

Aline Duarte Ferreira^{a,b} <https://orcid.org/0000-0003-0102-1738>Ercy Mara Cípulo Ramos^a <https://orcid.org/0000-0002-3310-7336>Iara B. Trevisan^a <https://orcid.org/0000-0003-0743-3231>Marceli R. Leite^c <https://orcid.org/0000-0002-6795-8948>Mahara Proença^a <https://orcid.org/0000-0002-6684-4922>Luiz Carlos Soares de Carvalho-Junior^c <https://orcid.org/0000-0001-9040-7282>Alessandra Choqueta Toledo^d <https://orcid.org/0000-0001-8117-5045>Dionei Ramos^a <https://orcid.org/0000-0002-2956-7399>

^a Universidade Estadual Paulista, Departamento de Fisioterapia, Programa de Pós-graduação em Fisioterapia. Presidente Prudente, SP, Brazil.

^b Universidade do Oeste Paulista, Departamento de Fisioterapia. Presidente Prudente, SP, Brazil.

^c Universidade Federal de São Carlos, Departamento de Fisioterapia, Laboratório de Fisioterapia Cardiopulmonar. São Carlos, SP, Brazil.

^d Universidade de São Paulo, Faculdade de Medicina, Instituto do Coração. São Paulo, SP, Brazil.

Contact:

Aline Duarte Ferreira

E-mail:

alineduarteferreira@hotmail.com

The study received financial support (Auxílio à Pesquisa - Regular) from Fundação de Amparo à Pesquisa do Estado de São Paulo (Process 12/12901-0).

The authors declare no conflict of interest.

The study is not based on a thesis or dissertation and was not presented at a scientific meeting.

Received: 06/03/2017

Revised: 12/07/2017

Approved: 19/07/2017

Lung function and nasal mucociliary clearance in Brazilian sugarcane cutters exposed to biomass burning

Função pulmonar e depuração mucociliar nasal de cortadores de cana-de-açúcar brasileiros expostos à queima de biomassa

Abstract

Objective: to evaluate the effects of sugarcane burning on lung function and mucociliary clearance in sugarcane workers. **Methods:** sixteen sugarcane workers were evaluated in two sequential periods: during the non-harvest season, in April/2011, and during the sugarcane burning harvest season, in October/2011. Mean values (standard deviation) of lung function and mucociliary clearance were evaluated through spirometry and the saccharin transit time (STT) test, respectively. **Results:** lung function decreased %FEF₂₅₋₇₅ [99.31 (23.79) to 86.36 (27.41); $p = 0.001$]; %FEV₁ [92.19 (13.24) to 90.44 (12.76); $p = 0.022$]; and FEV₁/FVC [88.62 (5.68) to 84.90 (6.47); $p = 0.004$] during the harvest season compared with the non-harvest season. A significant decrease was found in saccharin transit time during the harvest [3 (1) min] season compared with the non-harvest season [8 (3) min] ($p < 0.001$). **Conclusion:** sugarcane workers present a decrease in %FEF₂₅₋₇₅, %FEV₁, FEV₁/FVC ratio, and increase in nasal mucociliary transport velocity at the end of the harvest season.

Keywords: rural worker; occupational health; mucociliary clearance; spirometry.

Resumo

Objetivos: avaliar a função pulmonar e a depuração mucociliar nasal de cortadores de cana-de-açúcar. **Métodos:** foram avaliados dezesseis cortadores de cana-de-açúcar em dois períodos: durante o plantio da cana-de-açúcar, em abril/2011, e no final da safra, no período de queima e colheita manual da cana-de-açúcar, outubro/2011. A função pulmonar e a depuração mucociliar foram avaliadas por meio da espirometria e do teste de tempo de trânsito da sacarina (TTS), respectivamente. **Resultados:** a função pulmonar apresentou diminuição no %FEF₂₅₋₇₅ [99,31 (23,79) para 86,36 (27,41); $p = 0,001$]; %VEF₁ [92,19 (13,24) para 90,44 (12,76); $p = 0,022$] e VEF₁/CVF [88,62 (5,68) para 84,90 (6,47); $p = 0,004$] no período da colheita em comparação ao de plantio. Também houve uma diminuição significativa no resultado do teste do TTS na colheita [3 (1) min] em comparação ao plantio [8 (3) min] ($p < 0,001$). **Conclusão:** os cortadores de cana-de-açúcar apresentaram diminuição do %FEF₂₅₋₇₅, %VEF₁, do índice VEF₁/CVF, e aumento da velocidade do transporte mucociliar nasal no final do período de colheita.

Palavras-chave: trabalhador rural; saúde do trabalhador; depuração mucociliar; espirometria.

Introduction

Brazil is the largest sugarcane producer in the world and the state of São Paulo is responsible for about 60% of the sugar and ethanol production^{1,2}. Sugarcane burning is adapted to facilitate the manual harvest process, exposing workers to high concentrations of particulate matter (PM) throughout the season^{3,4}.

Sugarcane burning causes gas emissions and releases particles in much higher concentrations than the levels recommended by the World Health Organization (WHO)⁵. This pollution significantly leads to adverse effects on human health, both acutely and chronically⁴, especially on rural workers who inhale noxious gases and PM for approximately forty-four hours per week during the harvest season⁶.

Inhalation of pollutants produced by sugarcane burning along with the intense physical exertion require a higher ventilation rate. This results in a greater probability of nasal, pulmonary and systemic inflammation, and impaired mucociliary clearance (MC), known to be the main upper airway defense mechanism against inhaled particles and microorganisms. It affects the lower airways, which can lead to lung function impairment^{7,8}.

A study has shown that acute exposure to sugarcane burning causes decreased mucociliary transport time in workers⁹. However, long-term effects are expected to increase mucociliary transport time¹⁰ and decrease lung function¹¹.

Thus, it is very important to know the effects of atmospheric pollutants, which can affect both MC and lung function, eventually leading to an increase in lung diseases and nasal symptoms^{7,9-11}. The aim of this study was to evaluate the effects of sugarcane burning on sugarcane workers' lung function and mucociliary clearance.

Methods

The study followed a prospective cohort design, as an exploratory, preliminary approach to the problem. It was carried out with a small group of sugarcane workers from a Sugar and Ethanol Company located in the west of the state of São Paulo, Brazil, who volunteered to participate in the study. Only non-smokers were included, and the following conditions were defined as exclusion criteria; history of nasal surgery or trauma; any

lung disease; nasal septum deviation; or recent episodes of upper airway infection. Each subject gave a written informed consent according to the Declaration of Helsinki. This study was approved by the Research Ethics Committee (15/2010) of São Paulo State University (UNESP).

The study was held in a sugarcane field in two sequential periods: during the manual sugarcane cultivation (non-harvest) in April 2011, and during harvesting after sugarcane burning in October 2011. An interview was conducted to record general information on the working process and each subject's clinical history. During the non-harvest and harvest seasons, we evaluated lung function was through spirometry; nasal MC through the saccharin transit time (STT) test; and exhaled carbon monoxide (CO) levels, which were measured to confirm smoking abstinence.

Spirometry followed the American Thoracic Society and European Respiratory Society guidelines¹², using a portable flow spirometer, Spirobank-MIR (version 3.6, MIR, Rome, Italy). The following parameters were recorded: absolute values and percentage of predicted values for forced vital capacity (FVC and %FVC); forced expiratory volume in the first second (FEV₁ and %FEV₁), forced expiratory flow between 25% and 75% FVC (FEF₂₅₋₇₅ and %FEF₂₅₋₇₅), FEV₁/FVC ratio, and peak expiratory flow (PEF and %PEF). Reference values available for the Brazilian population were used for comparison¹³.

The technique for measuring nasal MC has been described elsewhere^{9,14,15}. In brief, granulated sodium saccharin (250 µg) was deposited under visual control about 2 cm inside the right nostril. A timer was used to measure the saccharin transit time (STT) test. The STT test records the time elapsed from the moment the particles were placed until the moment the subject reports disguising the sweet taste of saccharine. Subjects were allowed to swallow freely and were asked to maintain normal ventilation, avoiding deep breaths, talking, sniffing, sneezing, eating, or coughing. If no response was reported after sixty minutes, the test was concluded after confirming, by placing saccharin powder directly on the subject's tongue, that was able to disguise the sweet taste normally. Participants were instructed not to use pharmacological agents, such as anesthetics, analgesics, barbiturates, tranquilizers, and antidepressants, and to avoid the ingestion of alcohol and caffeine-based substances, during the twelve hours preceding the test¹⁴.

Exhaled CO levels were measured using a CO analyzer (Micro CO Meter, Cardinal Health, Basingstoke, UK)¹⁶. To confirm smoking abstinence, the sugarcane workers were instructed to hold their breath for twenty seconds and then exhale slowly from functional vital capacity through a mouthpiece¹⁷. Two successive recordings were produced and the highest value was used. A cutoff value of < 6 ppm was used to define abstinence from smoking¹⁸.

For statistical analysis we used SPSS – Statistical Package for the Social Sciences (version 16.0; SPSS Inc; Chicago, USA) and R statistical software (version 2.13.0; R Foundation for Statistical Computing). The Shapiro-Wilk test was applied to verify data normality. To analyze lung function and STT results between the two seasons, we used, respectively, the paired Student’s t test and the Wilcoxon test, considering a statistical significance of 0.05.

Results

Of the 40 recruited male workers who volunteered to participate in the study, 30 met the inclusion criteria and 10 smokers were excluded.

Eleven of the 30 men were excluded because they did not return for reevaluation at harvest time, and three workers were excluded due to upper airway infections during the second evaluation. Results are presented as mean (standard deviation). A final group of sixteen sugarcane workers aged 25 (4) years, body mass index (BMI) 24 (3) kg/m², with exhaled CO of 2.1 (1.5) ppm were then evaluated in both non-harvest and harvest seasons. The average time the participants had been working in sugarcane harvesting was 3 (2) years.

Table 1 shows the spirometry values of the 16 subjects evaluated during the non-harvest seasons and the subsequent periods. A statistically significant decrease in FEF₂₅₋₇₅ and %FEF₂₅₋₇₅ was observed during the harvest season compared with the non-harvest season (p = 0.002; p = 0.001), respectively. A significant decrease was also found in FEV₁/FVC ratio and %FEV₁ during harvest compared with the non-harvest season (p = 0.004; p = 0.022), respectively.

The STT test results showed a significant decreased time in the harvest season, 3 (1) minutes, compared with the non-harvest one, 8 (3) minutes (p < 0.001).

Table 1 Sugarcane cutters’ (n=16) lung function values [average (standard deviation)] comparison between non-harvest season and harvest biomass burning season in a region of São Paulo State, Brazil, 2011

	<i>Non-harvest</i>	<i>Harvest</i>	<i>p*</i>
FVC	4.49 (0.57)	4.46 (0.67)	0.874
%FVC	97.00 (14.43)	92.43 (12.56)	0.177
FEV ₁	3.97 (0.45)	3.79 (0.60)	0.221
%FEV ₁	92.19 (13.24)	90.44 (12.76)	0.022
FEF ₂₅₋₇₅	5.08 (1.05)	4.42 (1.30)	0.002
%FEF ₂₅₋₇₅	99.31 (23.79)	86.36 (27.41)	0.001
FEV ₁ /FVC	88.62 (5.68)	84.90 (6.47)	0.004
PEF	9.33 (1.24)	9.11 (1.82)	0.545
%PEF	92.19 (13.25)	89.81 (17.23)	0.507

FVC: forced vital capacity; FEV₁: forced expiratory volume in the first second; FEF₂₅₋₇₅: expiratory flow between 25% and 75% FVC; PEF: peak expiratory flow. *Paired Student’s t test, considering a statistical significance of 0.05.

Discussion

The results of this study showed that, despite the spirometry values being within normal limits during both evaluation periods, a significant decrease was observed in %FEF₂₅₋₇₅, %FEV₁, and FEV₁/FVC during the harvest season.

Recent studies have shown that %FEF₂₅₋₇₅ reduction represents the functional impairment of the peripheral airways, and even when the FEV₁ values are within normal limits, a decrease in %FEF₂₅₋₇₅ may detect initial alterations in lung function^{11,19}.

For nonsmokers, Long-term exposure to PM results in pulmonary retention of fine particles and small airway remodeling¹⁹. Our results show that even after a short period of exposure some lung function impairment can be detected.

The study by Prado et al.¹¹ observed a reduction in in sugarcane workers' mean values of FEV₁, FEV₁/FVC, and FEF₂₅₋₇₅ after the harvest season, despite all the values remaining within reference values. The most affected variable was the %FEF₂₅₋₇₅, with reductions of about 31.1%. This decrease indicates the development of an initial obstructive disturbance in healthy subjects exposed to pollution, as observed in other studies²⁰⁻²².

One study that assessed agricultural exposure as a risk factor for the development of chronic obstructive pulmonary disease found a decreasing tendency for FEV₁ in parallel to an increase in exposure²³. The fact that this study design involved only a small number of subjects with evaluations over only two periods of time does not allow any inference that the respiratory effects are progressive and irreversible. This hypothesis should be verified in a cohort study with a larger population and over a longer period of time.

Agarwal et al.²⁴ also demonstrated a reduction in FEV₁ in a study investigating the pulmonary effects of exposure to air pollution from wheat-residue burning on healthy inhabitants in Punjab, India. These results, along with those by Montaña et al.²⁵ and Po et al.²⁶, who showed a relationship between exposure to biomass burning pollution and airway disease, provide the basis for the hypothesis that chronic exposure to this kind of air pollution could be a significant risk factor for the development of chronic respiratory diseases.

A previous study also reported that firefighters who did not use protective respiratory equipment presented a progressive decline in %FEV₁ and

FEV₁/FVC²⁷. It is important to emphasize that the subjects in this study did not use protective respiratory equipment.

In our study, the STT was shorter during the harvest season. Nasal MC is recognized as the first line of defense in the respiratory system, responsible for removing inhaled airborne particles and microorganisms. The efficiency of MC depends on three major components, among them the ciliary beat frequency (CBF)²⁸. Acting as a barrier, the respiratory ciliary epithelium removes mucus from the airways, and regulates innate and adaptive immunity²⁹.

Inhalation of pollutants and physical exertion result in a greater probability of nasal, pulmonary^{9,30} and systemic inflammation^{31,32}, as well as MC impairment^{9,10}.

The study by Ferreira-Ceccato et al.⁹ evaluated the acute effects of exposure to particulate matter from biomass burning on nasal MC of sugarcane workers. The authors observed a significant decrease in STT, supposing that acute exposure to particulate matter might be associated with increased oxidative stress and production of nitric oxide by inflammatory cells, which would stimulate ciliary beat frequency. This reasoning may also explain our findings.

Riechelmann et al.³³ reported two possible mechanisms underlying the observed acceleration effect on mucociliary transport: an auto regulated increase in ciliary beat frequency in response to increased mucus load and epithelial lining fluid viscosity, and the activation of mucociliary transport as a consequence of mucosal irritation.

Unlike our findings, the study by Goto et al.¹⁰ evaluated 27 sugarcane workers and observed that the harvest season was associated with a reduction of 80% MC, that is, an average prolonged saccharin transit time of 7.83 min (1.88 – 13.78; 95% confidence interval). However, these evaluations were not made in the field and data for both seasons were collected after a 5-day working week.

In short, this study suggests that sugarcane workers present a decrease in FEF₂₅₋₇₅, %FEF₂₅₋₇₅, FEV₁/FVC ratio, and an increased nasal mucociliary transport velocity at the end of the harvest season. Such results may be further investigated by a prospective cohort design study involving a larger group of workers during a longer follow-up period.

Acknowledgments

To Instituto Nacional de Análise Integrada do Risco Ambiental (INAIRA) for the intellectual support to the development of this study.

Authors' contributions

The authors equally contributed to all steps of the research and to the conception and approval of the final version of the manuscript.

References

1. Rudorff BFT, Aguiar DA, Silva WF, Sugawara LM, Adami M, Moreira MA. Studies on the rapid expansion of sugarcane for ethanol production in São Paulo state (Brazil) using Landsat data. *Remote Sens*. 2010;2(4):1057-76.
2. Goldemberg J. Ethanol for a sustainable energy future. *Science* 2007;315(5813):808-10.
3. Luz VG, Zangirolani LTO, Vilela RAG, Corrêa Filho HR. Food consumption and working conditions in manual sugarcane harvesting in Sao Paulo state. *Saúde Soc*. 2014;23(4):1316-28.
4. Arbex MA, Cançado JED, Pereira LAA, Braga ALF, Saldiva PHN. [Biomass burning and its effects on health]. *J Bras Pneumol*. 2004;30(2):158-75. Portuguese.
5. World Health Organization. WHO air guidelines for particulate matter, ozone, dioxid and sulfer dioxid: global update 2005 [Internet]. Geneva: WHO; 2005 [cited on 2015 Jul 16]. Available from: http://apps.who.int/iris/bitstream/10665/69477/1/WHO_SDE_PHE_OEH_06.02_eng.pdf
6. França DA, Longo KM, Soares Neto TG, Santos JC, Freitas SR, Rudorff BFT, et al. Pre-harvest sugarcane burning: determination of emission factors through laboratory measurements. *Atmosphere*. 2012;3(1):164-80.
7. Arbex MA, Santos UP, Martins LC, Saldiva PHN, Pereira LAA, Braga ALF. Air pollution and the respiratory system. *J Bras Pneumol*. 2012;38(5):643-55.
8. Shusterman D. The effects of air pollutants and irritants on the upper airway. *Proc Am Thorac Soc*. 2011;8(1):101-5.
9. Ferreira-Ceccato AD, Ramos EM, Carvalho LC Jr, Xavier RF, Teixeira MF, Raymundo-Pereira PA, et al. Short-term effects of air pollution from biomass burning in mucociliary clearance of Brazilian sugarcane cutters. *Respir Med*. 2011;105(11):1766-8.
10. Goto DM, Lança M, Obuti CA, Barbosa CMG, Saldiva PHN, Zanetta DMT, et al. Effects of biomass burning on nasal mucociliary clearance and mucus properties after sugarcane harvesting. *Environ Res*. 2011;111(5):664-9.
11. Prado GF, Zanetta DM, Arbex MA, Braga AL, Pereira LA, Marchi MR, et al. Urnt sugarcane harvesting: particulate matter exposure and the effects on lung function, oxidative stress, and urinary 1-hydroxypyrene. *Sci Total Environ*. 2012;437:200-8.
12. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardization of spirometry. *Eur Respir J*. 2005;26(2):319-38.
13. Duarte AAO, Pereira CAC, Rodrigues SCS. Validation of new Brazilian predicted values for forced spirometry in Caucasians and comparison with predicted values obtained using other reference equations. *J Bras Pneumol*. 2007;33(5):527-35.
14. Stanley P, MacWilliam L, Greenstone M, Mackay I, Cole P. Efficacy of a saccharin test for screening to detect abnormal mucociliary clearance. *Br J Dis Chest*. 1984;78(1):62-5.
15. Ito JT, Ramos D, Lima FF, Rodrigues FM, Gomes PR, Moreira GL, et al. Nasal mucociliary clearance in subjects with COPD after smoking cessation. *Respir Care*. 2015;60(3):399-405.
16. Jarvis MJ, Belcher M, Vesey C, Hutchison DC. Low cost carbon monoxide monitors in smoking assessment. *Thorax*. 1986;4(11):886-7.
17. Middleton ET, Morice AH. Breath carbon monoxide as an indication of smoking habit. *Chest*. 2000;117(3):758-63.
18. Javors MA, Hatch JP, Lamb RJ. Cut-off levels for breath carbon monoxide as a marker for cigarette smoking. *Addiction*. 2005;100(2):159-67.
19. Pope CA 3rd, Dockery DW. Health effects of fine particulate air pollution: lines that connect. *J Air Waste Manag Assoc*. 2006;56(6):709-42.
20. Simon MR, Chinchilli VM, Phillips BR, Sorkness CA, Lemanske RF Jr, Szeffler SJ, et al. FEF₂₅₋₇₅ and FEV₁/FVC in relation to clinical and physiologic parameters in asthmatic children with normal FEV₁ values. *J Allergy Clin Immunol*. 2010;126(3):527-34.
21. Regalado J, Pérez-Padilla R, Sansores R, Páramo Ramirez JI, Brauer M, et al. The effect of biomass burning on respiratory symptoms and lung

- function in rural Mexican women. *Am J Respir Crit Care Med.* 2006;174(8):901-5.
22. Minelli C, Wei I, Sagoo G, Jarvis D, Shaheen S, Burney P. Interactive effects of antioxidant genes and air pollution on respiratory function and airway disease: a HuGE review. *Am J Epidemiol.* 2011;173(6):603-20.
 23. Bailey KL, Meza JL, Smith LM, Von Essen SG, Romberger DJ. Agricultural exposures in patients with COPD in health systems serving rural areas. *J Agromedicine.* 2007;12(3):71-6.
 24. Agarwal R, Awasthi A, Mittal S, Singh N, Gupta PK. Effects of air pollution on respiratory parameters during the wheat-residue burning in Patiala. *J Med Eng Technol.* 2010;34(1):23-8.
 25. Montaña M, Cisneros J, Ramírez-Venegas A, Pedraza-Chaverri J, Mercado D, Ramos C, et al. Malondialdehyde and superoxide dismutase correlate with FEV(1) in patients with COPD associated with wood smoke exposure and tobacco smoking. *Inhal Toxicol.* 2010;22(10):868-74.
 26. Po JY, Fitzgerald JM, Carlsten C. Respiratory disease associated with solid biomass fuel exposure in rural women and children: systematic review and meta-analysis. *Thorax.* 2011;66(3):232-9.
 27. Almeida AG, Duarte R, Mieiro L, Paiva AC, Rodrigues AM, Almeida MH, et al. [Pulmonary function in portuguese firefighters]. *Rev Port Pneumol.* 2007;13(3):349-64. Portuguese.
 28. Donnelley M, Morgan KS, Siu KKW, Parsons DW. Dry deposition of pollutant and marker particles onto live mouse airway surfaces enhances monitoring of individual particle mucociliary transit behavior. *J Synchrotron Radiat.* 2012;19(Pt 4):551-8.
 29. Pérez Bravo F, Méndez AG, Lagos AR, Vargas Munita SL. Dinámica y patología del barrido mucociliar como mecanismo defensivo del pulmón y alternativas farmacológicas de tratamiento. *Rev Méd Chile.* 2014;142(5):606-15.
 30. Torres-Duque C, Maldonado D, Pérez-Padilla R, Ezzati M, Viegi G, Forum of International Respiratory Studies (FIRS) Task Force on Health Effects of Biomass Exposure. Biomass fuels and respiratory diseases: a review of the evidence. *Proc Am Thorac Soc.* 2008;5(5):577-90.
 31. Van Eeden SF, Tan WC, Suwa T, Mukae H, Terashima T, Fujii T, et al. Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter air pollutants (PM₁₀). *Am J Respir Crit Care Med.* 2001;164(5):826-30.
 32. Seagrave J. Mechanisms and Implications of air pollution particle associations with chemokines. *Toxicol Appl Pharmacol.* 2008;232(3):469-77.
 33. Riechelmann H, Rettinger G, Weschta M, Keck T, Deutschle T. Effects of low-toxicity particulate matter on human nasal function. *J Occup Environ Med.* 2003;45(1):54-60.