CHEST

Original Research

COPD

Young "Healthy" Smokers Have Functional and Inflammatory Changes in the Nasal and the Lower Airways

Marina Lazzari Nicola, BSc; Heráclito Barbosa de Carvalho, MD, PhD; Carolina Tieko Yoshida, BSc; Fabyana Maria dos Anjos, PhD; Mayumi Nakao, MD; Ubiratan de Paula Santos, MD, PhD; Karina Helena Morais Cardozo, PhD; Valdemir Melechco Carvalho, MD, PhD; Ernani Pinto, PhD; Sandra Helena Poliselli Farsky, PhD; Paulo Hilario Nascimento Saldiva, MD, PhD; Bruce K. Rubin, MD, MEngr, MBA; and Naomi Kondo Nakagawa, PhD

Background: **Smoking is responsible for most COPD. Although people with COPD often have concomitant nasal disease, there are few studies that report physiologic or inflammatory changes in the upper airways in young asymptomatic smokers. We investigated physiologic and inflammatory changes in the nasal and lower airways of young smokers and if these changes were related to smoking history.**

Methods: **Seventy-two subjects aged between 18 and 35 years (32 healthy nonsmokers and 40 young smokers) participated in this study. We measured nasal mucociliary clearance (MCC), nasal mucus surface contact angle, cell counts, myeloperoxidase and cytokine concentrations in nasal lavage fluid, exhaled breath condensate (EBC) pH, and lung function.**

Results: **Smokers had faster MCC, an increased number of cells (macrophages, ciliated cells, and goblet cells), increased lavage myeloperoxidase concentration, and decreased EBC pH compared with nonsmokers. There was a significant inverse relationship between pack-year smoking history and EBC pH. There were no differences in lung function or mucus surface properties comparing smokers to nonsmokers.**

Conclusions: **Young adult smokers have functional and inflammatory changes in the nasal and lower airways and these correlate with smoking history. However, in these young smokers, smoking history was not associated with pulmonary function decline, probably because it is unlikely that spirometry detects early physiologic changes in the airways.**

Trial registry: **ClinicalTrials.gov; No.: NCT01877291; URL: www.clinicaltrials.gov**

CHEST 2014; 145(5):998–1005

Abbreviations: bpm = beats per minute; CO = carbon monoxide; EBC = exhaled breath condensate; MCC = mucociliary clearance; MPO = myeloperoxidase; NLF = nasal lavage fluid; ppm = parts per million; SNOT20 = 20-item Sino-Nasal Outcome Test; STT = saccharine transit time; TNF-α = tumor necrosis factor α

The upper airway epithelium is the first defense of the respiratory system against inhaled toxic agents and microorganisms. This protective barrier depends on the integrity of the epithelial surface. Long-term tobacco smoking alters this barrier by oxidative stress and airway inflammation.[1,2] These increase epithelium permeability,[3] impair ion transport,[4] deplete cilia,[5,6] induce metaplasia of goblet and squamous cells, and increase mucus secretion.[7] Thus, smoking may impair mucociliary clearance (MCC),[8,9] leading to airway obstruction,[2] decreased lung function,[10] increased susceptibility to respiratory infections,[11,12] and progression of COPD, particularly in predisposed long-term smokers.[13] Approximately 25% of long-term smokers develop COPD, and initial lung function alterations can be observed in some smokers as young as 35 years of age[14,15] with a smoking history of ≥ 20 pack-years.[15] Studies in smoking adults < 35 years of age suggest that tobacco smoking is an important risk factor for chronic cough and that this is related to pack-year smoking history.[16,17]

On the other hand, studies of younger smokers or those with less pack-year history also suggest that "light" smoking and environmental tobacco smoke exposure

is associated with faster nasal ciliary beat frequency and mucus clearance[18] and that the mucociliary transportability of lower airway mucus is faster than mucus from healthy nonsmokers. This declines with longer smoking history, however, is impaired in subjects with diagnosed COPD.[7] Because of the close association of COPD with nasal and sinus inflammation,[19] we investigated if smokers younger than age 35 years with short pack-year smoking history have physiologic or inflammatory changes in the upper and lower airways and if these changes are related to smoking history.

Materials and Methods

Over a period of six consecutive months, we recruited subjects aged between 18 and 35 years from the Faculdade de Medicina da Universidade de São Paulo. Subjects were invited by telephone to participate in the study; the study objectives and procedures were discussed, and subjects were included in the study after obtaining written informed consent. Exclusion criteria were the inability to understand and follow commands, previous nasal surgery, sinusitis or respiratory infections in the previous 30 days, and asthma. Subjects were screened with the aid of a self-reported asthma questionnaire[20] and diagnosed by medical examination, including pulmonary function testing when indicated by history. The healthy nonsmoker was defined as a subject who never smoked and had no diagnosis of acute or chronic diseases, with no use of medications except contraceptives, and had normal findings on physical examination. Self-reported nonsmokers with exhaled carbon monoxide (CO) levels >9 parts per million (ppm) and/or cotinine levels >10 ng/mL in nasal lavage fluid (NLF) were excluded from this study. Current smoking was defined according to the guidelines of the World Health Organization as subjects who have smoked ≥ 100 cigarettes and who currently smoke at least one cigarette a day.[21] The Consolidated Standards of Reporting Trials (CONSORT) diagram is given in Figure 1.

Manuscript received June 11, 2013; revision accepted November 10, 2013; originally published Online First December 5, 2013.
Affiliations: From the Department of Pathology (Mss Nicola and Yoshida and Drs Nakao, Saldiva, and Nakagawa), and Department of Physiotherapy (Mss Nicola and Yoshida and Dr Nakagawa), Communication Science and Disorders, Occupational Therapy, LIM 34, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Department of Preventive Medicine (Dr H. B. Carvalho), and Department of Clinical and Toxicological Analysis (Drs Anjos, Pinto, and Farsky), Faculty of Pharmaceutical Sciences, Universidade de São Paulo, São Paulo, Brazil; Pulmonary Division (Dr Santos), Heart Institute, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Fleury Medicine and Health Institute (Drs Cardozo and V. M. Carvalho), São Paulo, Brazil; and the Department of Pediatrics (Dr Rubin), Virginia Commonwealth University School of Medicine, Richmond, VA.
Funding/Support: This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo [FAPESP 13/13598-1 and 13/11401-6].
Correspondence to: Naomi Kondo Nakagawa, PhD, Faculdade de Medicina da Universidade de São Paulo, 455 Av. Dr. Arnaldo, room 1150, Cerqueira Cesar, São Paulo, Brazil, CEP 01246-903; e-mail: naomi.kondo@usp.br

DOI: 10.1378/chest.13-1355

This study received approval by the institutional review board of the Faculdade de Medicina da Universidade de São Paulo Ethical Committee (CEP 147/13) and is registered at ClinicalTrials.gov (NCT01877291). All clinical investigations were conducted according to the Declaration of Helsinki of the World Medical Association.

Clinical Assessment and Pulmonary Function

All subjects were assessed between 7:00 AM and 12:00 PM. Subjects completed a general health questionnaire. We also administered the 20-item Sino-Nasal Outcome Test (SNOT20)[22] for symptoms of airway discomfort. Heart rate, systolic and diastolic BP, respiratory rate, and pulse oxygen saturation were recorded. To measure exhaled CO, subjects were asked to orally exhale slowly from their total lung capacity through a Micro CO analyzer (Cardinal Health U.K.232 Ltd) over 15 to 20 s.

Pulmonary function was measured using the Koko Legend spirometer (nSpire Health Inc) and using the American Thoracic Society/European Respiratory Society Task Force guidelines[23,24] to determine FEV_1 and FVC. The percentages of predicted spirometry values were calculated from published Brazilian population data.[25]

Nasal MCC

We evaluated the nasal MCC by measuring nasal saccharine transit time (STT).[26] The subject was asked to avoid alcohol, tea, and coffee for 6 h and to eat or drink nothing for 2 h before the measurements. We first confirmed the subject's ability to taste saccharine by placing a small amount directly on the tongue. The STT assessment was performed in a quiet room at a temperature of 21°C to 22°C and relative humidity of 63% to 71%. Subjects sat in a chair and were asked to maintain regular breathing and to avoid deep breathing, coughing, sneezing, sniffing, or talking during STT measurements. Saccharine particles (2.5 mg) were deposited 2 cm from the anterior end of the inferior nasal turbinate of the nonobstructed nostril, and the timer was stopped at the first perception of sweet taste. The maximum delay between the deposition and perception was set at 60 min for nondetection.

Nasal Mucus Collection and Mucus Surface Contact Angle

The subject extended his or her neck approximately 30°, and a mucus sample was collected with the aid of a soft brush in the opposite nostril to that used for the STT. Mucus samples were kept in coded, sealed plastic containers and immediately stored at −80°C until analyzed. All samples were analyzed for mucus contact angle.[26,27] The contact angle measures the wettability of solid planar surface by a liquid and is measured at the liquid-air-solid interface. Surfaces that are very poorly wettable (like Teflon) are considered to be less "sticky." Mucus that has a larger contact angle is more difficult to clear by coughing or sneezing.[7,28] A 25-μL drop of mucus was dropped on a glass slide that had previously been treated with a sulfochromic solution to remove electrical charges and then washed with deionized water. The mucus was allowed to stabilize for 5 min, and the image was captured with the aid of a stereomicroscope (Stemi 2000C; Carl Zeiss) connected to a camera (Axiocam HSC; Carl Zeiss AG). The contact angle was measured using an image analysis program (Interactive AxionVison 4.7; Carl Zeiss AG).[27]

Exhaled Breath Condensate Collection and pH Analysis

It is difficult to noninvasively obtain specimens from the lower airways to assess inflammation. The exhaled breath condensate (EBC) provides information from the lower to the upper airways and can be repeatedly performed.[29] For the EBC sample collection,