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# Reversibility of impaired nasal mucociliary clearance in smokers following a smoking cessation programme

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## ABSTRACT

*Background and objective:* **Smoking cessation (SC) is recognized as reducing tobacco-associated mortality and morbidity. The effect of SC on nasal mucociliary clearance (MC) in smokers was evaluated during a 180-day period.**

*Methods:* **Thirty-three current smokers enrolled in a SC intervention programme were evaluated after they had stopped smoking. Smoking history, Fagerström's test, lung function, exhaled carbon monoxide (eCO), carboxyhaemoglobin (COHb) and nasal MC as assessed by the saccharin transit time (STT) test were evaluated. All parameters were also measured at baseline in 33 matched non-smokers.**

*Results:* **Smokers (mean age 49 ± 12 years, mean pack-year index 44 ± 25) were enrolled in a SC intervention and 27% ($n = 9$) abstained for 180 days, 30% ($n = 11$) for 120 days, 49.5% ($n = 15$) for 90 days or 60 days, 62.7% ($n = 19$) for 30 days and 75.9% ($n = 23$) for 15 days. A moderate degree of nicotine dependence, higher education levels and less use of bupropion were associated with the capacity to stop smoking ($P < 0.05$). The STT was prolonged in smokers compared with non-smokers ($P = 0.002$) and dysfunction of MC was present at baseline both in smokers who had abstained and those who had not abstained for 180 days. eCO and COHb were also significantly increased in smokers compared with non-smokers. STT values decreased to within the normal range on day 15 after SC ($P < 0.01$), and remained in the normal range until the end of the study period. Similarly, eCO values were reduced from the seventh day after SC.**

*Conclusions:* **A SC programme contributed to improvement in MC among smokers from the 15th day after cessation of smoking, and these beneficial effects persisted for 180 days.**

## SUMMARY AT A GLANCE

Mucociliary clearance was measured in smokers enrolled in a Smoke Cessation Intervention Programme. Impairment of nasal mucociliary transport in smokers was reversed from the 15th day after cessation of smoking, and these beneficial effects persisted for 6 months.

**Key words:** airway epithelium, mucociliary transport, public health, smoking cessation, tobacco.

## INTRODUCTION

Smoking of tobacco is the leading cause of preventable deaths worldwide.[1] Currently, approximately six million people worldwide die from smoking-induced diseases each year, and according to the World Health Organization, smoking-related deaths are projected to rise to eight million per year by 2030.[1] Exposure to tobacco smoke is a significant risk factor for the development of respiratory diseases, including COPD, and a number of other diseases. Besides, smoking of tobacco is associated with exacerbations of pulmonary diseases.[2–5] Tobacco smoke is a complex mixture of thousands of toxic chemical compounds, of which more than 200 are highly toxic.[6] High levels of oxidants and reactive oxygen species have been detected in both mainstream and sidestream smoke, resulting in a large oxidant burden at the epithelial surface of the lung.[7] Endogenous production of carbon monoxide (CO) is usually linked to oxidative stress.[8,9]

Exposure to cigarette smoke induces a significant decrease in host defence resulting from mucociliary clearance in the nose and airways. This mucociliary clearance mechanism provides essential protection against a wide variety of noxious inhaled particles and microorganisms by unidirectional transport towards the oropharynx.[10] The saccharin transit time (STT) test has been shown to be a simple, cheap, reproducible and effective method of assessing mucociliary clearance and its use is widely accepted.[11]

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Comprehensive smoking cessation programmes are regarded as the most effective method of reducing tobacco-related mortality and morbidity. In the absence of any assistance, only 6% of attempts to quit smoking are successful.[12,13] Smoking cessation programmes with 1 year of follow-up increase the rate of quitting by around 30%.[14] Measurements of exhaled CO have been shown to be useful for the detection and management of smoking status in these smoking cessation programmes.[15] Smoking cessation is associated with improved lung function and reduction in respiratory symptoms, airway inflammation and endothelial dysfunction.[16] Despite the clear benefits of smoking cessation for respiratory health, the specific effects on mucociliary transport have not been fully investigated. We hypothesized that mucociliary clearance is improved after smokers enrolled in a smoking cessation programme abstain from smoking. To test this hypothesis, STT was measured in ex-smokers, 12 h after cessation of smoking and during a 6-month follow-up period.

## METHODS

### Subjects

Individuals enrolled in the 'Anti-Tobacco Orientation and Awareness' programme at the Faculty of Sciences and Technology, São Paulo State University (FCT/UNESP), Brazil were invited to participate in this study. Participants gave written informed consent according to the Declaration of Helsinki of the World Medical Association. During the first month, patients underwent a weekly assessment of lung function, and measurements of exhaled CO and nasal mucociliary clearance. For the last 5 months of the study these measurements were performed on a monthly basis. Two groups of subjects were evaluated: 33 current smokers enrolled in the Anti-Tobacco Orientation and Awareness programme (smoking cessation group) and 33 non-smoking individuals (control group). Individuals with cystic fibrosis, bronchiectasis, immotile cilia syndrome, a history of nasal surgery or trauma, inflammation of the upper airways or smoking-related diseases were excluded. The study was approved by the Ethics and Research Committee of São Paulo State University.

### Study design and protocol

All individuals underwent an interview in order to obtain personal data, a detailed smoking history and a complete medical history. The Fagerström Tolerance Questionnaire was completed at the first appointment.[17] In the smoking cessation group, spirometry and measurements of exhaled CO, carboxyhaemoglobin (COHb) and nasal mucociliary transport by the STT test were performed at baseline (12 h after smoking the last cigarette) and again at 7, 15, 30, 60, 90, 120, 150 and 180 days after cessation of smoking and while individuals continued to abstain from smoking. A group of non-smokers with similar demographic characteristics was also evaluated at baseline.

### Smoking cessation programme

The smoking cessation programme was originally developed by the National Cancer Institute (INCA) in Brazil.[18] This programme is based on cognitive behavioural treatment, either with or without the aid of medical therapy. The behavioural programme consisted of 20 sessions that were offered to the participants during the course of 1 year. In the first 2 weeks, four 1-h sessions were offered to groups of 10–15 smokers. It was expected that the smokers would quit smoking after the fourth meeting. Sessions 4–10 were offered in the following 6 weeks, to guide subjects through the smoking cessation phase. Sessions 11–20 were offered on a monthly basis, to maintain abstinence from smoking for up to 1 year. During the programme, particular attention was paid to the benefits of smoking cessation, and ex-smokers had the opportunity to call a counsellor and/or receive individual support between meetings whenever it was necessary. Smoking cessation was considered successful when participants maintained their abstinence from smoking for at least 6 months.[19] An ex-smoker was defined as someone who had refrained from smoking for at least 1 year.

### Measurement of lung function and exhaled CO

Spirometry was performed according to the guidelines of the American Thoracic Society,[20] using a portable spirometer (Spirobank-MIR version 3.6, MIR, Rome, Italy). Reference values specific for the Brazilian population were used.[21] Exhaled CO levels were used as a biochemical marker for abstinence from smoking, and were used in combination with self-reported smoking status.[15] Exhaled CO and carboxyhaemoglobin (%COHb) were measured using a CO analyser (Micro CO Meter, Cardinal Health, Basingstoke, UK).[22] Subjects were instructed to hold their breath for 20 s and then to exhale slowly from functional vital capacity through a mouthpiece. Two successive recordings were performed and the highest value was used. A low threshold for exhaled CO levels (<8 ppm) was used in order to verify abstinence from smoking with a high degree of certainty.[23]

### Measurement of mucociliary clearance by the STT test

The technique for measuring nasal mucociliary clearance has been reported previously.[11] In brief, granulated sodium saccharin (250 μg) was deposited under visual control at a point about 2 cm inside the right nostril. Nasal mucociliary transport was measured as the time it took for subjects to perceive a sweet taste. If no response was reported at 60 min,

the test was concluded after confirming the subject had normal sweet taste perception by placing saccharin powder directly on the tongue. Individuals were instructed not to breathe deeply, talk, cough, sneeze or sniff during the test. Participants were also instructed not to use pharmacological agents such as anaesthetics, analgesics, barbiturates, tranquilizers and antidepressants, as well as alcohol and caffeine-based substances, during the 12 h preceding the test. Tests were conducted between 8 and 9 AM for all participants, to avoid variation in the analysed parameters.

### Statistical analyses

Statistical analyses were performed using Sigma Stat 10 software (Systat Software, Inc., San Jose, CA, USA). Comparisons of baseline values between non-smokers and the smoking cessation group were performed using *t*-tests. To compare determinants of quitting behaviour between individuals who dropped out and those who abstained from smoking for 180 days, with consideration of qualitative variables such as gender, educational level, Fagerström's test, medication use and the number of cigarettes smoked per day, the Goodman test was used. Comparisons of exhaled CO, COHb and STT test values at different times during the smoking cessation programme were performed by repeated measures analysis of variance, followed by the Holm-Sidak test for parametric data, or by analysis of variance on ranks followed by Dunn's test for non-parametric data. Differences were considered significant at $P < 0.05$.

## RESULTS

Thirty-three individuals enrolled in a smoking cessation group and 33 healthy subjects (control group) were included in this study. The mean ages of the smoking cessation group and the control group were 49 ± 12 years and 52 ± 14 years, respectively, and their mean BMI were 26 ± 3 kg/m$^2$ and 26 ± 4 kg/m$^2$ respectively. Demographic and spirometric data, as well as data on smoking behaviour and Fagerström's test for nicotine dependence, for subjects in the smoking cessation and control groups are presented in Table 1. There were no differences in demographic characteristics between the groups. Baseline lung function was significantly lower in smokers than in non-smokers ($FEV_1$ 2.6 ± 0.9 L and 3.4 ± 0.7 L, respectively, $P < 0.001$).

Nine subjects in the smoking cessation group (27%) abstained from smoking for the entire 180-day period. Twenty-three of the 33 patients were not smoking 2 weeks after starting the programme, 19 were not smoking after 30 days, 15 after 90 days, 11 after 120 days and 9 after 180 days. Individuals who were still abstaining after 180 days showed baseline characteristics of light to moderate levels of nicotine dependence, higher education levels and less use of bupropion, as compared with patients who started smoking again after an initial attempt to quit (Table 2).

Baseline values for the STT test were prolonged in smokers compared with non-smokers ($P = 0.002$) (Fig. 1a). Exhaled CO and COHb were also significantly increased in smokers compared with non-smokers (Fig. 1b,c, respectively, $P < 0.001$). Exhaled CO was detected in all subjects.

**Table 1** Demographic and spirometric data, tobacco smoking behaviour and Fagerström's test for nicotine dependence for subjects in the smoking cessation and control groups

| Characteristics | Smoking cessation group ($n = 33$) | Control group ($n = 33$) | *P*-value |
|---|---|---|---|
| Demographic | | | |
| Male gender, % | 55 | 36 | 0.14 |
| Age, years, mean ± SD | 49 ± 12 | 52 ± 14 | 0.44 |
| BMI, kg/m$^2$, mean ± SD | 26 ± 4 | 26 ± 4 | 0.43 |
| Spirometric values | | | |
| $FEV_1$/FVC, %, mean ± SD | 78 ± 8 | 81 ± 4 | 0.27 |
| FVC, L, mean ± SD | 3.5 ± 1.1 | 4.1 ± 0.8 | 0.67 |
| FVC, % predicted, mean ± SD | 100 ± 17 | 102 ± 14 | 0.66 |
| $FEV_1$, L, mean ± SD | 2.6 ± 0.9 | 3.4 ± 0.7 | 0.06 |
| $FEV_1$, % predicted, mean ± SD | 94 ± 17 | 104 ± 13 | <0.001 |
| Tobacco smoking behaviour | | | |
| Years smoked, mean ± SD | 21 ± 8 | — | |
| Pack-years index, mean ± SD | 44 ± 25 | — | |
| Cigarettes smoked per day, mean ± SD | 20 ± 10 | — | |
| Light smokers, % | 49 | — | |
| Moderate smokers, % | 30 | — | |
| Heavy smokers, % | 21 | — | |
| Fagerström's test, % | | | |
| Mild | 21 | — | |
| Moderate | 76 | — | |
| Severe | 3 | — | |

**Table 2** Demographic data, education, tobacco smoking behaviour, medication used to stop smoking and Fagerström's test for nicotine dependence for subjects in the smoking cessation and control groups

| Characteristics | Smokers who were abstaining at 180 days ($n = 9$) | Smokers who were not abstaining ($n = 24$) |
|---|---|---|
| Demographic | | |
| Male gender, % | 67 | 50 |
| Age, years, mean ± SD | 54 ± 12 | 47 ± 12 |
| BMI, kg/m$^2$, mean ± SD | 24 ± 4 | 26 ± 4 |
| Education, % | | |
| Elementary | 22 | 54 |
| High school | 11 | 33 |
| University | 67* | 13 |
| Tobacco smoking behaviour | | |
| Years smoked, mean ± SD | 17 ± 10 | 22 ± 7 |
| Pack-years index, mean ± SD | 20 ± 9* | 28 ± 14 |
| Cigarettes smoked per day, mean ± SD | 16 ± 11 | 21 ± 10 |
| Light smokers, % | 67 | 42 |
| Moderate smokers, % | 22 | 33 |
| Heavy smokers, % | 11 | 25 |
| Medication used to stop smoking, % | | |
| None | 22 | 33 |
| Antidepressant (bupropion) | 11* | 15 |
| Nicotine patch | 67 | 52 |
| Fagerström's test, % | | |
| Mild | 45 | 17 |
| Moderate | 55 | 79 |
| Severe | 0 | 4 |

* $P < 0.05$ compared with smokers who were not abstaining.

Baseline dysfunction in mucociliary clearance was similar in smokers who were or were not abstaining at 180 days (median STT 4.4 min (interquartile range 4.1–5.0) and 7 min (2.2–8.4), respectively, $P = 0.34$). There was a significant decrease in STT in smokers from the 15th to the 180th day after cessation of smoking, as compared with baseline values (Fig. 2a, $P < 0.01$). There was also a reduction in STT on day 7 as compared with day 60 ($P < 0.01$). Exhaled CO and COHb were significantly lower 7 days after cessation of smoking, as compared with baseline (Fig. 2b,c, $P < 0.001$). Similar results were observed 15, 30, 60, 90, 120 and 180 days after cessation of smoking, as compared with baseline.

## DISCUSSION

The present study demonstrated for the first time that smoking cessation, besides improving lung function and reducing the levels of exhaled CO and COHb, also improved nasal mucociliary clearance. The study also demonstrated that these effects were detectable from day 15 after cessation of smoking and were maintained for up to 6 months.

In this study, the quit rate at the end of 180 days was 27%. Notably, the individuals who abstained from smoking were those who were considered light smokers and had a higher level of education, which was similar to the findings from previous studies.[14,24] Also, in line with previous findings, nicotine dependence was related to the capacity to stop smoking.[25] Better adherence to the programme at 180 days was not correlated with greater use of bupropion therapy, as reported in a previous study.[26]

Earlier studies have demonstrated that tobacco smoking damages the respiratory epithelium and impairs host respiratory defences, thereby contributing to increased susceptibility to infections.[4,5,27] Mucociliary clearance is impaired in smokers compared with non-smokers, and the results from this study corroborate previous findings.[28] Swan *et al.* showed a consistent effect of smoking cessation on cytomorphological features, such as fewer columnar cells, less mucus and reduced epithelial cell metaplasia, in quitters as compared with non-quitters.[29] However, as far as we know, no previous study has focused on the effects of smoking cessation on nasal mucociliary clearance. The present study showed that impairment of nasal mucociliary clearance was reversible in smokers who enrolled in a smoking cessation programme. After cessation of smoking, nasal mucociliary clearance remained within the normal range from day 15 up to 6 months, suggesting that there was a significant improvement in epithelial function.

Mucociliary clearance is usually impaired by oxidative stress induced by exposure to cigarette smoke.[30] Oxidative stress induced by smoking disturbs cell differentiation, as well as repair and function of airway epithelial cells.[6] The gaseous phase of cigarette smoke contains high concentrations of free radicals (>$10^{15}$ molecules per puff),[6] resulting in increased oxidative

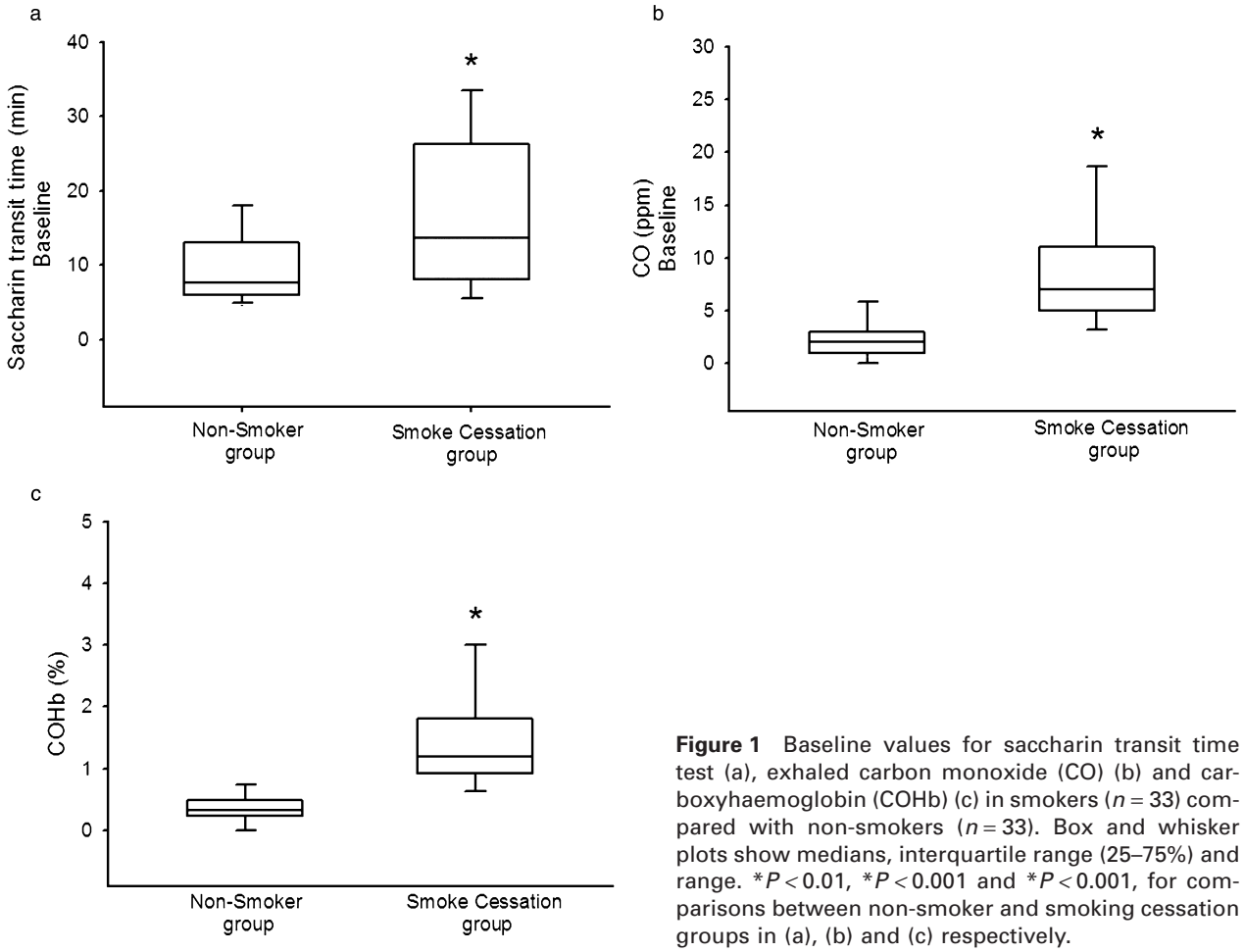


**Figure 1** Baseline values for saccharin transit time test (a), exhaled carbon monoxide (CO) (b) and carboxyhaemoglobin (COHb) (c) in smokers ($n = 33$) compared with non-smokers ($n = 33$). Box and whisker plots show medians, interquartile range (25–75%) and range. $*P < 0.01$, $*P < 0.001$ and $*P < 0.001$, for comparisons between non-smoker and smoking cessation groups in (a), (b) and (c) respectively.

stress with consequent changes in the function of the airway epithelium.[31] CO is synthesized in many tissues of the body as a product of haem degradation by the enzyme haem oxygenase (HO).[32] This enzyme is present in alveolar macrophages,[33] and is upregulated by oxidative stress,[30,34] nitric oxide (NO)[35] and inflammatory cytokines.[36] CO is also produced by oxidative metabolism of exogenous hydrocarbons, including dichloromethane, other dihalomethanes and carbon tetrachloride, and by the haem oxidase catalysed photo-oxidation of organic compounds, as well as auto-oxidation of phenols and lipid peroxidation of cell membrane lipids.[37,38] The CO that is produced diffuses into the blood, is transported by haemoglobin and excreted by the lungs.

Endogenous CO production is usually associated with oxidative stress.[8,9] Measurement of exhaled CO has been shown to be useful for the detection and management of smoking status during smoking cessation programmes. Measurement of CO concentrations in expired air may be useful during follow-up of smokers in the process of quitting, as well as allowing them to monitor their exhaled CO levels. Monitoring of exhaled CO levels may motivate individuals to abstain during smoking cessation programmes.[15] The present study showed that 7 days after cessation of smoking, exhaled CO and COHb levels declined to normal and remained in this range during the 6-month study period. Previous studies have reported that exhaled CO levels may be increased by up to 7 ppm in inflammatory pulmonary diseases such as asthma, primary ciliary dyskinesia and bronchiectasis, demonstrating that exhaled CO levels may indicate the induction of HO-1 and oxidative stress.[38,39] One explanation for the improvement in nasal mucociliary clearance after cessation of smoking is that the decline in exhaled CO may be partly mediated by a decrease in oxidative stress. Giuca *et al*. showed that exposure to cigarette smoke may alter the detoxification of hydrogen peroxide through decreased activity of the antioxidant enzyme, glutathione peroxidase, and this effect may be reduced after cessation of smoking.[40] Favourable effects on oxidant-antioxidant imbalance after smoking cessation may possibly increase ciliary beat frequency and improve mucociliary clearance.

A limitation of this study was that attendance at meetings and evaluation was not continued for subjects, who despite repeated telephone contact, dropped out of the study. Most subjects who started smoking again consumed fewer cigarettes per day than before their attempt to quit. Therefore, the study was not able to show whether exhaled CO, COHb and STT in these individuals returned to baseline levels immediately after they started smoking again.

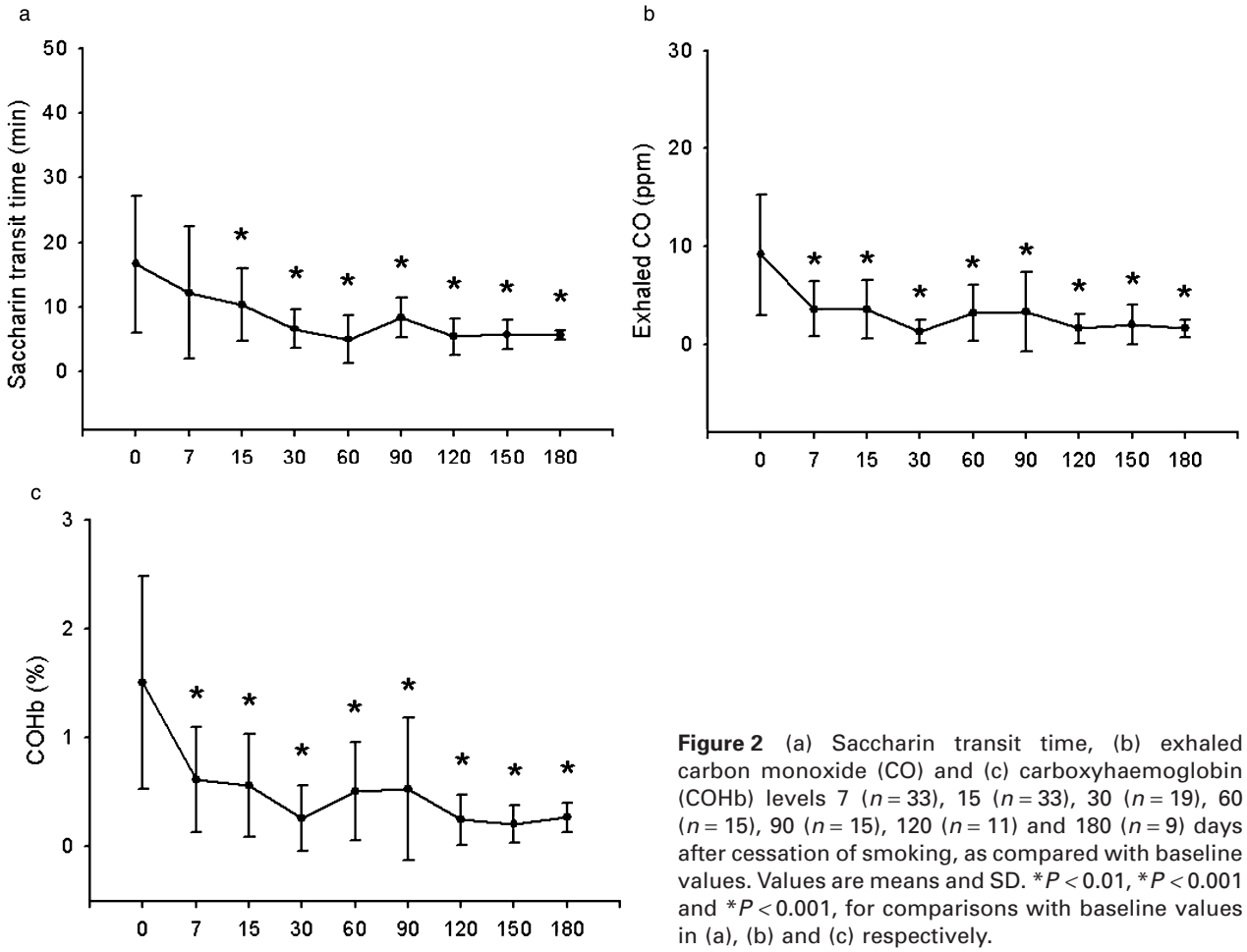


**Figure 2** (a) Saccharin transit time, (b) exhaled carbon monoxide (CO) and (c) carboxyhaemoglobin (COHb) levels 7 ($n = 33$), 15 ($n = 33$), 30 ($n = 19$), 60 ($n = 15$), 90 ($n = 15$), 120 ($n = 11$) and 180 ($n = 9$) days after cessation of smoking, as compared with baseline values. Values are means and SD. *$P < 0.01$, *$P < 0.001$ and *$P < 0.001$, for comparisons with baseline values in (a), (b) and (c) respectively.

In conclusion, this study has shown that smoking cessation contributes to improved nasal mucociliary clearance in smokers from day 15 after cessation, and that these effects are maintained for up to 6 months.

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