose difference in PC_{20} methacholine between air and ozone exposure. Ying reported that 46% (6/13) of his healthy subjects were insensitive to ozone, while Stolk reported that 30% of his asthmatic subjects were insensitive to ozone. Previous studies which showed a significant mean increase in ozone-induced bronchial reactivity, either pre-screened subjects based on PC_{20} sensitivity to ozone {i.e. > than 50% decrease in

PC100 specific airway resistance)(Ying, 1990)}, or the PC_{20} sensitive subjects greatly influenced the mean change in airway reactivity. Both of these examples would result in an overall significant mean increase in airway reactivity (Holtzman, 1979; Kreit, 1989). Also, these studies had a higher effective doses of ozone than our study. For example Ying (1990) used 400 ppb ozone for 2 hours with an exercise minute ventilation of 30 $L/min/m^2$ body surface area (approximately 60 l/min, using a body surface area of 2.0 m^2 for a 180 cm, 75 kg male). This exercise minute ventilation (60 l/min) approximately doubled our mean exercise minute ventilation of 30 l/min.

Our data suggest that asthmatics have a non-responsive subgroup of their population who do not demonstrate increased airway reactivity following an acute exposure to ozone. Of the 4 subjects who demonstrated airway hyperreactivity following ozone exposure, 2 had decrements in FEV_1 of greater than 15%.

Since we were unable to demonstrate a significant mean decrease in PC_{20} following ozone exposure in the asthmatic group, we could not demonstrate whether indomethacin had any significant protective effects on ozone-induced airway hyperreactivity in asthmatics. We determined from individual subject analysis that the majority of subjects (N=9) did not demonstrate a significant decrease in PC_{20} following ozone exposure, and that this was the likely explanation for our not finding a significant mean decrease in PC_{20} for the

asthmatic subject group as a whole. As a result we selected out subjects who did demonstrate airway hyperreactivity (N=4) after ozone exposure and examined them as a separate subgroup. Since this subject group demonstrated a significant PC_{20} ozone effect, we were able to explore whether indomethacin was able to inhibit ozone-induced airway hyperreactivity in these subjects. In these 4 subjects indomethacin did not inhibit ozone-induced increased airway reactivity. These data appear to support Ying's (1990) results, who examined only ozone sensitive subjects, (i.e. > 50% decline in PC100,SRaw for methacholine after ozone or a decrease in one concentration level of methacholine), and used a more robust indicator of PC100,SRaw change, i.e. percent change values, to determine whether indomethacin provided any protection from ozone-induced increased airway reactivity. Ying (1990) did not find a significant protective indomethacin effect as measured by PC100,SRaw. His data also suggested that no relationship existed between ozone sensitivity, as defined by PC100,SRaw, and the effect of indomethacin in healthy subjects.

Our data together with Ying's results (1990) suggest that indomethacin provides no protection against ozone-induced increased airway reactivity in both asthmatic and healthy subjects. Since only four of our asthmatic subjects showed a significant increase in airway reactivity following ozone, our data were underpowered to form any real conclusions regarding the role of cyclooxygenase metabolites in ozone-induced airway reactivity. It does appear

however, that in a small number of subjects, asthmatics like healthy subjects, receive no protection against ozone-induced airway hyperreactivity from indomethacin pretreatment. More asthmatic subjects need to be examined in order to conclusively demonstrate whether or not cyclooxygenase metabolites, which are sensitive to indomethacin inhibition, play a role in ozone-induced airway hyperreactivity.

CONCLUSION

Exposure to 400 ppb ozone for two hours produced decrements in maximum expiratory flows and volumes (FVC, FEV_1, $\dot{V}50$) in asthmatic subjects. Among all pulmonary function variables, those that reflect the status of the small airways appeared to be the most sensitive to the effects of ozone and indomethacin and this was not due to changes in FVC. Indomethacin pretreatment failed to significantly protect against decrements in pulmonary function (FVC, FEV_1, $\dot{V}50$) following 2 hours of ozone exposure. Specific time point analysis of FEV_1 data suggested that indomethacin was unable to sustain it's protective effects throughout the exposure period. This may have been due to an insufficient dose of indomethacin, since some attenuation of FVC and FEV_1 was present after one hour of exposure, but dissipated as exposure duration increased. Data also revealed that protection against FEV_1 decline, became progressively weaker throughout the exposure period. Also, a previous study on asthmatics has reported significant but partial

attenuation of ozone-induced decreases in FVC and FEV_1 using a larger dose of indomethacin than that used in this study.

Asthmatic subjects demonstrated significant increases in symptom reporting and symptom severity scores following an acute exposure to ozone. Indomethacin pretreatment provided partial but non-significant attenuation of increased symptom reporting and increased symptom severity. Our symptom results closely tracked the pulmonary function results we observed with respect to ozone exposure and the effect of indomethacin.

Ozone exposure did not cause a mean increase in airway reactivity in asthmatic and non-asthmatic subjects. Ozone exposure did cause a mean increase in airway reactivity in four asthmatic subjects who were defined as PC_{20} sensitive to ozone exposure. Indomethacin failed to inhibit ozone-induced increased airway reactivity in these four asthmatic subjects. Approximately 70% of the asthmatic subjects did not respond to acute ozone exposure with a drop in PC_{20},methacholine of greater than two doubling doses. Like healthy subjects, asthmatics appear to have a subgroup of their population who do not respond to ozone with an increase in airway reactivity. We conclude that more PC_{20} sensitive asthmatic subjects need to be examined in order to form any conclusions regarding the role of cyclooxygenase metabolites in airway reactivity following acute ozone exposure.

CHAPTER 4 TABLES

INDIVIDUAL FVC DATA
(LITERS)

ASTHMATICS

|------------------------ PLACEBO---------------------------| |--------------------------------INDOMETHACIN----------------------|

Subject	GENDER M=Male F=Female	PRE OZONE	OZONE Exposure At 2 HRS	% Change (pre-post/pre) x 100	PRE AIR	AIR Exposure At 2 HRS	% Change (pre-post/pre) x 100	DIFF IN % Change	PRE OZONE	OZONE Exposure At 2 HRS	% Change (pre-post/pre) x 100	PRE AIR	AIR Exposure At 2 HRS	% Change (pre-post/pre) x 100	DIFF IN % Changes	DIFF between % change DIFFS
4	M	4.58	4.09	10.7	N/AV	N/AV	N/AV	N/AV	4.66	4.38	6.7	N/AV	N/AV	N/AV	N/AV	N/AV
8	F	4.00	3.22	19.5	3.83	3.65	4.69	14.8	3.64	2.96	18.9	3.60	3.50	2.78	16.16	-1.36
9	M	5.11	4.06	20.5	5.20	5.36	-3.08	23.62	5.35	5.13	4.11	4.66	4.63	-1.76	5.87	17.76
11	M	4.61	4.38	4.98	4.82	4.89	-1.45	6.43	4.69	3.80	18.9	4.71	4.79	-1.69	20.67	-14.24
12	M	4.89	4.27	12.7	5.16	4.84	6.20	6.48	5.11	4.92	3.72	4.87	4.82	1.02	2.7	3.78
13	F	5.26	5.26	0.00	5.56	5.45	1.97	-1.98	5.53	5.43	1.81	5.49	5.29	3.64	-1.83	-0.15
14	F	3.09	2.88	6.79	3.24	3.47	-7.09	13.88	3.26	3.08	5.52	3.33	3.48	-4.50	10.02	3.86
15	F	4.32	3.38	21.8	4.11	4.05	1.46	20.3	4.31	3.54	17.87	4.11	4.24	-3.16	21.03	-0.73
16	M	6.06	5.61	7.42	5.60	5.79	-3.39	10.81	5.76	5.08	12.11	5.52	5.70	-3.26	15.37	-4.56
17	M	5.62	5.17	8.00	5.79	5.99	-3.45	11.46	5.76	5.86	-1.74	5.71	5.79	-1.40	-0.34	11.8
18	F	4.16	3.66	12.02	4.36	4.12	5.50	6.52	4.36	3.53	19.04	4.12	3.99	3.16	15.88	-9.36
19	F	3.55	2.41	32.11	3.51	3.20	8.83	23.28	3.65	1.97	46.03	3.45	3.47	-0.58	46.6	-23.32
22	F	4.26	4.21	1.17	4.66	4.26	8.58	-7.41	4.31	4.43	-2.78	4.85	4.31	11.13	-13.9	6.49
23	F	4.16	3.86	7.18	3.35	4.00	-19.40	26.58	4.13	3.98	3.63	4.00	4.16	-3.76	7.38	19.2
mean		4.55	4.03	11.9	4.55	4.54	-0.05	11.9	4.61	4.13	11.3	4.49	4.47	0.13	11.2	0.70
std error		0.23	0.26	2.57	0.25	0.25	2.14	2.80	0.23	0.31	3.66	0.22	0.22	1.19	4.08	3.35

Table 1 .Individual and group mean (+/-SEM) pre and post exposure FVC data in all exposure and treatment conditions in 14 asthmatic subjects.. Also shown the percent change differences between pre and post exposure and the difference between the percent change differences across exposure and treatment conditions; N/AV = Not Available; SEM = standard error of the mean

GROUP MEAN (+/-SEM) PULMONARY FUNCTION DATA IN ASTHMATICS
ALL PRETREATMENT AND EXPOSURE CONDITIONS

| |--------------------- PLACEBO -----------------------------| |----------------- INDOMETHACIN --------------------|

	OZONE		AIR		OZONE		AIR	
Spirometry Variable	Pre Exposure	Exposure At 2 HRS	Pre Exposure	Exposure At 2 HRS	Pre Exposure	Exposure At 2 HRS	Pre Exposure	Exposure At 2 HRS
FVC (L)	4.55 (0.23)	4.03 (0.26)	4.55 (0.25)	4.54 (0.25)	4.61 (0.23)	4.13 (0.31)	4.49 (0.22)	4.47 (0.22)
FEV1 (L)	3.43 (0.16)	3.03 (0.18)	3.43 (0.18)	3.52 (0.19)	3.49 (0.16)	3.18 (0.23)	3.48 (0.16)	3.48 (0.16)
V50 (L\S)	3.53 (0.29)	3.03 (0.29)	3.57 (0.32)	3.78 (0.35)	3.63 (0.29)	3.28 (0.34)	3.68 (0.26)	3.67 (0.29)
V25 (L/S)	1.47 (0.16)	1.27 ((0.13)	1.50 (0.18)	1.64 (0.21)	1.52 (0.16)	1.49 (0.20)	1.52 (0.14)	1.62 (0.22)
V40(F) (L\S)	2.57 (0.25)	2.29 (0.24)	2.77 (0.28)	2.86 (0.34)	2.77 (0.27)	2.62 (0.29)	2.84 (0.24)	2.78 (0.27)
V40(P) (L\S)	2.39 (0.25)	2.03 (0.19)	2.34 (0.21)	2.49 (0.28)	2.53 (0.21)	2.23 (0.21)	2.63 (0.24)	2.59 (0.23)
V75 (L\S)	6.39 (0.36)	5.45 (0.43)	6.49 (0.46)	6.62 (0.36)	6.86 (0.44)	5.77 (0.53)	6.99 (0.42)	6.69 (0.38)
PEFR (L\S)	8.35 (0.23)	7.20 (0.35)	8.39 (0.36)	8.24 (0.28)	8.50 (0.29)	7.22 (0.44)	8.47 (0.24)	8.29 (0.26)

Table 2. Group means (+/- SEM) for pulmonary function variables in all treatment and exposure conditions for N=13 asthmatic subjects; L= liters; L/S = liters per second; SEM = standard error of the mean

NUMBER OF GENERAL SYMPTOMS REPORTED FOLLOWNG A 2 HOUR EXPOSURE TO 400 PPB OZONE IN ASTHMATIC SUBJECTS

Subject	GENDER M=Male F=Female	PRE OZONE	OZONE Exp At 2 Hrs	PRE-2 HRS POST OZONE	PRE AIR	AIR Exp At 2 Hrs	PRE-2HRS POST AIR	(PRE-2HRS POST OZONE)-(PRE-2HRS POST AIR)	PRE OZONE	OZONE Exp At 2 Hrs	PRE-2HRS POST OZONE	PRE AIR	AIR Exp At 2 Hrs	PRE-2HRS POST AIR	(PRE-2HRS POST OZONE)-(PRE-2HRS POST AIR)	Placebo DIFF-Iindomethacin DIFF
					PLACEBO							INDOMETHACIN				
8	F	0	4	-4	0	0	0	4	2	2	0	0	0	0	0	-4
9	M	0	1	-1	0	0	0	1	0	1	-1	0	0	0	-1	0
11	M	0	1	-1	0	0	0	1	1	1	0	0	0	0	0	-1
12	M	0	2	-2	1	0	1	3	0	0	0	0	0	0	0	-3
13	F	0	2	-2	0	1	-1	1	0	1	-1	0	1	-1	0	-1
14	F	0	1	-1	0	0	0	1	0	1	-1	0	0	0	-1	0
15	F	0	1	-1	0	1	-1	0	0	3	-3	0	0	0	-3	3
16	M	0	1	-1	0	1	-1	0	0	2	-2	0	0	0	-2	2
17	M	0	0	-0	0	0	0	0	0	0	0	0	0	0	0	0
18	F	0	5	-5	0	1	-1	4	0	1	-1	0	0	0	-1	-3
19	F	0	2	-2	0	4	-4	-2	0	1	-1	2	3	-1	0	2
22	F	0	0	0	0	0	0	0	0	1	-1	0	0	0	-1	1
23	F	3	5	-2	3	4	-1	1	9	11	-2	1	2	-1	-1	0
mean		0.23	1.77	-1.54	0.31	0.92	-0.62	1.08	0.92	1.92	-1.0	0.23	0.46	-0.23	-0.77	-0.31
std error		0.23	0.61	0.48	0.24	0.40	0.33	0.47	0.69	0.79	0.25	0.17	0.26	0.12	0.26	0.56

Table 5. Individualn and group mean (+/-SEM) pre and post exposure general symptom reporting (SYMG) values in all exposure and treatment conditions in 13 asthmatic subjects. Also shown the differences between pre and 2 hr post exposure and the pre - post differences between the placebo and indomethacin condition; Exp = Exposure; SEM = standard error of the mean

131

NUMBER OF RESPIRATORY SYMPTOMS REPORTED FOLLOWING A 2 HOUR EXPOSURE TO 400 PPB OZONE IN ASTHMATIC SUBJECTS

Subject	GENDER M=Male F=Female	PLACEBO							INDOMETHACIN							
		PRE OZONE	OZONE Exp At 2 Hrs	PRE-2HRS POST OZONE	PRE AIR	AIR Exp At 2 Hrs	PRE-2HRS POST AIR	(PRE-2HR POST OZONE) - (PRE-2HR POST AIR)	PRE OZONE	OZONE Exp At 2 Hrs	PRE-2HRS Post ozone	PRE AIR	AIR Exp At 2 Hrs	PRE-2HRS POST AIR	(PRE-2HRS Post OZONE) - (PRE-2HRS POST AIR)	DIFF Placebo DIFF Indomethaci
8	F	0	2	-2	0	0	0	2	2	2	0	0	1	-1	-1	-3
9	M	0	1	-1	0	0	0	1	0	1	-1	0	0	0	1	0
11	M	0	1	-1	0	0	0	1	1	2	-1	0	0	0	1	0
12	M	0	2	-2	1	0	1	3	0	0	0	0	0	0	0	-3
13	F	0	1	-1	0	1	-1	0	0	3	-3	0	0	0	3	3
14	F	0	1	-1	0	0	0	1	0	1	-1	0	0	0	1	0
15	F	0	1	-1	0	0	0	1	0	3	-3	0	0	0	3	2
16	M	0	1	-1	0	0	0	1	0	1	-1	0	0	0	1	0
17	M	0	1	-1	0	0	0	1	0	1	-1	0	0	0	1	0
18	F	0	4	-4	0	0	0	4	0	2	-2	0	0	0	2	-2
19	F	0	4	-4	0	2	-2	2	0	1	-1	2	2	0	1	-1
22	F	0	0	0	0	0	0	0	0	1	-1	0	0	0	1	1
23	F	3	5	-2	3	3	0	2	9	9	0	1	2	-1	-1	-3
mean		0.23	1.85	-1.62	0.31	0.46	-0.15	-1.46	0.92	2.08	-1.15	0.23	0.38	-0.15	-1.0	-0.46
std error		0.23	0.42	0.33	0.24	0.27	0.19	0.31	0.69	0.63	0.28	0.17	0.21	0.10	0.34	0.53

Table 6. Individual and group mean (+/-SEM) pre and post exposure respiratory symptom reporting (SYMR) values in all treatment and exposure conditions in 13 asthmatic subjects.. Also shown the difference between pre and 2 hrs post exposure and the pre - post exposure differences for the placebo and indomethacin conditions; Exp = Exposure; DIFF = Difference; SEM = standard error of the mean

GENERAL SYMPTOM SEVERITY SCORES FOLLOWING A 2 HOUR EXPOSURE 400 PPB OZONE IN ASTHMATIC SUBJECTS

| | | |------- PLACEBO -------| | | | | | |------- INDOMETHACIN -------| | | | | |
Subject ID	GENDER M=Male F=Female	PRE Ozone	Ozone Exp At 2 Hrs	PRE-2 HRS POST Ozone	PRE AIR	AIR Exp At 2 Hrs	PRE-2HRS POST AIR	(PRE-2HR POST OZONE)-(PRE-2HR POST AIR)	PRE OZONE	OZONE Exp At 2 Hrs	PRE-2HRS POST OZONE	PRE AIR	AIR Exp At 2 Hrs	PRE-2HRS POST AIR	(PRE-2 HRS POST OZONE)-(PRE-2HRS POST AIR)	DI Pla -D In omet
8	F	0	1.8	-1.8	0	0	0	-1.8	1	2	-1	0	0	0	-1	-0
9	M	0	3	-3	0	0	0	-3	0	3	-3	0	0	0	-3	(
11	M	0	2	-2	0	0	0	-2	2	0	2	0	0	0	2	-4
12	M	0	3	-3	2	0	2	-5	0	0	0	0	0	0	0	-5
13	F	0	2	-2	0	2	-2	0	0	1	-1	0	4	-4	3	-3
14	F	0	3	-3	0	0	0	-3	0	3	-3	0	0	0	-3	0
15	F	0	3	-3	0	2	-2	-1	0	2.7	-2.7	0	0	0	-2.7	1.
16	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	M	0	3	-3	0	2	-2	-1	0	3.5	-3.5	0	0	0	-3.5	2.
18	F	0	2.8	-2.8	0	3	-3	0.2	0	3	-3	0	0	0	-3	3
19	F	0	3	-3	0	2.5	-2.5	-0.5	0	4	-4	2.5	2.7	-0.2	-3.8	2.
22	F	0	0	0	0	0	0	0	0	2	-2	0	0	0	-2	2
23	F	1	1.2	-0.2	1	2.2	-1.2	1	2.3	1.8	0.7	2	1	1	-0.3	1.
mean		0.08	2.14	-2.06	0.23	1.05	-0.82	-1.24	0.41	2.0	-1.58	0.35	0.59	-0.25	-1.33	0.0
std error		0.08	0.31	0.34	0.17	0.34	0.39	0.46	0.23	0.38	0.51	0.24	0.36	0.32	0.60	0.7

Table 7. Individual and gropu mean (+/-SEM) pre and 2 hr post exposure severity scores in all treatment conditions in 13 asthmatic subjects. Also shown are the pre - post exposure differences and the difference between the pre-post exposure differences in the placebo and indomethacin condition.; Borg Severity Scale: 0=no complaint; 1=minimal; 2=mild; 3=moderate; 4=severe; 5=incapacitating; Exp = Exposure; Difference; SEM = standard error of the mean

RESPIRATORY SYMPTOM SEVERITY SCORES FOLLOWING A 2 HOUR EXPOSURE TO 400 PPB OZONE IN ASTHAMTIC SUBJECTS

Subject ID	GENDER M=Male F=Femae	PLACEBO							INDOMETHACIN							DIFF Placebo - DII indomethaci
		PRE Ozone	Ozone Exp At 2 Hrs	PRE-2 HRS POST Ozone	PRE AIR	AIR Exp At 2 Hrs	PRE - 2HRS POST AIR	(PRE-2HR POST OZONE) - (PRE-2HR POST AIR)	PRE OZONE	OZONE Exp At 2 Hrs	PRE - 2HRS POST OZONE	PRE AIR	AIR Exp At 2 Hrs	PRE- 2HRS POST AIR	(PRE-2 HRS POST Ozone) - (PRE-2 HRS POST AIR)	
8	F	0	2	-2	0	0	0	-2	1	2	-1	0	2	-2	1	-3
9	M	0	2	-2	0	0	0	-2	0	2.5	-2.5	0	0	0	-2.5	0.5
11	M	0	2	-2	0	0	0	-2	2	3	-1	0	0	0	-1	-1
12	M	0	3	-3	0	0	0	-3	0	0	0	0	0	0	0	-3
13	F	0	3	-3	0	2	-2	-1	0	1.7	-1.7	0	0	0	-1.7	0.7
14	F	0	3	-3	0	0	0	-3	0	3	-3	0	0	0	-3	0
15	F	0	3	-3	0	0	0	-3	0	2.7	-2.7	0	0	0	-2.7	-0.3
16	M	0	3	-3	0	0	0	-3	0	3.5	-3.5	0	0	0	-3.5	0.5
17	M	0	3	-3	0	0	0	-3	0	2	-2	0	0	0	-2	-1
18	F	0	2.6	-2.6	0	0	0	-2.6	0	2.5	-2.5	0	0	0	-2.5	-0.1
19	F	0	2.8	-2.8	0	2.2	-2.2	-0.6	0	3.3	-3.3	3	2.3	0.7	-4	3.4
22	F	0	0	0	0	0	0	0	0	2	-2	0	0	0	-2	2
23	F	1	1.4	-0.4	1	2.5	-1.5	1.1	2.3	1.9	0.4	0	1	-1	1.4	-0.3
mean		0.08	2.37	-2.29	0.08	0.52	-0.44	-1.85	0.41	2.32	-1.91	0.23	0.1	-0.18	-1.73	-0.12
std error		0.08	0.25	0.28	0.08	0.27	0.24	0.37	0.23	0.25	0.34	0.23	0.26	0.18	0.45	0.46

Table 8 . Individual and group mean (+/-SEM) pre and 2 hr post exposure respiratory symptom severity scores in all treatmen conditions in 13 asthmatic subjects. Also shown the pre-post exposure differences and the differences between the pre-post exposure differences in the placebo and indomethacin condition.; Borg Severity Scale: 0=no complaint; 1=minimal; 2=mild; 3=moderate; 4=severe; 5=incapacitating; Exp = Exposure; DIFF = Difference; SEM = standard error of the mean

AIRWAY REACTIVITY IN ASTHMATIC SUBJECTS

PC20, METHACHOLINE (mg/ml)

ASTHMATICS

Subject	GENDER M=Male F=Female	PLACEBO							INDOMETHACIN							DIFF Placel - DIFF Indomethaci
		PRE OZONE Exp	Post OZONE Exp	PRE-POST OZONE Exp	PRE AIR Exp	Post AIR Exp	PRE-POST AIR Exp	(PRE-POST OZONE)-(PRE-POST AIR)	PRE OZONE Exp	Post OZONE Exp	PRE-POST OZONE Exp	PRE AIR Exp	Post AIR Exp	PRE-POST AIR	(PRE-POST OZONE)-(PRE-POST AIR)	
8	F	0.28	0.01	0.27	0.00	0.00	0.007	0.27	2.23	0.21	2.02	0.00	0.00	0.00	2.02	-1.75
9	M	0.29	0.33	-0.04	0.09	0.00	0.09	-0.13	2.00	0.50	1.50	3.20	0.03	3.17	-1.67	-1.54
11	M	0.25	0.08	0.17	0.18	0.50	-0.32	-0.13	0.19	0.09	0.10	0.16	0.18	-0.02	0.12	-1.67
12	M	0.38	0.19	0.19	0.59	0.32	0.27	-0.08	0.49	0.09	0.40	0.16	0.18	-0.02	0.42	0.37
13	F	0.24	1.68	-1.44	3.35	1.06	2.29	-3.73	0.15	0.18	-0.03	0.15	0.13	0.02	-0.05	-0.03
14	F	0.12	0.13	-0.01	0.10	0.08	0.02	-0.03	8.00	1.68	6.32	1.18	2.00	-0.82	7.14	-10.87
15	F	0.89	0.46	0.43	0.16	0.29	-0.13	0.56	0.14	0.14	0.00	0.09	0.16	-0.07	0.07	-0.10
16	M	0.25	0.59	-0.34	0.19	0.22	-0.03	-0.31	2.40	0.18	2.22	0.28	0.33	-0.05	2.27	-1.71
17	M	0.79	0.63	0.16	0.25	0.18	0.07	0.09	0.17	0.00	0.17	0.16	0.15	0.01	0.16	-0.47
18	F	0.94	1.88	-0.94	0.79	2.51	-1.72	0.78	1.56	2.70	-1.12	2.23	0.56	1.67	-2.79	2.88
19	F	0.89	0.05	0.84	0.63	0.53	0.10	0.74	3.20	5.60	-2.40	14.9	3.20	11.7	-14.10	14.88
22	F	1.00	0.42	0.58	2.00	1.99	0.01	0.57	0.39	0.06	0.33	0.50	0.95	-0.45	0.78	0.56
23	F	0.67	0.19	0.48	0.75	0.45	0.30	0.18	0.84	0.25	0.59	1.41	1.70	-0.29	0.88	-0.31
mean		0.54	0.51	0.03	0.69	0.62	0.07	-0.04	1.70	0.94	0.76	1.89	0.73	1.16	-0.33	-0.36
std error		0.09	0.17	0.17	0.27	0.27	0.23	0.32	0.73	0.45	0.76	1.12	0.27	0.92	-0.40	1.51

Table 9. Individual and group mean (+/- SEM) pre and post exposure PC-20 data in all exposure and treatment conditions in 13 asthmatic subjects. Pre Exposure is 1 day prior to exposure and post exposure is 90 minutes post exposure. Also shown is the pre - post exposure difference and the difference between pre - post expsoure differences in the placebo and indomethacin conditio; SEM = standard error of the mean.

CHAPTER 4 FIGURES

PULMONARY FUNCTION RESPONSES IN ASTHMATICS

DIFFERENCE IN MEAN (+/-SEM) BASELINE CHANGES BETWEEN OZONE AND AIR EXPOSURE
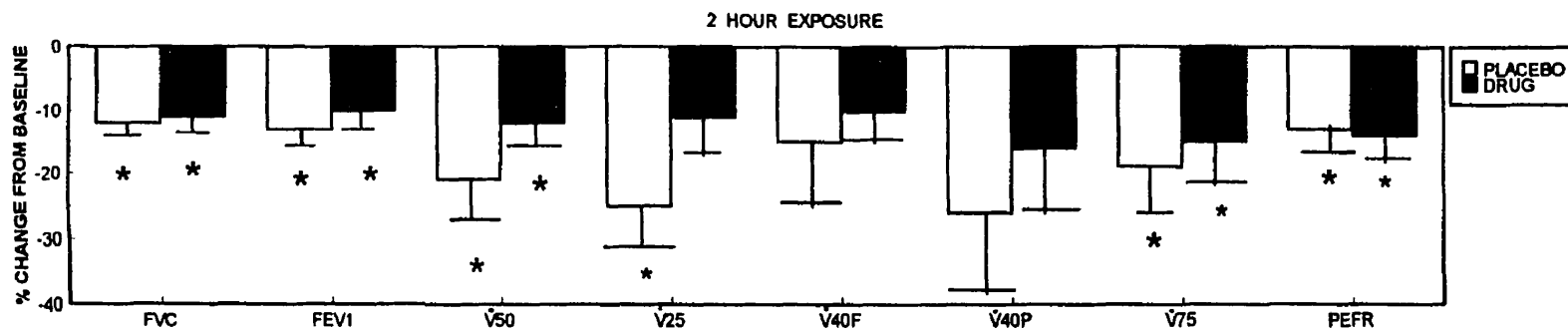
2 HOUR EXPOSURE

Figure 1A. Difference in mean (+/-SEM) baseline changes for pulmonary function variables between ozone (400 ppb) and air exposure with placebo (clear bar) and indomethacin (shaded bar) in 13 asthmatic subjects. * significant difference between ozone and air at the 0.05 level. Baseline changes are % changes from baseline; SEM = standard error of the mean
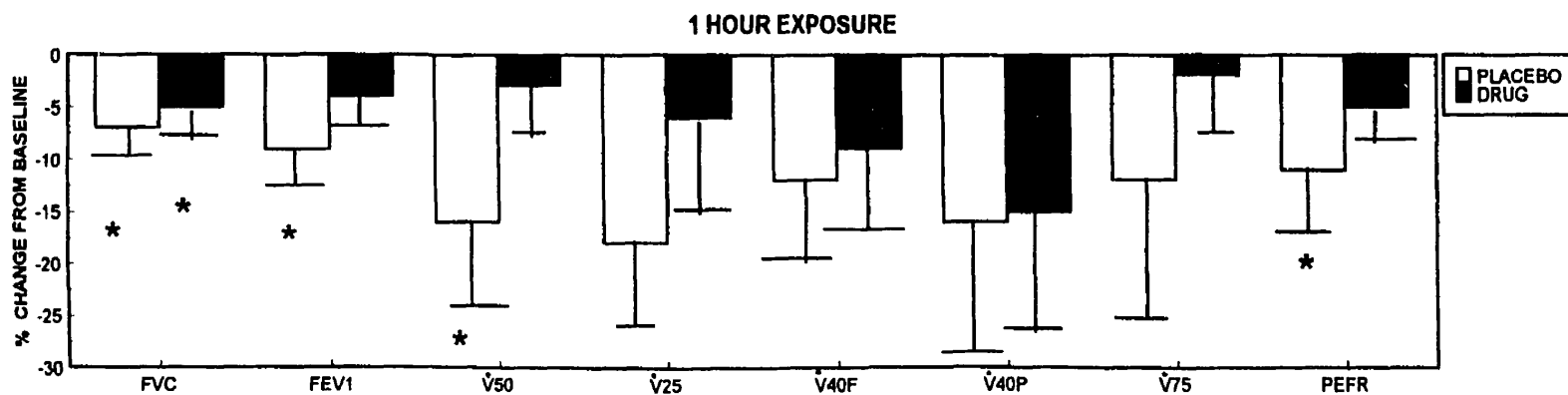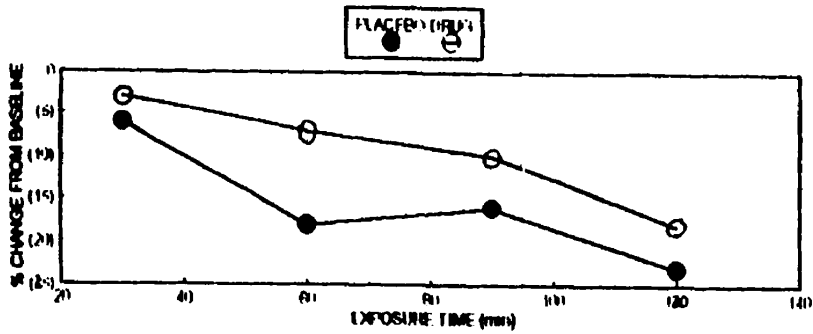
1 HOUR EXPOSURE

Figure 1B. Difference in (mean (+/-SEM) baseline changes for pulmonary function variables between ozone (400 ppb) and air exposure with placebo (clear bar) and indomethacin (shaded bar) in 13 asthmatic subjects. * significant difference between ozone and air at the 0.05 level. Baseline changes are % changes from baseline; SEM = standard error of the mean
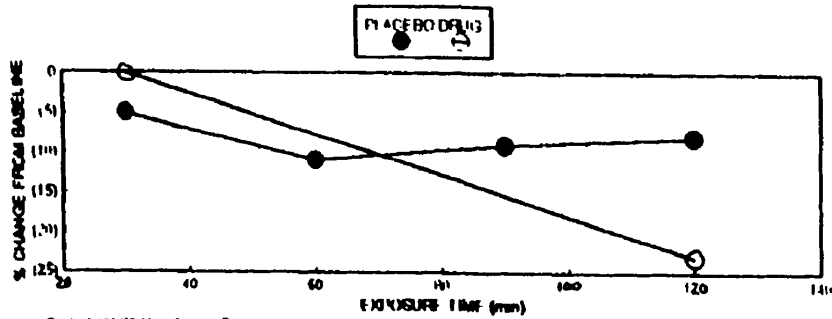
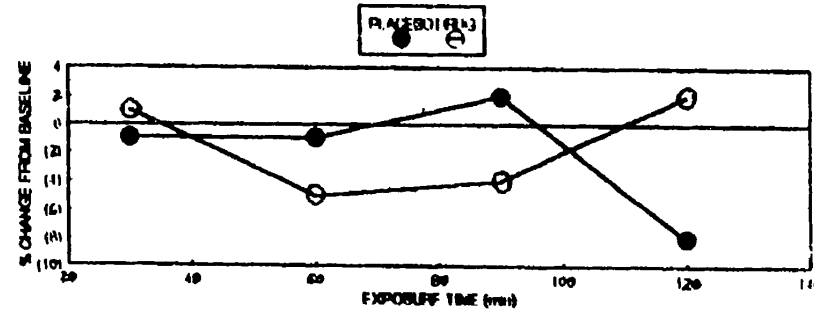# INDIVIDUAL FEV1 RESPONSES AT 30, 60, 90 AND 120 MINUTES OF OZOI EXPOSURE IN ASTHMATIC SUBJECTS

2 (A) SUBJECT 8: FEV1 responses at 30, 60, 90 and 120 minutes of exposure to 400 ppb ozone
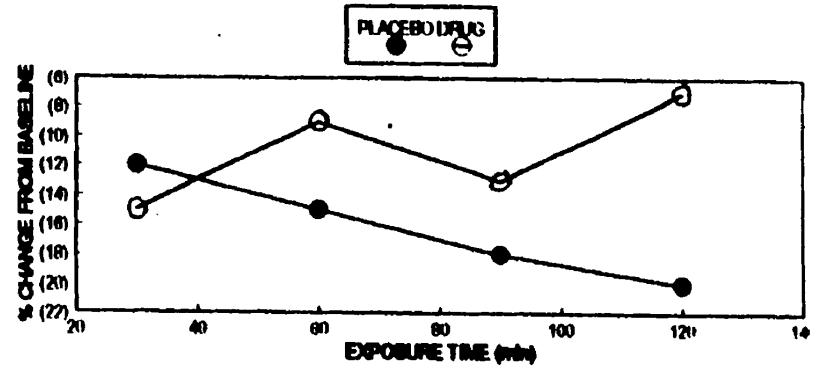
2 (B) SUBJECT 9: FEV1 responses at 30, 60, 90 and 120 minutes of exposure to 400 ppb ozone

2 (C) SUBJECT 11: FEV1 responses at 30, 60, 90 and 120 minutes of exposure to 400 ppb ozone

2 (D) SUBJECT 12: FEV1 responses at 30, 60, 90, and 120 minutes of exposure to 400 ppb ozone

2 (E) SUBJECT 13: FEV1 responses at 30, 60, 90, and 120 minutes of exposure to 400 ppb ozone

2 (F) SUBJECT 14: FEV1 responses at 30, 60, 90, and 120 minutes of exposure to 400

INDIVIDUAL FEV1 RESPONSES AT 30, 60, 90 AND 120 MINUTES OF OZO[
EXPOSURE IN ASTHMATIC SUBJECTS

2. (G) SUBJECT 15: FEV1 responses at 30, 60, 90, and 120 minutes of exposure to 400 ppb ozone

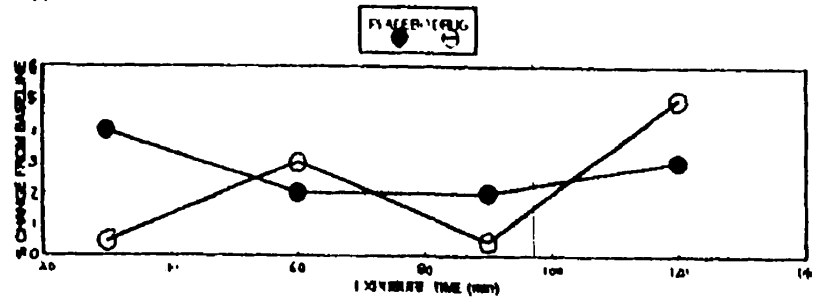2. (H) SUBJECT 16: FEV1 responses at 30, 60, 90 and 120 minutes of exposure to 400 ppb ozone
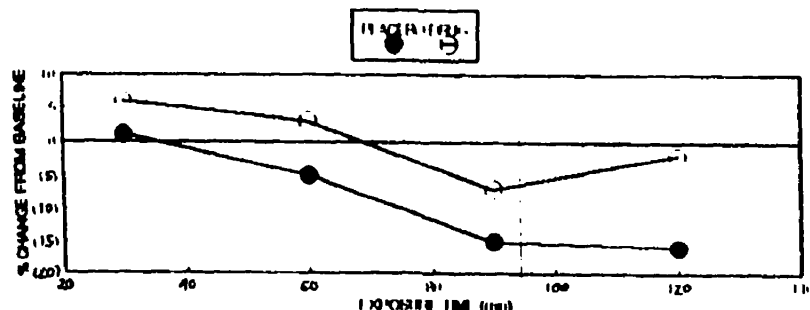
2. (I) SUBJECT 17: FEV1 responses at 30, 60, 90 and 120 minutes of exposure to 400 ppb ozone
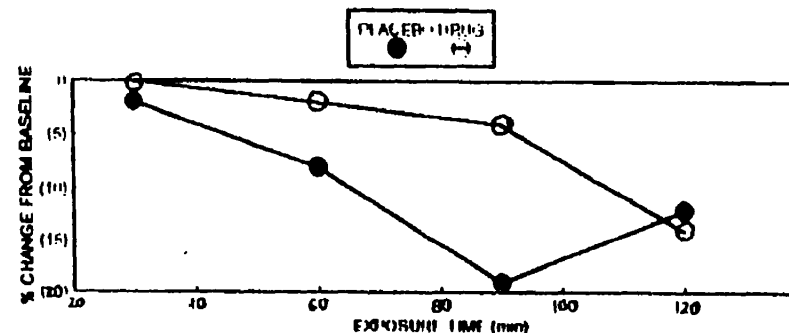
2. (J) SUBJECT 18: FEV1 responses at 30, 60, 90 and 120 minutes of exposure to 400 ppb ozone
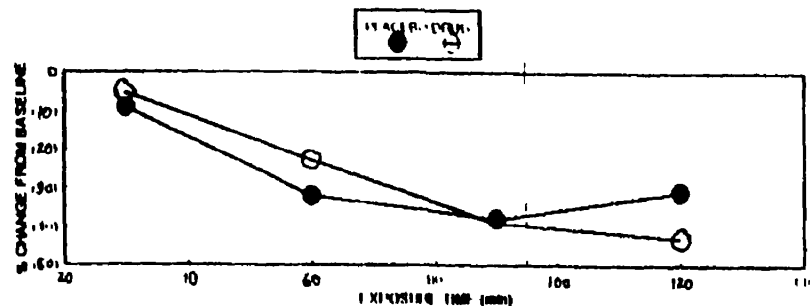
2. (K) SUBJECT 19: FEV1 responses at 30, 60, 90 and 120 minutes of exposure to 400 ppb ozone
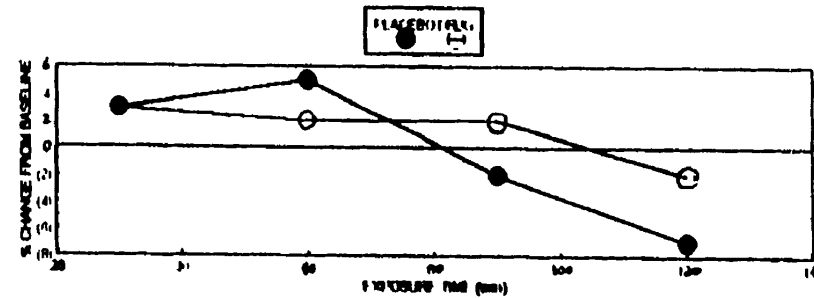
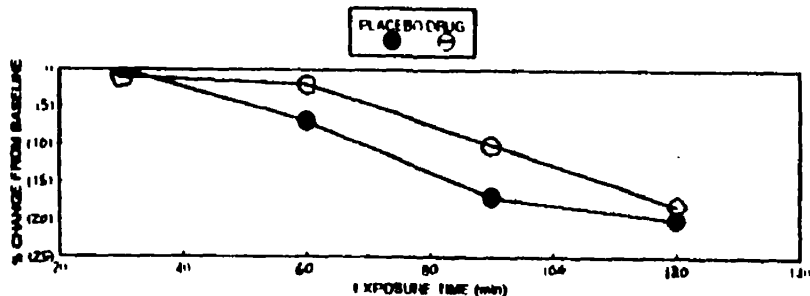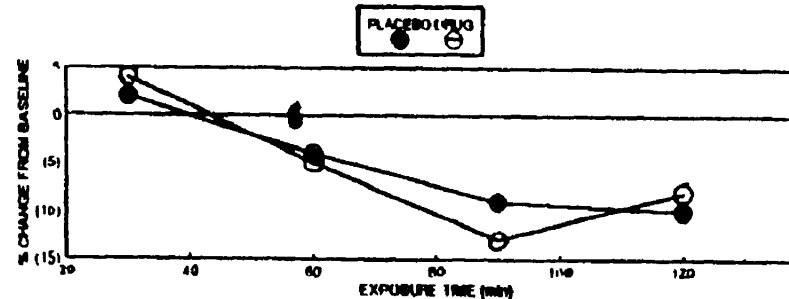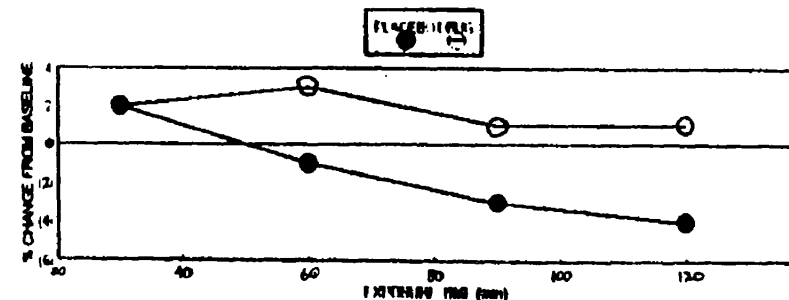2. (L) SUBJECT 22: FEV1 responses at 30, 60, 90 and 120 minutes of exposure to 400 ppb ozone

INDIVIDUAL FEV1 RESPONSES AT 30, 60, 90 AND 120 MINUTES OF OZONE EXPOSURE IN ASTHMATIC SUBJECTS
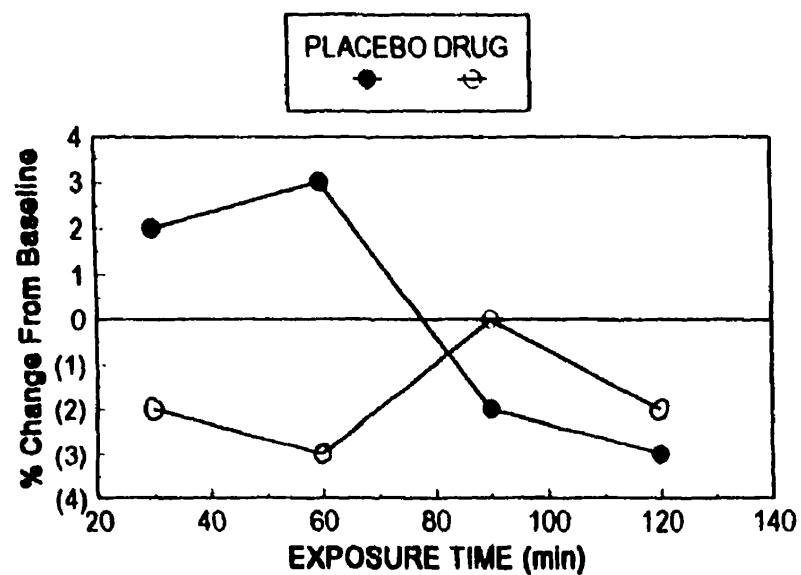
2. (M) SUBJECT 23 FEV1 responses at 30, 60, 90 and 120 minutes of exposure to 400 ppb ozone.

RELATIONSHIP BETWEEN OZONE-INDUCED SPIROMETRIC DECLINE AND PROTECTION AGAINST SPIROMETRIC DECLINE BY INDOMETHACIN

Figure 3a

Figure 3b

Figure 3c

Figure 3d

protection=(baseline change in FVCplacebo) - (baseline change in FVCindomethacin)/ baseline change in FVCplacebo x 100%; yes: -ve value indicates protection; no: +ve value indicates no protection
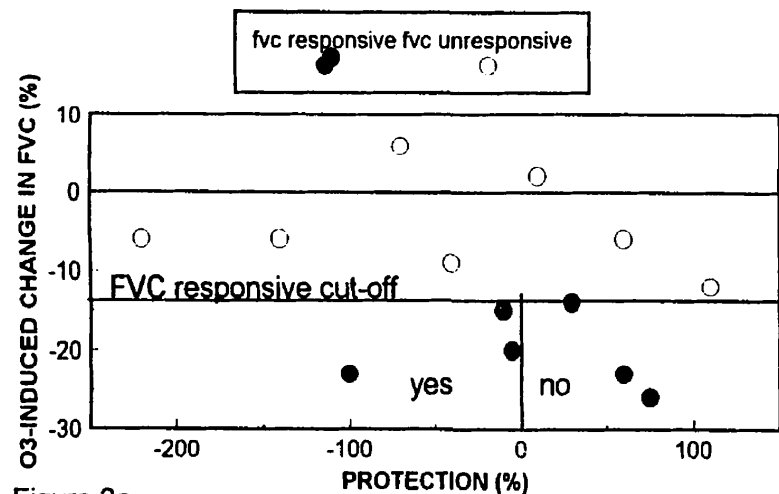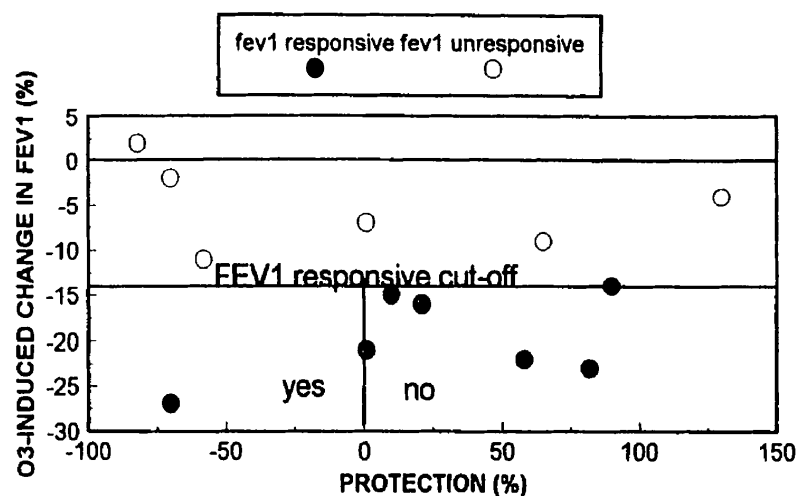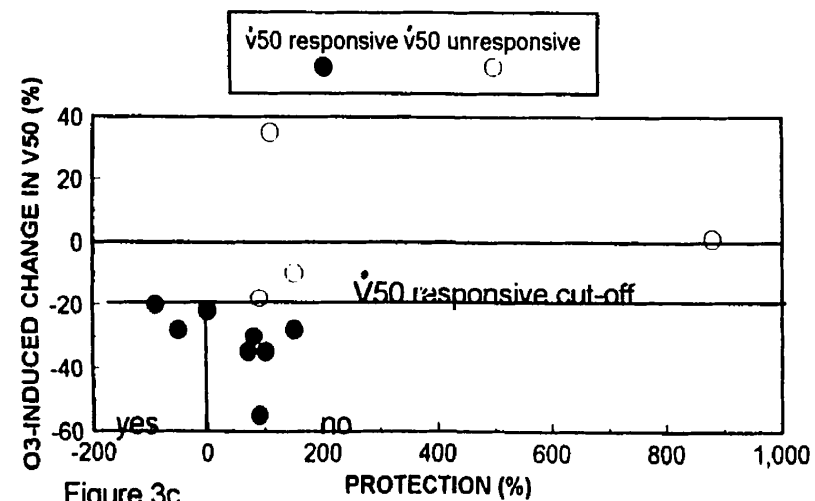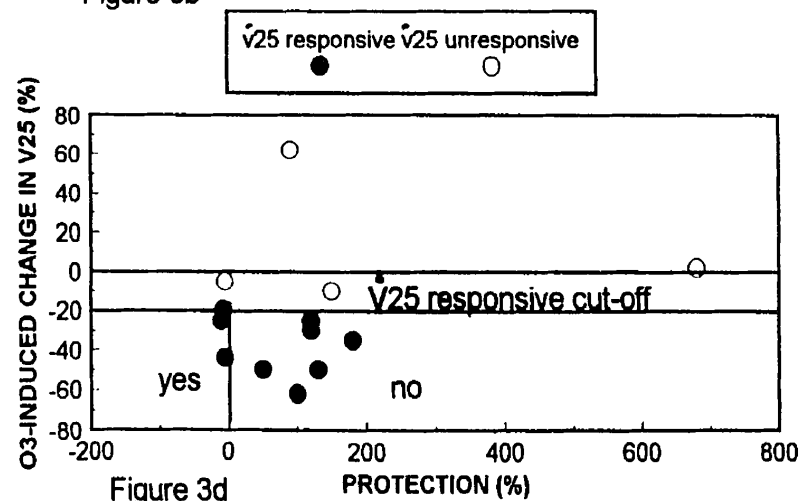
SYMPTOM REPORTING IN ASTHMATICS

DIFFERENCE IN MEAN (+/- SEM) BASELINE CHANGES BETWEEN OZONE AND AIR EXPOSURE
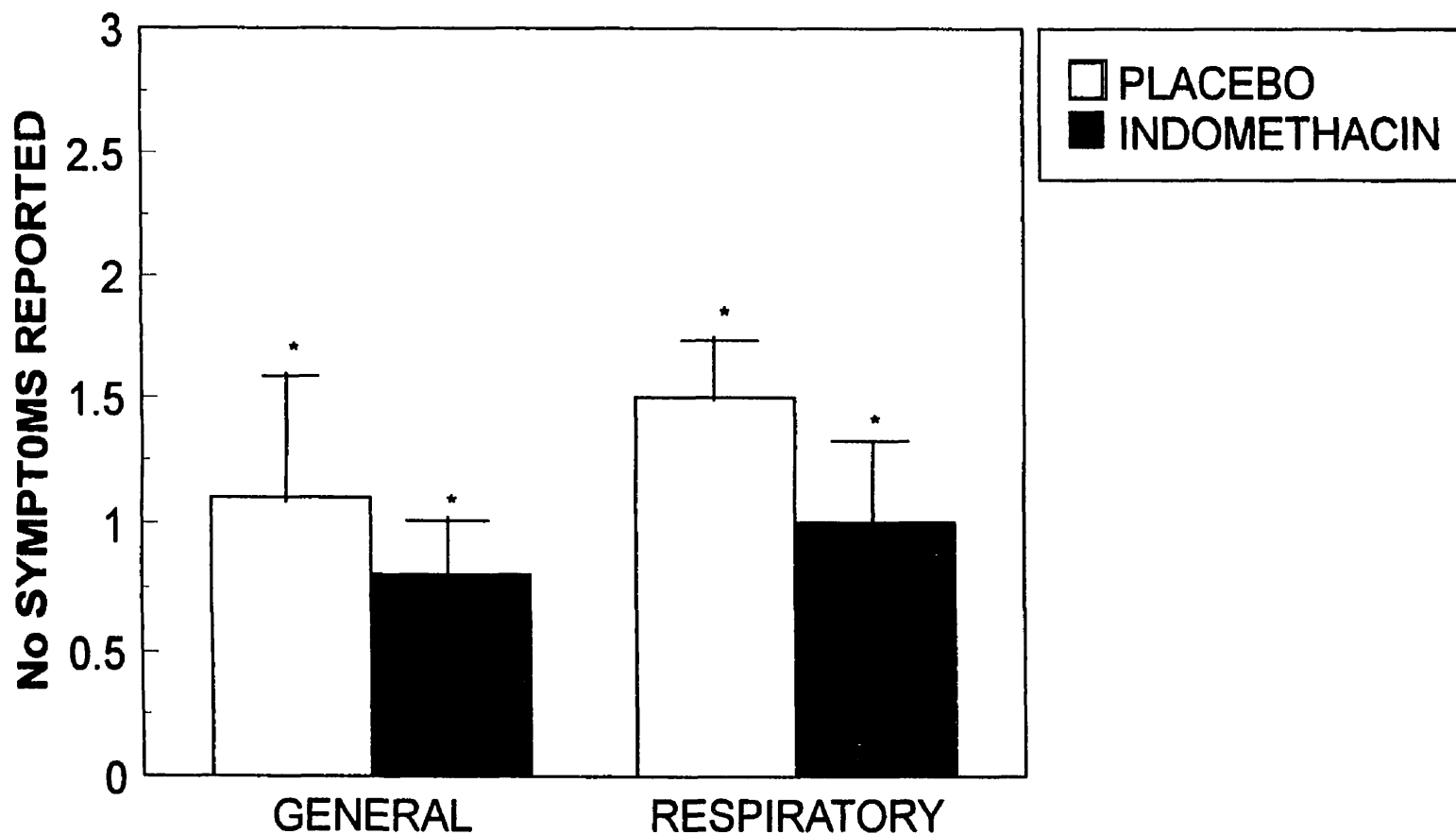
Figure 4. Difference in mean (+/- SEM) absolute baseline changes for symptom reporting between ozone and air exposure with placebo (clear bar) and indomethacin (shaded bar) in 13 asthmatic subjects. * Significant difference between ozone and air exposure at the 0.05 level; SEM = standard error of the mean

SYMPTOM SEVERITY SCORES iN ASTHMATIC SUBJECTS

DIFFERENCE IN MEAN (+/-SEM) BASELINE CHANGES BETWEEN OZONE AND AIR EXPOSURE

Figure 5. Difference in mean (+/-SEM) absolute baseline changes for symptom severity scores between ozone and air exposure with placebo (clear bar) and indomethacin (shaded bar) in 13 asthmatic subjects. * Significant difference between ozone and air exposure at the 0.05 level; SEM = standard error of the mean
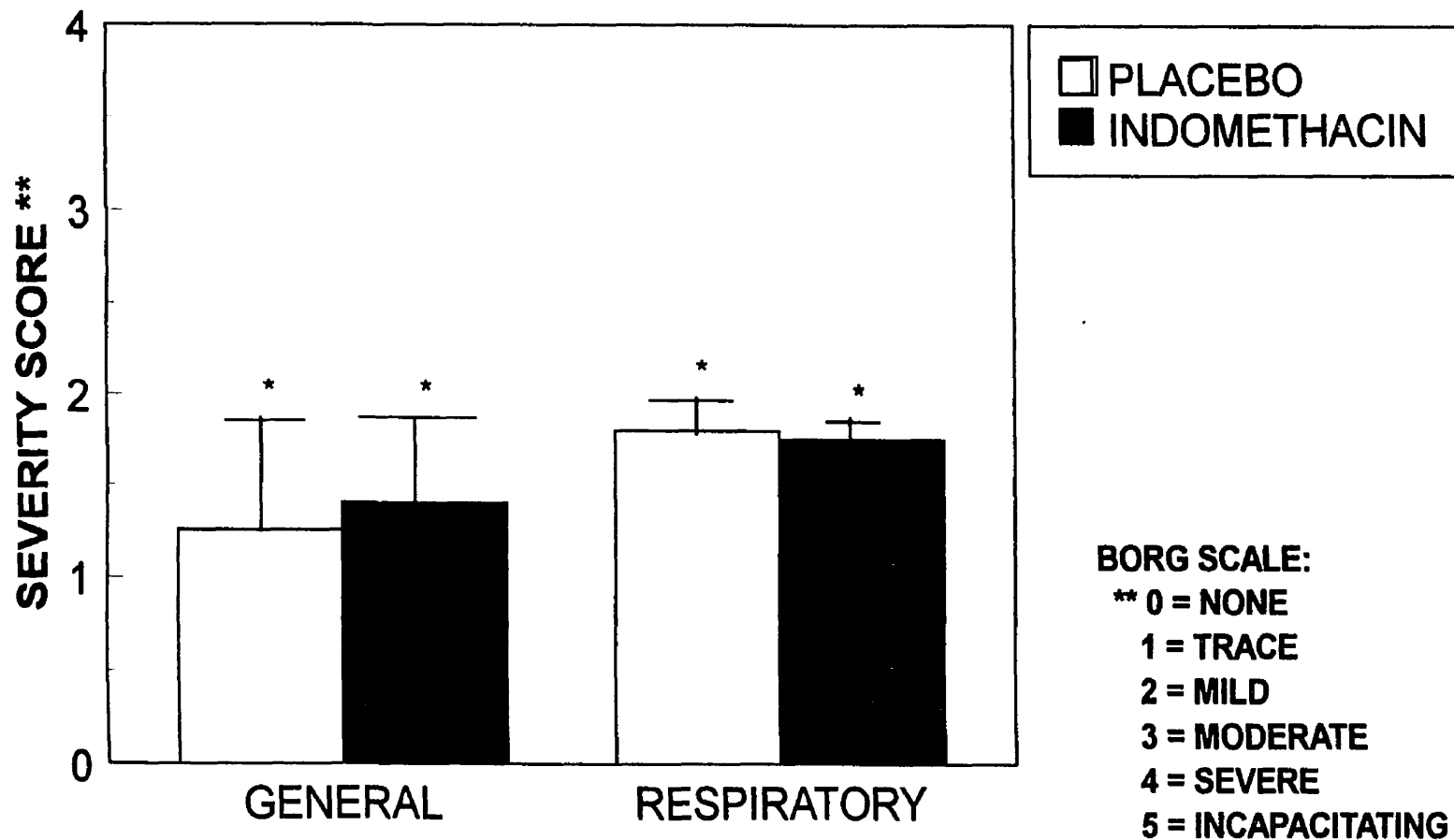
DIFFERENCE IN MEAN (+/- SEM) BASELINE CHANGES BETWEEN OZONE AND AIR EXPOSURE
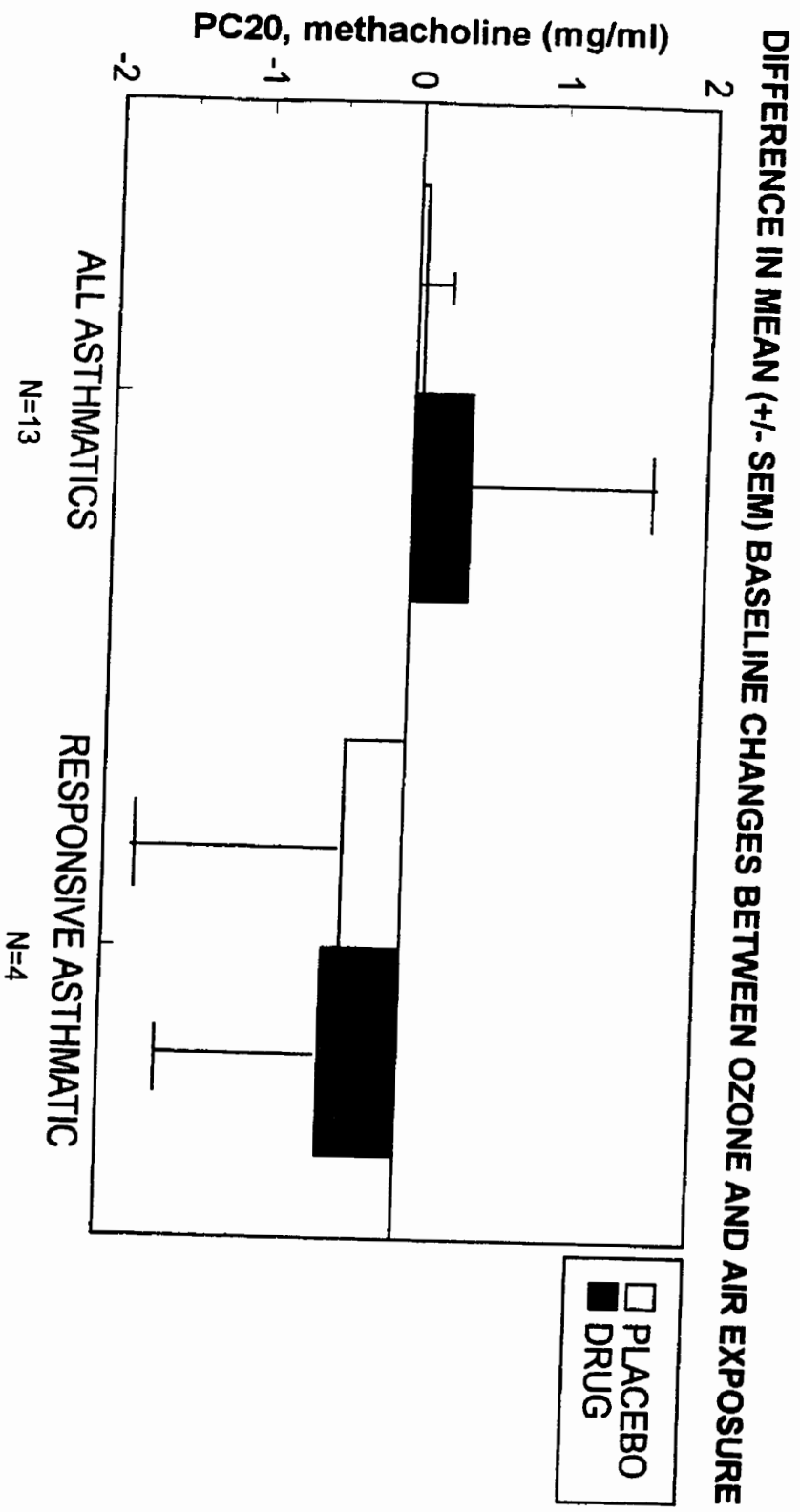
Figure 6. Difference in mean (+/-SEM) baseline changes (+/- SEM) for PC20, methacholine between ozone and air exposure with placebo (clear bar) and indomethacin (shaded bar) in 13 asthmatic subjects. * 4 subjects were defined as ozone responsive asthmatics, i.e. >2 doubling dose fall in PC20 following ozone exposure; SEM = standard error of the mean

CHAPTER 4 APPENDIX

ANTHROPOMETRIC DATA AND BASELINE PULMONARY FUNCTION TESTS

ASTHMATICS

SUBJECT ID	GENDER	HEIGHT (CM)	WEIGHT (KG)	AGE (YRS)	%FVC	%FEV1	%V50	%V25
4	M	170	71	23	102	89	79	57
9	M	178	76	24	104	83	62	42
11	M	175	74	20	121	107	80	77
12	M	183	122	20	87	80	65	62
16	M	186	83	21	98	75	49	35
17	M	179	77	22	104	87	66	44
8	F	158	52	22	114	100	82	60
15	F	173	77	20	110	101	80	59
13	F	179	70	26	126	122	93	95
14	F	163	68	19	81	92	64	37
18	F	155	68	28	105	97	78	65
19	F	169	82	20	112	117	120	116
22	F	163	63	20	125	129	174	163
23	F	163	64	22	117	100	82	58
MEAN		171	75	22	108	99	84	70
STD ERROR		2.7	4.6	0.7	3.8	4.5	8.9	9.9
RANGE		155-186	52-83	19-28	81-126	75-129	49-174	35-163

Table A1. Individual and group mean (+/-SEM) anthropometric data and baseline pulmonary function tests for percent predicted of normal values for 13 asthmatic subjects. % PREDICTED NORMAL VALUES ACCORDING TO: FVC: GOLDMAN & BECKLAKE (1959); FEV1: MORRIS, TEMPLE & KOSKI (1973); V50, V25: LAP & HYATT (1967); SEM = standard error of the mean

EFFECT OF INDOMETHACIN ON BASELINE FEV1

Spirometry VariableE	Indomethacin Condition Pre-Post ingestion	Placebo Condition Pre-Post ingestion	Indomethacin - Placebo (difference)	Degrees of Freedom (N=13)	Paired T-Test	P<0.05
Mean FEV1 (liters)	0.11	0.13	-0.03	17	-0.91	N.S.
SEM			0.03			

Table A2. Effect of indomethacin pretreatment on FEV1. N.S. = not significant at the 0.05 level. SEM = standard error of the mean

MEAN (+/- SEM) EXERCISE MINUTE VENTILATION (Vimin) AND RESPIRATOR' FREQUENCY (f(R) VALUES

ASTHMATICS

EXPOSURE/ TREATMENT CONDITION	Vi min (exercise) l/min (Std Error)	Vi min (rest) l/min (Std Error)	f(R) - exercise breaths/minute (Std Error)	f(R)- rest breaths/minute (Std Error)
AIR / PLACEBO	31 (1)	10 (1)	21 (2)	13 (1)
AIR/ INDOMETHACIN	31 (1)	11 (1)	21 (2)	13 (1)
OZONE / PLACEBO	31 (1)	9 (1)	21 (2)	12 (1)
OZONE / INDOMETHACIN	30 (1)	10 (1)	22 (2)	13 (1)

Table A3. Mean (+/- SEM) inspiratory exercise minute ventilation (Vimin) and respiratory frequency (f(R) values in all exposure and treatment conditions 13 asthmatic subjects; L/MIN = litres per minute; Std Error = Standard error of the mean. Exercise = alternating 15 minute exercise/rest periods on station bicycle at a cadence of 60 revolutions per minute. Rest = seated position.

EFFECT OF EXERCISE ON PULMONARY FUNCTION IN ASTHMATICS

PLACEBO

PULMONARY FUNCTION VARIABLE	PRE - POST AIR EXPOSURE	STD ERROR	P VALUE	SIGNIFICANT AT P<0.05 LEVEL YES/NO
FVC (L)	-0.11	0.08	0.19	NO
FEV1 (L)	0.08	0.09	0.43	NO
V̇50 (L/S)	0.31	0.21	0.19	NO
V̇25 (L/S)	0.24	0.18	0.23	NO
V̇40F (L/S)	0.28	0.22	0.25	NO
V̇40P (L/S)	0.30	0.37	0.44	NO
V̇75 (L/S)	0.19	0.36	0.60	NO
PEFR (L/S)	-0.28	0.34	0.44	NO

Table A4A. Paired T-Test comparison between pre filtered air (with exercise) expsoure and post filtered air (with exercise) exposure in the placebo pretreatment condition in 13 asthmatic subjects

INDOMETHACIN

PULMONARY FUNCTION VARIABLE	PRE - POST AIR EXPOSURE	STD ERROR	P VALUE	SIGNIFICANT AT P<0.05 LEVEL YES/NO
FVC	-0.04	0.07	0.61	NO
FEV1	0.02	0.06	0.71	NO
V̇50	0.02	0.13	0.88	NO
V̇25	0.19	0.10	0.10	NO
V̇40F	0.05	0.16	0.76	NO
V̇40P	0.27	0.28	0.37	NO
V̇75	-0.14	0.18	0.45	NO
PEFR	-0.06	0.18	0.75	NO

Table A4B. Paired T-Test comparison between pre filtered air (with exercise) exposure and post filtered air(with exercise) exposure in the indomethacin pretreatment condition in 13 asthmatic subjects; Air Exposure = filtered air exposure with 15 minute intermittent exercise periods (Vimin = 30L/min)

CHAPTER 4 REFERENCES

Aizawa, H., Chung, KF., Leikauf, GD., Ueki, I., Bethel, RA., O'Byrne, PM., Hirose, T., Nadel, JA. Significance of thromboxane generation in ozone-induced airway hyperresponsiveness in dogs. J Appl Physiol 59: 1918-1923, 1985

Aris, RM., Christian, D., Hearne, PQ., Finkbeiner, WE., Balmes, JR. Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. Am Rev Respir Dis 148: 1363-1372, 1993

Balmes, J., Chen, L., Scannell, C., Tager, I., Christian, D., Hearne, P., Kelly, T., Aris, R. Ozone-induced decrements in FEV1 and FVC do not correlate with measures of inflammation. Am J Respir Crit Care Med, 153: 904-9, 1996

Beckett, WS., McDonald, WF., Horstman, DH., House, DE. Role of the parasympathetic nervous system in acute lung response to ozone. J Appl Physiol 59: 1879-1885, 1985

Bouhnys A., Hunt, VR., Kim, BM., Zapletal, A. Maximum expiratory flow rates in induced bronchoconstriction in man. J clin Invest 48: 1159-1168, 1969

Coleridge, JCG., Coleridge, HM. Schelegle, ES., Green, JF. Acute inhalation of ozone stimulates bronchial C-fibers and rapidly adapting receptors in dogs J Appl Physiol 74: 2345-2352, 1993

Dehaut, P., Rachiele, A., Martin, RR., Malo, JL. Histaine dose response curves in asthma: reproducibility and sensitivity of different indicies to assess responses. Thorax; 38: 516-22, 1983

Dimeo, MJ., Glenn, MG., Holtzman, MJ., Sheller, JR., Nadel, JA., Boushey, HA. Threshold concentration of ozone causing an increase in bronchial reactivity in humans and adaptation with repeated exposures Am Rev Respir Dis; 124: 245-8, 1981

Eschenbacher, WC., Ying, RC., Kreit, JW., Gross, KB. Ozone-induced lung function changes in normal and asthmatic subjects and the effect of indomethacin. In T. Schneider, SD, Lee, GJR, Wolters, and LD Grant (Eds), Atmospheric Ozone Research and Its Policy Implications. Elsevier, Amsterdam, P493, 1989

Fairfax, AJ., Hanson, JM., Morley, J. The late reaction following bronchial provocation with house dust mite allergen. Dependence on arachidonic acid metabolism. Clin Exp Immunol 52: 393-398, 1983

Folinsbee, LJ., McDonnell, WF., Horstman, DH. Pulmonary function and symptom response after 6.6 hour exposure to 0.12 ppm ozone with moderate exercise JAPCA 38, 28, 1988

Folinsbee, LJ., Silverman, F., Shephard, RJ. Exercise responses following ozone exposure. J Appl Physiol 38, 996, 1975

Folinsbee, LJ., Silverman, F., Shephard, RJ. Decrease of maximum work performance following ozone exposure. J Appl Physiol: Respirat Environ Exercise Physiol 42 (4) 531-536, 1977

Gertner, A. Bromberger-Barnea, B., Traystman, R., Berzen, D., Menkes, H. Responses of the lung peripheree to ozone and histamine. J Appl Physiol, 54: 640-6, 1983

Golden, JA., Nadel, JA., Boushey, HA. Bronchial hyperirritability in healthy subjects after exposure to ozone. Am Rev Respir Dis 118, 287, 1978

Goldman, HI., Becklake, MR: Respiratory function tests: Normal values at median altitudes and the prediction of normal results. Am Rev Tuberc; 79: 456-467, 1959

Gong, H. Jr., Bradley, P., Simmons, M., Tashkin, D. Impaired exercise performance and pulmonary function in elite cyclists during low-level ozone exposure in a hot environment. Am Rev Respir Dis; 134, 726-733, 1986

Habib, MP., Pare, PD., Engel, LA. Variability of airflow responses to inhaled histamine in normal subjects. J Appl Physiol: Respirat Environ Exercise Physiol 47: 51-58, 1979

Hackney, JD., Linn, WS., Mohler, JG., Perderson, EE., Breisacher, P., Russo, A. Experimental studies on human health effects of air pollution. Arch Environ Health, 30, 359, 1975

Hargreave, FE. Lab protocol for histamine and methacholine inhalation test (unpublished) Firestone Regional Chest and Allergy Unit, St. Josephs Hospital, 50 Charlton, Ave. East, Hamilton, Ontario, 1984

Hazucha, MJ., Bates, DV., Bromberg, P. Mechanism of action of ozone on the human lung. J Appl Physiol 67(4) 1535-1541, 1989

Hazucha, M., Madden, M., Pape, G., Becker, S., Devlin, R., Koren, H., Kehrl, H., Bromberg, P. Effects of cyclo-oxygenase inhibition on ozone-induced respiratory inflammation and lung function changes. Eur J Appl Physiol, 73: 17-27, 1996

Holtzman, MJ., Cunnigham, JH., Sheller, JR., Irsigler, GB., Nadel, JA., Boushey, H. Effect of ozone on bronchial reactivity in atopic and nonatopic subjects. Am Rev Respir Dis 120, 1059, 1979

Holtzman, MJ., Aizawa, H., Nadel, J., Goetz, EJ. Selective generation of leukotriene B4 by tracheal epithelial cells from dogs. Biochem Biophys Res Commun 114: 1071-6, 1983

Horstman, DH., Folinsbee, LJ., Ives, PJ., Abdul, S., McDonnell, WF. Ozone concentration and pulmonary response relationships for 6.6

hours with 5 hours of moderate exercise to 0.08, 0.10 and 0.12 ppm. Am Rev Resp Dis 142 1158, 1990

Hu, S.C., Ben-Jebria, A., Ultman, J.S. Longitudinal distribution of ozone absorption in the lung: quiet respiration in healthy subjects. J Appl Physiol 73 (4): 1665-1661, 1992.

Juniper, EF., Frith, PA., Dinnett,C., Cockcroft, DW., Hargreave, FE. Reproducibility and comparison of responses to inhaled histamine and methacholine. Thorax: 33, 705-10, 1978

Koenig, JQ., Covert, DS., Marshall, SG., Van Belle, G., Pierson, WE. The effects of ozone and nitrogen dioxide on pulmonary function in healthy and in asthmatic adolescents. Am Rev Respir Dis, 136, 1152, 1987

Koenig, JQ., Covert, DS., Morgan, MS., Horike, M., Horike, N., Marshall, SG., Pierson, WE. Acute effects of 0.12 ppm ozone or 0.12 ppm nitrogen dioxide on pulmonary function in healthy and asthmatic adolescents. Am Rev Respir Dis 132, 648, 1985

Koren, H., Devlin, RB., Graham, D., Mann, R., McGee, M., Horstman, DH., Kozumbo, WJ., Becher, S., House, DE., McDonnell, WF., Bromberg, P. Ozone-induced inflammation in the lower airways to human subjects Am Rev Respir Dis 139, 407, 1989

Kreit, JW. Gross, KB., Moore, TB., Lorenzen, TJ., D'Arcy, J., Eschenbacher, WL. Ozone induced changes in pulmonary function and bronchial responsiveness in asthmatics. J Appl Physiol 60, 217, 1989

Lap, NL., Hyatt, RE: Some factors affecting the relationship of maximal expiratory flow to lung volume in health and disease. Diseases of the chest, 51: 475-481, 1967

Lee, LY., Bleeker, ER., Nadel, JA. Effect of ozone on the bronchomotor response to inhaled histamine aerosol in dogs J Appl Physiol; 43: 626-31, 1977

Li, Z., Daniel, EE., Lane, CG., Arnout, MA., O'Byrne, PM. Effect of an anti-Mol MAb on ozone-induced airway inflammation and airway hyperresponsiveness in dogs. Am J Physiol (Lung Cell Mol Physiol) 263: L723-L726, 1992

Linn, WS., Jones, MP., Bachmyer, EA., Spier, CE,. Mazur, SF., Avol, EL., Hackney, JD. Short term respiratory effects of polluted air: A laboratory study of volunteers in a high oxidant community. Am Rev Respir Dis 121, 243, 1980

Linn, WS., Buckley, RD., Spier, CE, Blessey, RL., Jones, MP., Fisher, DA., Hackney, JD. Health effects of ozone exposure in asthmatics. Am Rev Respir Dis 117: 835, 1978

Linn, WS., Avol, EL., Shamoo, DA., Peng, RC,. Valencia, LM., Little, DE, Hackney, JD. Repeated laboratory ozone exposure of volunteers Los Angeles residents: An apparent seasonal variation variation in response Toxicol Ind Health 4, 504, 1988

Mcdonnell, WF., Smith, MV. Description of acute ozone responses as a function of exposure rate and total inhaled dose J Appl Physiol 76(6): 2776-2784, 1994

McDonnell, WF. Individual variability in the magnitude of acute respiratory responses to ozone exposure. In M.J. Utell and R. Frank, editors. Susceptibility to inhaled pollutants. ASTM, Philadelphia, 75-88, 1989

McDonnell, WF., Horstman, DH., Abdul, S., Ragio, LS. Green, JA. The respiratory responses of subjects with allergic rhinitis to ozone exposure and the relationship to nonspecific airway reactivity. Toxicol Ind Health, 3, 507, 1987

Miller, FJ., Overton, JH., Jaskott, RH., Menzel, DB. A model of the regional uptake of gaseous pollutants in the lung. Toxicol Appl Pharmacol 79: 11-27, 1985

Molfino, N., Wright, S., Katz, I., Tarlo, S., Silverman, F., McClean, P., Szalai, J., Raizenne., Slutsky, A., Zamel., N. Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. Lancet 338: 199-220, 1991

Morgan, MS., Meyer, R., Holub, R., Frank, R. Overall and regional lung function in dogs exposed acutely to ozone. Environ Research 41: 546-557, 1986

Morris, JF., Temple, WP., Koski, A: Normal values for the ratio of one-second forced expiratory volume to forced vital capacity. Am Rev Respir Dis: 108: 1000-1003, 1973

Murlas, CG. Enviornmental airway injury: mucosoal changes and airway hyperreactivity. In SG Farmer and DNP Hay, eds. The airway epithelium. Marcel Dekker, New York 213-253

O'Byrne, PM., Junes, GL. The effect of indomethacin on exercise-induced bronchoconstriction and refractoriness after exercise. Am Rev Respir Dis 134: 69-72, 1986

O'Byrne, PM., Walters, EH., Fabbri, LM., Holtzman, MJ., Nadel, JA. Indomethacin inhibits the airway hyperresponsiveness but not the neutrophil influx induced by ozone in dogs. Am Rev Respir Dis 130: 220-224, 1984

Ryan, G., Dolovich, MB., Boberts, Rs., Frith, PA., Juniper, EF., Hargreave, FE., Newhouse, MT. Standardization of inhalation provocation tests: Two techniques of aerosol generation and

inhalation compared. Am Rev Respir Dis; 123: 195-199, 1981

Salmon, M., Kato, H., Baines, PJ., Chung, KF. Ozone exposure causes increases proliferation in airway epithelium: Effects of corticosteroids and antioxidants (Abst) Am J Respir Crit Care Med Vol 153 No 4 April 1996

Scannell, C., Chen, L., Aris, R,. Tager, I., Christian, D., Ferrando, R., Welch, B., Kelly, T., Balmes, J. Greater ozone-induced inflammatory responses in subjects with asthma. Am J Respir Crit Care Med 154: 24-9, 1996

Schelegle, ES., Adam, WC., Siefkin, AD. Indomethacin pretreatment reduces ozone-induced pulmonary function decrements in human subjects Am Rev Respir Dis, 136, 1350-1354, 1987

Schelegle, ES., Siefkin, AD., McDonald, RJ. Time course of ozone-induced neutrophilia in normal humans. Am Rev Respir Dis 143: 1353-1358, 1991

Seltzer, J., Bigby, BG., Stulburg, M., Holtzman, J., Wadel, JA. Ozone-induced change in bronchial reactivity to methacholine and airway inflammation in humans J Appl Physiol 60, 1321, 1986

Shephard, EG., Malan, L., MacFarlane, CM., Mouton, W. Joubert, JR. Lung function and plasma levels of thromboxane B2, 6-keto-prostaglandin F1a and B-Thromboglobulin in antigen-induced asthma before and after indomethacin pretreatment. Br J Clin Pharmacol 19: 459-470, 1985

Silverman, F., Shephard, RJ., Folinsbee, L. Effects of physical activity on lung responses to acute ozone exposure. Procedings of the fourth international clean air congress, 15-9, 1977

Stolk, J., Alberts, B., Hiltermann, T., Van Bree, L., Sterk, P. Repeatablility of ozone-induced airway hyperresponsiveness to methacholine in mild asthmatics (Abst) Am J Respir & Crit Care Med Vol 153, No 4, April, 1996

Tepper, JS., Costa, DL., Lehmann, JR. Extrapolation of animal data to humans: homology of pulmonary physiological responses with ozone exposure. In DE Gardner, editor. Toxicology of the lung. Raven Press, New York, 217-251

Walters, EH. Effects of inhibition of prostagland synthesis on induced bronchial hyperresponsiveness. Thorax 38: 195-199, 1983

Weinmann, G., Liu, MC., Proud, D., Hubbard, W., Frank, R. Repeated ozone exposure in humans: Inflammatory, small and peripheral airway responses (Abst) Am J Respir Crit Care Med Vol 151 No 4 April, 1995

Wong, HH., Liu, J., Bouchey, HA., Fahy, JV. The effect of ozone

exposure on markers of inflammation in induced sputum. (Abst) Am J Respir Crit Care Med vol 151, No 4 April, 1995

Ying, RL., Gross, KB., Terzo, TS., Eschenbacher, WC. Indomethacin does not inhibit the ozone-induced increase in bronchial responsiveness in human subjects. Am Rev Respir Dis 142, 817, 1990

Zamel, N. Threshold of airway response to inhaled methacholine in healthy men and women. J Appl Physiol: Respirat Environ Exercise Physiol 56: 129-133, 1984

CHAPTER 5

INFLAMMATORY RESPONSE AND USE OF INDUCED SPUTUM WITH HYPERTONIC SALINE: EFFECTS OF INDOMETHACIN PRETREATMENT

INTRODUCTION

Airways inflammation is a key response to acute ozone exposure with leukocyte infiltration into the airway submucosa accompanied by increased levels of mediators, proteins and cytokines. This response has been demonstrated in both animal models and in human subjects. With respect to human ozone exposure studies, the extent, time course and constitutive elements of the inflammatory response have been described using mainly bronchoalveolar lavage (BAL) samples in healthy subjects (Aris, 1993; Devlin, 1991; Schelegle, 1991; Koren, 1989; Seltzer, 1986). Cellular infiltration into airway walls and onto the airway surface is dominated by neutrophil influx (Koren, 1989; Seltzer, 1986) which peaks at approximately 4-6 hours post exposure (Schelegle, 1991). Activated neutrophils at the site of injury can also release oxygen free radicals and proteolytic enzymes capable of causing cell damage (Hazucha, 1996). Accompanying neutrophil influx into the airways are increased levels of several eicosanoids which are derived from arachidonic acid metabolism via cyclooxygenase (COX) activity (Kleeberger, 1988). These include Prostaglandin F2- alpha (PGF2-alpha), Prostaglandin E2 (PGE2), and Thromboxane B2 (TXB2) (Weinmann, 1994; Devlin, 1991; Hazucha, 1991; McDonnell, 1990; Koren, 1989; Seltzer, 1986). Each of the cells involved in mediating upper and lower airway inflammation release a distinct profile of lipid mediators (eiocosanoids) after stimulation. Neutrophils do not release COX metabolites but release Leukotriene B4 (LTB4), a 5-lipoxygenase metabolite, and alveolar macrophages release LTB4 and TXA2

(Henderson, 1987). Exposure of cultured epithelial cells to O_3 produces PGE2 and PGF2-alpha (Noah, 1991). Also found in increased quantities in lung fluid following ozone exposure are cytokines Interleukin 8 (IL-8), Interleukin 6 (IL-6), and Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) (Madden, 1991; Malek, 1995; Devlin, 1991; Gwizdala, 1993; Scannell, 1994). Also reported are significant decreases in BAL macrophages (Weinmann, 1995; Koren, 1989; Torres, 1995), increased BAL eosinophils (Torres, 1995), increased proximal airway lavage (PAL) epithelial cells (Aris, 1993), and increased levels of lactate dehydrogenase (LDH), a marker of cell damage.

PGF2-alpha is a principal cyclooxygenase metabolite of arachidonic acid metabolism. Its level in airway fluid samples will give an indication of the degree of inhibition of the cyclooxygenase pathway by indomethacin, or conversely the degree of activation. Its mechanism of formation in tissue is mainly oxidation followed by endoperoxide reduction of prostaglandin H2 (PGH2), but also includes reduction of prostaglandin D2 (PGD2) and prostaglandin E2 (PGE2) (Hamberg, 1975). PGF2-alpha is a potent constrictor of airway smooth muscle (Hamberg, 1975) and may also cause heightened reflex bronchoconstriction by sensitizing airway nerve endings (O'Byrne, 1984) or directly stimulating them (Coleridge, 1976).

IL-8 is an 8000 Dalton protein of approximately 72 amino acids. Structurally the presence of 2 disulphide bonds are important to

retain its biologic activity. IL-8 is produced by monocytes, fibroblasts and keratinocytes in response to stimulation by lipopolysaccharides, Interleukin-1 (IL-1) or Tumor Necrosis Factor alpha (TNF alpha). IL-8 stimulates a number of functions, its primary one being the chemotaxis of human neutrophils. IL-8 can also induce liposomal enzyme release and oxidative burst by neutrophils, inducing chemotaxis and degranulation at nanomolar concentrations by interacting with a specific plasma membrane receptor. Since IL-8 is a potent chemotactic agent for neutrophils it is a good candidate for being involved in the ozone response. Evidence to support its role comes from a study in which healthy subjects were exposed to O_3 and increased levels of IL-8 mRNA were found in respiratory epithelium (Devlin, 1994). In vitro work has also implicated a role for IL-8 and ozone. BEAS-2B cells exposed to ozone produced increased IL-8 mRNA and increased amounts of IL-8 protein (Devlin, 1994). In this study, IL-8 will act as an additional marker for neutrophil involvement in the ozone response, and indicate the activity state of cells that produce IL-8, such as bronchial epithelial cells.

Only 2 studies in addition to this one have examined the inflammatory airway response to acute ozone exposure using induced sputum with hypertonic saline. Wong (1995) compared ozone exposure (400 ppb, 2 hours) versus filtered air (FA) exposure and reported increased percent neutrophils with ozone (80%) versus air (51%), increased levels of myeloperoxidase (MPO), and a trend toward

higher levels of IL-6 and IL-8 in ozone (1497 pg/ml) versus FA (1153 pg/ml) exposure in ten healthy subjects. Vagaggini (1996) also used induced sputum samples and compared ozone (400 ppb, 2 hours) and FA exposures in 9 asthmatics, and reported increased percent neutrophils in ozone (70%) versus FA (27%) exposures. Alexis (1995) reported preliminary data from this study and compared inflammatory cell responses with changes in pulmonary function, symptom reporting and airway reactivity following ozone in asthmatic subjects pretreated with indomethacin. Neither Wong (1995) or Vagaggini (1996) examined potential mechanisms of the inflammatory response following ozone exposure.

The mechanism behind ozone-induced airway inflammation is not fully understood. Several studies have examined the role of pulmonary function changes and changes in airway reactivity in the inflammatory response. Recent studies suggest that inflammation is not related to spirometric changes (Balmes, 1996; Kulle, 1985; Weinmann, 1993), while other studies report that the presence of neutrophils is not essential for ozone induced airway hyperreactivity, although species differences existed in these animal studies (Murlas, 1991; Li, 1992). In vitro evidence has suggested that the source of the increased BAL constituents following ozone exposure are lung cells or cells recruited from peripheral sites through increased epithelial permeability. Recent in vitro studies of exposure of macrophages to extremely high ozone levels (1000 ppb) have shown them to release many of the compounds

found in BAL fluid (Driscoll, 1988; Becker, 1991; Devlin, 1991).
Other studies have also demonstrated airway epithelial cells to
release prostaglandins and leukotrienes (Hunter, 1985; Henke, 1988;
Leikauf, 1988), cytokines (Devlin, 1994; Henke, 1988; Standiford,
1990) and chemotactic factors (Koyama, 1991), compounds typically
found in BAL fluid from ozone exposed subjects. Only one study to
date has examined the role of cyclooxygenase metabolites in the
ozone-induced inflammatory response in humans. Hazucha (1996)
pretreated healthy subjects with the cyclooxygenase inhibitor
ibuprofen, and reported significant inhibition of ozone-induced
FEV_1 decrement and significant inhibition of ozone-induced
increases in PGE2, TXB2 and IL-6, while PMN influx in BAL fluid was
unaffected. Although Hazucha's (1996) study is similar to this one,
important differences exist between the type of pharmacologic
blocking agent he used, i.e. ibuprofen, and the type of airway
sampling technique he used, i.e. BAL.

One major difference of ibuprofen versus indomethacin is that
ibuprofin in addition to blocking cyclooxygenase activity, also
blocks some lipoxygenase activity as well (Humes, 1983). Ibuprofin
also has anti-oxidant properties, is an inhibitor of oxygen-free
radical generation, inhibits neutrophil degranulation (Kaplan,
1984), and inhibits COX2 and COX1 equally (Smith, 1993).

With respect to the sampling technique of BAL, the method of sputum
induction with hypertonic saline has several advantages over more

traditional sampling methods like BAL when conducting a controlled human ozone exposure study. The technique of induced sputum (IS) with hypertonic saline has been shown to be a responsive (Pin, 1992; Gibson, 1992; Gibson, 1995) valid (Gibson, 1989; Pin, 1992; Pizzichini, 1995; Fahy, 1993) and reproducible (Pizzichini, 1995; Spanevello, 1996)) method for measuring lower airways inflammation. Several studies have compared leukocyte counts from IS, BAL, bronchial washings and bronchial biopsies and shown different results between sampling methods (Pizzichini, 1996; Maestrelli, 1995; Fahy, 1995). These studies concluded that the different results are due to the different sites of the airways sampled by the different procedures, and that IS primarily samples the lower airways region excluding alveolar sites. This is advantageous for ozone exposure since uptake and distribution studies in humans suggest that the lower conducting region has the highest ozone uptake under conditions of exercise (Hu, 1994; Biscardi, 1993; Gerrity, 1988). Further, Pizzichini (1996) and Fahy (1995) reported that IS samples are more concentrated in cells and fluid phase chemicals than bronchial wash or BAL due to the natural concentration of secretions lining the airways as they are swept from the periphery to the central airways. This is advantageous when measuring chemicals present in low concentrations in airway secretions. This is particularly important with respect to the subjects in this study who have mild asthma, since subjects with more severe asthma have a greater concentration of inflammatory mediators in their airway secretions (Bousquet, 1991). In addition,

compared to BAL or bronchial washes (BW), samples collected by IS have potentially less dilution by saliva and hypertonic saline than BAL instillate. This is because both BAL and BW sample relatively small volumes of secretions lining distal airways. The small volumes of secretions in this area are considerably diluted by the instillate of saline required by lavage.

To date, sputum induction with hypertonic saline has been used to assess lower airway inflammation in a variety of subjects including mild and exacerbated atopic asthmatics (Pin, 1992; Pin, 1992; Pizzichini, 1995), subjects with chronic bronchitis (Gibson, 1989), healthy subjects (Fahy, 1993), and subjects with occupational asthma (Balkissoon, 1995; Maestrelli, 1994). Controlled exposure studies with sputum induction have included sensitized subjects exposed to allergen (Pin, 1992) and isocyanates (Maestrelli, 1994), and in subjects exposed to ozone (Vagaggini, 1996; Alexis, 1995; Wong, 1995). Other advantages of the sputum induction technique include less invasiveness, no requirement for hospital admission, no use of anesthetic and no need for a significant recovery period following induction.

PURPOSE

First, to safely use the technique of induced sputum with hypertonic saline to examine airways inflammation in mild asthmatics following a controlled exposure to ozone. Second, to

determine whether asthmatics respond with neutrophilic inflammation following exposure to ozone. Third, to determine whether cyclooxygenase metabolites sensitive to indomethacin inhibition, play a role in the ozone-induced inflammatory response in asthmatics.

HYPOTHESIS

First, induced sputum with hypertonic saline will successfully and safely measure airways inflammation in asthmatics following a controlled exposure to ozone. Second, asthmatics will respond with neutrophilic inflammation following ozone exposure. Third, indomethacin pretreatment will significantly attenuate ozone-induced increases in sputum inflammatory cells, specifically neutrophils, and IL-8 in asthmatics.

OBJECTIVES

First, safely collect adequate sputum samples (i.e. > 75 mg, containing less than 20% squamous epithelial cells), and an adequate volume of supernatant (400 ul) to measure fluid phase components. Second, to demonstrate a significant increase in sputum total leukocytes, percent neutrophils, PGF2-alpha and IL-8 following a 2 hour exposure to 400 ppb ozone. Third, to determine whether indomethacin can block the increase in sputum total cells, neutrophils, PGF2-alpha and IL-8.

METHODS

Subjects

Thirteen asthmatic subjects were used for inflammatory cell analysis. Seven asthmatic subjects were used for biochemical marker analysis.

Induced Sputum With Hypertonic Saline

The method of induced sputum (IS) with hypertonic saline was taken from Pin (1992). Since 1992 three significant revisions of the method have been evaluated and introduced into the literature by Dr. F. Hargreave (Hamilton, Ontario) (Efthimiadis, 1995). This study used the original method throughout the study protocol in order to consistently compare early results with results obtained at the end of the study. FEV_1 and vital capacity (VC) were measured on a wedge spirometer connected to an x-y recorder before and 10 minutes after salbutamol inhalation. An ultrasonic nebulizer was filled with 9 cc of 3% hypertonic saline to begin the test. The nebulizer was set to the maximum output setting and the subject was instructed to latch his/her mouth onto the nebulizer mouthpiece. The power was turned on and hypertonic saline was nebulized through the mouthpiece in a jet stream and inhaled for three separate seven minute periods for up to 21 minutes at three saline concentrations. The subject was instructed to breath at normal tidal volume while

on the mouthpiece and was encouraged to come off the mouthpiece to cough if a sputum sample from the lower airways (i.e. not from the back of the throat) was ready for expectoration. Prior to expectoration, subjects were asked to blow their nose and clear their throat to avoid the inclusion of non-airway fluid samples. During tidal breathing on the mouthpiece, the nose was not occluded. The sample was expectorated into a sterile specimen jar and capped. Following the measurement of FEV_1 after each inhalation period, the concentration of saline was increased from 3% to 4% to 5%, provided the FEV_1 fell by < 10% from the post-bronchodilator value. A volume of 9cc filled the nebulizer well on each occasion. If the FEV_1 fell between 10-20% of the post bronchodilator value, the test was continued but at the same concentration of saline. If the FEV_1 fell by > 20% or if troublesome symptoms occurred, the nebulization was discontinued and salbutamol was available if necessary. Troublesome symptoms included cough, chest tightness, general discomfort. The nebulization was stopped after 21 minutes or earlier if a sputum sample of good quality was obtained.

SPUTUM ANALYSIS

Immediately following the conclusion of sputum induction, sample processing begins. The sample was transferred to a Petri dish and its characteristics recorded in terms of colour, consistency and degree of salivary contamination. The sputum plugs were manually separated from surrounding saliva with sterile forceps and

transferred to a preweighed Eppendorf tube. This sample volume was recorded and weighed. One thousand microliters (ul) of 0.1% Dithiothreitol (DTT) was then added to the sputum sample. The sample was aspirated with a pipette, vortexed and agitated (bench rocked) for 20 minutes. Following filtration through a 52 micron nylon mesh the sample was separated by centrifugation at 1500 rpm. Approximately 400-600 ul of the supernatant was extracted and stored frozen at -20 °C for future study of its soluble constituents. The cell pellet was resuspended in Delbecos Phosphate Buffer Saline (DPBS) with a volume equal to that extracted for the supernatant (400-600 ul), and that volume was recorded. Total cell count was performed by filling two hemacytometer chambers with 10 ul of sample stained with Trypan Blue. Viability was determined by Trypan Blue exclusion. Viable total cells excluding squamous cells were counted and averaged from the two chambers, and expressed as the absolute number of cells and the number of cells/original wet weight (mg) of sputum. The remaining cell suspension was then prepared for cytospinning and diluted if necessary to a maximum concentration of 750,000 cells/ml. The cytospin slides were air dried and Wright stained using an automated system. Cells identified with the Wright stain included neutrophils, macrophages, eosinophils, lymphocytes, bronchial epithelial cells and squamous epithelial cells. Differential cell counts were performed by counting a total of 400 nucleated cells (200 cells per slide) and taking the average out of 100 and expressing the count as a percentage of 100 cells. Salivary contamination was expressed as

the proportion of squamous cells among nucleated cells. A squamous cell count of >20% rendered a slide unacceptable.

INTERLEUKIN-8 (IL-8) ANALYSIS

Frozen supernatant samples were thawed and analyzed with a standard commercial Enzyme Linked Immunosorben Assay (ELISA) kit purchased from Amersham Life Science, Mississauga, Ontario (1995) specific for human IL-8 (Interleukin-8 [(h)IL-8]). The assay used the quantitative immunometric "sandwich" enzyme technique where a monoclonal antibody specific for IL-8 was coated on the bottom of a microtitre plate. IL-8 standards and samples were added to the wells and any IL-8 present in the sample was bound to the immobilized antibody. Following a wash step, an enzyme-linked polyclonal antibody specific for IL-8 was added to the well and was bound to the IL-8 that was bound from the first reaction. Following a wash step, the final substrate solution was added to the wells and colour developed in proportion to the amount of IL-8 present. A standard curve was plotted with optical density on the ordinate versus IL-8 concentration in picograms (pg) on the abscissa. The sensitivity of the assay was 0.5 pg with a standard range of 3-200 pg/well. IL-8 concentrations were expressed in pg/milliliter (ml) and adjusted for the dilution effect of the sputum analysis procedure.

PROSTAGLANDIN F2-ALPHA (PGF2-alpha) ANALYSIS

Frozen supernatant samples were thawed and analyzed with a standard commercial radioimmunoassay kit purchased from Amersham Life Science, Missassauga, Ontario (1995) specific for PGF2-alpha. The method has been described previously (Seltzer, 1986). The assay is based upon competition between unlabeled PGF2-alpha and a fixed quantity of tritium labeled PGF2-alpha for binding to a limited quantity of an antibody which has specificity and affinity for PGF2-alpha. The amount of radioactive ligand bound by the antibody will be inversely proportional to the concentration of added non-radioactive ligand. Measurement of the protein-bound radioactivity enables the amount of unlabeled PGF2-alpha in the sample to be determined. PGF2-alpha was expressed in pg/ml and all measurements were adjusted for the dilution effect of the sputum analysis procedure. This factor was 8 fold and this resulted from the addition of DTT and buffer solution to the sputum sample.

STATISTICAL ANALYSES

Paired T-Test analyses were performed to compare whether baseline changes for cell and mediator variables were significantly different with ozone and air exposure (the ozone response), and whether the ozone response was significantly different with placebo or indomethacin (the indomethacin interaction effect). Multiple linear regression analysis was a preferred method for determining

exposure and treatment interaction effects if missing data points significantly reduced the number of paired data points in the paired T-Test analysis.

The reproducibility of sputum cell counts and soluble fluid phase compounds between consecutive days and the reliability of the induced sputum method were examined by multiple linear regression analysis. Pearson correlation coefficients (r) determined the former and the coefficient of reliability (R) - the ratio of the variance of cell counts or soluble factors within subjects to the total variance in cell counts or soluble factors (within subject variance + error variance) - determined the latter, with higher values indicating higher reliability of the method (Kramer, 1981). Test-retest cell and soluble factor reproducibility were examined from sputum specimens collected from the same subject on two consecutive days. Multiple linear regression analysis was performed and plots of day 1 versus day 2 cell and mediator variables are shown with Pearson correlation coefficients (r). Two tailed unpaired T-Tests were also used to compare day 1 and day 2 cell and mediator means.

Pearson correlation coefficients (r) were used to assess the inter-observer variation among three readers for differential cell counts on a selection of randomly coded slides. Significance was accepted at the 95% level for all statistical analyses.

RESULTS

Cell Responses

Individual and group mean (+/- SE) pre and post exposure values for Total Cell Counts (TCC), Total Cell Counts per milligram wet weight sputum (TCC/mg sputum), % Polymorphonuclear Neutrophils (%PMNs) and % Macrophages (MACs) are shown for all exposure and treatment conditions in Tables 1, 2, 3 and 4 (pg185-188), respectively. Also shown in Tables 1-4 are the pre minus post exposure differences in all exposure and treatment conditions. Data for the other differential cell types enumerated are not shown because the mean cell responses for eosinophils, lymphocytes, and bronchial epithelial cells following ozone exposure were all less than 3%.

Figures 1 and 2 (pg192) show the difference in baseline changes for total cells (TCC), % PMNs and % MACs between ozone and air exposure with placebo (clear bar) and indomethacin (shaded bar) in 13 asthmatic subjects. An * indicates a significant difference between ozone and air exposure, i.e. Ozone response, at the 0.05 level. The indomethacin interaction effect is defined as: (ozone response with placebo) - (ozone response with indomethacin). Significant ozone responses with placebo were found for TCC (p=0.05) and % PMNs (p=0.02). Significant ozone responses with indomethacin were found

TCC, % PMNs or % MACs (p>0.05), however a smaller mean ozone response for %PMNs was observed with indomethacin.

Figures 3A-3D (pg193) show scatter diagrams of the ozone response versus % protection by indomethacin for PMNs, MACs, TCC and TCC/mg, respectively. Protection is defined as the % inhibition of the ozone response and is calculated as: protection = (ozone response$_{placebo}$) - (ozone response$_{indomethacin}$)/ozone response$_{placebo}$ x 100%. Arbitrary cut-off points defining "responsive" subjects are shown for each variable, and protection is shown as YES (> 0) or NO (< 0) for PMNs, TCC and TCC/mg. For MACs, protection is YES (< 0) or NO (> 0). Using these limits, "responsive" subjects are divided into two quadrants, those demonstrating protection, and those who do not. For PMNs, twice as many subjects (4) appeared in the protection quadrant, and for MACs almost equal numbers of subjects appeared in both the protection and no protection quadrants. For TCC and TCC/mg, 100% of responsive subjects appeared in the protection quadrant.

Biochemical Response

Table 5 (pg189) shows the individual and group mean (+/- SEM) pre and 2 hours post exposure PGF2-alpha values in asthmatic subjects in all exposure and treatment conditions. Also shown are the pre minus post exposure differences in all exposure and treatment conditions and the difference of the pre minus post exposure

differences between placebo and indomethacin. The pre-exposure group means (+/-SEM) are also shown for all conditions giving an indication of the level of airways inflammation prior to each exposure. Table 6 (pg190) shows the individual and group mean (+/-SEM) pre and post exposure values for IL-8 in asthmatic subjects in all exposure and treatment conditions.

Figures 4A and 4B (pg194) show the difference in baseline changes for IL-8 and PGF2-alpha between ozone and air exposure with placebo (clear bar) and indomethacin (shaded bar). No significant differences between ozone and air were observed for IL-8 or PGF2-alpha with placebo or indomethacin (p>0.05), although both compounds showed an increase from pre exposure baseline following ozone exposure with placebo. No significant indomethacin interaction effects were reported for IL-8 (p=0.5) or PGF2-alpha (p=0.9).

Figure 5 (pg195) shows the mean pre-exposure sputum PGF2-alpha levels (including pre air exposures) in asthmatic subjects with placebo and indomethacin. Mean (+/-SEM) PGF2-alpha levels were 115 pg/ml (22) in the placebo condition and 65 pg/ml (14) with indomethacin. Two tailed unpaired T-Test analyses between the placebo and indomethacin condition demonstrated no significant difference between placebo and indomethacin (p>0.05).

Results on cell reader variation (Table A7, pgA202), cell and

biochemical marker reproducibility (Figures A7-A12, pgA203-204) and sputum processing appear in the Appendix following this chapter.

DISCUSSION

Our results showed that in asthmatics pretreated with placebo, ozone caused a significant increase from pre-exposure baseline in total leukocytes and percent neutrophils in induced sputum samples. With respect to the PMN response, our results support two recent studies where PMN responses were examined in sputum samples following exposure to 400 ppb ozone. Vagaggini (1996) examined asthmatic subjects and Wong (1995) examined healthy subjects and both reported ozone-induced increases in PMNs. Our ozone-induced increase in neutrophils of 23 % points in asthmatics was lower than Vagaggini's (44% points), but very similar to Wong's (29% points). One possible reason our neutrophil results differed from Vagaggini's yet are similar to Wong's normals, may be because our subjects were mild asthmatics and therefore had less severe pre-existing airway inflammation compared to more severe asthmatics. More severe asthmatic subjects tend to have higher concentrations of inflammatory cells and mediators in their airway secretions than mild asthmatics (Bousquet, 1991). Our data also support previous studies where the ozone-induced inflammatory response was measured in bronchial wash fluids in normal and asthmatic subjects. These studies used similar exposure conditions to this study and reported increases in bronchial wash PMNs of 27% points in asthmatics

(Scannell, 1996) and 25% points in ozone sensitive (FEV_1) normal subjects (Balmes, 1996). These data together with our own, suggest that asthmatics and normals do not differ substantially in their neutrophil response to acute ozone exposure, and that sputum provides similar information to bronchial wash samples. In fact, Fahy (1995) reported that the cellular and biochemical characteristics of induced sputum more closely resemble the cellular and biochemical characteristics of bronchial wash than of BAL. These findings indicate that sputum induction and bronchial wash measure secretions from similar lung compartments and that this compartment is likely the lower airways (excluding the alveolar region). Secretions from sputum induction therefore, unlike BAL are not diluted by fluid lining the alveolar spaces. This is advantageous in samples where the expected concentration of cell or chemical is low. It can be argued that any observed increase in sputum inflammatory cells, i.e. neutrophils, is enhanced by the hypertonicity of the inhaled saline thereby making it difficult to differentiate ozone-induced increases in cells from those of the sputum induction technique. Popov (1995) compared inflammatory cells counts following normal saline and hypertonic saline sputum inductions. He reported no difference in differential leukocyte counts.

As a result we believe the increases in cells we observed following ozone exposure, were indeed induced by ozone and not the hypertonic saline.

Our results showed that compared to the placebo condition, the mean (+/-SEM) ozone response for PMNs was not as strong with indomethacin pretreatment {11% points (15) vs 23% points (22)}, but this inhibitory effect was not statistically significant (p=0.56).

Our inability to show a statistically significant inhibitory effect on the neutrophil response to ozone, compares to Hazucha (1996) who pretreated healthy subjects with ibuprofin (a cyclooxygenase blocking agent) and exposed them to 400 ppb ozone for 2 hours. Hazucha reported that ibuprofin did not affect O_3-induced cellular changes in BAL fluid in healthy subjects. In fact, Hazucha (1996) reported almost identical levels of PMNs in both the placebo (11%) and drug (12%) condition. One difference however between Hazucha's results and ours, is that we appeared to show a trend toward a difference between the placebo and indomethacin condition (12 % points or 52% inhibition), although it did not reach statistical significance. The relatively high standard error associated with our neutrophil measurements may be responsible for our inability to demonstrate a significant indomethacin interaction effect. The difference between partial, but non-significant inhibition on PMNs by a cyclooxygenase blocker in asthmatics, compared to absolutely no inhibition on PMNs from a cyclooxygenase blocker in normal subjects, suggests the possibility that asthmatics and non-asthmatics possess different PMN sensitivities to cyclooxygenase inhibition by indomethacin. The different magnitude of PMN responses found in our study versus Hazucha's, may highlight the

different locations that our respective sampling techniques measured. BAL fluid originates from distal airway fluid surfaces and represents alveolar fluid contributions, regions where ozone is not expected to penetrate and generate an inflammatory response. Sputum samples on the other hand originate from less distal airway regions, i.e. in the lower conducting airways, where ozone is expected to penetrate and generate an inflammatory response.

From our results, we suggest that although indomethacin did not significantly reduce the neutrophil ozone response in asthmatics, this may not have been due to the fact that indomethacin had no real inhibitory effect on neutrophils. Rather, it is more likely the high variability associated with our sputum neutrophil counts made it difficult to achieve a statistically significant indomethacin interaction effect.

We speculated whether those subjects who demonstrated the largest cell responses to ozone, would also derive the most benefit from indomethacin pretreatment. In other words, are the most ozone-PMN responsive subjects also the most responsive to indomethacin pretreatment? Our scatter diagram for PMNs showed that twice as many ozone-responsive subjects demonstrated protection versus no protection. For total cells and total/mg sputum, 100% of ozone-responsive subjects demonstrated protection. These results suggest that subjects who demonstrate the highest cell responses to ozone, are also the most sensitive to the effects of indomethacin and may

therefore derive the most benefit from the drug.

IL-8 is a potent chemotaxic agent for neutrophils (Richman-Eisenstat, 1993) and has been shown to be correlated with neutrophil levels in the sputum of exacerbated asthmatics (Fahy, 1995). As a result, we expected IL-8 to track the neutrophil response following ozone exposure with placebo and indomethacin. Our results in the placebo condition showed that relative to air exposure, IL-8 tracked the PMN response quite well by demonstrating an overall increase from pre-exposure baseline following ozone. The overall increase in IL-8 however, was not statistically significant. This was most likely due to the high standard error associated our IL-8 measurements, as well as the relatively small sample size. Unfortunately, we were unable to compare our results with Vaggagini (1996) who did not measure IL-8 in his asthmatic subjects, nor could we directly compare our change from baseline results with Wong (1995) since he reported only post exposure values for IL-8. Our mean post ozone exposure IL-8 value was 2 times higher than Wong's (3259 pg/ml +/- 1885 vs 1497 pg/ml +/- 452), but of the same molar concentration order of magnitude (10^{-9}M). Previous studies have shown that IL-8 has minimal chemotactic activity for neutrophils at 10^{-10} M, significant activity at 10^{-9}, and maximal activity at approximately 10^{-8} M, both in vitro (Yoshimura, 1987) and in airways in vivo (Jorens, 1992). Since our post ozone exposure IL-8 levels were of the same molar concentration order of magnitude as Wong's (1995), and represented

significant chemotactic concentrations, our similar neutrophil recovery percentages were not surprising. With indomethacin, IL-8 tracked the reduced PMN response by declining 905 pg/ml (482) from baseline.

With indomethacin pretreatment, the baseline change following ozone exposure was negative compared to air exposure and this coincided with a decrease in percent neutrophils. These results give indirect evidence to suggest that reduced IL-8 levels contributed to the reduced neutrophil response by reducing chemotaxis for neutrophils. Since other mediators with chemotactic activity for neutrophils have been identified in sputum from patients with chronic airway diseases (Schuster, 1991), Il-8 cannot be assumed to be the only mediator responsible for neutrophil chemotaxis following ozone exposure. Mediators such as leukotriene B4 produce neutrophil chemotaxis in sputum and BAL from cystic fibrosis patients (Cromwell, 1981; Sampson, 1990), and in patients with chronic bronchitis (Ozaki, 1992). Although neutrophil chemotaxis in sputum following ozone exposure has not been directly measured, there is evidence to indicate that IL-8 plays a significant role in recruiting neutrophils to the airways in inflammatory diseases. Richman-Eisenstat (1993) demonstrated 98% inhibition of neutrophil chemotaxis in asthmatics versus 9% inhibition in normal subjects by using a monoclonal antibody specific to IL-8 $(F(ab')_2$. Our data suggest that cyclooxygenase metabolites may play a role in the ozone-induced neutrophil response by increasing IL-8 concentrations

in the airways of asthmatics.

The overall increase in sputum IL-8 following ozone exposure indicates increased cell activity of cells producing IL-8 in the airways (Richman-Eisenstat, 1993). Many cells in the airways produce IL-8, including stimulated alveolar macrophages (Baggiolini, 1989), lymphocytes, neutrophils (McCain, 1994) and epithelial cells (Kwon, 1994; Nakamura, 1992). Airway epithelial cells have been reported to increase gene expression of IL-8 when stimulated with IL-1, tumour necrosis factor (Nakamura, 1991) and neutrophil elastase (Nakamura, 1992). Ozone has also been implicated to cause bronchial epithelial cell shedding resulting in increased epithelial permeability (Kehrl, 1987) as evidenced by increased proteins in BAL fluid (Koren, 1989) leading ultimately to increased airway reactivity.

With respect to pre-exposure PGF2-alpha concentration, our results showed a lower level with indomethacin than placebo, but the difference was not statistically significant. One reason for not observing a statistically significant difference may have been that the full dose of indomethacin had not been ingested one day prior to exposure when pre-exposure PGF2-alpha was measured. This would have prevented maximal inhibition of PGF2-alpha from occurring. A second reason for not observing a significantly lower pre-exposure PGF2-alpha level with indomethacin, may have been that our subjects received a lower total dose of indomethacin compared to previous

ozone studies where indomethacin successfully blocked the formation of cyclooxygenase metabolites (Ying, 1990; Eschenbacher, 1989). These studies used approximately 150 mg/day of indomethacin for 3-5 days which resulted in plasma concentrations of 1.9-2.3 ug/ml (Schoog,1981; Verbesselt, 1983). Plasma concentrations of indomethacin in this range have been shown to be sufficient to inhibit cyclooxygenase metabolism from 50% to 90% in vivo (Hamberg, 1972; Higgs, 1976; Randall, 1980; Higgs, 1982). Since we did not measure plasma levels of indomethacin in our subjects, the true levels in our subjects is unknown. However, we can form an estimate based on approximately half the dose used in the studies where plasma levels were measured (Ying, 1990; Eschenbacher, 1989). Since we used 75 mg/day for 3 days we estimate that we induced 25-45% inhibition of cyclooxygenase metabolism. In the placebo condition, our pre-exposure data show an absolute mean PGF2-alpha level of 115 pg/ml versus 65 pg/ml with indomethacin. This represents 43% inhibition which falls in the range of our estimate based on previous studies. Compared to the placebo condition (where ozone induced an increase from pre-exposure baseline of 49 pg/ml of PGF2-alpha relative to air exposure), ozone induced an increase from pre-exposure baseline of 53 pg/ml of PGF2-alpha relative to air exposure with indomethacin. The ozone response with indomethacin was 8% greater than placebo. Indomethacin therefore, failed to exact any inhibition on increased PGF2-alpha levels from baseline following ozone exposure. Considering that PGF2-alpha is a potent bronchoconstrictor, and that indomethacin did not inhibit its

levels in the airways, suggests that the ozone-induced decline in FVC and FEV_1 we observed in our subjects while pretreated with indomethacin, may be due to elevated levels of PGF2-alpha in the airways.

CONCLUSION

Acute ozone exposure caused a significant increase in sputum total leukocytes and percent neutrophils in the airways of asthmatic subjects. Indomethacin pretreatment failed to significantly attenuate the cellular responses, but subjects demonstrated a smaller increase in percent neutrophils with indomethacin. IL-8 responses following ozone appeared to track the PMN responses when subjects were pretreated with placebo or indomethacin. Pre-exposure PGF2-alpha levels showed that asthmatics likely experienced an appreciable level of cyclooxygenase inhibition prior to ozone exposure, and this was estimated to be approximately 43%. Based on the dose of indomethacin used in this study, the maximum cyclooxygenase inhibition we could expect was 45%. Since indomethacin achieved 96% of this prior to ozone exposure, we suggest not enough indomethacin remained in the airways to inhibit further increases in cyclooxygenase activity consequent to ozone exposure. Indomethacin did not attenuate increased PGF2-alpha levels relative to baseline following ozone exposure, in fact an increase in PGF2-alpha was observed. We suggest that lack of suppression of PGF2-alpha levels following ozone exposure, may have

contributed to the reduced FVC and FEV_1 we observed in our subjects while pretreated with indomethacin.

A chief limitation of this data is the relatively small number of subjects (N=7) we used. Therefore, more asthmatic subjects need to be studied in order to confirm or contradict the results obtained in this study.

CHAPTER 5 TABLES

TOTAL CELL COUNTS
(X 1000)

ASTHMATICS

|----------------- PLACEBO ----------------| |----------------- INDOMETHACIN ----------------|

Subject	GENDER M=Male F=Female	PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	PRE-POST AIR	(PRE-POST OZONE)-(PRE-POST AIR)	PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	PRE-POST AIR	(PRE-POST OZONE)-(PRE-POST AIR)
8	F	0*	36	-36	0*	642	-642	606	115	331	-216	0*	0*	-	-
9	M	1389	2282	-893	472	661	-189	-704	740	1535	-795	0*	8	-	-787
11	M	323	337	-14	899	377	522	-536	286	386	-100	284	328	-44	-58
12	M	0*	179	-179	0*	229	-229	50	148	657	-509	0	235	-235	-274
13	F	745	611	134	51	110	-59	193	56	0*	56	0*	217	-217	273
14	F	85	167	-82	1457	135	1322	-1404	289	759	-470	62	100	-38	-432
15	F	142	119	23	1438	270	1168	-1145	683	958	-275	168	216	-48	-227
16	M	35	126	-91	116	427	-311	220	228	263	-35	211	211	0	-35
17	M	0*	10	-10	298	145	153	-163	85	471	-386	0	93	-93	-293
18	F	482	407	75	65	463	-398	473	528	146	382	71	182	-111	493
19	F	11	295	-284	612	178	434	-718	485	518	-33	339	306	33	-66
22	F	275	512	-237	480	724	-244	7	994	1596	-602	1258	611	647	-1249
23	F	89	561	-472	123	160	-37	-435	73	94	-21	111	335	-224	203
mean		275	434	-158.94	462.4	347.8	114.6	-273.5	362.3	593	-231	208	237	29	-260
std error		11.2	163	75.9	142.6	60.7	165.0	170.2	83.1	141	88	101	44	83	138.3

Table 1. Individual and group mean (+/- SEM) pre and post exposure total cell counts (x 1000) in all exposure and treatment conditions in 13 asthmatic subjects.. Also shown are the pre - post exposure differences in all exposure and treatment conditions. Pre exposure = 1 day prior to exposure; Post exposure = 4-6 hrs post exposure; * sample not processed due to insufficient volume of sample plug; - = missing data point; DIFF=Difference; SEM = standard error of the mean

TOTAL CELL COUNT PER MILLIGRAM WET WEIGHT SPUTUM (X 1000)

ASTHMATICS

Subject	GENDER M=Male F=Female	PLACEBO PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	PRE-POST AIR	(PRE-POST OZONE)-(PRE-POST AIR)	INDOMETHACIN PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	PRE-POST AIR	(PRE-POST OZONE)-(PRE-POST AIR)
8	F	0*	1.40	-1.4	0*	1.29	-1.29	0.11	0.64	1.60	-0.96	0*	0*	-	-
9	M	9.40	1.10	8.30	3.42	4.29	-0.87	9.17	8.20	1.20	7.10	0*	0.38	-0.36	7.48
11	M	0.81	0.84	-0.03	2.63	1.02	1.61	-1.64	0.36	3.34	-2.98	1.01	1.19	-0.18	-2.80
12	M	0*	1.77	-1.77	0*	2.60	-2.6	-0.83	0.45	2.62	-2.17	0	1.29	-0.95	-1.22
13	F	8.20	7.67	0.53	1.22	3.29	-2.07	2.60	0.57	0*	0.57	0*	2.04	-2.04	2.61
14	F	0.24	0.53	-0.29	8.20	1.10	7.10	-3.39	0.77	1.30	-0.53	0.26	0.42	1.22	-1.75
15	F	0.74	0.54	0.20	4.79	0.89	3.90	-3.70	0.85	3.63	-2.78	1.19	1.36	-0.19	-2.59
16	M	0.49	0.73	-0.24	0.58	2.77	-2.19	1.95	3.42	1.69	1.73	0.94	0.82	0.32	1.41
17	M	0*	0.03	-0.03	1.13	0.84	0.29	0.28	5.36	2.25	3.11	0	0.89	0.59	2.63
18	F	1.78	0.89	0.89	0.236	1.66	-1.80	2.49	3.18	0.72	2.46	0.42	0.44	1.80	0.66
19	F	0.04	3.35	-3.31	2.10	0.93	1.17	-4.48	0.69	1.54	-0.85	1.92	1.32	0.60	-1.45
22	F	0.68	4.80	-4.12	2.20	3.85	-1.45	-2.87	7.40	8.20	-0.8	2.84	1.54	1.10	-1.90
23	F	0.45	2.30	-1.85	0.36	0.59	-0.23	-1.62	0.31	0.31	0	0.36	1.06	-0.70	0.7
mean		1.76	2.00	-0.24	2.07	1.93	0.14	-0.38	2.48	2.18	0.3	0.73	1.05	-0.32	0.62
std error		0.88	0.59	0.62	0.65	0.34	0.78	1.01	0.79	0.58	0.77	0.25	0.21	0.16	0.85

Table 2. Individual and group mean (+/- SEM) pre and post exposure total cell counts per mg wet weight sputum t (x 1000) in all exposure and treatment conditions in 13 asthmatic subjects. Also shown are the pre post exposure difference s in all exposure and treatment conditions. Pre exposure = 1 day prior to exposure; Post exposure = 4-6 hrs post exposure; * sample not processed due to insufficient volume of sample plu[s] . = missing data point; DIFF=Difference;SEM = standard error of the mean

POLYMORPHONUCLEAR NEUTROPHILS
(%)

ASTHMATICS

|--------------------------- PLACEBO---------------------------| |-------------------INDOMETHACIN---------------------------|

Subject	GENDER M=Male F=Female	PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	PRE-POST AIR	(PRE-POST OZONE)-(PRE-POST AIR)	PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	PRE-POST AIR	(PRE-POST OZONE)-(PRE-POST AIR)
8	F	0*	0**	-	0*	38	-38	38	31	62	-31	0*	0*	-	-31
9	M	78	83	-5	34	78	-44	39	0**	0**	-	0*	0**	-	-
11	M	10	46	-36	70	11	59	-95	73	45	28	20	41	-21	49
12	M	0*	0**	-	0*	0	0	0	11	62	-51	2	23	-21	-30
13	F	0	35	-35	84	0**	84	-119	41	0*	41	0*	44	-44	85
14	F	9	34	-25	0**	0**	-	-25	8	25	-17	30	2	28	-45
15	F	5	23	-18	38	6	32	-50	20	73	-53	29	13	16	-69
16	M	19	5	14	6	10	-4	18	0**	20	-20	35	3	32	-52
17	M	0*	0***	-	0	18	-18	18	0**	23	-23	0***	20	-20	-3
18	F	12	69	-57	7	73	-66	9	54	44	10	12	37	-25	35
19	F	0**	72	-72	68	53	15	-87	0	78	-78	4	21	-17	-59
22	F	54	80	-26	58	66	-8	-18	64	80	-18	48	42	6	-22
23	F	0	0***	0	0***	79	-79	79	0***	5	-5	17	24	-7	-2
mean		21	50	-29	30	36	-6	-23	25	43	-18	18	25	-7	-11
std error		8.9	9.2	8.6	9.6	9.3	15.4	22	7.8	8.0	9.7	4.8	4.5	7	14.8

Table 3. Individual and group mean (+/- SEM) pre and post exposure neutrophils (%) in all exposure and treatment conditions. Also shown are the pre - post exposure difference s in exposure and treatement conditions. Pre exposure = 1 day prior to exposure; Post exposure = 4-6 hrs post exposure; * sample not processed due to insufficient volume of sample plug.; ** sample slide not readable due to poor slide quality; *** slide disqualified due to > 20% squamous cells - = missing data point; SEM = standard error of the mean

MACROPHAGES (%)

ASTHMATICS

Subject	GENDER M=Male F=Female	PLACEBO							INDOMETHACIN						
		PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	PRE-POST AIR	(PRE-POST OZONE)-(PRE-POST AIR)	PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	PRE-POST AIR	(PRE-POST OZONE)-(PRE-POST AIR)
8	F	0*	0**	-	0*	32	-32	32	39	27	12	0*	0**	-	12
9	M	6	3	3	55	9	46	-43	0**	12	-	0*	0**	-	-
11	M	13	20	-7	22	88	-48	39	0	2	-2	67	48	19	-21
12	M	0*	0**	-	0*	84	-84	84	2	2	-	0*	77	-	-
13	F	71	46	23	14	0**	14	-9	11	21	40	40	30	-30	37
14	F	77	31	46	0*	0**	0*	46	22	0*	22	47	58	-8	48
15	F	56	52	4	62	83	-21	25	86	41	47	48	76	-30	67
16	M	20	79	-59	44	37	7	-63	74	20	54	63	63	-13	46
17	M	0*	0**	-	80	79	11	-11	0**	82	-62	36	0***	-27	35
18	F	64	1	63	47	13	34	29	42	9	33	81	60	21	24
19	F	0**	9	-9	9	17	-8	-1	19	8	11	33	42	-9	12
22	F	41	20	21	34	32	2	19	28	20	8	44	57	-13	30
23	F	21	18	3	0**	5	-5	8	11	23	-12	20	41	-21	21
mean		37	28	9	31	38	-7	18	8	8	-12	8	54	-15	23
std error		9	7.8	10	8.5	9.3	10	11.4	9	8	8	8	4.4	21	9.5

Table 4. Individual and group mean (+/- SEM) pre and post exposure macrophages (%) in all exposure and treatment conditions in 13 asthmatic subjects. Also shown are the pre - post exposure differences in all exposure and treatment conditions. Pre exposure = 1 day prior to exposure; Post exposure = 4-6 hrs post exposure. * sample not processed due to insufficient volume of sample plug; ** sample slide not readable due to poor slide quality; *** slide disqualified due to > 20% squamous cells. - = missing data point; SEM = standard error of the mean

PROSTAGLANDIN F2-ALPHA
(PG/ML)

ASTHMATICS

|----------------------------- PLACEBO-----------------------------| |----------------------INDOMETHACIN--------------------|

Subject	GENDER M=Male F=Female	PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	PRE-POST AIR	(PRE-POST OZONE)-(PRE-POST AIR)	PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	(PRE-POST AIR)	(PRE-POST OZONE)-(PRE-POST AIR)
3	F	22	24	-2	2	4	-2	0	16	15	1	39	30	9	-8
4	M	121	64	57	89	112	-23	80	6	88	-82	40	30	9	-91
5	F	107	39	68	208	88	120	-52	0 *	36	-36	66	51	15	-51
6	M	65	104	-39	44	65	-21	-18	65	79	-14	65	139	-74	60
7	M	112	64	48	136	190	-54	102	64	216	-152	160	128	52	-204
10	F	46	62	-16	90	77	13	-29	133	132	1	43	59	-16	17
11	F	63	47	16	504	64	440	-424	64	59	5	128	32	96	-91
mean		77	58	19	153	86	68	-49	50	89	-39	80	67	13	-53
std error		14	10	15	63	21	65	66	18	25	22	20	18	20	33

Table 5. Individual and group mean (+/- SEM) pre and post exposure PGF2-ALPHA (pg) values in all exposure and treatment conditions in asthmatic subjects. Also shown are the pre - post exposure differences in all exposure and treatment conditions. Pre exposure = 1 day prior to exposure; Post exposure = 4-6 hrs post exposure; * sample not processed due to insufficient volume of sample plug; : DIFF=Difference; SEM = standard error of the mean

INTERLEUKIN 8 (PG/ML)

ASTHMATICS

		PLACEBO							INDOMETHACIN						
Subject	GENDER M=Male F=Female	PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	PRE-POST AIR	(PRE-POST OZONE)-(PRE-POST AIR)	PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	PRE-POST AIR	(PRE-POST OZONE)-(PRE-POST AIR)
3	F	283	61	222	37531	0*	37531	-37309	444	432	12	400	1996	-1596	1608
4	M	70	3122	-3052	5048	10000	-4952	1900	0*	598	-598	165	0*	165	-763
5	F	5273	0*	5273	68	5738	-5888	10941	1280	1288	-8	2016	0*	2016	-2024
6	M	2863	1863	1000	2984	736	2228	-1228	5422	851	4571	4411	1875	2535	2038
7	M	3088	0*	3088	6118	2083	4035	-947	1063	2212	-1159	528	3183	-2666	1507
10	F	52	5636	-5584	3431	4217	-786	-4798	3903	610	3292	1438	1889	-452	3744
11	F	3742	198	3546	2199	2786	-587	4133	3139	3000	139	2775	2312	463	-324
mean		2196	1554	642	8194	3651	4543	-3901	2177	1284	893	1876	1609	66	828
std error		785	818	1452	4945	1291	5655	5878	760	367	815	579	449	700	703

Table 6. Individual and group mean (+/- SEM) pre and post exposure IL-8 (pg/ml) values in all exposure and treatment conditions in 7 asthmatic subjects. Also shown are the pre - post exposure differences in all exposure and treatment conditions. Pre exposure = 1 day prior to exposure; Post exposure = 4-6 hrs post exposure; * sample not processed due to insufficient volume of sample plug; DIFF=Difference; SEM = standard error of the mean

190

CHAPTER 5 FIGURES

INFLAMMATORY CELL RESPONSE IN ASTHMATICS

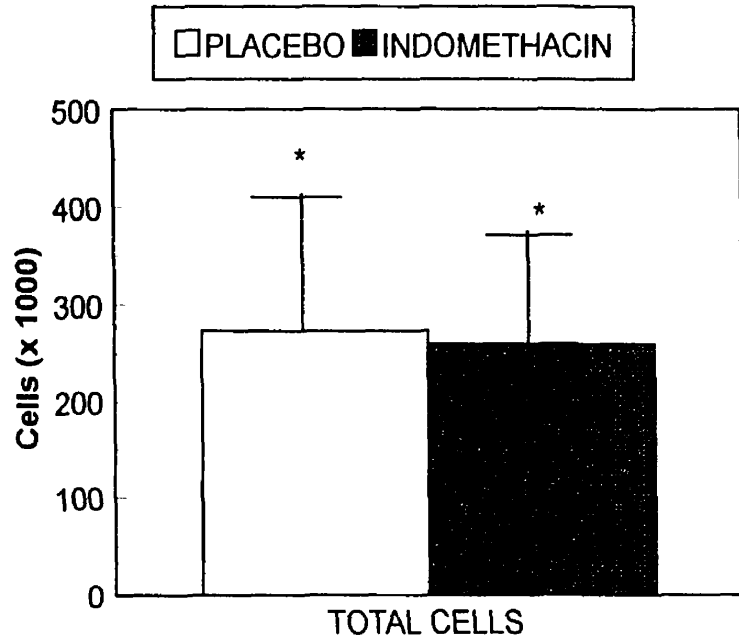Difference In Mean (+/- SEM) Baseline Changes Between Ozone and Air Exposure

Figure 1. Difference in mean (+/- SEM) baseline changes for total cells (TCC) betweenozone and air exposure with placebo (clear bar) and indomethacin (shaded bar) in 13 asthmatic subjects. * Significant difference between ozone and air at the 0.05 level. SEM = standard error of the mean
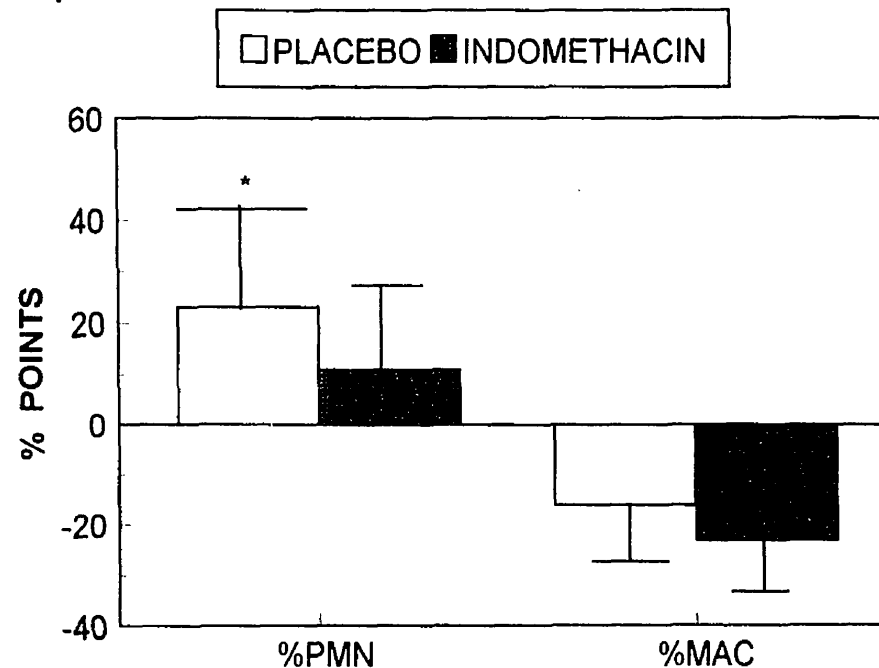
Figure 2. Difference in mean (+/- SEM)baseline changes for neutrophils (PMNs) and macrophages (MACs) between ozone and air exposure with placebo (clear bar) and indomethacin (shaded bar) in 13 asthmatic subjects. * Significant difference between ozone and air at the 0.05 level; SEM = standard error of the mean

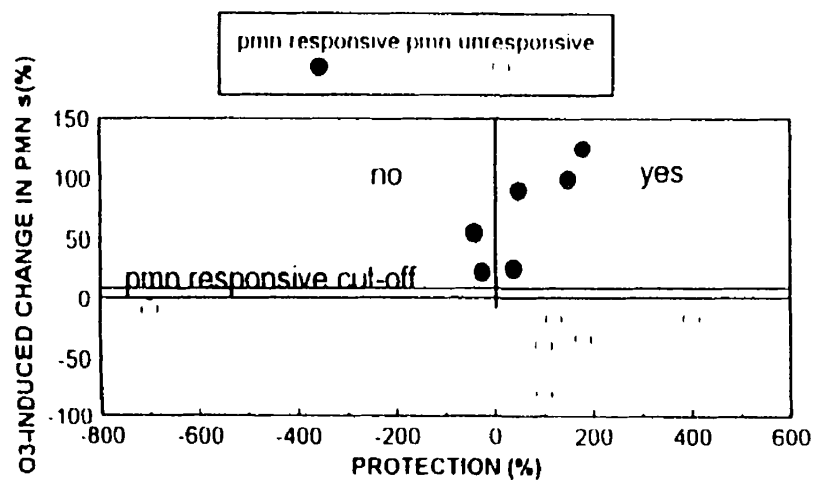# RELATIONSHIP BETWEEN OZONE-INDUCED CHANGES IN CELLS AND PROTECTION AGAINST THESE CHANGES BY INDOMETHACIN
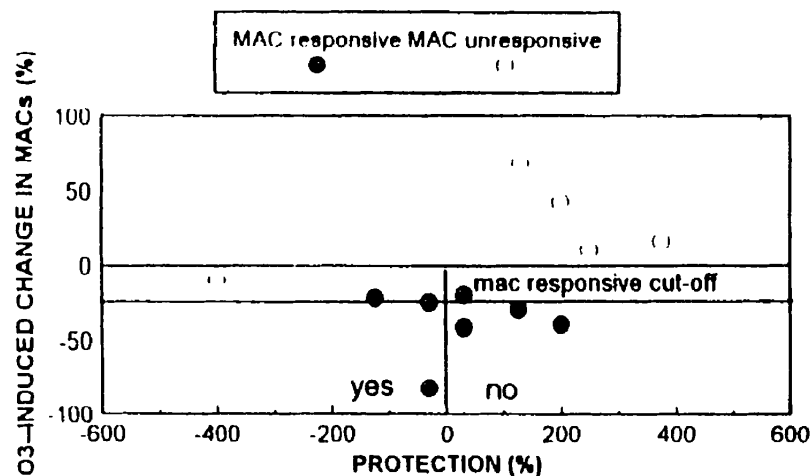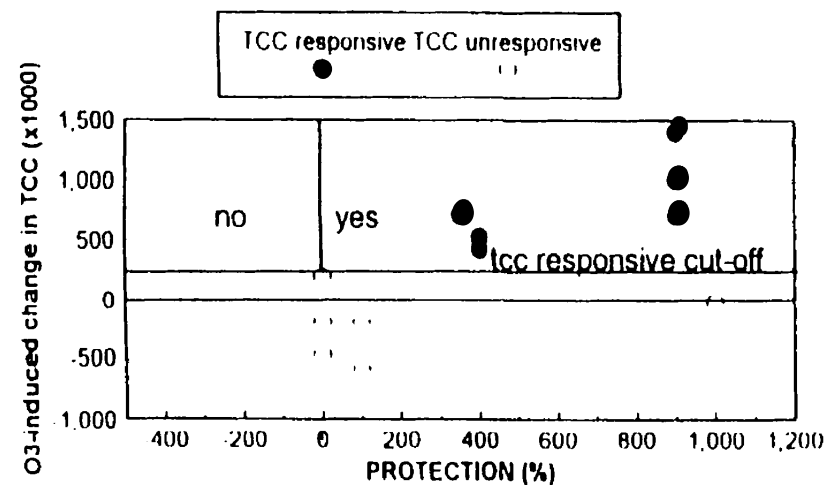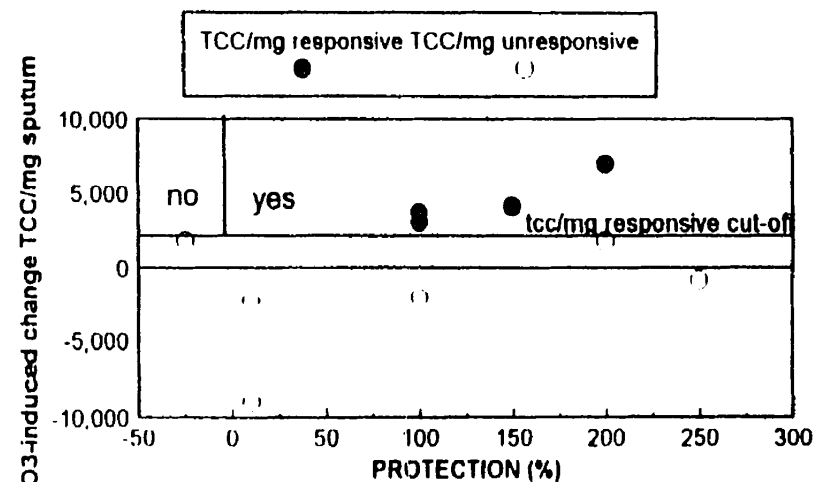

Figure 3A

Figure 3B

Figure 3C

Figure 3D

PROTECTION = (BASELINE CHANGE IN CELLS(PLACEBO)) - (BASELINE CHANGE IN CELLS[INDOMETHACIN])/BASELINE CHANGE IN CELLS(PLACEBO) X 100%
YES INDICATES PROTECTION AGAINST OZONE EFFECT. NO INDICATES NO PROTECTION AGAINST OZONE EFFECT.

BIOCHEMICAL MARKER RESPONSE IN ASTHMATICS

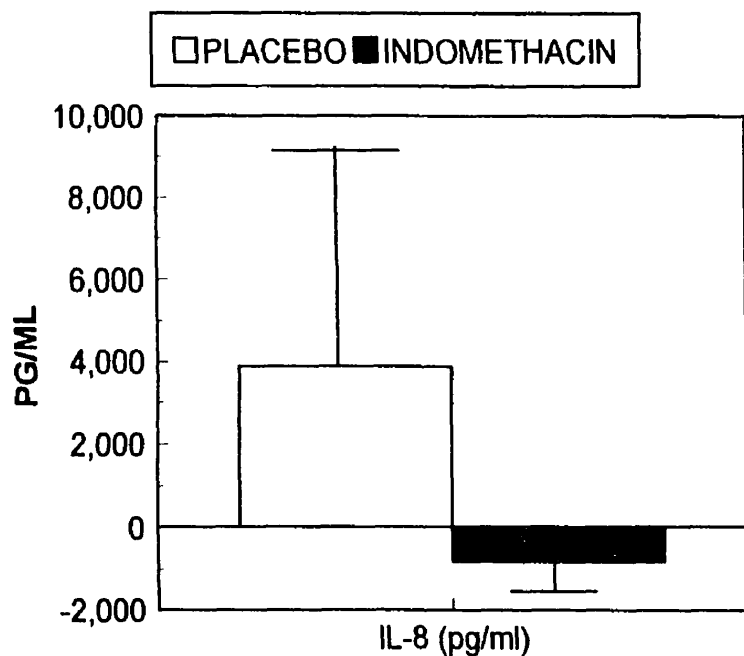## Difference In Mean (+/- SEM) Baseline Changes Between Ozone and Air Exposure

Figure 4A. Difference in mean (+/-SEM)baseline changes between ozone and air exposure for IL-8 with placebo (clear bar) or indomethacin (shaded bar) in 7 asthmatic subjects. Pre-exposure is 1 day prior to exposure. Post-exposure is 4-6 hrs post exposure. IL-8 = Interleukin 8; SEM = standard error of the mean
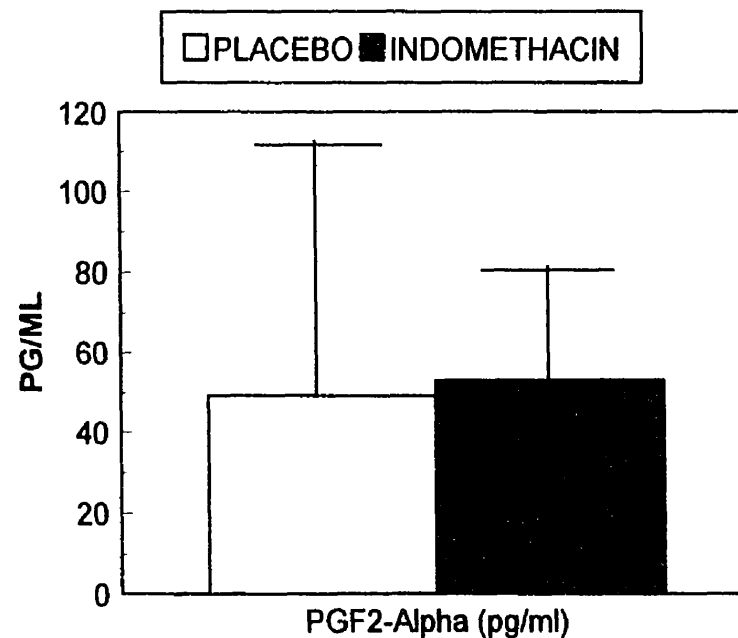
Figure 4B. Difference in mean (+/- SEM) baseline changes between ozone and air exposure for PGF2-alpha with placebo (clear bar) or indomethacin (shaded bar) in 7 asthmatic subjects. Pre- exposure is 1 day prior to exposure. Post-exposure is 4-6 hrs post exposure. PGF2-ALPHA = Prostaglandin F2-alpha; SEM = standard error of the mean

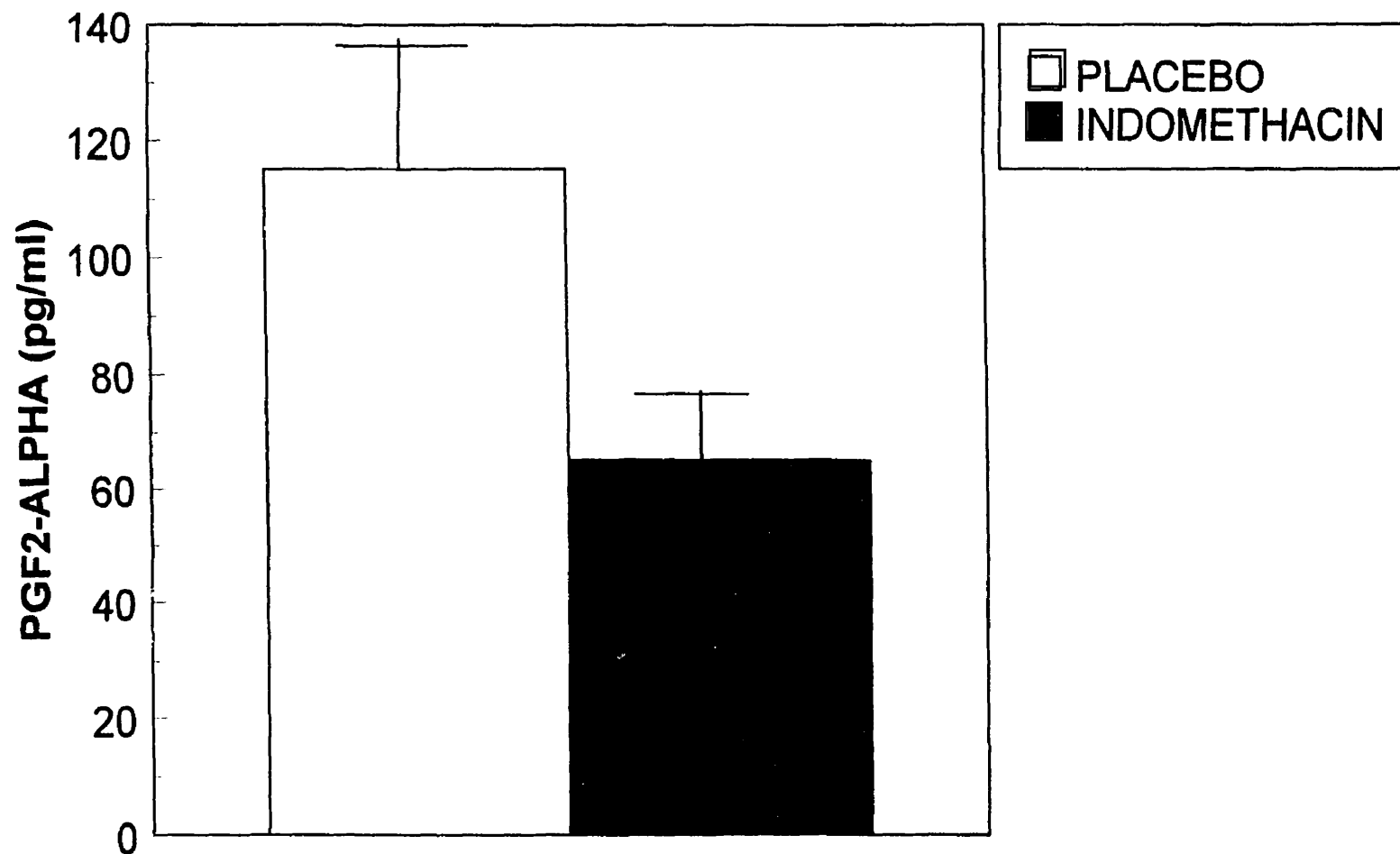# PRE-EXPOSURE MEAN (+/- SEM) PGF2-ALPHA LEVELS IN SPUTUM OF ASTHMATICS

Figure 5. Pre-exposure mean (+/-SEM) sputum PGF2-alpha levels with placebo (clear bar) or indomethacin (shaded bar) in 7 asthmatic subjects. No significant difference was found between placebo and indomethacin (p>0.05); SEM = standard error of the mean

CHAPTER 5 APPENDIX

Reproducibility of Sputum Cell Counts

Test-retest reproducibility was determined from sputum samples collected from the same subjects on two consecutive days. Plots of day 1 versus day 2 sputum total cells, total cells/mg sputum, % polymorphonuclear neutrophils (PMNs), % macrophages (MACs), interleukin 8 (IL-8) and prostaglandin F2-alpha (PGF2-alpha) are shown in Figures A7-12 (pgA205-A206). Significant (p<0.05) correlation coefficients (r) between day 1 and day 2 ranged from 0.7 (p=0.05) in %PMNs to 0.9 (p=0.002) for total cells. Two tailed unpaired T-Test analysis determined that mean +/- Standard Error (SE) cell responses on day 1 versus day 2 were not significantly different for total cells (208,000 +/- 101,000 vs 237,000 +/- 44,500; p=0.81), total cells/mg sputum (730 +/- 250 vs 1050 +/- 210; p=0.31); %PMNs (18 +/- 5 vs 25 +/-5; p=0.23); %MACs (39 +/- 8 vs 54 +/- 4; p=0.09); IL-8 (1638 pg/ml +/- 509 vs 1983 pg/ml +/- 584; p=0.39); and PGF2-alpha (78 pg/ml +/- 17 vs 71 pg/ml +/- 14; p=0.23). The Reliability coefficient (R=within subject variance/total variance) was 0.34 for total cells, 0.36 for total cells/mg sputum, 0.66 for %PMNs, 0.94 for %MACs, 0.46 for % lymphocytes (LYM), 0.31 for % bronchial epithelial cells (Brep), 0.38 for IL-8 and 0.47 for PGF2-alpha.

Observer Variation

To determine the accuracy of the investigator (coded A) with

respect to reading differential cell counts, Pearson Correlation Coefficients (r) were determined with two other independent readers. Eleven or thirteen stained cytospin slides were randomly chosen, coded and given to an experienced hematologist (coded as V) at the Toronto Western Hospital, as well as to a respirologist (coded as B) at the GAGE Research Institute, experienced in performing differential leukocyte counts from BAL samples. V and B were blinded to the conditions of the study. V and B were asked to perform a differential cell count, and express the cells as a percentage of total cells. The blinded readers were asked to count a minimum of 400 cells and report the average number. The same slides were read by A with the same conditions, and statistical comparisons were made. Table A7 (pgA202) reports r values ranging from 0.44 for Brep to 0.96 for MACs.

Sputum Processing

Each subject underwent 8 sputum inductions (2 per week) over the course of 4 study weeks for a total of 104 sputum inductions. Three subjects had successful inductions on all eight occasions, i.e. produced a minimum mucus plug weight of 75 milligrams (mg) for total cell counting, and useable cytospin slides for differential cell counting. Ten subjects had at least one unsuccessful induction out of eight. Of 104 total inductions, 26 (25%) were rejected for any of the following reasons: 1. insufficient weight of mucus plug i.e. < 75mg, to perform a total cell count (TCC) (* Table 1); 2.

unable to read or distinguish leukocyte cell types with Wright stain and use of a magnified microscope (** Tables 3,4); 3. > 20% squamous cell contamination disqualified a slide (*** in Tables 3,4). The rejection breakdown was as follows: * = 10; ** = 11; *** - 5. For * - 10, 9 out of 10 (90%) were in the pre-exposure condition. There were no reported respiratory incidents during sputum induction, i.e. no occasions where a subject's FEV_1 fell below the test termination value of 20% or bothersome symptoms occurred to terminate the procedure. On 3 occasions in 2 subjects the FEV_1 fell greater than 10%, but less than 20%, so that the concentration of hypertonic saline was not increased. Viability of non squamous cells was always greater than 50% as determined by Trypan Blue exclusion and averaged approximately 80%.

We examined cell counts and fluid phase IL-8 and PGF2-alpha reproducibility on two consecutive days within a week, in samples of induced sputum from the same subjects. Results showed that total cells demonstrated high day 1 to day 2 reproducibility (r=0.9), while neutrophils demonstrated relatively low day 1 to day 2 reproducibility (r=0.4). Apart from neutrophils, the day to day reproducibility was quite good (r>0.6) for all other variables examined, including IL-8 (r=0.6) and PGF2-alpha (r=0.6). Our results also showed that the mean values for cells, IL-8 and PGF2-alpha were not statistically significantly different on day 1

versus day 2 (p>0.05). This supports earlier studies by Pizzichini (1995) who examined consecutive day reproducibility within a week in sputum cell counts and showed no significant difference between days for mean total cells, neutrophils and macrophages. Our reliability coefficients (R) for total cells (R=0.34), neutrophils (R=0.70), macrophages (R=0.94), and lymphocytes (R=0.46) compares closely with Pizzichini's (1995) reliability results for sputum total cells (R=0.39), neutrophils (R=0.74), macrophages (R=0.81) and lymphocytes (R=0.43). For fluid phase markers, we observed R values of 0.4 for IL-8 and 0.5 for PGF2-alpha. Although Pizzichini (1995) did not measure IL-8 or PGF2-alpha, she did report higher R values than our study for fluid phase chemicals such as ECP (R=0.85), IL-5 (R=0.78) and tryptase (R=0.65). Our results show that the method of sputum processing used in this study gives reliable cell counts for total cells, neutrophils and macrophages, and somewhat less reliable but adequate readings for IL-8 and PGF2-alpha levels in sputum.

Our results on observer variation with differential cell count readings, show good between reader correlations for neutrophils (mean r=0.89) and macrophages (mean r=0.93), and relatively poor correlation for bronchial epithelial cells (mean r=0.62). Since bronchial epithelial cells were not a major cell type involved in our ozone responses, we did not consider the low r value to be significantly important. Our high r values for neutrophils and macrophages however, demonstrated that these cell types were read

with a high degree of accuracy. This was important since these two cell types were significantly affected following ozone exposure.

CELL READER COMPARISONS

CELL TYPE	A vs V			A vs B			B vs V		
	N	r	P VALUE	N	r	P VALUE	N	r	P VALUE
NEUTROPHIILS	11	0.93	0.0001	13	0.84	0.0003	11	0.90	0.0001
MACROPHAGES	11	0.96	0.0001	13	0.88	0.0001	11	0.94	0.0001
EOSINOPHIILS	11	0.80	0.003	13	0.81	0.001	11	0.87	0.004
BRONCHIAL EPITHELIAL CELLS	11	0.44	0.20	13	0.74	0.004	11	0.67	0.02

Table A7. Pearson correlation coefficients (r) for between reader comparisons of differential cells. Statistically significant comparisons if $P < 0.05$. A = Investigator; V = Heamatologist ; B = Respirologist N= number of slides read;

NOTE TO USERS

Page(s) not included in the original manuscript are unavailable from the author or university. The manuscript was microfilmed as received.

A203

UMI

TEST-RETEST REPRODUCIBILITY OF IL-8 AND PGF2-ALPHA IN ASTHMATIC AND NON-ASTHMATIC SUBJECTS
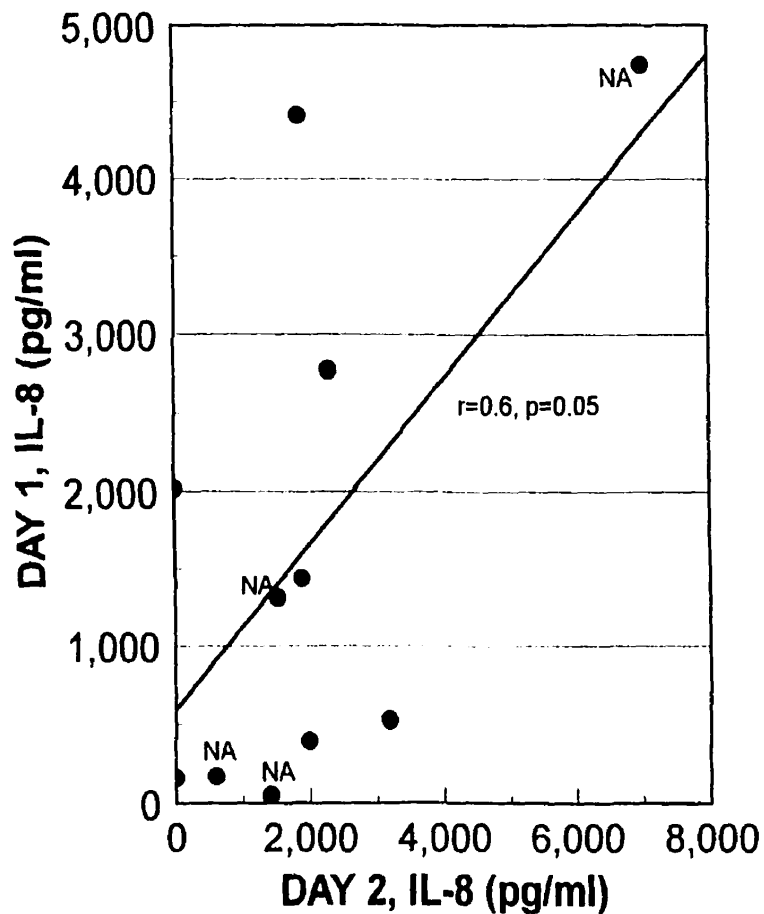

Figure A11. plot of sputum IL-8 (pg) from specimens collected from the same subject on two consecutive days; NA = Non-Asthmatic
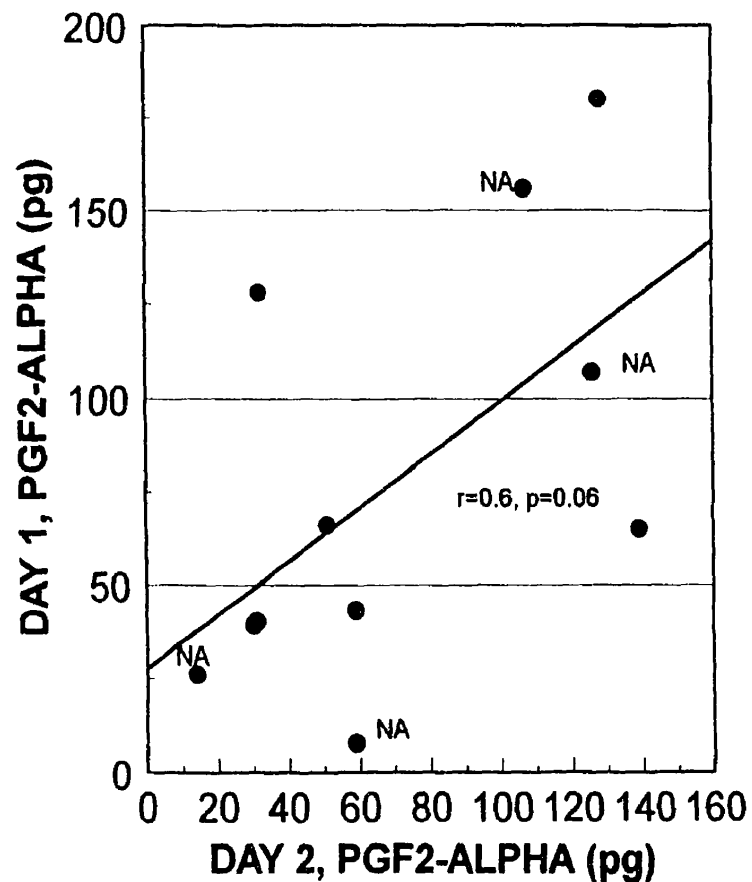
Figure A12. plot of sputum PGF2-alpha (pg) from specimens collected from subjects on two consecutive days; NA = Non-Asthmatic

CHAPTER 5 REFERENCES

Alexis, N,. Silverman, F., Tarlo, S., Corey, P. Effects of indomethacin on acute ozone exposure in asthmatics (Abst) Am J Resp Crit Care Med Vol 151, No 4 April, 1995

Aris, RM., Christian, D., Hearne, PQ., Finkbeiner, WE., Balmes, JR. Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. Am Rev Respir Dis 148: 1363-1372, 1993

Baggiolini, M., Walz, A., Kunkel, S. Neutrophil-activating peptide-1/interleukin-8, a novel cytokine that activates neutrophils. J Clin Invest 84: 1045-1049, 1989

Balkisoon, RC., Clelland, RN., Girgis-Gebardo, A., Popov, T., Dolovich, J., Hargreave, FE. Sputum inflammatory indicies in occupational asthma (OA) (Abst)

Balmes, J., Chen, L., Scannell, C., Tager, I., Christian, D., Hearne, P., Kelly, T., Aris, R. Ozone-induced decrements in FEV1 and FVC do not correlate with measures of inflammation. Am J Respir Crit Care Med, 153: 904-9, 1996

Becker, S., Madden, MC., Newman, SC., Devlin, RB., Koren, HS. Modulaton of human aleolar macrophage properties by ozone exposure in vitro. Toxicol Appl Pharmacol 110: 403-415, 1991

Biscardi, F., Gerrity, T., Strong, A., Garlington, R., Bromberg, P. Ozone uptake in human airways. Am Rev Resp and Crit Care Med. (Abst) Vol 147 No 4 April 1993.

Bousquet, J., Chanez,P., Lacoste, JV., Enander, I., Benge, P., Peterson, C., Ahlstedt, S., Michel, FB., Godard, P. Indirect evidence of bronchial inflammation assessed by titration of inflammatory mediators in BAL fluid of patients with asthma. J Allergy Clin Immunol 88: 1991

Cox, G., Crossely, J., Xing, Z. Macrophage engulfment of apoptotic neutrophils contributes to the resolution of pulmonary inflammation In Vitro. Am J Respir Cell Mol Biol Vol 12, 232-237, 1995

Cromwell, O., Walport, MJ., Morris, HR., Taylor, GW., Hodson, ME., Batten, J., Kay, AB. Identification of leukotrienes D and B in sputum from cystic fibrosis patients. Lancet 2: 164-165, 1981

Devlin, RB., McDonnell, WF., Mann, R., Becker, J., House, DE., Schreinemachers, D., Koren, HS. Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. Am J Respir Cell Mol Biol 4: 72-81, 1991

Devlin, RB., McKinnon, KP., Noah, T., Becker, S., Koren, HS. Ozone-induced release of cytokines and fibronectin by alveolar macrophages and airway epithelial cells. Am J Physiol L66 (Lung Cell Mol Physiol 10) L612-L619, 1994

Driscoll, KE., Schlessinger, RB. Alveolar macrophage stimulated neutrophil and monocyte migration: effects of in vitro ozone exposure. Toxicol Appl Pharmacol 93: 312-318, 1988

Driscoll, KE., Vollmuth, TA., Schlessinger, RB. Acute and subchronic ozone inhalation in the rabbit: response of alveolar macrophages. J Toxicol Environ Health 21: 27-43, 1987

Efthimiadis, A., Pizzichini, E., Kidney, JC., Dolovich, J., Hargreave, FE. Evaluation of an evolving protocol for sputum examination (Abst) J Allergy Clin Immunol Vol 95, No 1, Part 2, 1995

Fahy, JV., Liu, J., Wong, A., Boushey, HA. Comparison of markers of inflammation in induced sputum, bronchial wash and bronchoalveolar lavage from healthy and asthmatic subjects. Am J Respir Crit Care Med; 149: A5571, 1993

Fahy, JV., Wong, H., Liu, J., Boushey, HA. Comparison of samples collected by sputum induction and bronchoscopy from asthmatic and healthy subjects. Am J Respir Crit Care Med 152: 53-8, 1995

Gerrity, T.r., Weaver, R.A., Bernstein, J., House, D.E., O'Neil, J.J. Extrathoracic and intrathoracic removal of ozone in tidal breathing humans. J. Appl Physiol 65:393-400, 1988.

Gibson, PG., Hargreave, FE., Girgis-Gebardo, A., Morris, M., Denburg, JA., Dolovich, J. Chronic cough with eosinophilic bronchitis and examination for variable airflow obstruction and response to corticosteroid. Clin Exp Allergy, 25: 121-32, 1995

Gibson, PG., Girgis-Gebardo, A., Morris, MM., Mattoli,S., Dolovich,J et al. Cellular characteristics of sputum from patients with asthma and chronic bronchitis. Thorax: 44: 693-9, 1989

Gibson, PG., Wong, BJ., Hepperie, MJ., Kline, P., Girgis-Gebardo, A., Guyatt, G., et al. A research method to induce and examine a mild exacerbation of asthma by withdrawl of inhaled corticosteroid. clin Exp Allergy 22: 525-32, 1992

Gwizdala, CJ., Basha, MA., Gross, KB., Haider, AU., Popvich, J. Airway inflammation in asthmatic and healthy volunteers after controlled exposure to ozone and filtered air. (Abst) Am Rev Respir Dis Vol 147, No 4, April 1993

Hamberg, M. Inhibition of prostaglandin synthesis in man. Biochem Biophys Res Commun; 49: 720-6, 1972

Hazucha, MJ., Pape, G., Madden, M., Koren, H., Kehrl, H., Bromberg, P. Effects of cyclooxygenase inhibition on ozone-induced respiratory inflammation and lung function changes (Abst). Am Rev Respir Dis 143: A701, 1991

Hazucha, MJ., Folinsbee, LJ., Seal, E. Jr. Effects of steady state and variable ozone concentration profiles on pulmonary function Am Rev Respir dis 146: 1487-1493, 1992

Henke, D., Danilowicz, RM., Curtis, JF., Boucher, RC. Eling, MJ. Metabolism of arachidonic acid by human nasal and bronchial epithelial cells. Arch Biochem Biophys 267: 426-436, 1988

Higgs, GA., Harvey, EA., FErreira, SH., Vane, TR. The effect of antiinflammatory drugs on the production of prostaglandins in vivo. In: Samuelson B, Paoletti, R, eds. Advances in prostaglandins and thromboxane research, Vol 1. New York, Raven Press, 105-110, 1976

Higgs, GA., Bax, CMR., Moncada, S. Inflammatory properties of lipoxygenase products and the effects of indomethacin and BW755C on prostaglandin production, leukocyte migration and plasma exudation in rabbit skin. In: Samuelson, B., Paoletti, R. eds Leukotrienes and other lipoxygenase products. New York: Raven Press, 331-9, 1982

Hu, S.C., Ben-Jebria, A., Ultman, J.S. Longitudinal distribution of ozone absorption in the lung: effects of respiratory flow. J Appl Physiol 77 (2): 574-583, 1994.

Hunter, JA. Finkbeiner, WE., Nadel, JA., Goetzl, EJ., Holtzman, MJ. Predominant generation of 15-lipoxygenase metabolism of arachidonic acid by epithelial cells form human trachea. Proc Natl Acad Sci USA 82: 4633-4637, 1985

Hurley, JV. Termination of acute inflammation. I. Resolution of inflammation. In Acute Inflammation 2nd edition, JV Hurley, ed. Chruchill Livingstone, London, 109-117, 1983

Jorens, PJ., Richmann-Eisenstat, J., Housset, B., Graf, P., Ueki, I., Olesch, J,. Nadel, J. Interleukin-8 induces accumulation but nont protease secretion in the canine trachea. Am J Physiol 263 (Lung Cell Mol Physiol 7) L708-L713, 1992

Kehrl, HR., Vincent, LM., Kowalsky, RJ., Horstman, DH., O'Neil, J., McCartney, WA., Bromberg, PH. Ozone exposure increases respiratory epithelial permeability in humans. Am Rev Respir Dis 135, 1174, 1987

Koren, H., Devlin, RB., Graham, D., Mann, R., McGee, M., Horstman, DH., Kozumbo, WJ., Becher, S., House, DE., McDonnell, WF., Bromberg, P. Ozone-induced inflammation in the lower airways to human subjects Am Rev Respir Dis 139, 407, 1989

Koyama, S., Rennard, SI., Leikauf, GD., Stoji, S., Von Essen, L., Claasen, Robbins, RA. Endotoxin stimulates bronchial epithelial cells to release chemotactic factors for neutrophils. J Immunol 147: 4293-4301, 1991

Kulle, TJ., Sauder, LR., Hebel, JR., Chatham, MD. Ozone response relationships in healthy nonsmokers. Am Rev Respir Dis 132, 36, 1985

Kwon, OJ., Au, BT., Collins, PD., Baranuick, JN., Adcock, IM., Chung, KF., Barnes, P. Inhibition of IL-8 expression by dexamethasone in human cultured airway epithelial cells. Immunology 81: 389-394, 1994

Leikauf, GD., Driscoll, KE., Wey, HE. Ozone-induced augmentation of eicosanoid metabolism in epithelial cells from bovine trachea. Am Rev Respir Dis 137: 435-442, 1988

Li, Z., Daniel, EE., Lane, CG., Arnout, MA., O'Byrne, PM. Effect of an anti-Mol MAb on ozone-induced airway inflammation and airway hyperresponsiveness in dogs. Am J Physiol (Lung Cell Mol Physiol) 263: L723-L726, 1992

Maestrelli, P., Saetta, D., DiStefano, A., Caleagni, P., Turato, G., Mapp, LE., Fabbri, LM. Comparison of cell counts in sputum, bronchoalveolar lavage (BAL) and bronchial biopsies in asthmatics (Abst) J Allergy Clin Immunol, 1995

Maestrelli, P., Calcagni, PG., Saetta, M., DiStefano, A., Hosselet, JJ., Santonastaso, A., Fabbri, LM., Mapp, CE. Sputum eosinophelia after asthmatic responses induced by isocyanates in sensitized subjects. Clin Exp Allergy Vol 24, 1994

Malek, M., Utell, MJ., Looney, RJ., Morrow, PE, Levy, PL., Tsai, Y., Barth, R., Frampton, MW. Does exposure to ozone induce systemic acute phase response in humans (Abst) Am J Respir Crit Care Med vol 151 No 4, April 1995

McCain, RW., Holden, EP. Blackwell, TR., Christmas, JW. Leukotriene B4 stimulates human polymorphonuclear leukocytes to synthesize and release IL-8 in vitro. Am J Respir Cell Mol Biol 10: 651-657, 1994

McDonnell, W., Koren, H., Devlin, R., Salaam, A., Ives, P., O'Neil, J. Biochemical and cellular correlates of changes in pulmonary function and symptoms in humans exposed to ozone. Am Rev Respir Dis 141: A72, 1990

Murlas, CG. Enviornmental airway injury: mucosoal changes and airway hyperreactivity. In SG Farmer and DNP Hay, eds. The airway epithelium. Marcel Dekker, New York 213-253

Nakamura, H., Yoshimura, K., Jaffe, H., Crystal, RG. Interleukin-8 gene expression in human bronchial epithelial cells. J Biol Chem 266: 19611-19617, 1991

Nakamura, H., Yoshimura, K., McElvaney, NG., Crystal, RG. Neutrophil elastase in respiratory epithelial lining fluid of

individuals with cystic fibrosis induces IL-8 gene expression in a human bronchial epithelial cell line. J Clin Invest 89: 1478-1484, 1992

Oosting, RS., Van-Golde, LM., Van-Bree, L. Species differences in impairment and recovery of alveolar macrophage function following single and repeated ozone exposures. Toxicol Appl Pharmacol 110: 170-178, 1991

Ozaki, T., Hayashi, TH., Tani, K., Ogushi, F., Yasuoka, S, Ogura, T. Neutrophil chemotactic factors in the respiratory tract of patients with chronic airway diseases or idiopathic pulmonary fibrosis. Am Rev Respir Dis 145: 85-91, 1992

Pin, I., Gibson, PG., Kolendowicz, R., Girgis-Gebardo, A., Denburg, J., Hargreave, FE., et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. Thorax: 47, 25-9, 1992

Pin, I., Freitag, AP. O'Byrne, P., Girgis-Gebardo, A., Watson, RM., Dolovich, J et al., Changes in the cellular profile of induced sputum after allergen-induced asthmatic responses. Am Rev Respir Dis 145: 1265-9, 1992

Pizzichini, MMM., Pizzichini, E., Kidney, MB., Popov, T., Efthimiadus, A., Kolendowicz, R., Dolovich,J., Hargreave, FE. Induced sputum reproducibility of cell counts on cytospins (Abst) J Allegy Clin Immunol vol 95, No 1, Part 2, 1995

Pizzichinni, E., Kidney, J., Popov, T., Hussack, P., Efthimiadis, A., Cox, G., O'Byrne, PM., Dolovich, J., Hargreave, FE. Indicies of airway inflammation in asthma: Comparison between induced sputum and bronchoalveolar lavage (Abst) Am J Respir Crit Care Med Vol 153, No 4, April, 1996

Popov, TA., Pizzichinni, MM., Pizzichinni, E., Kolendowicz, R., Punthakee, Z., Dolovich, J,. Hargreave, FE. Some technical factors influencing the induction of sputum for cell analysis. Eur Respir J, 8:559-565; 1995

Randall, RW., Eakins, KE., Higgs, GA., Salmon, JA., Tateson, JE. Inhibition of arachidonic acid cyclooxygenase and lipoxygenase activities of leukocytes by indomethacin and compound BW 755C. Agents Actions; 10:553-5, 1980

Richmann-Eisenstat, J., Jorens, P., Hebert, L., Ueki, I., Nadel, J. Interleukin-8: an important chemoattractant in sputum of patients with chronic inflammatory airways. Am J Physiol 264(Lung Cell Mol Physiol 8): L413-L418, 1993

Sampson, AP., Spencer, DA., Green, LP., Piper, PJ., Price, JF. Leukotrienes in the sputum and urine of cystic fibrosis children. Br J Clin Pharmacol 30: 861-869, 1990

Scannell, CH., Aris, R., Christian, D., Hearne, P., Tager, I., Ferrando, R., Chen, L., Balmes, JR. Ozone-induced airway inflammation is not correlated with physiological responses (Abst) Am J Respir Crit Care Med vol 149 No 4 April, 1994

Scannell, C., Chen, L., Aris, R,. Tager, I., Christian, D., Ferrando, R., Welch, B., Kelly, T., Balmes, J. Greater ozone-induced inflammatory responses in subjects with asthma. Am J Respir Crit Care Med 154: 24-9, 1996

Schelegle, ES., Siefkin, AD., McDonald, RJ. Time course of ozone-induced neutrophilia in normal humans. Am Rev Respir Dis 143: 1353-1358, 1991

Schoog, M., Laufen, H., Pessain, P. A comparison of the pharmikokinetics of piroxicam with those of plain and slow release indomethacin. Eur J Rheumatol Inflamm; 4: 298-302, 1981

Schuster, A., Ueki, I., Nadel, JA. Cystic fibrosis sputum induces a secretatory response from tracheal glandserous cells that can be inhibited by antiproteases. Pediatr Pulmonol 56: 299, 1991

Seltzer, J., Bigby, BG., Stulburg, M., Holtzman, J., Wadel, JA. Ozone-induced change in bronchial reactivity to methacholine and airway inflammation in humans J Appl Physiol 60, 1321, 1986
Kramer, MS., Feinstein, AR. Clinical biostatistics LIV: The biostatistics of concordance. Clin Pharmacol Ther: 29: 111-23, 1981

Seltzer, J., Bigby, BG., Stulburg, M., Holtzman, J., Wadel, JA. Ozone-induced change in bronchial reactivity to methacholine and airway inflammation in humans J Appl Physiol 60, 1321, 1986

Spanevello, A., Sharara, AM., Migliori, GB., Bridge, P., Myiris, JD., Bianchi, A., Ballandini, L., Neri, M., Ind, PW. Reproducibility of induced sputum in asthmatic and normals (Abst) Am J Respir Crit Care Med Vol 153, No 4, April, 1996

Torres, A., Utell, MJ., Morrow, PE., Voter, KZ., Tsai, Y., Barth, R., Frampton, MW. Airway inflammatory responses to ozone: relationship to lung function changes in smokers and nonsmokers. (Abst) Am J Respir Crit Care Med Vol 151 No 4 April, 1995

Vagaggini, B., Taccola, M., Giannini, D., Di Franco, H., Cannevali, S. Gianchetti, S., Bacci, E., Bancalari, L., Bartoli, ML., Dente, FL., Pagiaro, PL., Giantini, C. The effects of ozone exposure in the inflammatory cell percentages in the induced sputum of asthmatic subjects (Abst). Am J Respir Crit Care Med Vol 153 No 4 April 1996

Verbesselt, R., Tjandranga, TB., Mulllie, A. et al. Comparative multiple dose kinetics of two formulations of indomethacin. Eur J Clin Pharmacol; 24: 563-8, 1983

Weinmann, GG., Lin, M., Gerbase, MW., Foster, WM., Frank, R. Baseline peripheral airways resistance is significantly associated with spirometric responsiveness to ozone in humans. (Abst) Am Rev Respir Dis Vol 147, No 4, 1993

Weinmann, G., Liu, MC., Proud, D., Hubbard, W., Frank, R. Repeated ozone exposure in humans: Inflammatory, small and peripheral airway responses (Abst) Am J Respir Crit Care Med Vol 151 No 4 April, 1995

Weinmann, GG., Liu, M., Proud,D., Hubbard, WC., Frank, R. Dissociation between peripheral airways resistance and inflammatory response to ozone in humans (Abst) Am J Respir Crit Care Med Vol 149 No 4 April 1994

Wong, HH., Liu, J., Bouchey, HA., Fahy, JV. The effect of ozone exposure on markers of inflammation in induced sputum. (Abst) Am J Respir Crit Care Med vol 151, No 4 April, 1995

Yoshimura, T., Matsushima, S., Tanaka, S., Robinson, E., Appella, E., Oppenheimer, JJ., Leonard, EJ. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. Proc Natl Acad Sci USA 84: 9233-9237, 1987

CHAPTER 6

OUTCOME MEASURE ASSOCIATIONS: EFFECTS OF INDOMETHACIN PRETREATMENT

INTRODUCTION

Several recent studies have shown that human ozone exposures capable of causing decrements in pulmonary function, also produce cellular and biochemical evidence of inflammation in broncho-alveolar lavage (BAL), proximal airway lavage (PAL) (Balmes, 1996; Aris, 1993; Koren, 1989; Seltzer, 1986) and sputum samples (Vagaggini, 1996; Wong, 1995). Several studies have examined the relationship between pulmonary function changes and markers of inflammation following ozone exposure (Balmes, 1996; Hazucha, 1996; Morrison, 1995; Aris, 1993; Schelegle, 1991; Scannell, 1994; Weinmann, 1993; Koren, 1989; Frampton, 1994). Most recently, Hazucha (1996) demonstrated no correlation between ozone-induced spirometric decrements and changes in BAL fluid components, such as neutrophils, PGE2, or TXB2 in healthy subjects exposed to 400 ppb ozone. Hazucha used 2 hour exposures with a mean exercise minute ventilation of 60 litres/minute. Recently, Balmes (1996) concluded that levels of ozone-induced symptoms and BAL inflammatory markers, including Interleukin 8 (IL-8), were not correlated with the magnitude of decrements in Forced Expiratory Volume in 1 second (FEV_1) and Forced Vital Capacity (FVC). Balmes also concluded that FEV_1 sensitivity to ozone was not related to inflammatory markers. An early report by Schelegle (1989) also found no correlation between plasma PGF2-alpha and declines in FVC or FEV_1. Weinmann (1994) recently reported that changes in BAL cell counts and inflammatory mediators including Prostaglandin F2-alpha (PGF2-alpha), were not correlated with peripheral airway resistance.

Although the majority of studies in the literature indicate that no positive relationship exists between ozone-induced decreases in pulmonary function and increases in inflammatory cells and biochemical markers, some recent studies have suggested otherwise. Morrison (1995) examined the relationship between pulmonary function changes, BAL inflammation, epithelial permeability and oxidant status in the airspaces of non-smokers exposed for 1 hour to 400 ppb O_3. He reported that O_3-induced bronchoconstriction (9% fall in FEV_1) was associated with PMN influx into the airspaces. Recently, Keatings (1996) reported a significant correlation between sputum neutrophils and the percent predicted FEV_1 in patients with Chronic Obstructive Pulmonary Disease (COPD)(r = -0.62, p<0.05), and McBride (1994) demonstrated a significant correlation between inflammatory cells and biochemial markers of upper airways inflammation in asthmatics exposed for 90 minutes to 240 ppb ozone. He demonstrated a significant correlation between IL-8 levels and total cells/ml (R=0.8) in nasal lavage samples taken 10 minutes post exposure. Apart from these few recent studies, several inverse trends between pulmonary function decline and increased cell responses following ozone have been demonstrated. For example, Balmes (1996) observed a trend toward (r=-0.16) an inverse relationship between FEV_1 decrement and the bronchial fraction of the BAL neutrophil response to ozone. Schelegle (1991) showed a trend (r=-0.8, p<0.1) toward an inverse relationship between maximal decrement in FEV_1 and peak neutrophilia in bronchial fraction samples taken 6 hours post ozone

exposure (300 ppb, 1 hour) in 5 healthy subjects. These results appeared in studies where inflammatory markers were measured in the bronchial fraction of the BAL fluid, and not the entire BAL fluid sample itself. As stated earlier, the bronchial fraction of BAL fluid samples segments of the airways more proximal to the lower conducting airways where the majority of ozone is absorbed.

Evidence from Seltzer (1986) and Schelegle (1987) has suggested that prostaglandins, including PGF2-alpha, PGE2, and Thromboxane B2 (TXB2), are released in the airways following acute ozone exposure, and those that cause bronchoconstriction such as PGF2-alpha and PGE2 contribute to reductions in inspiratory capacity. This leads to reductions in FVC and FEV_1. Hazucha (1989) demonstrated that ozone-induced decreases in FEV_1 and FVC are neurally mediated events that involve activation of submucosal irritant receptors and stimulation of afferent C-fibers which lead to involuntary inhibition of inspiratory effort. PGF2-alpha has been found to stimulate pulmonary neural afferents that induce several responses characteristic of ozone exposure, such as altered ventilatory pattern (Coleridge, 1976; Roberts, 1985). Considering the above evidence, we speculated whether ozone-induced changes in PGF2-alpha were related to ozone-induced changes in FVC or FEV_1.

Currently the weight of evidence suggests that the magnitude of the pulmonary function decline following ozone exposure, is not related to the increase in lower airway inflammatory indices. Our study

will explore new relationships between pulmonary outcome measures not previously reported, ie. small airways flow variables, and inflammatory markers following ozone exposure. Since these relationships are exploratory in nature and serve only to guide future research questions, we have made no adjustment for multiple statistical comparisons and acknowledge a certain degree of intercorrelation among outcome measures.

PURPOSE

First, to explore whether markers of airway inflammation are associated with changes in pulmonary function following ozone exposure. Second, to determine whether cyclooxygenase metabolites play a role in any of the observed associations.

HYPOTHESIS

First, no significant associations will be found between the cellular and biochemical responses to acute ozone exposure and the ozone-induced changes in pulmonary function. Second, indomethacin pretreatment will not significantly affect any observed associations between outcome measures.

OBJECTIVES

Derive Pearson correlation coefficients (r) to measure the

association between ozone-induced changes in cellular and biochemical inflammatory end points and changes in pulmonary function following acute ozone exposure. Use multiple linear regression models to determine whether relationships between outcome measures are significantly different with placebo and indomethacin.

METHOD

Subjects

For cellular outcome measures, 13 asthmatic subjects were used for comparative analyses. For biochemical outcome measures, 7 asthmatic subjects were used for comparative analyses.

Sputum Induction with Hypertonic Saline

The method of sputum induction (SI) with hypertonic saline and analysis of subsequent sputum samples is described in detail in Chapter 6.

Statistical Analysis

Multiple linear regression analysis and Pearson correlation coefficients (r) were used to determine all associations between dependent variables. Paired T-Tests and multiple linear regression

analysis were used to determine whether changes from pre exposure baseline were significantly different with ozone and air (ie. Ozone response), and whether the ozone response was significantly different with indomethacin and placebo. Multiple linear regression analysis was used to determine whether an association between two ozone responses was significantly different with placebo or indomethacin. The regression models and SAS program appears in the Appendix at the end of this chapter.

RESULTS

Figures 1-5 (pg226-227)show scatter plot diagrams (with best fitting lines) of the associations between ozone induced decreases in pulmonary function, and ozone-induced changes in inflammatory cells (Figures 1-3) and PGF2-alpha (Figures 4-5). Pearson correlation coefficients (r) with accompanying p values are shown for each best fitting line of the data points. A * indicates a significant r value at the 0.05 level, while a # symbol indicates if the outcome measure comparisons differed significantly between placebo and indomethacin at the 0.05 level.

With placebo, no significant associations (p>0.05) were found for any set of variable comparisons between inflammatory cells and pulmonary function; however a trend toward a significant positive association was found between the ozone-induce decline in $\dot{V}40P$ and the ozone-induced increase in %PMNs (r=0.6, p=0.06) (Figure 2).

With indomethacin pretreatment, significant positive associations (*) were found between the ozone-induced increase in TCC/mg sputum and the ozone-induced declines in $\dot{V}50$ (r=0.6, p=0.02) (Figure 1) and PEFR (r=0.6, p=0.04)(Figure 3).

Significant differences (#) between placebo and indomethacin were found for $\dot{V}40P$ vs %PMN (p<0.05) (Figure 2), and TCC/mg sputum vs PEFR (p<0.01) (Figure 3).

Figures 4 and 5 show significant inverse associations between the ozone-induced increase in PGF2-alpha and the ozone-induced fall in $\dot{V}50$ (r=-0.97, p=0.0002) (Figure 4), and $\dot{V}25$ (r=-0.91, p=0.005) (Figure 5). A significant difference (#) between placebo and indomethacin was found for PGF2-alpha vs $\dot{V}50$ (p<0.02) (Figure 4).

Figure 6 (pg230) shows a scatter plot diagram of the association between pulmonary function protection and the ozone-induced changes in total inflammatory cells. Protection is defined as the difference in the ozone response between placebo and indomethacin. A significant inverse association was found for $\dot{V}50$ protection vs TCC/mg (r=-0.5, p=0.003).

DISCUSSION

Our results demonstrated no significant association between decrements in FEV_1 or FVC and sputum neutrophils following acute

ozone exposure with placebo or indomethacin. These results support several recent studies which demonstrated no association between decrements in FEV_1 and FVC and BAL fluid neutrophils following acute ozone exposure (Hazucha, 1996; Balmes, 1996; Aris, 1993; Schelegle, 1991; Scannell, 1994; Weinmann, 1993; Koren, 1989; Frampton, 1994). With respect to other indicators of spirometric performance however, we report significant positive associations between ozone-induced declines in expiratory flows, ($\dot{V}50$, PEFR) and increased total cells/mg with indomethacin. We also report a trend toward a significant positive association between ozone-induced decreased small airways flow ($\dot{V}40P$) and increased percent neutrophils (p=0.06) with placebo. In each case the ozone-induced decline in expiratory flow was positively associated with the ozone-induced increase in inflammatory cells. This occurred in the presence of placebo for V40P vs neutrophils, and indomethacin for $\dot{V}50$ vs TCC/mg, and PEFR vs TCC/mg. For $\dot{V}40P$ vs neutrophils and PEFR vs TCC/mg, indomethacin significantly changed the slope of the association between the two outcome measures in the opposite direction. This suggests that whether or not cyclooxygenase metabolites affect individual cell types or individual pulmonary function measurements following ozone exposure, they do appear to play a significant role when the relationship between airway response variables is examined. It is interesting to observe that neither FVC or FEV_1 were directly associated with any changes in inflammatory cells, whereas expiratory flow rates were related to changes in cells following ozone. One explanation for this may be

that the mechanism governing the FEV_1 and FVC response to ozone is a neurally mediated one affecting inspiratory effort (Hazucha, 1989). As a result, the level of lower respiratory tract inflammation may have less of a direct association with inspiratory capacity and more of a direct association with the airways themselves. For example, a decrease in small airways flow may be due to a decrease in airway caliber from mucosal edema which correlates with increased inflammation. In addition, inflammation may have a stronger temporal relationship with changes in expiratory flow rates, since recovery of flow rates, especially small airways flow rates following ozone exposure is slower than FVC or FEV_1 (Weinmann, 1995). Inflammation, as well as decrements in small airways flow, can persist for up to 24 hours post exposure (Weinmann, 1995).

Our data showed an inverse relationship between the decline in small airways flow ($\dot{V}50$, $\dot{V}25$) and the increase in PGF2-alpha following ozone exposure. This relationship was maintained when the outlying subject was removed from the anlysis. The inverse association may reflect the different measurement time points of pulmonary function and mediators. Pulmonary function measurements reflect a momentary functional status of the airways and were performed immediately at the end of two hours of exposure, whereas PGF2-alpha levels were measured 4 hours post exposure and are likely in a constant state of flux. Declines in expiratory flow rates immediately following ozone exposure have just started to

recover to baseline and therefore closely reflect the magnitude of the ozone response on the airways. Since PGF2-alpha is locally released in the airways consequent to ozone exposure, it also better reflects the magnitude of the ozone response immediately post exposure versus 4-6 hours later. Therefore, the different measurement time points could represent very different airway states. Our results did not show any significant associations involving IL-8 and pulmonary function.

We speculated whether protection against ozone-induced pulmonary function decline was associated with ozone-induced increases in inflammatory cells. Our data revealed that with respect to $\dot{V}50$, spirometric protection was significantly positively associated with a smaller increase in total cells/mg sputum. These data may have important implications on the chronic effects of ozone exposure in asthmatics since they already have a relatively higher pre-existing state of airway inflammation than healthy subjects. Methods to limit the level of airway inflammation by protecting declines in expiratory flow rates should be considered to reduce the risk of chronic health effects from ozone exposure in asthmatics.

CONCLUSION

Decrements in FVC and FEV_1 following acute ozone exposure are not associated with ozone-induced increases in neutrophils. However, ozone-induced decrements in large airways expiratory flow (PEFR)

appeared to be related to increases in total cells, and ozone-induced reductions in small airways expiratory flows ($\overset{\bullet}{V}$40P, $\overset{\bullet}{V}$50) appeared to be related to increases in neutrophils and total cells respectively. Cyclooxygenase metabolites of arachidonic acid appear to play a role in the association between these outcome measures, as indomethacin significantly changed the slope of the relationship between $\overset{\bullet}{V}$40P and PMNs and PEFR and TCC/mg. We reported an inverse association between PGF2-alpha and $\overset{\bullet}{V}$50 and PGF2-alpha and $\overset{\bullet}{V}$25 following ozone exposure. The inverse nature of the associations were unexpected and not explained biologically, and may reflect different measurement times of flow rates and PGF2-alpha levels. Finally, our data showed that protecting the decline in expiratory flow rates following ozone exposure, results in smaller inflammatory cell increases from baseline following ozone exposure. We suggest this may have important chronic health implications in asthmatics who already have pre-existing airway inflammation as a major feature of their disease.

CHAPTER 6 FIGURES

ASSOCIATION BETWEEN CHANGES IN PULMONARY FUNCTION AND CHANGES IN INFLAMMATORY CELLS FOLLOWING OZONE EXPOSURE

Figure 1. Ozone-induced decline in in V50(%) vs ozone-induced increase Total Cells/mg sputum with indomethacin following a 2 hr exposure to 400 ppb ozone. r = correlation coefficient (p value). * significant at 0.05 level.
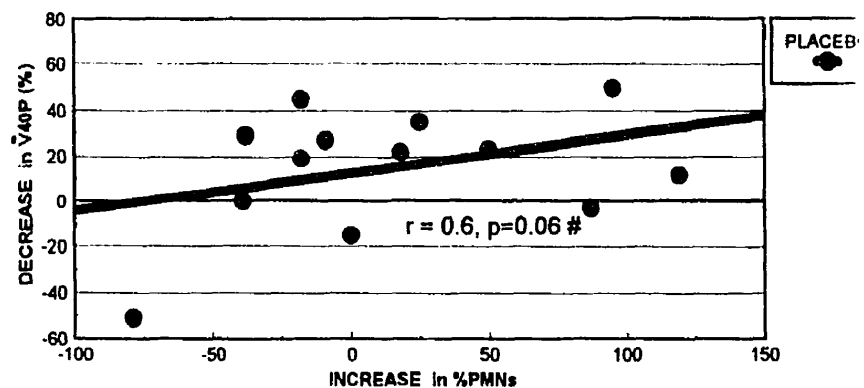
Figure 2. Ozone-induced decline in V40P(%) vs ozone-induced increase in %PMNs with placebo (closed circle) following a 2 hr exposure to 400 ppb ozone. r = correlation coefficient, p value. # significant difference between placebo and indomethacin (p=0.04)
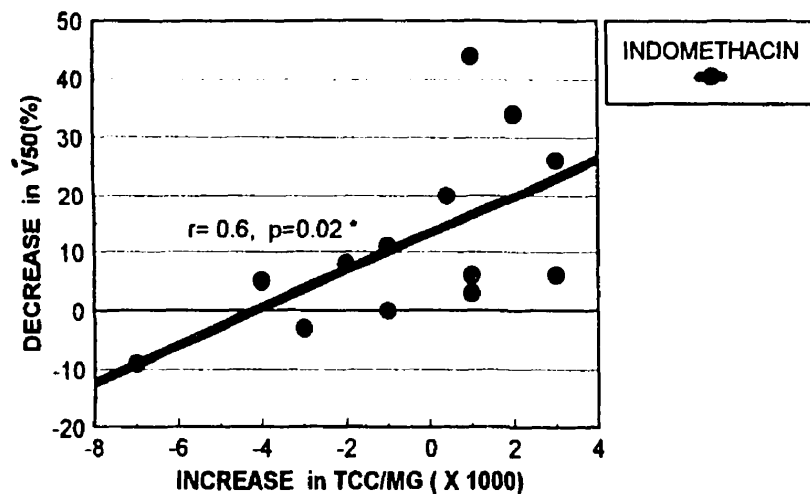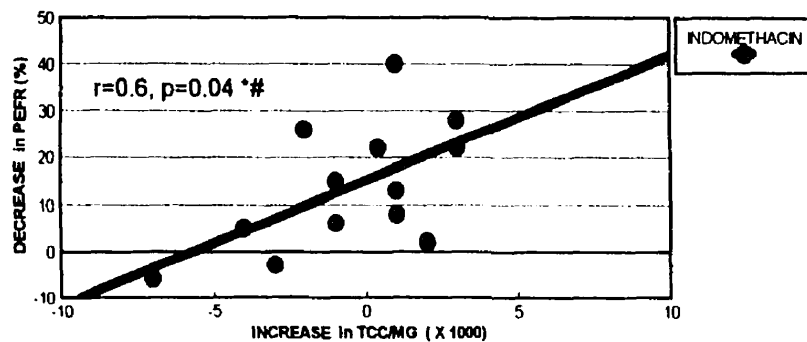

Figure 3. Ozone-induced decline in PEFR (%) vs ozone-induced increase in Total Cells/mg sputum with indomethacin following a 2 hr exposure to 400 ppb ozone. r=correlation coefficient, p value. * significant at the 0.05 level. # significant difference between placebo and indomethacin (p=0.01)

ASSOCIATION BETWEEN CHANGES IN PULMONARY FUNCTION AND CHANGES IN PGF2-ALPHA FOLLOWING OZONE EXPOSURE

PLACEBO

r = -0.9, p=0.0002 *#
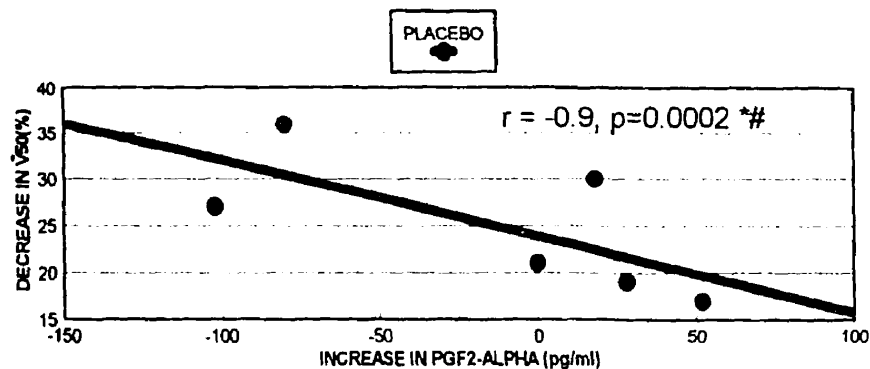
Figure 4. Ozone-induced decline in V̇50 (%) vs ozone-induced increase in PGF2-ALPHA (pg/ml) following a 2 hr exposure to 400 ppb ozone with placebo. r = correlation coefficient p value. * significant at the 0.05 level. # Significant difference between placebo and indomethacin at the 0.05 level.

PLACEBO

r = - 0.9, p= 0.005 *
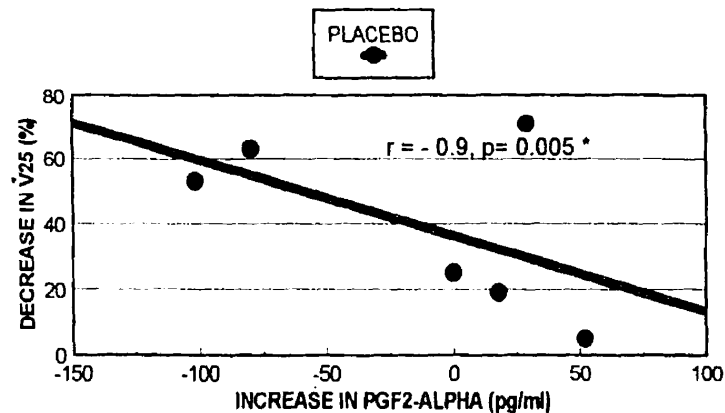
Figure 5. Ozone-induced decline in V̇25(%) vs ozone-induced increase in PGF2-ALPHA (pg/ml) following a 2 hr exposure to 400 ppb ozone with placebo . r= correlation coefficient p valure. * significant at the 0.05 level.

ASSOCIATION BETWEEN PULMONARY FUNCTION PROTECTION AND OZONE-INDUCED INFLAMMATORY CELL RESPONSES
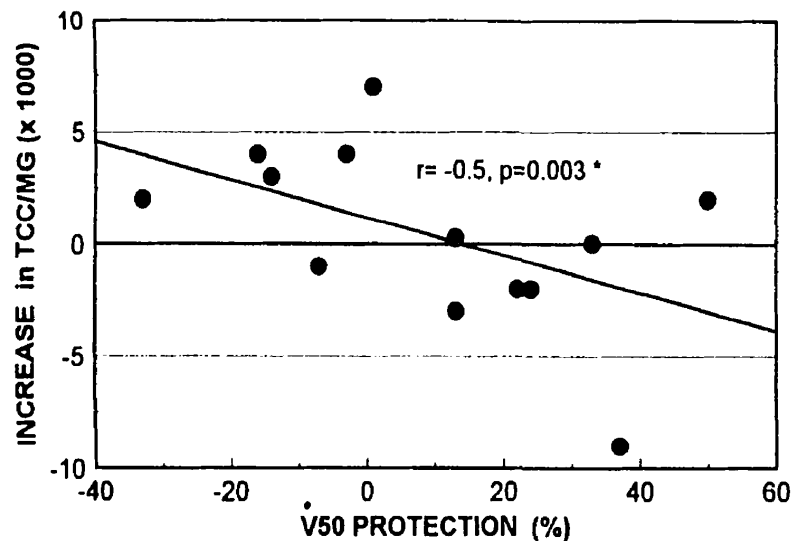
Figure 6. Protection against ozone-induced decline in V̇50(%) vs ozone-induced increase in Total Cells/mg sputum following a 2 hr exposure to 400 ppb ozone. r= correlation coefficient, p value. * significant at the 0.05 level.

Protection = [ozone response(placebo) - [ozone response(indomethacin)/ozone response (placebo) x 100%]

CHAPTER 6 APPENDIX

Two regression models (denoted as Model 1 and Model 2 below) were used to derive two different error sum of squares terms (SSE1, SSE2) and one mean square error term from model 1 (MSE1). These terms were used to calculate a T statistic (equation shown below) which determined whether the associations in the placebo and indomethacin condition were significantly different, ie. the associations with placebo and indomethacin were not described on parallel lines. Significance was accepted at the 0.05 level for all analyses.

If PPMN = pre-post ozone exposure for neutrophils
If Y = difference between placebo and drug for any variable, ie.
FEV_1

Model 1: $Y = \{alpha_{(drug)} - alpha_{(placebo)}\} + beta_{(drug)} PPMN_{(drug)} - beta_{(placebo)} PPMN_{(placebo)}$

Model 2: $Y = \{alpha_{(drug)} - alpha_{(placebo)}\} + beta\{PPMN_{(drug)} - PPMN_{(placebo)}\}$

df = n-3, where 3= number of terms in model

$$T = \sqrt{\frac{SSE2 - SSE1}{\frac{SSE1}{n-3}}} \quad --> MSE1$$

SAS program: proc glm; model Y = X;

CHAPTER 6 REFERENCES

.

Aris, RM., Christian, D., Hearne, PQ., Finkbeiner, WE., Balmes, JR. Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. Am Rev Respir Dis 148: 1363-1372, 1993

Balmes, J., Chen, L., Scannell, C., Tager, I., Christian, D., Hearne, P., Kelly, T., Aris, R. Ozone-induced decrements in FEV1 and FVC do not correlate with measures of inflammation. Am J Respir Crit Care Med, 153: 904-9, 1996

Frampton, MW., Voter, KZ., Fogarty, JP., Morrow, PE., Gibb, FR., Speers, DM., Tsai, Y., Utell, MJ. Airway inflammation and functional responsiveness to ozone in humans. (Abst) Am J Respir Crit Care Med vol 149 No 4 April 1994

Koren, H., Devlin, RB., Graham, D., Mann, R., McGee, M., Horstman, DH., Kozumbo, WJ., Becher, S., House, DE., McDonnell, WF., Bromberg, P. Ozone-induced inflammation in the lower airways to human subjects Am Rev Respir Dis 139, 407, 1989

Morrison, D., Langridge, F., Rahman, I., MacNee, W. Epithelial permeability, inflammation and oxidant status in the airspaces of non-smokers exposed to ozone. (Abst) Am J Respir Crit Care Med Vol 151, No 4 April 1995

Scannell, CH., Aris, R., Christian, D., Hearne, P., Tager, I., Ferrando, R., Chen, L., Balmes, JR. Ozone-induced airway inflammation is not correlated with physiological responses (Abst) Am J Respir Crit Care Med vol 149 No 4 April, 1994

Schelegle, ES., Siefkin, AD., McDonald, RJ. Time course of ozone-induced neutrophilia in normal humans. Am Rev Respir Dis 143: 1353-1358, 1991

Seltzer, J., Bigby, BG., Stulburg, M., Holtzman, J., Wadel, JA. Ozone-induced change in bronchial reactivity to methacholine and airway inflammation in humans J Appl Physiol 60, 1321, 1986

Vagaggini, B., Taccola, M., Giannini, D., Di Franco, H., Cannevali, S. Gianchetti, S., Bacci, E., Bancalari, L., Bartoli, ML., Dente, FL., Pagiaro, PL., Giantini, C. The effects of ozone exposure in the inflammatory cell percentages in the induced sputum of asthmatic subjects (Abst). Am J Respir Crit Care Med Vol 153 No 4 April 1996

Weinmann, GG., Lin, M., Gerbase, MW., Foster, WM., Frank, R. Baseline peripheral airways resistance is significantly associated with spirometric responsiveness to ozone in humans. (Abst) Am Rev Respir Dis Vol 147, No 4, 1993

Wong, HH., Liu, J., Bouchey, HA., Fahy, JV. The effect of ozone exposure on markers of inflammation in induced sputum. (Abst) Am J Respir Crit Care Med vol 151, No 4 April, 1995

CHAPTER 7

PILOT STUDY ON THE EFFECTS OF INDOMETHACIN PRETREATMENT IN NON-
ASTHMATICS FOLLOWING ACUTE OZONE EXPOSURE

INTRODUCTION

Schelegle (1987) first demonstrated cyclooxygenase metabolite involvement in ozone-induced spirometric decrement in normal subjects. He showed that indomethacin compared to placebo attenuated declines in FVC and FEV_1 following a 1 hour exposure to 350 ppb ozone. Since indomethacin acts on the cyclooxygenase pathway of arachidonic acid, this suggested that the ozone effect on inspiratory capacity (IC) was mediated in part by the release of cyclooxygenase products. Eschenbacher (1989) and Ying (1990) confirmed Schelegle's results by successfully reducing declines in FVC and FEV_1 with indomethacin in normal healthy exercising ($\dot{V}e_{min}=60$ L/min.) subjects following a 2 hour exposure to 400 ppb ozone. This evidence suggested that in normals, products of the cyclooxygenase pathway sensitive to indomethacin inhibition, play an important role in the pulmonary function changes following acute ozone exposure. The following chapter will present data on 9 non-asthmatic subjects who underwent a two hour exposure to 400 ppb ozone while pretreated with indomethacin or placebo. This study served as the pilot study for the main thesis investigation previously discussed. Both studies were performed at the GAGE Research Institute by this investigator using identical exposure and assessment methodologies. The pilot study data on non-asthmatics is presented at this point in the thesis because the data appears to shed some light on the asthmatic thesis data presented in the previous chapters.

PURPOSE

To determine whether indomethacin would attenuate decreases in pulmonary function, increases in airway reactivity, and increases in inflammatory cells and biochemical markers of inflammation {Prostaglandin F2-alpha (PGF2-alpha); Interleukin 8 (IL-8)} following a 2 hour exposure to 400 ppb ozone.

HYPOTHESES

Indomethacin will significantly inhibit decreases in pulmonary function and increases in airway reactivity following a 2 hour exposure to 400 ppb ozone. Indomethacin will not significantly attenuate ozone-induced changes in inflammatory cells and biochemical markers of inflammation.

OBJECTIVES

First, to measure changes from baseline in maximum expiratory flows and volumes, airway reactivity, and inflammatory cells and biochemical markers, and compare these changes following a 2 hour exposure to 400 ppb ozone or clean air. Second, compare the differences observed between ozone and air when subjects are pretreated with indomethacin or placebo.

METHOD

SUBJECT SELECTION

Nine non-asthmatic volunteers were recruited to the study. Inclusion/exclusion criteria were the same as the asthmatic study and are described in detail in Chapter 3. Non-asthmatics had no history of physician diagnosed asthma or presented with asthmatic symptoms upon medical examination during baseline week. All exposure and assessment methodologies were identical to the asthma study and are described in detail in the preceding relevant chapters.

STUDY PROTOCOL

Five out of the 9 non-asthmatic subjects underwent a *1x2* randomized factorial design protocol where they were exposed to ozone (400 ppb) for 2 hours on two occasions while pretreated with placebo or indomethacin. Pretreatment with placebo or indomethacin was given in a randomized double-blind fashion. Both exposures were separated by a minimum of 2 weeks. Four out of the 9 non-asthmatic subjects underwent a more extensive *2x2* randomized factorial design protocol where they underwent a total of 4 exposures, two with ozone (400 ppb) and two with clean air, each exposure lasting 2 hours. Subjects were either pretreated with placebo or indomethacin such that four exposure/treatment conditions were filled: 1. ozone/placebo; 2. ozone/indomethacin; 3. air/placebo; 4.

air/indomethacin. The placebo and indomethacin capsules were given in a randomized double blind fashion. Exposures were given in a single blind, randomized fashion with a minimum of 2 weeks separating each exposure. All subjects underwent a week of baseline testing prior to the study periods as described in Chapter 4.

Assessment of pulmonary function and airway reactivity were performed on all 9 subjects. Assessment of inflammatory cells and biochemical markers was performed on 4 subjects.

RESULTS

Baseline Week

Anthropometric data and baseline pulmonary function tests appear in Table 1 (pg248). Compared to the asthmatics, the mean weight of the non-asthmatics was 9kg lighter, but this difference was not statistically significant (p=0.18).

Ozone Response With Placebo and Indomethacin

Pulmonary Function

Table 2 (pg249) [like Table 1 (pg128) for asthmatics], gives an example using FVC of how individual pre and post exposure data was used to calculate baseline changes and the difference in baseline

changes between placebo and indomethacin (shown as "DIFF IN %
Changes" in Table 2). Table 3 (pg250) shows the group mean pre and
post exposure pulmonary function data for all spirometric indices
with placebo and indomethacin following ozone exposure.

Table 4 below shows the mean (+/-SEM) ozone-induced percent change
differences from baseline in maximum expiratory flows and volumes
following 2 hours of exposure in 9 non-asthmatic subjects
pretreated with placebo or indomethacin.

TABLE 4

**MEAN (+/-SEM) BASELINE CHANGES (%) IN PULMONARY FUNCTION
FOLLOWING A 2 HOUR OZONE EXPOSURE IN 9 NON-ASTHMATIC SUBJECTS**

	PLACEBO	P VALUE	INDOMETHACIN	P VALUE
FVC	-10 (2)	0.009	-5 (2)	0.05
FEV$_1$	-11 (3)	0.01	-6 (3)	0.04
V50	-16 (5)	0.01	-8 (5)	0.13
V25	-12 (12)	0.41	-14 (7)	0.12
V40F	-14 (7)	0.13	-9 (6)	0.13
V40P	-21 (7)	0.02	-11 (7)	0.13
V75	-15 (5)	0.008	-12 (5)	0.02

SEM = standard error of the mean

Data from Table 4 appears in graph form in Figure 1 (pg258). In
Figure 1, significant mean (+/-SEM) differences from baseline in 9
non-asthmatics are indicated with an asterisk (*), and significant

(p<0.05)mean (+/-SEM) differences between placebo and indomethacin are indicated with a crossed bar (#). Figure 1 indicates that significant differences from baseline (*) were found for FVC, FEV_1, $\dot{V}50$, $\dot{V}40P$ and $\dot{V}75$ with placebo (clear bar) and FVC and FEV_1 with indomethacin (shaded bar). Significant differences between placebo and indomethacin (#) were found for FVC, FEV_1 and $\dot{V}50$. Individual subject analysis demonstrated that in 7 of 9 non-asthmatic subjects, indomethacin protected against ozone-induced decrements in FVC and FEV_1. With the exception of $\dot{V}25$, indomethacin appeared to attenuate the ozone-induced decrease from baseline for all variables. Variables representing the status of the small airways had the largest ozone-induced decline from pre-exposure baseline ($\dot{V}40P$ = 21%; $\dot{V}50$ = 16%) versus FVC (10%) and FEV_1 (11%).

Inflammatory Cells and Biochemical Mediators

Tables 5-10 (pgs251-256) show the individual pre and post ozone exposure data with placebo and indomethacin, for Total Cells (TCC), Total Cells/mg sputum (TCC/mg), %Neutrophils (PMNs), %Macrophages (MAC), Prostaglandin F2-alpha (PGF2-alpha) and Interleukin-8 (IL-8), respectively.

Figures 2 and 3 (pg259) show the mean (+/-SEM) difference in baseline changes between ozone and air exposure with placebo (clear bar) and indomethacin (shaded bar) for total cells (Figure 2) and differential cells (%PMN, %MAC) (Figure 3) in 4 non-asthmatic

subjects. A significant difference in baseline changes (*) between ozone and air was observed for %PMNs with indomethacin (p=0.04), while a trend (p=0.09) toward a significant difference was observed with placebo.

Figure 4 shows the pre-exposure mean (+/-SEM) PGF2-alpha levels in sputum with placebo (clear bar) and indomethacin (shaded bar) in 4 non-asthmatic subjects. The mean (+/- SEM) levels were 54 pg/ml (11) and 59 pg/ml (19) for placebo and indomethacin, respectively. Figures 5 and 6 (pg261) show the difference in mean (+/-SEM)baseline changes between ozone and air exposure for IL-8 (pg/ml) and PGF2-alpha (pg/ml) with placebo (clear bar) and indomethacin (shaded bar) in 4 non-asthmatic subjects. No significant (p>0.05) differences were observed between ozone and air exposure for IL-8 and PGF2-alpha.

DISCUSSION

Our data on non-asthmatics demonstrated that ozone caused a significant decrease from pre-exposure baseline in several maximum expiratory flows and volumes, including FVC, FEV_1 and $\dot{V}50$. Maximum decrements appeared in $\dot{V}50$ and $\dot{V}40P$. Compared to previous studies that assessed ozone-induced pulmonary function decrement in healthy subjects (Schelegle, 1987; Eschenbacher, 1989; Ying, 1990; Hazucha, 1996), the magnitude of decline in our subjects was smaller. Compared to our mean decline in FEV_1 of 11%, Schelegle,

Eschenbacher and Hazucha showed declines of 25%, 24%, and 18%, respectively, following ozone exposure. This was most likely due to the fact that higher effective doses of ozone were used in these studies. For example, Eschenbacher used 400 ppb for 2 hours at 30 L/min/m² body surface area with intermittent exercise (approximately 60 L/min, using a body surface area of 2.0 m² for a 180 cm, 75 kg male). Compared to the asthmatic results in Chapter 4, declines in FVC and FEV₁ in non-asthmatics were very similar, differing by no more than 2 percentage points for each variable.

Our data showed that indomethacin pretreatment significantly inhibited ozone-induced declines in FVC, FEV₁ and V̇50.

When comparing results between asthmatics and non-asthmatics, it appears that an equivalent dose of indomethacin provided an unequal amount of protection against pulmonary function decline in asthmatics versus non-asthmatics. This data may shed some light on likely explanations for our inability to demonstrate significant attenuation of FVC and FEV₁ decline in asthmatics exposed to ozone. With respect to our asthmatic subjects receiving an insufficient dose of indomethacin, our non-asthmatic results support this notion. The effective dose of indomethacin in the airways of our subjects is dependent on such factors as absorption, distribution and metabolic rate of the drug (Hazucha, 1996). Factors such as weight affect these variables. The dose of indomethacin administered to both subject groups was not normalized for weight.

Asthmatics had a mean (+/-SE) weight of 75 kg (5) compared to 66 kg (4) in normals. It's possible that the concentration of indomethacin at the site of action in the airways, and consequently the degree of inflammation at the site of action, differed between the two groups due to the different absorption rates of the drug. As a result of differing effective doses of indomethacin, different levels of cyclooxygenase activity could also be expected in the airways of the two groups. Since we did not measure the concentration of indomethacin in the plasma or sputum of our subjects, we do not know if the levels of indomethacin differed prior to exposure. However, we were able to form an estimate of cyclooxygenase inhibition based on known doses and measured levels of indomethacin in plasma from previous studies. Doses of indomethacin used in previous studies (approximately 2 fold higher than ours) produced mean plasma concentrations of 1.9-2.3 ug/ml (Hamburg, 1972; Higgs, 1976). These plasma concentrations have been shown to inhibit cyclooxygenase metabolism from 50-90%. Based on these figures, we could expect at the most half that range of inhibition (25-45%) based on the concentration of indomethacin ingested by our subjects. Since we showed that asthmatics had already undergone 43% inhibition prior to ozone exposure, 96% of the maximum inhibition we could expect had been achieved prior to ozone exposure. In addition to estimating cyclooxygenase inhibition based on indomethacin activity, we compared PGF2-alpha levels between the two groups both before and after exposure to give us an indication of the level of pre-existing cyclooxygenase activity,

and how effective indomethacin was at preventing its increase following ozone.

Our results show that with placebo, mean pre-exposure levels of PGF2-alpha were 2.1 times higher in asthmatics (113 pg/ml) versus non-asthmatics (54 pg/ml). This suggests a higher baseline state of cyclooxygenase activity in asthmatics. With indomethacin, pre-exposure levels of PGF2-alpha were very similar between subject groups, with asthmatics demonstrating a mean (+/-SEM) level of 65 pg/ml (14) versus 59 pg/ml (19) for non-asthmatics. Therefore, at the beginning of the ozone exposure it was likely that the production of cyclooxygenase metabolites exceeded the amount of indomethacin on board in the airways to suppress further increases in cyclooxygenase metabolism. Without measuring plasma levels of indomethacin, it is difficult to know whether indomethacin was nearly completely metabolized and removed from the airways prior to exposure as a function of its suppressive activity. Regardless of its levels in the airways, our data suggests that in asthmatics, indomethacin did not inhibit PGF2-alpha production (cyclooxygenase activity) following ozone exposure. These data demonstrate three important things. First, asthmatics had undergone an appreciable level cyclooxygenase inhibition (43%) prior to ozone exposure versus non-asthmatics. Second, both subject groups had relatively equal concentrations of PGF2-alpha in their airways prior to ozone exposure while pretreated with indomethacin. Third, due to the relatively higher concentration of soluble PGF2-alpha

prior to ozone exposure with placebo, asthmatics would have been expected to have greater ozone-induced reductions in FVC and FEV$_1$ versus non-asthmatics. The reason for this expectation is the fact that PGF2-alpha is a potent bronchoconstrictor of airway smooth muscle, and has been shown to be involved in the ozone response of humans (Schelegle, 1987). Since this was not the case, it raises the possibility of non-cyclooxygenase pathways contributing to the asthmatic response.

In contrast, non-asthmatics appeared to demonstrate a low level of PGF2-alpha suppression prior to ozone exposure, suggesting that indomethacin had not achieved its maximum inhibitory effect and therefore an adequate concentration of drug may have been on board to deal sufficiently with the number of pathways producing cyclooxygenase metabolites following ozone exposure. This may explain why PGF2-alpha levels remained high in asthmatics and suppressed in non-asthmatics following ozone exposure. In non-asthmatics with indomethacin, post ozone exposure PGF2-alpha levels decreased relative to baseline by 5 pg/ml, while asthmatics demonstrated an appreciable increase from baseline of 53 pg/ml. These data suggest that indomethacin effectively suppressed cyclooxygenase activity in non-asthmatics exposed to ozone, but did not protect asthmatics in the same way. It appears that a possible explanation for this was that asthmatics had a higher pre-existing state of cyclooxygenase activity and therefore insufficient quantities of indomethacin were present in the airways to inhibit

excess cyclooxygenase metabolism. It appears in non-asthmatics that enough indomethacin was present in the airways to inhibit the level of cyclooxygenase metabolism consequent to ozone exposure.

CONCLUSION

Indomethacin pretreatment significantly attenuated the reductions in pulmonary function, (i.e. FVC, FEV_1, $\dot{V}50$) caused by ozone exposure. Compared to asthmatic subjects, non-asthmatics demonstrated markedly similar declines in FVC and FEV_1 following ozone exposure. Asthmatics appeared to have a higher level of pre-existing cyclooxygenase activity as measured by sputum PGF2-alpha levels prior to ozone exposure. We suggest this may have been ultimately responsible for indomethacin's inability to significantly inhibit ozone-induced pulmonary function declines in asthmatics. We suggest that the high level of cyclooxygenase activity was not matched with a sufficient quantity of indomethacin prior to ozone exposure and as a result insufficient concentrations of indomethacin were present to combat ensuing increases in PGF2-alpha. The end result was that PGF2-alpha levels remained high in asthmatics following ozone exposure and this lead to persistent reductions in FVC and FEV_1 through stimulation of irritant receptors, activation of C-fibers and inhibition of inspiratory capacity. Finally, we suggest that more non-asthmatic subjects need to be studied with respect to biochemical indices of inflammation

in order to confirm results we obtained with relatively few subjects. Also, in order for a proper comparison to be made between the asthmatic and non-asthmatic airway response to ozone, a carefully designed study must be performed with matched subjects equally represented in all conditions of the study

CHAPTER 7 TABLES

ANTHROPOMETRIC DATA AND BASELINE PULMONARY FUNCTION TESTS

NON-ASTHMATICS

SUBJECT ID	GENDER	HEIGHT (CM)	WEIGHT (KG)	AGE (YRS)	%FVC	%FEV1	%V50	%V25
1	M	163	61	25	118	110	118	98
2	M	180	84	27	112	83	69	69
3	F	155	49	32	115	116	143	121
5	M	175	75	22	110	113	138	141
8	F	168	58	28	100	104	121	102
7	M	174	75	39	121	102	85	74
10	M	168	59	20	81	85	122	92
20	F	169	82	28	112	117	120	116
21	F	180	52	20	115	128	155	210
MEAN		168	66	25.9	109	107.6	118.8	113
STD ERROR		2.6	4.3	2.2	4.0	4.4	8.9	14.2
RANGE		155-180	52-84	20-39	81-121	85-128	69-155	69-210

Table 1. Individual and group mean (+/-SEM) anthropometric data and baseline pulmonary function tests for percent predicted of normal values for non-asthmatic subjects (N=9) % PREDICTED NORMAL VALUES ACCORDING TO: FVC: GOLDMAN & BECKLAKE (1959); FEV1: MORRIS, TEMPLE & KOSKI (1973); V50, V25: LAP & HYATT (1967); SEM = standard error of the mean

INDIVIDUAL FVC DATA
(LITERS)

NON-ASTHMATICS

		I------------- PLACEBO----------------I			I---------------INDOMETHACIN---------------I		
Subject	GENDER M=Male F=Female	PRE OZONE	OZONE EXPOSURE AT 2 HRS	% Change (pre-post/ pre) x 100	PRE OZONE	OZONE EXPOSURE AT 2 HRS	% Change (pre-post /pre) x 100	DIFF IN % Changes
1	M	4.98	4.23	15	5.05	4.50	11	4
2	M	5.68	4.31	24	5.42	4.50	17	7
3	F	3.61	3.44	5	3.71	3.66	1	4
5	M	6.06	5.39	11	5.67	5.79	-2	13
6	F	3.44	2.99	13	3.43	3.36	2	11
7	M	5.20	5.05	3	5.23	5.13	2	1
10	M	3.78	3.75	1	3.99	3.77	6	-5
20	F	3.91	3.37	14	3.66	3.42	7	7
21	F	3.24	3.09	5	3.12	2.97	5	0
mean		4.43	3.96	10.1	4.36	4.12	5.44	4.67
std error		0.35	0.28	2.43	0.32	0.31	1.92	1.86

Table 2. Individual and group mean (+/- SEM)pre and 2 hr post exposure FVC data in both pretreatment conditions and one (ozone) exposure condition in 9 non-asthmatic subjects. Also shown are the percent change differences from pre exposure baseline and the difference between the percent change differences (Diff in % change) across both pretreatment conditions; SEM = standard error of the mean

GROUP MEAN (+/- SEM) PULMONARY FUNCTION DATA FOR NON-ASTHMATICS IN ALL PRETREATMENT CONDITIONS AND ONE EXPOSURE (OZONE) CONDITION

NON-ASTHMATICS

	PLACEBO		INDOMETHACIN	
Spirometry Variable FVC, FEV1 = LITERS FLOW(V) VARIABLES = LITERS/ SECOND	Pre-Ozone Exposure	Ozone Exposure At 2 HRS	Pre Ozone Exposure	Ozone Exposure At 2 HRS
FVC	4.43 (0.35)	3.96 (0.28)	4.36 (0.32)	4.12 (0.31)
FEV1	3.77 (0.23)	3.32 (0.19)	3.75 (0.21)	3.49 (0.19)
V50	5.14 (0.38)	4.40 (0.5)	5.14 (0.39)	4.76 (0.46)
V25	2.28 (0.21)	2.07 (0.36)	2.52 (0.23)	2.17 (0.28)
V40(F)	3.89 (0.28)	3.41 (0.42)	3.99 (0.33)	3.62 (0.36)
V40(P)	4.17 (0.27)	3.32 (0.39)	4.35 (0.34)	3.80 (0.34)
V75	8.33 (0.41)	7.09 (0.59)	8.33 (0.44)	7.34 (0.56)
PEFR	8.96 (0.39)	7.83 (0.41)	8.98 (0.40)	8.34 (0.46)

Table 3. Group means (+/- SEM) for pulmonary function variables in all treatment and one exposure (ozone) condition for N=9 non-asthmatic subjects; L= liters; L/S = liters per second; SEM = standard error of the mean

TOTAL CELL COUNTS
(X 1000)

NON-ASTHMATICS

| | | |—————— PLACEBO ———————| | |—————— INDOMETHACIN —————| | | | | |
Subject	GENDER M=Male F=Female	PRE OZONE	POST OZONE	PRE - POST OZONE	PRE AIR	POST AIR	PRE - POST AIR	(PRE - POST OZONE) - (PRE - POST AIR)	PRE OZONE	POST OZONE	PRE - POST OZONE	PRE AIR	POST AIR	PRE - POST AIR	(PRE - POST OZONE) - (PRE - POST AIR)
7	M	594	1346	-752	493	1743	-1250	2002	607	1123	-516	627	322	305	-821
10	M	640	713	-73	258	448	-190	117	305	429	-124	1153	986	167	-291
20	F	-	1612	-	321	422	-101	-	871	471	400	292	230	62	338
21	F	95	149	-54	175	182	-7	-47	408	1299	-891	329	350	21	-912
mean		443	955	-512	312	699	-387	-125	548	831	-283	600	472	139	-422
std error		174	328	230	67	353	290	260	125	223	276	199	173	63	288

Table 5 . Individual and group mean (+/- SEM) pre and post exposure total cell counts (x 1000) in all exposure and treatment conditions in 4 non-asthmatic subjects.. Also shown are the pre - post exposure differences in all exposure and treatment conditions. Pre exposure = 1 day prior to exposure; Post exposure = 4-6 hrs post exposure; * sample not processed due to insufficient volume of sample plug; - = missing data point; DIFF=Difference ; SEM = standard error of the mean

TOTAL CELL COUNT PER MILLIGRAM WET WEIGHT SPUTUM
(X 1000)

NON-ASTHMATICS

|------------------------ PLACEBO------------------------------| |-------------------------INDOMETHACIN------------------------|

Subject	GENDER M=Male F=Female	PRE OZONE	POST OZONE	PRE - POST OZONE	PRE AIR	POST AIR	PRE - POST AIR	(PRE - POST OZONE) - (PRE - POST AIR)	PRE OZONE	POST OZONE	PRE - POST OZONE	PRE AIR	POST AIR	(PRE - POST AIR	(PRE - POST OZONE) - (PRE - POST AIR)
7	M	4.000	7.056	-3.056	2.450	9.220	-6.770	3.714	4.000	7.290	-3.290	3.013	3.013	0	-3.290
10	M	6.620	3.500	3.120	2.500	2.260	.240	2.880	.930	2.020	-1.090	5.800	9.600	-3.800	2.710
20	F	-	4.800	-	.960	1.130	-.170	-	1.900	1.100	.800	1.100	.510	.590	.210
21	F	.580	1.140	-.560	.470	.880	-.410	-.150	.750	1.970	-1.220	1.800	.790	1.010	-2.230
mean		3.733	4.124	-.391	1.595	3.373	-1.778	1.387	1.895	3.095	-1.200	2.928	3.478	-.550	-.650
std error		1.749	1.237	1.794	.518	1.972	1.669	1.174	.746	1.414	.836	1.036	2.116	1.103	1.338

Table 6 . Individual and group mean (+/- SEM) pre and post exposure total cell counts/mg sputum in all exposure and treatment conditions in 4 non-asthmatic subjects.. Also shown are the pre - post exposure differences in all exposure and treatment conditions. Pre exposure = 1 day prior to exposure; Post exposure = 4-6 hrs post exposure; * sample not processed due to insufficient volume of sample lug;l DIFF=Difference; SEM = standard error of the mean

POLYMORPHONUCLEAR NEUTROPHILS (%)

|--------- PLACEBO ---------| NON-ASTHMATICS |--------- INDOMETHACIN ---------|

Subject	GENDER M=Male F=Female	PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	PRE-POST AIR	(PRE-POST OZONE)-(PRE-POST AIR)	PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	PRE-POST AIR	(PRE-POST OZONE)-(PRE-POST AIR)
7	M	71	87	-16	69	91	-22	4	13	68	-55	70	77	-7	-48
10	M	28	77	-49	17	32	-15	-34	0	50	-50	11	1	10	-60
20	F	40	76	-36	24	12	12	-48	50	70	-20	50	43	7	-27
21	F	7	60	-53	2	0	2	-55	13	24	-11	28	27	1	-12
mean		37	75	-39	28	34	-8	-33	19	53	-34	40	37	3	-37
std error		13	6	8	14	20	8	13	11	11	11	13	18	4	11

Table 7. Individual and group mean (+/- SEM) pre and post exposure neutrophils (%) in all exposure and treatment conditions in 4 non-asthmatic subjects. Also shown are the pre - post exposure difference s in exposure and treatment conditions. Pre exposure = 1 day prior to exposure; Post exposure = 4-6 hrs post exposure;SEM = standard error of the mean

MACROPHAGES
(%)

NON-ASTHMATICS

|——————— PLACEBO ———————| |————— INDOMETHACIN —————|

Subject	GENDER M=Male F=Female	PRE OZONE	POST OZONE	PRE - POST OZONE	PRE AIR	POST AIR	PRE - POST AIR	(PRE - POST OZONE) - (PRE - POST AIR)	PRE OZONE	POST OZONE	PRE - POST OZONE	PRE AIR	POST AIR	PRE - POST AIR	(PRE - POST OZONE) - (PRE - POST AIR)
7	M	18	6	12	18	3	15	-3	80	20	60	22	13	9	51
10	M	65	21	44	79	51	28	16	83	9	74	86	82	4	70
20	F	48	18	30	42	19	23	7	29	11	18	37	14	23	-5
21	F	7	12	-5	40	31	9	-14	45	40	5	28	36	-8	13
mean		35	14	20	45	28	19	2	59	20	39	43	36	7	32
std error		13	3	11	13	10	4	6	13	7	16	15	16	8	17

Table 8 Individual and group mean (+/- SEM) pre and post exposure macrophages (%) in all exposure and treatment conditions in 4 non-asthmatic subjects. Also shown are the pre - post exposure differences in exposure and treatment conditions. Pre exposure = 1 day prior to exposure; Post exposure = 4-6 hrs post exposure; * sample not processed due to insufficient volume of sample plug; - = missing data point; DIFF=Difference; SEM = standard error of the mean

PROSTAGLANDIN F2-ALPHA
(PG/ML)

NON-ASTHMATICS

|----------------------PLACEBO----------------------||----------------------INDOMETHACIN----------------------|

Subject	GENDER M=Male F=Female	PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	PRE-POST AIR	(PRE-POST OZONE)-(PRE-POST AIR)	PRE OZONE	POST OZONE	PRE-POST OZONE	PRE AIR	POST AIR	(PRE-POST AIR)	(PRE-POST OZONE)-(PRE-POST AIR)
1	M	54	112	-58	42	51	-9	-49	99	97	2	156	107	49	-47
2	M	113	75	38	76	81	-5	43	49	52	-3	107	126	-19	18
8	F	62	25	37	31	15	16	21	18	16	2	26	14	12	-10
9	F	43	88	-45	12	30	-18	-27	11	0*	11	8	59	-51	62
mean		68	75	-7	40	44	-4	-3	44	41	3	74	77	-2	5
std error		15	18	26	13	14	7	21	20	22	3	35	25	21	23

Table 9. Individual and group mean (+/- SEM) pre and post exposure PGF2-ALPHA (pg) values in all exposure and treatment conditions in non-asthmatic subjects.. Also shown are the pre - post exposure differences in all exposure and treatment conditions. Pre exposure = 1 day prior to exposure; Post exposure = 4-6 hrs post exposure; **sample not processed due to insufficient volume of sample plug;t: DIFF=Difference; SEM = standard error of the mean

INTERLEUKIN 8
(PG/ML)

|--------------------- PLACEBO ---------------------||--------------------- INDOMETHACIN ---------------------|

NON-ASTHMATICS

Subject	GENDER M=Male F=Female	PRE OZONE	POST OZONE	PRE - POST OZONE	PRE AIR	POST AIR	PRE - POST AIR	(PRE - POST OZONE) - (PRE - POST AIR)	PRE OZONE	POST OZONE	PRE - POST OZONE	PRE AIR	POST AIR	PRE - POST AIR	(PRE - POST OZONE) - (PRE - POST AIR)
1	M	905	16000	-17095	7179	10397	-3218	-13677	817	1227	-410	4746	7009	-2263	1853
2	M	433	5155	-4722	3927	4424	-497	-4252	330	551	-221	174	598	-424	203
8	F	2750	42	2708	1507	1758	-251	2959	1480	1159	321	51	1414	-1363	1684
9	F	2037	1779	258	1087	617	450	-192	1177	961	216	1312	1530	-218	434
mean		1531	6244	-4713	3420	4299	-879	-3841	951	875	-24	1571	2638	-1067	1044
std error		527	4060	4407	1402	2184	805	3657	247	152	174	1096	1472	470	423

Table 10. Individual and group mean (+/- SEM) pre and post exposure IL-8 (pg/ml) values in all exposure and treatment conditions in 4 non-asthmatic subjects. Also shown are the pre - post exposure differences in all exposure and treatment conditions. Pre exposure = 1 day prior to exposure; Post exposure = 4-6 hrs post exposure; * sample not processed due to insufficient volume of sample plug; DIFF=Difference; SEM = standard error of the mean

CHAPTER 7 FIGURES

PULMONARY FUNCTION RESPONSES IN NON-ASTHMATICS

Mean (+/-SEM) Difference From Baseline Following Ozone Exposure

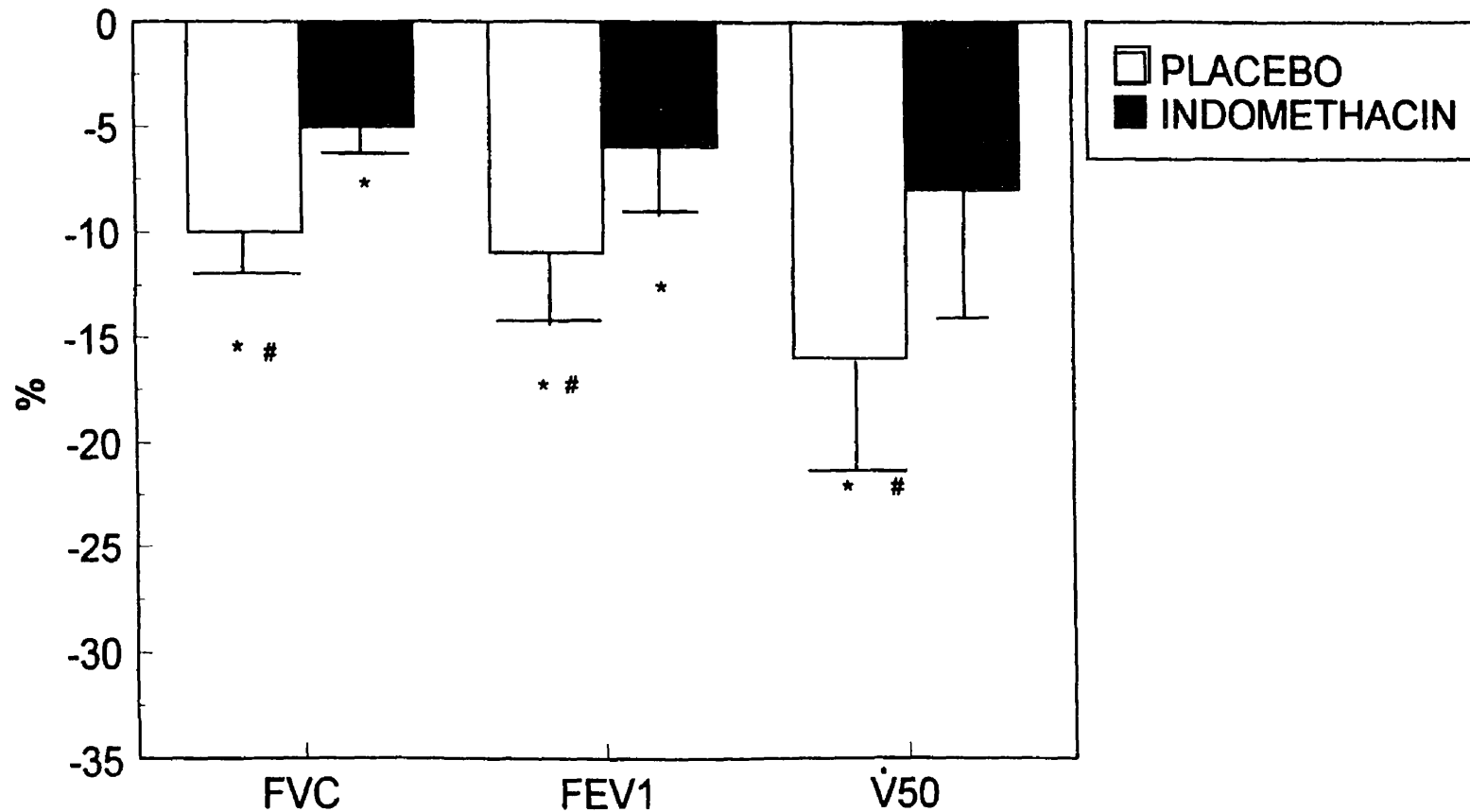Figure 1. Mean (+/-SEM) difference from baseline in maximum expiratory flows and volumes following ozone exposure with placebo (clear bar) or indomethacin (shaded bar). * significant change from baseline at the 0.05 level. # Significant difference between indomethacin and placebo at the 0.05 level; SEM = standard error of the mean.

INFLAMMATORY CELL RESPONSES IN NON-ASTHMATIC SUBJECTS

Difference In Mean (+/-SEM) Baseline Changes Between Ozone and Air Exposure
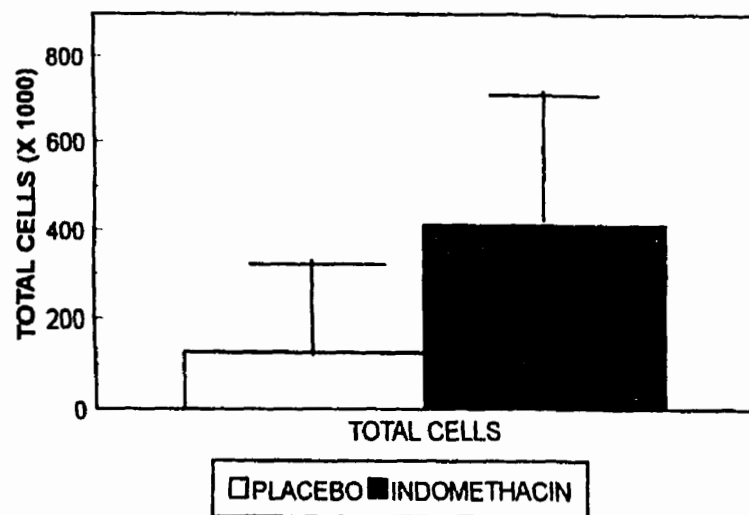
Figure 2. Difference in mean (+/-SEM) baseline changes for total cells (TCC) between ozone and air exposure with placebo (clear bar) or indomethacin (shaded bar) in 4 non-asthmatic subjects; SEM = standard error of the mean
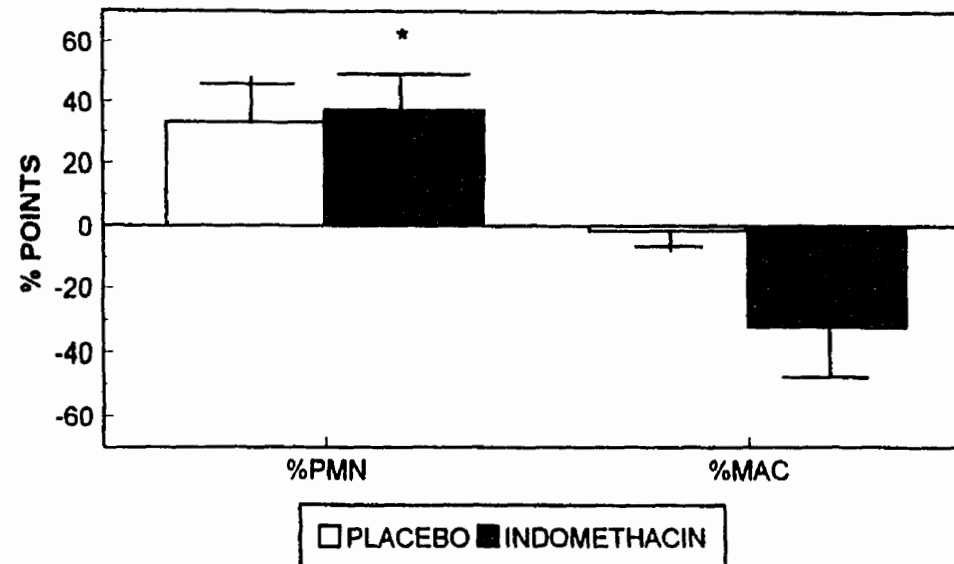

Figure 3. Difference in mean (+/-SEM) baseline changes (% points) for %PMNs and %MACs between ozone and air exposure with placebo (clear bar) or indomethacin (shaded bar) in 4 non-asthmatics . * significant difference between ozone and air exposure (p=0.04). PMN =Poly-morphonuclear Neutrophils; MAC = Macrophages; SEM = standard error of the mean

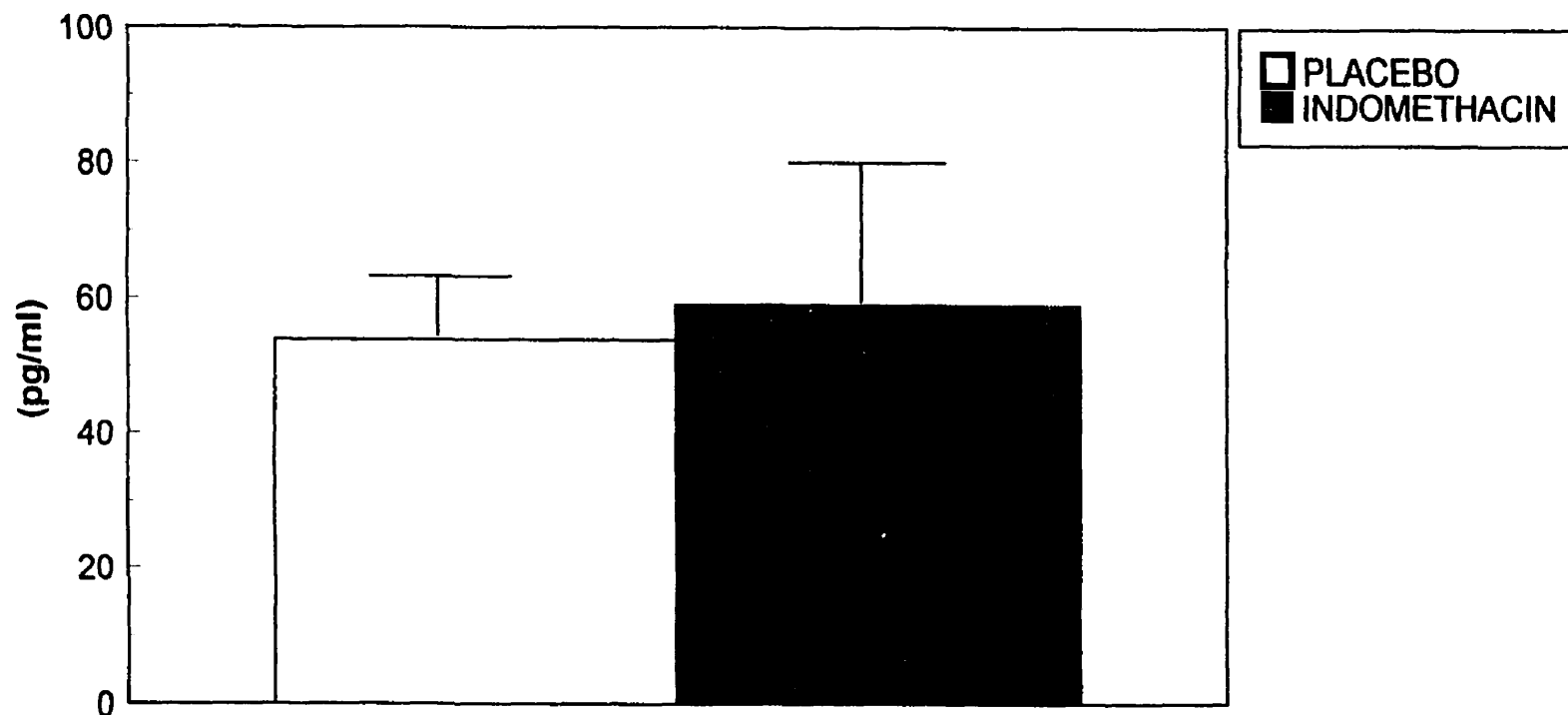# PRE EXPOSURE MEAN (+/- SEM) PGF2-ALPHA LEVELS IN SPUTUM OF NON-ASTHMATICS

Figure 4. Pre-exposure mean (+/- SEM) PGF2-alpha levels in sputum with placebo (clear bar) and indomethacin (shaded bar) in 4 non-asthmatic subjects; SEM = standard error of the mean

BIOCHEMICAL MARKER RESPONSE IN NON-ASTHMATICS

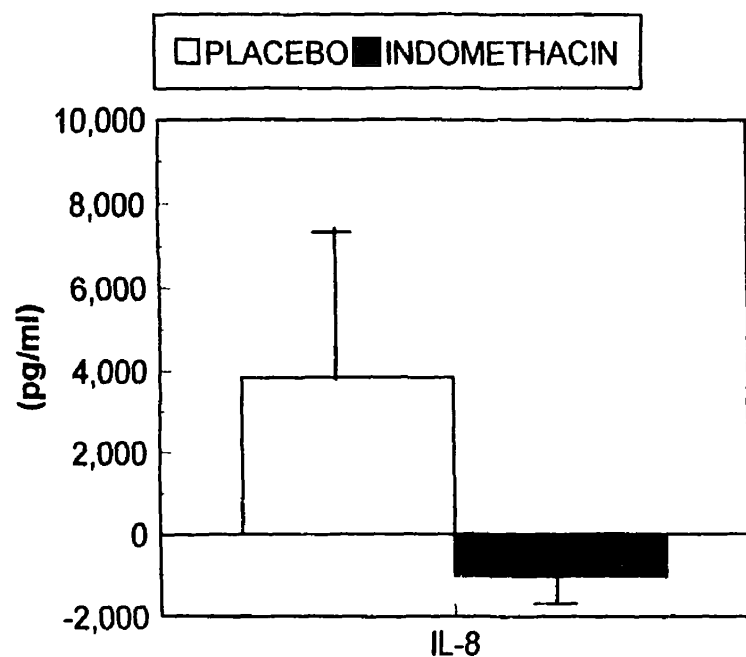## Difference In Mean (+/-SEM) Baseline Changes Between Ozone and Air Exposure

Figure 5. Difference in mean (+/-SEM) baseline changes between ozone and air exposure for IL-8 (pg/ml) with placebo (clear bar) or indomethacin (shaded bar) in 4 non-asthmatic subjects. Pre exposure is 1 day prior to exposure. Post exposure is 4-6 hrs post exposure. IL-8 = Interleukin 8; SEM = standard error of the mean
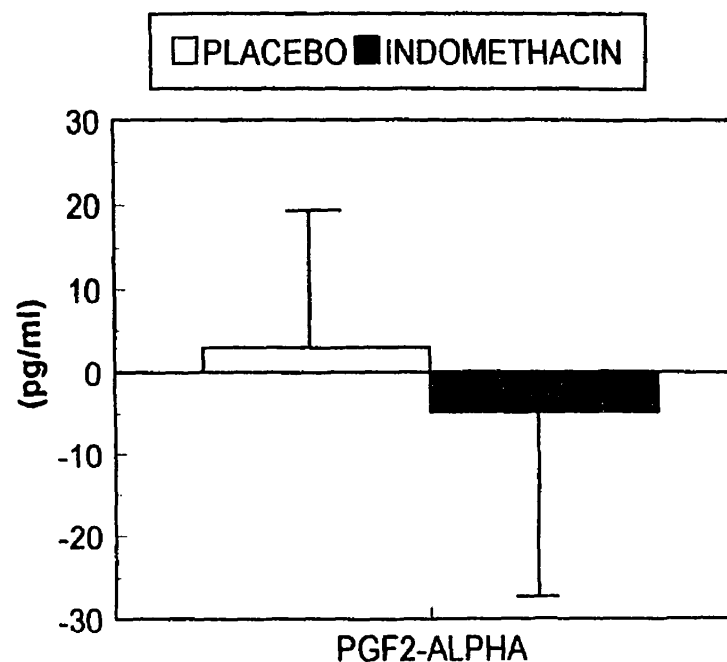
Figure 6. Difference in (+/-SEM) baseline changes between ozone and air exposure for PGF2-alpha (pg/ml) with placebo (clear bar) or indomethacin (shaded bar) in 4 non- asthmatic subjects. Pre exposure is 1 day prior to exposure. Post exposure is 4-6 hrs post exposure. PGF2-ALPHA = Prostaglandin F2-alpha; SEM = standard error of the mean

CHAPTER 7 REFERENCES

Eschenbacher, WC., Ying, RC., Kreit, JW., Gross, KB. Ozone-induced lung function changes in normal and asthmatic subjects and the effect of indomethacin. In T. Schneider, SD, Lee, GJR, Wolters, and LD Grant (Eds), Atmospheric Ozone Research and Its Policy Implications. Elsevier, Amsterdam, P493, 1989

Hamburg, M. Inhibition of prostaglandin synthesis in man. Biochem Biophys Res Commun; 49: 720-6, 1972

Hazucha, M., Madden, M., Pape, G., Becker, S., Devlin, R., Koren, H., Kehrl, H., Bromberg, P. Effects of cyclo-oxygenase inhibition on ozone-induced respiratory inflammation and lung function changes. Eur J Appl Physiol, 73: 17-27, 1996

Higgs, GA., Harvey, EA., Ferreira, SH., Vane, JR. The effects of anti-inflammatory drugs on the production of prostaglandins in vivo. In: Samuelsson B, Paoletti R, eds. Advances in prostaglandin and thromboxane research. Vol 1 New York: Raven Press; 105-10, 1976

Schelegle, ES., Adam, WC., Siefkin, AD. Indomethacin pretreatment reduces ozone-induced pulmonary function decrements in human subjects Am Rev Respir Dis, 136, 1350-1354, 1987

Ying, RL., Gross, KB., Terzo, TS., Eschenbacher, WC. Indomethacin does not inhibit the ozone-induced increase in bronchial responsiveness in human subjects. Am Rev Respir Dis 142, 817, 1990

CHAPTER 8

SUMMARY OF CRITICAL FINDINGS

ASTHMATICS

PULMONARY FUNCTION

1. Significant differences in baseline changes (changes from baseline) were observed between ozone and air exposure (ie. Significant ozone response) for all pulmonary function variables following 2 hour exposures. All changes from baseline were decrements, and maximum decrement occurred in small airways flow variables.

2. Indomethacin did not significantly inhibit ozone-induced declines in maximum expiratory flows and volumes. Compared to all other variables, indomethacin provided the most protection against ozone-induced declines in small airways flow variables.

3. Following 1 hour of ozone exposure, indomethacin showed a trend toward attenuating the ozone-induced declines in FVC and FEV_1.

4. There appeared to be no relationship between the magnitude of pulmonary function decline, ie. Ozone sensitivity as measured by FEV_1, and the ability of indomethacin to inhibit pulmonary function decline following ozone exposure.

SYMPTOMS

1. Significant baseline changes between ozone and air exposure were observed for general and respiratory symptom reporting and symptom

severity scores following 2 hours of exposure. All baseline changes were increases.

2. Indomethacin did not significantly inhibit the increased symptom reporting or increased symptom severity for general and respiratory symptoms following ozone exposure.

AIRWAY REACTIVITY

1. A mean significant increase in airway reactivity, as measured by a decline of greater than 2 doubling doses of methacholine, was not observed following a 2 hour exposure to ozone.

2. Individual subject analysis revealed that forty-six percent of subjects were PC_{20} insensitive to ozone as defined by a response of less than a two doubling dose concentration fall in methacholine following ozone exposure.

3. Four subjects were identified as ozone sensitive by demonstrating a greater than 2 doubling dose decline in methacholine following ozone. Indomethacin did not significantly inhibit the increased airway reactivity in these subjects.

INFLAMMATORY RESPONSE

1. A significant difference in baseline changes between ozone and

air exposure was observed for total inflammatory cells and neutrophils following 2 hour exposures. The changes from baseline were increases.

2. With indomethacin, the ozone-induced baseline changes for total cells and neutrophils were smaller than placebo, but this difference did not reach statistical significance.

3. Pre exposure PGF2-alpha levels were appreciably (2.1 x) higher with placebo versus indomethacin, but this difference was not statistically significant.

4. Sputum induction is a safe, reproducible, and reliable method for examining airway inflammatory cells and biochemical markers of inflammation in asthmatic subjects following an acute exposure to ozone.

OUTCOME MEASURE ASSOCIATIONS

1. Decreases in FVC and FEV_1 were not associated with increases in neutrophils following ozone exposure.

2. A significant positive association was observed between decreased expiratory flow rates ($\dot{V}50$, PEFR) and increased Total Cell Counts/mg sputum; and decreased small airways flow rate ($\dot{V}40P$)

and increased neutrophils following ozone exposure.

3. Indomethacin significantly changed the slopes of the following relationships following ozone exposure: a)decrease in $\dot{V}40P$ vs increase in PMNs; b) decrease in PEFR vs increase in TCC/mg sputum.

4. Protection against ozone-induced decreases in expiratory flow rates is related to smaller increases in inflammatory cells following ozone exposure.

PILOT STUDY (NON-ASTHMATICS)

1. Significant decreases from baseline were observed for several maximum expiratory flows and volumes, including FVC, FEV_1 and $\dot{V}50$ following ozone exposure.

2. Indomethacin significantly attenuated the ozone-induced decreases in FVC, FEV_1 and $\dot{V}50$.

3. Following ozone exposure, PGF2-alpha levels were suppressed to levels lower than baseline when subjects were pretreated with indomethacin versus placebo, but this difference was not statistically significant.

ASTHMATICS VERSUS NON-ASTHMATICS

1. Compared to asthmatics, non-asthmatics showed markedly similar declines in FVC and FEV_1 following ozone exposure.

2. Equal ingested doses of indomethacin by asthmatics and non-asthmatics did not produce equal degrees of protection against pulmonary function decline.

3. Compared to non-asthmatics, data on asthmatics suggested higher (two fold) mean PGF2-alpha concentrations prior to exposure with placebo (although this was not statistically significant). With indomethacin, the mean pre-exposure levels were quite similar between asthmatics and non-asthmatics.

4. Asthmatics were estimated to have undergone 96% of their expected cyclooxygenase inhibition (as measured by pre-exposure PGF2-alpha levels), prior to ozone exposure, whereas non-asthmatics did not demonstrate much cyclooxygenase inhibition prior to ozone exposure.

5. With indomethacin pretreatment, PGF2-alpha levels in asthmatics were not inhibited following ozone exposure, whereas in non-asthmatics, PGF2-alpha levels decreased relative to baseline following ozone exposure.

CHAPTER 9

SUMMARY CONCLUSIONS

ASTHMATICS

PULMONARY FUNCTION

Indomethacin pretreatment failed to significantly protect against decrements in pulmonary function (FVC, FEV_1, $\overset{\bullet}{V}50$) following 2 hours of ozone exposure. Specific time point analysis of FEV_1 data (i.e. exposure at 1 hour versus 2 hours), showed that some protection against FEV_1 decline occurred at one hour of exposure, but not at two. This suggests that the dose of indomethacin administered to the subjects was insufficient to effectively inhibit cyclooxygenase production for the entire two hour ozone exposure. The relatively higher baseline (pre-exposure) state of cyclooxygenase activity of the asthmatics compared to non-asthmatics, likely contributed to a higher overall level of cyclooxygenase production after the two hour exposure period. A study on asthmatics with indomethacin and ozone successfully demonstrated significant attenuation of FVC and FEV_1 decline using a larger dose of indomethacin than that used in this study.

SYMPTOM RESPONSES

Symptom reporting and symptom severity closely tracked the pulmonary function results we observed with respect to ozone exposure and the effect of indomethacin. Namely, symptom reporting and severity scores increased following ozone exposure, and indomethacin did not reduce these responses.

AIRWAY REACTIVITY

We did not observe a significant mean increase in airway reactivity following ozone exposure. As a result, we were unable to fully examine the effect of indomethacin on ozone-induced airway hyper-reactivity. In four subjects, we observed a reduction of greater than two doubling doses of methacholine following ozone exposure. In these subjects, indomethacin did not significantly reduce the increased airway reactivity.

INFLAMMATORY CELLS AND BIOCHEMICAL MARKERS OF INFLAMMATION

We observed a significant increase in total inflammatory cells and percent neutrophils following ozone exposure relative to air exposure. IL-8 and PGF2-alpha also increased relative to air exposure, but the difference was not statistically significant. Indomethacin pretreatment failed to significantly attenuate inflammatory cell and biochemical marker responses to ozone. IL-8 responses following ozone appeared to track neutrophil responses when subjects were pretreated with placebo or indomethacin. Pre-exposure PGF2-alpha levels indicated that asthmatics likely experienced an appreciable level of cyclooxygenase inhibition prior to ozone exposure, since levels with indomethacin were almost half the levels with placebo. The amount of cyclooxygenase inhibition

achieved prior to exposure based on PGF2-alpha concentrations, was estimated to be 43%. According to previous studies where subjects ingested approximately twice as much indomethacin as our subjects, and achieved a plasma molar concentration that historically produces 90% inhibition of cyclooxygenase activity, the upper limit of inhibition we could expect based on our dose of indomethacin was 45%. We estimated therefore, that prior to ozone exposure 96% of the potential cyclooxygenase inhibition had already occurred.

Indomethacin did not attenuate increased PGF2-alpha levels relative to baseline following ozone exposure. We suggest that lack of suppression of PGF2-alpha levels following ozone exposure, may have contributed to the reduced FVC and FEV_1 we observed in our asthmatic subjects while pretreated with indomethacin.

OUTCOME MEASURE ASSOCIATIONS

Decrements in FVC and FEV_1 following acute ozone exposure are not related to ozone-induced increases in neutrophils. Ozone-induced decrements in large and small airways expiratory flows ($\dot{V}40P$, $\dot{V}50$, PEFR) are associated with increases in total cells and neutrophils, respectively following ozone exposure. Cyclooxygenase metabolites of arachidonic acid appear to play a role in the association between certain expiratory flows and inflammatory cells. Finally, our data showed that protecting the decline in expiratory flow

rates following ozone exposure, results in smaller inflammatory cell increases from baseline. We suggest this has important chronic health implications for asthmatics who have small airways dysfunction since pre-existing airway inflammation is a major feature of their disease.

NON-ASTHMATICS: PILOT STUDY

Asthmatics Versus Non-Asthmatics

Indomethacin significantly attenuated the declines in FVC, FEV_1 and $\dot{V}50$ following ozone exposure. Compared to asthmatic subjects, non-asthmatics demonstrated a similar magnitude of decline in FVC and FEV_1 following ozone exposure. Asthmatics compared to non-asthmatics however, appeared to have a higher level of pre-exposure cyclooxygenase activity as measured by sputum PGF2-alpha levels. We suggest this may have been partially responsible for the lack of pulmonary function protection by indomethacin in asthmatics. Based on the dose of indomethacin administered to our subjects, we estimated that over 95% of the expected cyclooxygenase inhibition occurred prior to ozone exposure, therefore the amount of inhibition during exposure was likely minimal. As a result, PGF2-alpha levels were not inhibited following ozone exposure, and this likely contributed to the persistent reductions in FVC and FEV_1 we observed in asthmatics pretreated with indomethacin. Finally, we demonstrated that asthmatics and non-asthmatics had similar ozone-

induced decrements in FVC and FEV₁, despite the fact that asthmatics had higher levels of pre-exposure cyclooxygenase activity as measured by PGF2-alpha levels in sputum. This suggests that other mechanisms besides arachidonic acid derived cyclooxygenation, may be active in the airways of asthmatics exposed to ozone.

OVERALL CONCLUSIONS

Equivalent ingested doses of indomethacin by asthmatics and non-asthmatics did not provide equivalent protection against pulmonary function decline following ozone exposure. In order for asthmatic subjects to receive protective benefits from indomethacin, they may require relatively higher pretreatment doses than non-asthmatics in order to compensate for higher baseline levels of cyclooxygenase activity. Although it appears likely that an insufficient dose of indomethacin contributed toward our inability to achieve significant inhibition against ozone-induced pulmonary function decline in asthmatics, it does not explain the fact that despite higher levels of cyclooxygenase activity prior to ozone exposure with placebo, equivalent decrements in FVC and FEV₁ were observed in asthmatics and non-asthmatics. The possibility exists therefore, that non-cyclooxygenase pathways are involved in the asthmatic airway response to ozone.

CHAPTER 10

THESIS LIMITATIONS

One limitation of the thesis is the relatively small sample size we used for the biochemical end points. More asthmatic subjects need to be investigated to confirm some of the significant results we found, as well as confirm some of the trends our data suggested. In general, sample size is a problem common to all human controlled exposure studies where multiple exposures are necessarily spaced out by weeks to avoid potential carry over effects. Our original subject population of 14 asthmatic subjects was actually larger than is the rule for studies of this kind. Our adjusted sample size of 13 gave us adequate power at 0.05 level to detect significant changes in pulmonary function, symptom responses, airway reactivity and inflammatory cells with placebo and indomethacin following acute ozone exposure. However, it is possible that our failure to detect small significant differences between baseline and post exposure, between ozone and air, or between placebo and indomethacin, was due to a Type II error because an insufficient number of subjects were studied.

A second limitation of this thesis is not knowing the actual concentration of indomethacin in plasma or in airway fluid. Knowing the true concentration allows one to confirm subject compliance with ingesting the drug, determine what level of the drug still remains in the airways prior to and post ozone exposure, and more accurately estimate the level of cyclooxygenase inhibition experienced by the subjects.

A third limitation of this thesis is the use of a first generation methodology for sputum induction and analysis. Since the beginning of the study (1992), the sputum induction method has been revised and improved several times resulting in a lower variability around cell recovery and differential cell count reading.

A fourth limitation of this study is the possibility that inhalation of methacholine 2 hours prior to sputum induction affected the concentration of inflammatory markers measured in the sputum samples. One recent report suggested that methacholine (up to 76.8 mg/ml) caused a significant change in sputum eosinophils and macrophages when compared to placebo challenge in asthmatics 4 hours after methacholine challenge (Hiltermann, 1996). Other recent reports have also suggested that the sputum induction procedure itself is pro-inflammatory with respect to neutrophils (Nightingale, 1997; Holz, 1997). This is important since it is assumed in this study that ozone caused the observed changes in inflammatory markers relative to air exposure.

A fifth limitation of this study is really a caution rather than a limitation. It is the use of Dithiothreitol (DTT) (sputolysin) as a lysing agent to help separate saliva from mucous plugs. DTT breaks disulphide bonds and IL-8 contains 2 disulphide bonds which are important for its biological activity. Although we did not measure IL-8's biological activity and only measured the concentration of IL-8 in sputum, we did not expect DTT to affect

the concentration of IL-8 in sputum. Preliminary assays with IL-8 confirmed that DTT had no significant effect on the concentration of IL-8 in sputum. In retrospect IL-8 samples should have been split with one receiving no DTT treatment and the other processed as normal. Ideally one should avoid using chemical reagents that in any way affect an outcome measure.

NOTE TO USERS

Page(s) not included in the original manuscript are unavailable from the author or university. The manuscript was microfilmed as received.

280 & 281

UMI

to ozone.

Future research in the area of health effects of air pollution should concentrate on pollutant *mixtures* and not single air contaminants in isolation. Considering the environment as "one atmosphere" and studying it as such in the laboratory, would allow more correlations be drawn with current epidemiologic data.

Finally, epidemiologic field studies and occupational health studies should make use of sputum induction as a relatively non-invasive method to collect and analyse airway fluid samples. Its relative simplicity, low cost and portability make it an ideal procedure to use in field studies and occupational settings to obtain biological samples under outdoor or occupational exposure conditions.

Contributions To The Literature

This thesis added to our understanding of the airway inflammatory response in *asthmatics* following an acute ozone exposure. Prior to this thesis a substantial health effects of ozone data base had been built mainly on data from normal, healthy subjects, but very little data had been accumulated using asthmatics. This was primarily due to the invasive nature of the techniques used to assess inflammation. These techniques presented an element of risk to asthmatic subjects especially those who had just undergone an

assess inflammation. These techniques presented an element of risk to asthmatic subjects especially those who had just undergone an exposure to an irritant gas such as ozone. This thesis incorporated the use of a new non-invasive research procedure (induced sputum) and demonstrated that it could safely and reliably be performed on asthmatic subjects who had just undergone a two hour ozone exposure. This thesis showed that the induced sputum procedure produced valuable information on the airways inflammatory response in asthmatics. As a result it has contributed to our understanding of the role airways inflammation plays in the pathophysiology of the ozone response, and allows us to make better comparisons of the asthmatic population to the healthy population.