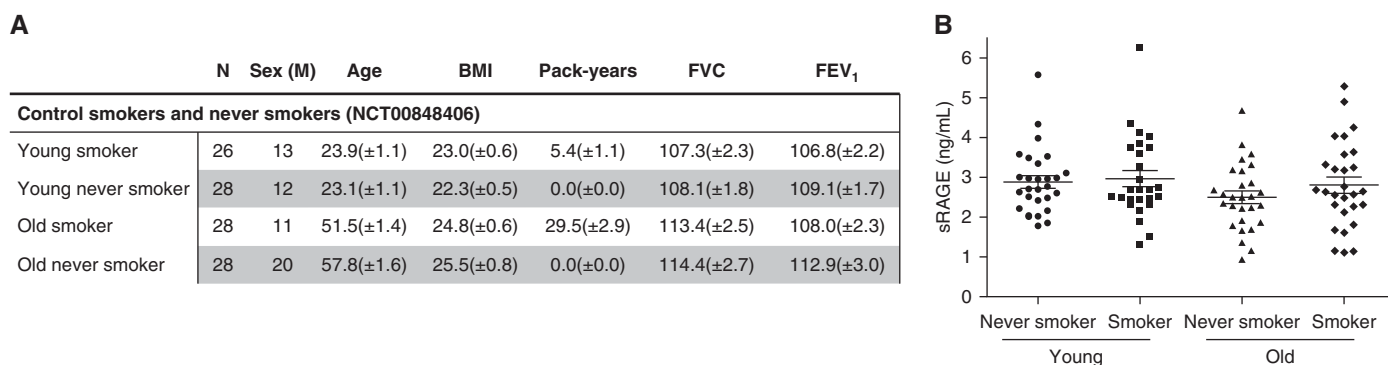## Reply to Biswas

From the Authors:

In our recent research letter focusing on the acute effects of smoking on the serum levels of sRAGE (soluble receptor for advanced glycation end products), we showed that smoking three cigarettes within 1 hour significantly decreases serum sRAGE levels within 2 hours (1). In addition, we also determined the effect of chronic cigarette smoke exposure on serum sRAGE levels by comparing smokers with never smokers (originally reported as "data not shown"). Here, we did not find any difference in serum sRAGE levels, which is in line with previous studies (2). In contrast, as rightfully mentioned in the response to our research letter, Biswas and colleagues previously reported that serum sRAGE levels were increased in smokers compared with nonsmokers (3). Biswas explains the discrepancies between their study and other studies by noting that most of the studies were not specifically designed to explore the effect of smoking on sRAGE in healthy individuals, whereas their study was (4). Further, in his original paper, he states that the differences may also be explained by the fact that his study population was of overall younger age compared with those in the other studies (3). Indeed, characteristics of the study population may affect the outcomes of sRAGE measurements; however, other factors,

including the method and timing of serum preparation, the method of sRAGE quantification, and, most importantly, the timing of the last smoked cigarette before blood sampling may also drive the observed differences in serum sRAGE levels. Although our initial research letter only showed data of serum sRAGE levels in healthy control subjects versus patients with chronic obstructive pulmonary disease (COPD) (1), our study was also designed to investigate the chronic effects of smoking in healthy individuals. Specifically, to investigate the effect of chronic smoke exposure on the serum levels of sRAGE, we used a well-controlled cohort (ClinicalTrials.gov Identifier: NCT00848406) of young (18–40 yr old) and old (>40 yr old) smokers and never smokers without airway obstruction (Figure 1A) (5). To adequately measure serum sRAGE levels, we used the highly sensitive and selective simplified immunoprecipitation in 96-well ELISA format–coupled liquid chromatography–mass spectrometry assay, which we recently demonstrated to be superior to the commonly used sRAGE ELISA (6). When we focused on the healthy control subjects, we found that there were no differences in serum sRAGE levels between smokers and never smokers, whether old or young (Figure 1B). Of note, the definition of "nonsmokers" in our study and the one used by Biswas are not exactly the same. Whereas our nonsmokers had never smoked, the nonsmokers in Biswas's study included subjects who had not smoked during the last

A

	N	Sex (M)	Age	BMI	Pack-years	FVC	FEV$_1$
Control smokers and never smokers (NCT00848406)							
Young smoker	26	13	23.9(±1.1)	23.0(±0.6)	5.4(±1.1)	107.3(±2.3)	106.8(±2.2)
Young never smoker	28	12	23.1(±1.1)	22.3(±0.5)	0.0(±0.0)	108.1(±1.8)	109.1(±1.7)
Old smoker	28	11	51.5(±1.4)	24.8(±0.6)	29.5(±2.9)	113.4(±2.5)	108.0(±2.3)
Old never smoker	28	20	57.8(±1.6)	25.5(±0.8)	0.0(±0.0)	114.4(±2.7)	112.9(±3.0)

B

Figure 1. Serum sRAGE (soluble receptor for advanced glycation end products) levels in never smokers and smokers. (A) Patient characteristics. BMI = body mass index (kg/m^2); N = number of group participants; sex (M) = number of males in a group. Data are shown as mean ± SEM. (B) Levels of sRAGE were measured in serum of young (18–40 yr old) smokers (n = 26) and nonsmokers (n = 28), and age-matched old (>40 yr old) smokers (n = 28) and nonsmokers (n = 28) without airway obstruction using immunoprecipitation in 96-well ELISA format–coupled liquid chromatography–mass spectrometry. Data are shown as individual measurements and mean ± SEM.

5 consecutive years. Moreover, the age of the study subjects does not influence the serum sRAGE levels. Indeed, our data show no differences in serum sRAGE levels between young (average 23.5 yr) and old (average 54.7 yr) subjects in either the never-smokers group or the smokers group (Figure 1B). In addition, our young subjects were even younger than the study population of Biswas, which had an average age of 34.1 years. Our data therefore indicate that neither age nor chronic smoke exposure affects serum sRAGE levels. The discrepancy in study results when comparing the serum sRAGE levels in smokers and nonsmokers may be explained by our finding that smoking before blood sampling acutely decreases serum sRAGE levels (1). Therefore, controlling or monitoring smoking behavior before blood sampling may be used as a precautionary measure to decrease the variability between measurements and increase the value of sRAGE as a biomarker for COPD. Lastly, we agree with Biswas that more research is needed regarding the effect of smoking on serum sRAGE levels and the underlying mechanisms before sRAGE can be clinically used as a biomarker for COPD. ∎

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Socioeconomic Disparities and Health Outcomes ◌

To the Editor:

The ability to link data from sources such as the U.S. Census is now enabling researchers to direct their focus toward reporting neighborhood and contextual characteristics that increase the risk for adverse health outcomes and are independent of patient-level attributes. This is all the more important because disparities in health outcomes likely arise as a result of both individual exposures and contextual factors (1). Research regarding disparities have until recently been challenging because of the high response bias associated with collecting individual-level socioeconomic measures (2). However, area-based measures from the U.S. Census's American Community Survey and the National Center for Health Statistics Urban-Rural Classification Scheme can be used to gain insight into the role of area-based measures as independent risk factors for diseases, as demonstrated in the work by Raju and colleagues (3). Understanding area-based risk factors could help researchers design, target, monitor, and assess public health programs, including prevention interventions.

First, some limitations of Raju and colleagues' analysis need to be emphasized. Although the authors used census tract–based determinants as area-based measures, it is important to acknowledge the possibility of ecological fallacy, and that these determinants provide information regarding the neighborhood that is not reducible to the individual level (4). Although the authors have defined neighborhoods as census tracts, nearby neighborhoods may also influence health outcomes and disparities.

Second, data structures arising from both individual and neighborhood levels are inherently hierarchical and correlated. To account for geographical correlation, it is important to analyze such data using multilevel models. Multilevel models can account for a lack of independence, evaluate multivariate associations, incorporate covariates at both individual and geographic levels, and model interactions between variables (5). Multilevel models have been used to evaluate health disparities and to describe the relationship between geographic exposures for a wide variety of health outcomes. They can also help researchers quantify the proportion of variability associated with being in a specific neighborhood.

The authors are to be applauded for taking the research on chronic obstructive pulmonary disease risk factors a step further by investigating