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Recovery of Acquired Cystic Fibrosis Transmembrane Conductance Regulator Dysfunction after Smoking Cessation

To the Editor:

Smoking is a key contributor to airway disease in addition to nonpulmonary disorders (1–4). One proposed mechanism involves acquired dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) protein, as cigarette smoke has been demonstrated to reduce CFTR activity both *in vitro* and *in vivo* (5–7) and is associated with clinical symptoms such as chronic bronchitis (7–10). Although direct smoke exposure to airway epithelial cells has been shown to cause respiratory CFTR dysfunction, it has only recently been observed that cigarette smoke is also associated with extrapulmonary (i.e., systemic) CFTR dysfunction, as detected by sweat chloride (9). This finding was confirmed in a distinct cross-sectional study using β-adrenergic sweat secretion rate as an alternative method to measure mild abnormalities in CFTR function (8). We hypothesized that smoking cessation will lead to improved systemic CFTR function, indicating a causal link in humans.

Methods

Smoking cessation program. Human protocols were approved by the University of Alabama at Birmingham's Institutional Review Board, and all subjects provided written informed consent. Eligible participants were otherwise healthy smokers, 19–70 years old, who were willing to quit smoking. Required smoking intensity before enrollment was at least 20 cigarettes per day for 6 months or longer. Spirometry was performed according to American Thoracic Society criteria, and FEV_1 and FEV_1/FVC were required to be above the lower limit of normal. Subjects were evaluated at screening and then after smoking cessation (Figure 1A). Serum cotinine and exhaled carbon monoxide (CO) measurements were used to confirm abstinence. If a patient resumed smoking after cessation, further assessments were discontinued. Healthy smokers and healthy nonsmokers were used as controls with otherwise similar inclusion criteria. Controls had three repeated measures of sweat evaporimetry, with each test separated by at least 1 day (median, 10.5 d).

Sweat testing. Sweat evaporimetry was used to measure exocrine function of the sweat gland, and sweat chloride was used to determine ductular function of CFTR. Sweat evaporimetry was considered the primary endpoint because of its proposed sensitivity for minimal abnormalities in CFTR function (8, 11). Sweat rates were calculated as the maximal stable rate observed after β-adrenergic injection minus the lowest observed rate after the preceding atropine injection, as previously described (8, 11). The rate of evaporative water loss (kg water loss/m^2/h) is expressed as evaporimeter units. Sweat chloride was measured by quantitative pilocarpine iontophoresis, using the Macroduct system (Westcor Inc., Logan, UT) (12–14).

Genetic testing. Genetic testing (50 mutation analysis) for CFTR mutations was performed by using a commercially accredited facility (Baylor Medical Genetics, Houston, TX). Patients with a CFTR mutation were excluded from the analysis. This test accounts for approximately 85% of the most common alleles found in the US population.

Statistics. Sweat tests were compared using repeated-measures analysis of variance or paired *t* test. Post hoc tests for multiple comparisons were calculated using Fisher's least significant difference. All statistical tests were two-sided and performed at a 5% significance level, using GraphPad Prism (La Jolla, CA). For points containing missing data because of subject discontinuation (n = 1), available data were analyzed; last observed values were carried forward.

Results

Nine subjects provided consent for the study. Seven met inclusion criteria and were enrolled in the smoking cessation program. One subject resumed smoking 10 days into the study and was subsequently withdrawn, but was included in the analysis with available data. Baseline characteristics are provided in Table 1. The median age was 47 years (range, 27–59 yr), and 43% were female. The median FEV_1 and FEV_1/FVC ratios were 3.94 L (101% predicted) and 0.76, respectively, reflecting near-normal lung function. Smoking intensity was relatively

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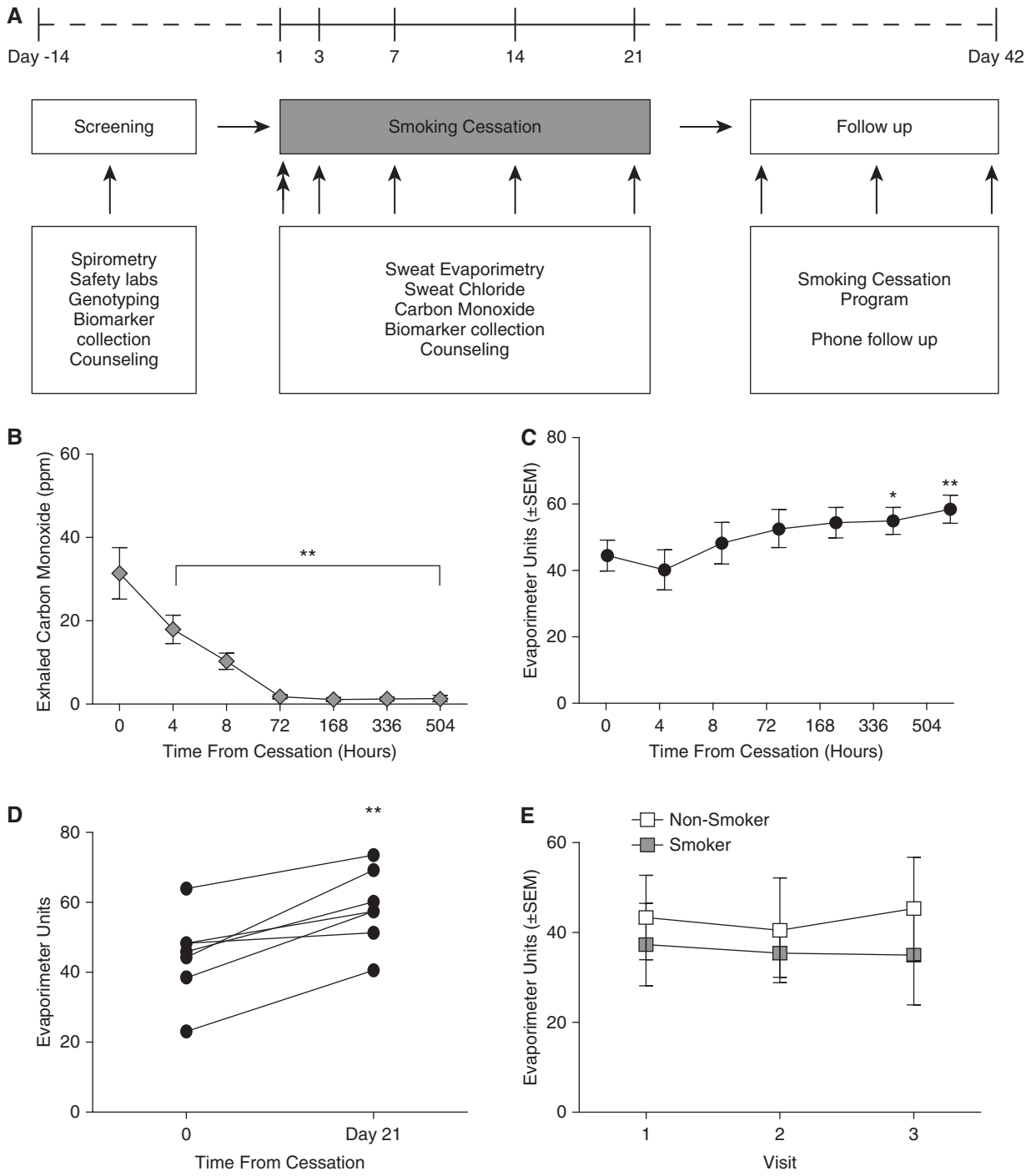

Figure 1. The effect of smoking cessation on cystic fibrosis transmembrane conductance regulator (CFTR) function. (*A*) Study design. (*B*) Exhaled carbon monoxide measured at each point after smoking cessation. (*C*) CFTR-dependent β-adrenergic sweat secretion rate measured at each point after smoking cessation. (*D*) β-Adrenergic sweat secretion rate for each study subject that completed the study, before and 21 days after smoking cessation. (*E*) CFTR-dependent β-adrenergic sweat secretion rate measured at three points 1–7 days apart in healthy smokers and normal healthy nonsmokers who did not alter smoking habits during the study. *White*, nonsmokers; *gray*, smokers. *$P < 0.05$; **$P < 0.01$.

heavy, with a median history of 19 pack-years (range, 12–43 pack-years) and a current use of 1.5 packs/day (range, 1–2 packs/d), and was greater than prior populations studied previously (8). CO levels dropped rapidly after smoking cessation (Figure 1B).

The mean (± standard deviation) CFTR-dependent sweat secretion rate for healthy smokers was 44.5 ± 12.3 at the start of cessation and increased to 58.5 ± 10.9 on Day 21 ($P < 0.005$; Figures 1C and 1D). A statistically significant difference from time 0 was observed by Day 14 (55.0 ± 10.8; $P < 0.05$) and persisted at

Table 1. Subject Characteristics

Subject	Age (*yr*)	Packs/d	Pack-Years	Sex	Race
1	57	1.5	64.5	Male	White
2	47	1	17	Female	African American
3	36	1	17	Female	White
4	37	2	38	Male	White
5	59	1.5	61.5	Male	African American
6	27	1	12	Female	White
7	56	1.5	57	Male	African American

Day 21. Findings remained significant when the subject who resumed smoking was omitted from the analysis. Sweat chloride was normal (18.4 ± 12.0 mEq/L) at baseline and did not significantly change after smoking cessation. Similarly, evaporative sweat loss induced by cholinergic (non–CFTR dependent) stimulus was not affected by smoking cessation (70.6 ± 12.7 Day 0 vs. 70.1 ± 6.3 Day 21), consistent with prior cross-sectional studies (8).

In comparison to changes in evaporimetry on smoking cessation among healthy smokers, healthy smokers (n = 4) who did not participate in the smoking cessation program and who maintained stable smoking habits (median age, 43 yr [range, 27–61 yr]; 50% female) exhibited no change in CFTR-dependent sweat secretion rate (Figure 1E). Similarly, normal nonsmokers (n = 5; median age, 41 yr [range, 34–49 yr]; 40% female) also had stable sweat evaporimetry (Figure 1E).

Discussion

Acquired CFTR dysfunction in smoking-related lung disease was only recently described to be present beyond the airway (8, 14). Although this has potentially significant ramifications as a result of the association of cigarette smoking with other disorders in which CFTR has an etiologic role (e.g., pancreatitis, male infertility, diabetes mellitus), these studies were limited by their cross-sectional design and could not determine causality in humans. This study represents the most viable alternative to asking patients to begin smoking, allowing causality to be inferred in the setting of smoking cessation. As such, these data represent the first to demonstrate that systemic CFTR dysfunction induced by cigarette smoke can also recover (8). This observation is significant, as it solidifies the mechanistic link between cigarette smoke exposure and systemic CFTR dysfunction. Moreover, these results indicate that β-adrenergic sweat rate can be a sensitive marker to monitor changes in CFTR dysfunction among individuals and is stable over time, suggesting its potential as a biomarker for measuring the recovery of CFTR function in the setting of therapeutic trials with an agent intended to augment CFTR activity in patients with chronic bronchitis (7, 15). Of interest, in this fashion it performed superior to sweat chloride, suggesting sweat rate may be a relatively dynamic measure; however, this relatively small population may not have been representative, as sweat chloride was relatively low compared with higher values observed in a two larger studies (8, 9); this points out the heterogeneity of sweat chloride abnormalities among smokers. ■

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Whether to Breathe Fast or Not: What Is Wrong with Breathing Control in Patients with Mild Obstructive Pulmonary Disease?

To the Editor:

In their very interesting study recently published in the *Journal*, Elbehairy and colleagues established that V_D is increased during exercise in smokers with mild obstructive pulmonary disease, leading to a compensatory increase in minute ventilation to keep alveolar ventilation constant (1). This in turn resulted in early dyspnea and exercise limitation. We would like to present the view that this study reveals an intriguing picture in which the breathing pattern adopted by these patients, and thus the way their breathing is regulated, contributes to the observed high ventilatory output.

1. The patients display a significant increase in their breathing frequency at any given work rate (1). This is true at 60 W, but also at 100 W, averaging 23 versus 42 c/min in the control and the patient group, respectively. The consequences on V_D ventilation ($\dot{V}_D$) must be considered regardless of the V_D values: Breathing at 23 versus 42 cycles (c)/min at a CO_2 production ($\dot{V}_{CO_2}$) of about 1.5 L/min (100 W) requires the same alveolar ventilation (50 L/min for the sake of simplicity) to keep Pa_{CO_2} constant. This implies that if the control subjects and patients had the same V_D (200 ml), an additional minute ventilation of 4.6 L/min, corresponding to $\dot{V}_D$ (200 ml × 23 c/min), would be required when breathing at 23 c/min. An additional minute ventilation of 8.4 L/min would be needed if breathing at 42 c/min (200 ml × 42 c/min) to maintain the same Pa_{CO_2} (Figure 1; *see* Reference 2 for more information). For an additional increase in V_D of 100 ml (the difference in V_D between the two groups is about 50 ml on average), an increase in minute ventilation of 12.6 L/min is required to keep isocapnia when breathing at 42 c/min (300 ml × 42 c/min). These 12.6 L/min represent a threefold increase in $\dot{V}_D$ when compared with breathing at a frequency of 23 c/min and with a V_D of 200 ml/min, or a minute ventilation of 62.6 in the fast breathers versus 54.6 L/min in the slow breathers, as illustrated in Figure 1.
2. Perhaps more intriguingly, the patient group displays levels of Pa_{CO_2} lower (by several millimeters of mercury) than in the control group throughout the entire period of exercise (1). This, *per se*, constitutes an additional "unnecessary" burden for the respiratory system, challenging the idea that the respiratory control system optimizes its output to keep Pa_{CO_2} homeostasis in response to any increase in V_D in these patients (3). A decrease in Pa_{CO_2} by 4 mm Hg requires by itself an additional increase in alveolar ventilation by about 10% and thus a much higher level of minute ventilation when breathing at a high frequency (Figure 1). The fundamental question is, therefore, what stimuli could be involved in the regulation of exercise hyperpnea leading to this rather inefficient pattern of breathing (high frequency and low P_{CO_2} "set point").

Whether patients with moderate chronic obstructive pulmonary disease have no other "choice" than reducing respiratory sensations related to the mechanical constraints of breathing at a higher FRC

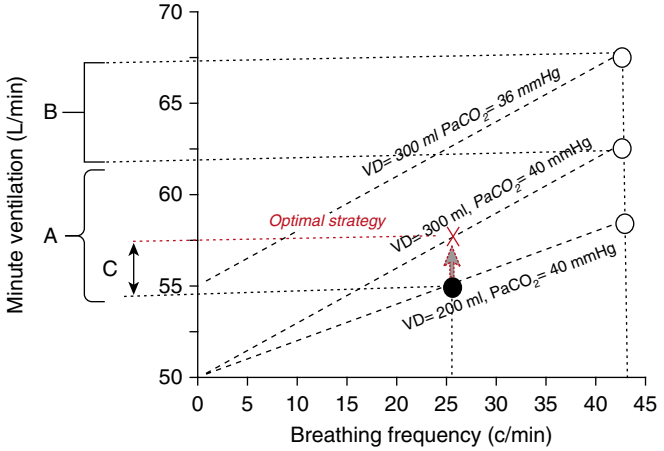

Figure 1. Schematic representation of the levels of ventilation required to maintain a given level of Pa_{CO_2} as a function of breathing frequency. The breathing frequencies reported by Elbehairy and colleagues (1) in the control group (26 cycles [c]/min, *solid circle*) and in the group of patients with mild obstructive pulmonary disease (42 c/min, *open circles*) exercising at 100 W ($\dot{V}_{CO_2}$ = 1.5 L/min) have been used for this computation. By the very fact of their adopting a higher frequency, the group of patients must develop a larger increase in ventilation than the control group to keep Pa_{CO_2} constant, even if the V_D were unchanged. Note that breathing at 42 c/min with an increase in V_D of only 100 ml requires a much higher level of ventilation (*A*) than if only V_T was increased by 100 ml (*red X*, *C*) and breathing frequency kept at 26 c/min. Maintaining a level of Pa_{CO_2} around 36 mm Hg (rather than 40) represents an even bigger burden in terms of ventilatory requirement (*B*). As a result, the level of minute ventilation (*A* and *B*) required by the group of patients breathing at 42 c/min while keeping their Pa_{CO_2} several millimeters of mercury lower than in the control group is considerably higher than what would be required to keep Pa_{CO_2} constant while breathing at a lower rate ("optimal strategy," *C*).