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Title: Estimating parameters in the two-compartment model of exhaled nitric oxide

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Body: INTRODUCTION: The fractional concentration of exhaled nitric oxide (FeNO) is a biomarker of airway inflammation. FeNO decreases with increasing flow, which has led to the development of mathematical models whose parameters quantify proximal and distal sources. FeNO measured at the conventional 50 ml/s flow rate is primarily from proximal sources, so the parameters may provide additional insight. Parameters are estimated from FeNO data measured at multiple flow rates, but there is no standard estimation method. AIMS AND OBJECTIVES: To evaluate and compare a comprehensive set of existing and novel regression-based estimators of parameters from the two-compartment model of exhaled nitric oxide. METHODS: We used simulated multiple flow datasets to assess the statistical properties of the estimators and multiple flow datasets from 1507 schoolchildren from the Southern California Children's Health Study (CHS) to investigate regression model fit and the sensitivity of estimates across models. RESULTS: A novel non-linear least squares model with log transformation fit CHS participant datasets very well (median adjusted R2 = 0.99), satisfied model assumptions (homoskedasticity), and produced estimators with good statistical properties (e.g., negligible bias). In the CHS, alveolar NO concentration (CaNO) estimates were only moderately correlated across estimation methods whereas bronchial flux estimates were highly correlated. CONCLUSIONS: Since CaNO estimation is a key result of multiple flow FeNO analysis, the sensitivity of CaNO estimates highlights the need for standardized methods. No gold standard is available to validate parameter estimators but our work can guide the selection of estimators in future studies.