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Daily Life Negative Mood and Exhaled Nitric Oxide in Asthma

Running title: Daily Negative Mood and Nitric Oxide

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Highlights

- Longitudinal association of mood with exhaled nitric oxide (FeNO) is unexplored
- Asthma patients recorded mood, FeNO, and lung function for 1-2 months
- Negative mood predicted concurrent and subsequent increases in FeNO
- Higher FeNO was related to higher same-day lung function longitudinally
- Stronger FeNO – lung function association in young patients and low asthma control

Abstract

Background: Psychosocial stress and negative affect have been linked to asthma exacerbations, but longitudinal studies demonstrating a daily life association between negative affect and airway nitric oxide are missing.

Objective: The longitudinal association between negative mood fluctuations, exhaled nitric oxide, and lung function in asthma was examined.

Methods: Self-assessments of the fraction of exhaled nitric oxide (FeNO), spirometry (forced expiratory volume in the first second, FEV₁), negative mood, and daily activities were obtained from 20 patients with asthma for 2 months, resulting in 1108 assessments for the analyses (approximately 55 per patient). Concurrent and prospective associations between FeNO, FEV₁, and negative mood were analyzed using mixed effects regression models for longitudinal data.

Results: Negative mood was positively associated with changes in FeNO during the same day, and to a stronger extent when prior day negative mood was included in the prediction. FeNO and negative mood were positively associated with same-day FEV₁, with the latter relation being partially mediated by changes in FeNO. Associations between FeNO and FEV₁ were stronger in younger patients, with earlier onset of asthma, or with lower asthma control. Findings were not changed when controlling for physical activity, medication, cold symptoms, air pollution, and hours spent outside.

Conclusion: Daily life changes of negative mood in asthma are positively associated with FeNO changes and FeNO increases are associated with a mild bronchodilation. These findings indicate that psychological influences need to be considered when using FeNO as indicator of airway inflammation and guide for treatment decisions.

Keywords: Asthma; Exhaled Nitric Oxide; Negative Mood; Home monitoring; Airway Inflammation; Lung Function; Longitudinal observation

Introduction

Psychosocial factors significantly impact asthma pathogenesis, pathophysiology, and disease management (Lehrer, Isenberg, & Hochron, 1993; Marshall, 2004; Miller & Wood, 2003; Ritz, Meuret, Trueba, Fritzsche, & von Leupoldt, 2013; Wright, 2010). In animal studies, various forms of stress have been found to be associated with exacerbation of allergic inflammatory markers (Forsyth, Ebeling, Gordon, Befus, & Vliagoftis, 2004; Joachim, Sagach, Quarcoo, Dinh, Arck, & Klapp, 2004; Kang & Weaver, 2010; Okuyama et al., 2007). Studies have also examined the impact of psychosocial stress on allergic inflammation using systemic (Chen, Fisher, Bacharier, & Strunk, 2003; Kang et al., 1997) or sputum markers following allergen challenge (Liu, Coe, Swenson, Kelly, Kita, & Busse, 2002) in humans. However, few studies have examined the spontaneous association of stress and emotion with markers of airway inflammatory activity, in particular the fraction of nitric oxide (NO) in exhaled breath (FeNO) (Vijverberg et al., 2011) in asthma (Höglund et al., 2006; Kullowatz et al., 2008). Increases in FeNO have been observed following laboratory stress (Chen, Strunk, Bacharier, Chan, & Miller, 2010; Ritz, Ayala, Trueba, & Vance, Auchus, 2011) but nothing is known about the longitudinal association of negative mood with FeNO in asthma. The recent development of portable FeNO assessment techniques (Alving, Jansson, & Nordvall, 2006; Pijnenburg, Floor, Hop, & De Jongste, 2006) offers the opportunity for patients to self-monitor their airway status at home. We employed these techniques in a proof-of-concept study to examine whether daily life fluctuations in negative mood are related to FeNO changes in asthma. Given prior findings, we expected that stronger negative mood would be associated with FeNO increases in longitudinal time series spanning two months. In addition, we expected that negative mood states would be related to FeNO increases on subsequent days, similar to earlier findings on negative life events and asthma exacerbations in children (Sandberg, Jarvenpaa, Penttinen, Paton, & McCann, 2004). We also studied the association of FeNO changes with lung function, which was self-monitored by patients using electronic spirometers with diary functions. The findings of this study could contribute to our understanding of the daily life dynamics of airway FeNO and its long-term home monitoring.

Methods

Participants

We recruited asthmatic participants through college courses, online ads, and flyers between August 2006 and November 2007. Patients had a positive physician diagnosis of asthma, were non-smokers (<10 life-time pack-years), and were in stable mental and physical health (by questions from a psychiatric interview [First, Spitzer, Gibbon, & Williams, 1994]). Systemic corticosteroid treatment in the previous 3 months was an exclusion criterion. Participants were instructed to stay on a stable dose of their inhaled corticosteroids during the study if possible, although adjustments were allowed in case of stronger symptoms or exacerbations. Prior to the initial laboratory assessment, short-acting bronchodilators were discontinued for 8 hours, long-acting beta-agonists for 12 hours, and leukotriene modifiers for three days.

The study was approved by the Southern Methodist University and University of Texas Southwestern Medical Center Institutional Review Boards and participants gave written informed consent.

Instruments and Measurements

FeNO (in ppb) was measured with a handheld electrochemical gas analyzer (NIOX MINO; Aerocrine, Solna, Sweden) (Alving, Jansson, & Nordvall, 2006; Pijnenburg, Floor, Hop, & De Jongste, 2006) according to current guidelines (American Thoracic Society, European Respiratory Society, 2005). Spirometry (light-weight pocket device, AM2, CareFusion/Jaeger, Höchberg, Germany) followed FeNO measurements and forced expiratory volume in the first second (FEV₁ percent of predicted) was extracted from the best of three valid forced expiratory efforts. Before spirometry, patients answered questions on their current mood (“depressed”, “frustrated/angry”, and “tense/anxious”, each rated 0 to 10), and activities, including “hours of outdoor activities” and “physical activity in previous 30 min” (scored as none, mild, moderate, or heavy) in the electronic diary of the AM2. These or similar ad-hoc questions have been used successfully in earlier ambulatory monitoring studies and have been found to be sensitive to daily mood fluctuations (Ritz, Rosenfield, DeWilde, & Steptoe, 2010; von Leupoldt, Ehnes, & Dahme, 2006). A paper diary was used for supplementary information on medication use and cold symptoms.

At the initial laboratory visit, patients also completed questionnaires including general demographics, asthma-relevant information (history, manifestation, management), and the Asthma Control Questionnaire (ASQ [Juniper, O'Byrne, Guyatt, Ferrie, & King, 1999]). Asthma control and severity were rated according to guidelines (National Heart, Lung, and Blood Institute, National Asthma Education and Prevention Program, 2007) using an ad-hoc questionnaire, FEV₁ percent predicted, and ASQ information on symptoms, nighttime awakening, functional limitations, and bronchodilator use. Severity was not rated for patients on maintenance medication, because this would require knowledge about the level of medication needed to achieve good asthma control (National Heart, Lung, and Blood Institute, National Asthma Education and Prevention Program, 2007). Longitudinal data on optimal medication regimens necessary for well-controlled asthma was not available for these patients recruited from the community.

Because air pollution has shown associations with asthma exacerbations (O'Connor et al., 2008), we recorded the following pollutant levels from the closest regional monitoring station: O₃, CO, SO₂, PM_{2.5}, NO, and NO₂.

Home-monitoring protocol

Participants were instructed to measure FeNO twice, mood with the AM2 diary, and FEV₁ every evening for up to two months. Additional laboratory visits were scheduled after 1 and 2 months with pulmonary function assessments and exploration of possible medication changes and cold symptoms. Patients were also contacted by phone at approximately 2 weeks and 6 weeks into the self-monitoring period to boost adherence, discuss any issues with the self-assessments, and to explore medication and symptoms.

Data Analysis

Means, standard deviations, and ranges were calculated for basic sample description. For mood, a composite negative mood score was computed by summing up the three negative mood items (depressed, frustrated/angry, and tense/anxious; internal consistency indicator Cronbach's $\alpha=.79$). We used the average of the two daily measurements for FeNO, and the highest value from the three forced expiratory maneuvers for FEV₁. For daily records of medication use, a short-term bronchodilator variable was created assigning values 6 to 0, where 6 represented the first hour and

0 the 7th hour following inhaler application. For long-term bronchodilator use, a similar variable was created assigning values from 12 to 0. Maintenance medication changes were coded with + 1 for any day with a reported increase in medication and for all days thereafter, -1 for any day with a reported decrease and all days thereafter, and 0 for all remaining days. Changes in maintenance medication occurred either through physician's prescription or variations in patients' intake behavior as reported by them. In addition, we coded days with cold symptoms (from diary entries and interviews) with 1s and all other days with 0s. Daily assessments were analyzed using mixed effects regression models (MRMs), the recommended analytic technique for longitudinal repeated measures data in psychophysiology as it allows for varying numbers of daily entries between participants, and easily accommodates missing data (Hamer & Simpson, 2009; Kristjansson, Kircher & Webb, 2007). Predictors of outcome over time (e.g., negative mood) were modeled as time-varying predictors of outcome (Singer & Willett, 2003) thereby calculating the relation between the predictors and outcome within subjects over time. The following control variables were also included: recent physical activity, use of short-acting and long-acting bronchodilators, and changes in maintenance medication. [Footnote 1]. Cold symptoms, daily hours outside, air pollution indicators, and interaction of the latter with hours outside were investigated as additional covariates. Because the reliability and precision of the level 1 MRM regression coefficients is proportional to the number of data points per participant rather than the total number of participants (Singer & Willett, 2003), our estimates of the relations between negative affect, FeNO, and FEV₁ should be highly reliable and precise because we averaged 55 assessments per participant (a total of 1108 assessments for the 20 participants). Although there were a number of control variables, we had 1108 data points, so the number of covariates was small compared to the number of data points.

For each reported effect, we show the unstandardized regression coefficient (the *b*), the associated t-statistic with degrees of freedom [*t(df)*], and the *p*-value (significance) associated with the t-statistic (*p*). We also tested whether FeNO mediates the effect of negative mood on FEV₁. Following our earlier paper findings on monitoring FeNO, FEV₁, and mood at two time points (3 months apart), we expected that negative mood would impact airway inflammatory processes, which could result in a change in lung function. We used the program PRODCLIN (MacKinnon, Fritz, Williams, & Lockwood, 2007), which performs the asymmetric distribution of products test on the mediated pathway to determine the 95% confidence interval for the indirect, mediated

pathway from negative mood to FeNO to FEV₁. If the 95% CI does not include 0, the mediated pathway is considered to be significant.

Results

Basic Sample Characteristics

Among the 20 participants of the final sample, 19 were women. Basic characteristics are provided in Table 1. Most participants had seasonal variations in asthma, problems with allergies (60% reported having undergone skin tests with positive results), and nighttime symptoms. Asthma onset was before the age of 12 in 50%. 75% of participants took inhaled corticosteroids in combination with (45%) or without long-acting bronchodilators, 65% short-acting bronchodilators, 25% leukotriene modifiers, and 50% antihistamines. None of the patients fell into the category of “very poorly controlled” asthma. Two participants were not taking any current medication and one took only bronchodilators. For these three participants, asthma severity was rated as intermittent, mild persistent, and moderate persistent, respectively. Only one person reported an emergency treatment in the previous year.

Daily Monitoring Data and Compliance with Instructions

Twenty participants returned sufficiently valid data, 10 more were initially enrolled but had only performed measurements sporadically or not at all (because patients were not approached directly to participate there were no approach or decline rates to report). Thirteen of the 20 final participants completed 2 months of self-monitoring, the rest completed 1 month of monitoring (2 of these were terminated by the experimenter because of low interest by the participant to continue). On average, participants performed 55 daily measurements of FeNO and FEV₁ (range: 25 to 70). No partial assessments were produced by patients because ratings, spirometry, and FeNO were always measured in conjunction. Table 2 provides means and distributional characteristics of mood and airway function variables across the observation period.

Concurrent Associations between Negative Mood, FeNO, and Spirometric Lung Function

A more negative mood was significantly related to a higher level of FeNO (Table 3). On average, the association was small, but for the two patients with the strongest association it reached 4.6 and 2.4 ppb increase in FeNO for one scale unit of negative mood increase (recalculated to a

0-10 scale) (Figure 1). Similarly, a more negative mood was associated with higher FEV₁, and higher FEV₁ with higher FeNO, $b=.48$, $t(955)=2.26$, $p=.024$ and $b=.14$, $t(506)=3.44$, $p<.001$, respectively. Again, although the association between FeNO and FEV₁ was small on average, it reached 7.4 and 4.0% FEV₁ change for a 10 ppb increase in FeNO for two individual patients with the strongest association (Figure 2).

We also explored the relations between the three individual negative mood items and both FeNO and FEV₁. When participants were more “tense”, they showed higher FeNO levels, $b=.20$, $t(805)=2.51$, $p=.012$. Similarly, higher anger was related to higher FEV₁, $b=.35$, $t(933)=2.14$, $p=.033$.

FeNO changes partially mediated the effects of negative mood on FEV₁ (see Figure 3a). Negative mood was positively related to FeNO (as reported above), $p=.012$, and FeNO was positively related to FEV₁, controlling for negative mood, $b=.13$, $t(504)=3.26$, $p=.001$. The mediation program RMediation (Tofighi & MacKinnon, 2011) demonstrated that the mediated pathway from negative mood to FEV₁ through FeNO was significant, $a*b=.04$, 95% CI:[.01,.09], $p<.05$. Approximately 8% of the association between negative mood and FEV₁ was mediated by FeNO. On the other hand, there was no confirming evidence that FEV₁ mediated the effects of negative mood on FeNO (see Figure 3b). Although negative mood was positively related to FEV₁ (as reported above), FEV₁ was not a significant predictor of FeNO when controlling for negative mood, $p=.190$. Hence, the mediated pathway from negative mood to FEV₁ to FeNO was not significant since RMediation indicated that the 95% confidence interval for this indirect, mediated pathway included 0, $a*b=.01$, 95% CI:[-.01,.04], $p=.21$.

Contribution of Prior Day Negative Mood to Associations with FeNO and Lung Function

Averaging negative mood for the current and the prior day (day-1) yielded a stronger association with FeNO than just using the current day mood (Table 3). The average of three days (the current, day-1, and day-2) was still related to FeNO in tendency. Negative mood on prior days alone without the current day (lag1, and lag2) did not predict FeNO significantly. Taken together, these results indicate that negative mood more than one day prior had little effect on current FeNO. There was also no evidence for reverse relations of prior days FeNO predicting negative mood ($ps>.111$). There were no significant prospective associations between negative mood and FEV₁ in either direction, $ps>.13$.

Averaging FeNO for the prior day and the current day was a substantial predictor of current FEV₁, $b=.15$, $t(220)=2.76$, $p=.006$, even when controlling for previous day's FEV₁. Prior day's FeNO alone (lag 1) was also related to current FEV₁, $b=.11$, $t(432)=2.50$, $p=.013$, but, in contrast to the average of prior day and current FeNO, not when controlling for prior day FEV₁, $p=.122$, suggesting, if at all, only weak prospective associations. There was no evidence for the reverse relations of FEV₁ on the prior days predicting current FeNO. [Footnote 2]

Moderator Effects of Demographics, Asthma-Related Variables, and Psychological Characteristics

Age and age of onset moderated the relation between FeNO and FEV₁, such that older patients and those with a later onset of asthma displayed a weaker relation between FeNO and FEV₁, $bs=-.01$, $ts(618 \text{ and } 730)=-2.24$, $ps=.025$. Indeed, for those over 40 or with an onset of asthma after 25 years of age, there was no significant relation between FeNO and FEV₁. The ACQ also moderated the association of FeNO with FEV₁, $b=.04$, $t(553)=2.50$, $p=.013$, such that patients with higher ACQ scores (indicating lower control) had stronger associations. No evidence of significant moderation of associations between negative mood, FeNO, and FEV₁ was found for inhaled corticosteroids use or long-acting or short-acting bronchodilators ($ps>.221$).

Effects of Control Variables

Maintenance medication intake was decreased in 5 patients (25% of our sample) and increased in 6 patients (30%) during their homemonitoring periods. Seven participants (35%) took their long-acting bronchodilators on one or more days within 12 hours before the evening self-assessments (overall range: 0-39 days) and 4 (20%) reported taking their short-acting bronchodilators within 6 hours. Physical activity was reported on one or more days as at least "mild" by 16 (80%) participants (mean: 11.8 days, range: 0-65 days). Cold symptoms were reported by 10 (50%) participants with an average duration of episodes being 3.7 days. On average, participants spent 1.09 ± 1.03 hours outside per day (range: 0-9 hours). None of the control variables, including measures of ambient pollution levels and their interaction with hours outside, were related to daily FeNO, lung function, or negative mood.

Discussion

This study demonstrated for the first time a longitudinal association between negative mood and FeNO under naturalistic conditions of home monitoring. Negative mood contributed systematically to daily fluctuations in FeNO, confirming prior findings that have linked acute laboratory negative affect to increases in FeNO (Chen et al., 2010; Ritz et al., 2011). The strength of this study lies in the extended time period of monitoring FeNO over months and the real-life setting of assessment that allowed for an ecologically valid exploration of the association between negative affect and FeNO. In addition to a concurrent, same-day positive association between negative mood and FeNO, we observed that including prior day negative mood in the prediction produced a stronger association with FeNO suggesting a build-up of effects on FeNO within a narrow time window of two to three days. Current day FeNO remained the most important component of this prediction, suggesting that negative mood effects appear rather short-lived, with some contribution of prior day mood levels. Previous research with children has found that negative life events are associated with asthma exacerbations within 1-2 days (Sandberg, Jarvenpaa, Penttinen, Paton, & McCann, 2004). However, asthma exacerbations in this study were defined by a combination of peak flow decline below 70% of the child's typical value and an increase in symptoms, and thus did not inform about airway NO levels.

Our study could suggest that elevated negative mood across two days may set the stage for asthma exacerbations with worsening of airway inflammation. However, we also found that higher FeNO was associated with higher FEV₁ and that negative mood was associated with higher FEV₁ on the same day. This would counter the idea that the types of negative mood states recorded in this study were linked to a decline in asthma control. Although changes in FEV₁ were relatively small on average, they reached levels of up to 7.4% FEV₁ change per 10 ppb FeNO change in individual asthmatics. The findings appear at odds with the expectation that higher FeNO levels indicate less asthma control. Indeed, the actual function of elevated NO levels in asthma is not well understood (Sanders, 1999; Ricciardolo et al., 1996) and a number of pathways that link psychological factors with FeNO need to be considered (Ritz & Trueba, 2014). Among these are adrenergic effects that increases FeNO, bronchoprotective effects of elevated NO secretion, and enhanced NO release from the surface of dilated airways. First, direct adrenergic effects on the airway epithelium could enhance the release of NO, similar to NO release from the urophelium observed under such conditions (Birder, Apodaca, De Groat, & Kanai, 1998). Adrenergic effects would also account for the observed concurrent bronchodilation. Second, bronchoprotective

effects have been observed through neuronal r NO synthase (NOS) activity (Ricciardolo, Sterk, Gaston, & Folkerts, 2004).. The positive association between FeNO and FEV₁ would be compatible with well-documented bronchodilatory effects of neuronal NOS activity (Belvisi, Stretton, Yacoub, & Barnes 1992; Que et al., 2005) and also with vasodilatory and protective activity of NO derived from the vascular endothelium (Förstermann & Münzel, 2006), rather than with inducible NOS activity linked to allergic processes. The observed mediation of FEV₁ by FeNO supported this idea, suggesting that increases in FeNO partially accounted for the higher FEV₁ under more negative mood. Third,

A, it has been suggested that dilated airways provide a larger surface for NO release into the exhaled air (Silkoff et al., 1999), which would also imply a positive association of FeNO with FEV₁. Although mediation by lung mechanics appears plausible, it should be noted that evidence for this relationship has been mixed across studies (Ho, Woods, Robson, Innes, Greening, 2000; Yates, Kharitonov, & Barnes, 1997) and that its demonstration has relied on beta-adrenergic bronchodilator application, which could also emulate an endogenous adrenergic pathway that favors NO release. In addition, our mediation analysis findings argue against a significant role of FEV₁ in mediating the association between negative mood and FeNO, but rather support the idea of a partial mediation of mood-associated FEV₁ change by FeNO. 1.

Although the observed FeNO changes related to negative mood were generally small, , with one unit of increase on a 0-10 scale of negative mood associated with 0.9 ppb increase in FeNO, changes were more pronounced in individual participants, reaching as high as 4.6 ppb per one scale unit of negative mood (Figure 1). The range of negative mood observed in our study is typical for daily mood diary studies, in which the majority of the ratings usually cover the lower third of the rating scale due to a positivity bias (Watson, Clark, & Tellegen, 1988). With regard to FeNO change, it is currently suggested that changes >10 ppb should be viewed as significant for patients with a FeNO level ≤50 ppb, and changes >20 ppb for patients with FeNO >50 ppb (Silkoff et al., 2006). However, these thresholds have been determined from FeNO studies that most likely measured NO resulting from inducible NOS activity due to allergic inflammation, whereas much smaller amounts of NO are known to be biologically potent when resulting from neuronal or endothelial NOS activity (Silkoff et al., 2006; Esch, Stefano, Fricchione, & Benson,

2002). Information translating biological outcomes of these NOS activities into FeNO changes is missing in the literature.

It also has to be considered that the observed NO fluctuations are due to changes in inducible NOS activity in epithelial cells (Lane et al., 2004), which produce NO constitutionally as part of the innate immune defense against pathogens (Proud, 2005). However, comparison with cold symptom effects recorded by patients was difficult, with no systematic overall association between cold symptoms and FeNO observed in our study. Trajectories for individual patients showed that the direction of association was inconsistent and plausible interpretations were possible for both directions, e.g., FeNO increases could lag brief episodes of perceived symptoms, creating the impression of a negative association (Online Supplement Figure 1, Participant #5), or cold symptoms could be associated with dramatic surges of FeNO leading to strong positive associations (Online Supplement Figure 1, Participant #6). Associations for maintenance medication change were equally inconsistent: Lower medication levels could facilitate exacerbations with high FeNO values (Online Supplement Figure 2, Participant #5), leading to an overall negative association, or increase in maintenance medication may be needed to counteract an ongoing exacerbation with high FeNO values (Online Supplement Figure 2, Participant #7), creating an overall positive association.

The observed associations between negative mood and FeNO may not necessarily be causal. Findings of our study only demonstrate associations or temporal precedence. Unidentified third variables could also explain the observed associations. Both respiratory tract infections (Dressel et al., 2008; Ricciardolo, Timmers, Sont, Folkerts, & Sterk, 2003) and bronchodilator use (Silkoff et al., 1999) could be suspected as potential modifiers of FeNO that may also be associated with negative mood, but both factors were accounted for in our set of control variables. Due to our measurement protocol, we also cannot rule out that concurrent associations are due to inflammatory processes that increased FeNO and also elevated negative mood on the same day. However, our result excluded the possibility that higher FeNO was associated with elevated negative mood on the following day. Future studies need to expand the focus onto other indicators of airway function to clarify possible mechanisms underlying the observed associations. Whereas sampling of sputum- or blood-based inflammation indicators will not be feasible in extended home-monitoring protocols, exhaled breath condensate may provide a practical and noninvasive

alternative for sampling various inflammatory markers from the central airways. The technique has been successful in basic research applications, including demonstrations of its sensitivity to stress-induced fluctuations in airway inflammatory markers (Trueba et al., 2013). However, challenges to standardization and reproducibility have to be overcome before clinical application of this sampling technique (Loukides, Kontogianni, Hillas, & Horvath, 2011). It remains to be determined to what extent our findings may point to complications in using FeNO for asthma management. Attempts to demonstrate the benefits of FeNO monitoring for improvement of asthma management have not been uniformly successful (Petsky et al., 2012). However, medication management strategies using FeNO have traditionally relied on absolute thresholds and effects of smaller fluctuations of FeNO could be mitigated this way. Nevertheless, stronger fluctuations, as seen in our cases with particularly prominent associations of negative mood with FeNO, could very well interfere with specific treatment algorithms. It should be noted though that these fluctuations would only constitute an interference if the mechanism of mood-induced FeNO change was not linked to allergic activation of inducible NOS.

The findings must be interpreted in the light of some limitations. Because diary functions were linked to the electronic spirometer, lung function assessments themselves were not able to affect concurrent mood, but we could not rule out that FeNO self-assessments may have affected concurrent mood. However, even if such influences were possible concurrently, it would be more difficult to understand significant associations in which prior day negative mood contributed substantially to the prediction of FeNO. Another limitation was the motivation and effort dependency of spirometry, potentially contributing to the positive association of negative mood with lung function, as also observed with PEF, an index more effort-dependent than FEV₁ (Paggiaro, Moscato, Giannini, Di Franco, & Gherson, 1997). Prior research on mood and spirometry diaries has remained equivocal, with large between-individual variations in the strength and direction of longitudinal associations between spirometry indices and mood (von Leupoldt, Ehnes, & Dahme, 2006; Apter et al., 1997; Hyland 1990; Steptoe & Holmes, 1985; Smyth, Soefer, Hurewitz, Kliment, & Stone, 1999). A host of intervening variables could account for these findings in an ambulatory environment. In a recent study we observed an interaction with time-of-day, in that positive associations between PEF and negative mood were observed for morning and evening measures and negative associations for afternoon measurements. This is consistent with findings in our present study, because patients performed spirometric measurements in the

evening (Ritz, Rosenfield, DeWilde, & Steptoe, 2010). Future studies would profit from the use of direct measurements of mechanical lung function by respiratory resistance (Ritz et al., 2002), for which hand-held interrupter technique devices (Bridge, Lee, & Silverman, 1996) could be used in the ambulatory context. The sample of our study was also rather small, but the primary intention of the study was to demonstrate preliminary evidence for longitudinal associations, which would justify a subsequent examination in a larger sample. It should be noted that our sample size was not unusual for this type of longitudinal design and some of the previously published research on mood – lung function associations has comparable or smaller sample sizes combines with shorter assessment durations (von Leupoldt, Ehnes, & Dahme, 2006; Apter et al., 1997; Hyland 1990; Steptoe & Holmes, 1985; Smyth, Sofer, Hurewitz, Kliment, & Stone, 1999). Although lack of statistical power was not a problem of this study given the longitudinal design with multiple data points, the composition of our sample, which lacked cases of uncontrolled asthma and was overwhelmingly female, relatively young, and well-educated, limits generalizability to a subpopulation of asthma patients. Recruitment of men for this longitudinal monitoring study proved to be rather difficult. We also did not systematically monitor menstrual cycle phase as a control variable in women, which has been shown to affect FeNO levels over time (Mandhane et al., 2009). Thus, we cannot rule out that some of the negative mood – FeNO associations were mediated by fluctuations of hormonal levels. However, four of our 19 female participants reported taking birth control medication which eliminates such FeNO fluctuations and including an additional birth control/male gender moderator variable did not alter our findings or yield a significant effect of this variable.

Future larger studies need to examine the role of various possible sources of the measured FeNO as a critical factor. NO could be recruited from multiple cells, including presynaptic terminals of nerve cells, endothelial cells, epithelial cells, or immune cells such as macrophages and eosinophils (Belvisi, Stretton, Yacoub, & Barnes 1992), and FeNO from these sources may not be equally related to psychological factors. However, our present findings are the first step in elucidating longitudinal influences of psychological factors on FeNO. They also indicate that using FeNO as a marker of disease activity in daily life asthma management decisions may require the consideration of additional psychological influences. Monitoring of the psychological status of susceptible patients may be indicated in the clinical long-term monitoring of FeNO.

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Footnote

1. An example of the MRM models used in the analyses is:

$$FEV_{1ij} = b_{0i} + b_{1i} * NEGMOOD_{ij} + b_{2i} * PHYSACT_{ij} + b_{3i} * SAB_{ij} + b_{4i} * LAB_{ij} + b_{5i} * MEDCHG_{ij} + \epsilon_{ij}$$

Where FEV_{1ij} is the FEV_1 for subject i at assessment j , $NEGMOOD_{ij}$ is subject i report of negative mood at assessment j , $PHYSACT_{ij}$ is physical activity, SAB_{ij} is use of short acting inhaler, LAB_{ij} is use of long acting inhaler, $MEDCHG_{ij}$ is change in medication, and ϵ_{ij} is the error in prediction, all for subject i at assessment j .

2. We also examined peak expiratory flow (PEF) rather than FEV_1 as dependent variable. Negative mood significantly predicted concurrent PEF, $b=.71$, $p<.05$, but FeNO did not ($p>.30$). Lags of negative mood and FeNO do not predict PEF, either as separate lags or as cumulative amounts (prior 2 days or prior 3 days).

Figure captions

Figure 1. Examples for associations between negative mood and FeNO across consecutive days of home-monitoring in two individual participants with asthma, with scatterplots of the associations to the right. For the concurrent day association and recalculated to a 0-10 scale, one scale unit of negative mood increase resulted in a 4.6 ppb increase in FeNO in Participant 1, and in a 2.4 ppb increase in FeNO in Participant 2. (Note that units of FeNO were divided by 10 for illustration purposes.)

Figure 2. Examples for associations between FeNO and FEV₁ across consecutive days of home-monitoring in three individual participants with asthma. Scatterplots of the associations are presented on the right hand panels. For the concurrent day association, each 1 ppb change in FeNO was related to a 0.7% change in FEV₁ in Participant 3 and to a 0.4% change in FEV₁ in Participant 4. (Note that units of FeNO and FEV₁ % of predicted were divided by 10 for illustration purposes.)

Figure 3. Models for mediation of the association of, (a) negative mood with FEV₁ by FeNO and, (b) negative mood with FeNO by FEV₁. Significant partial mediation was observed for model (a).

Figure 1

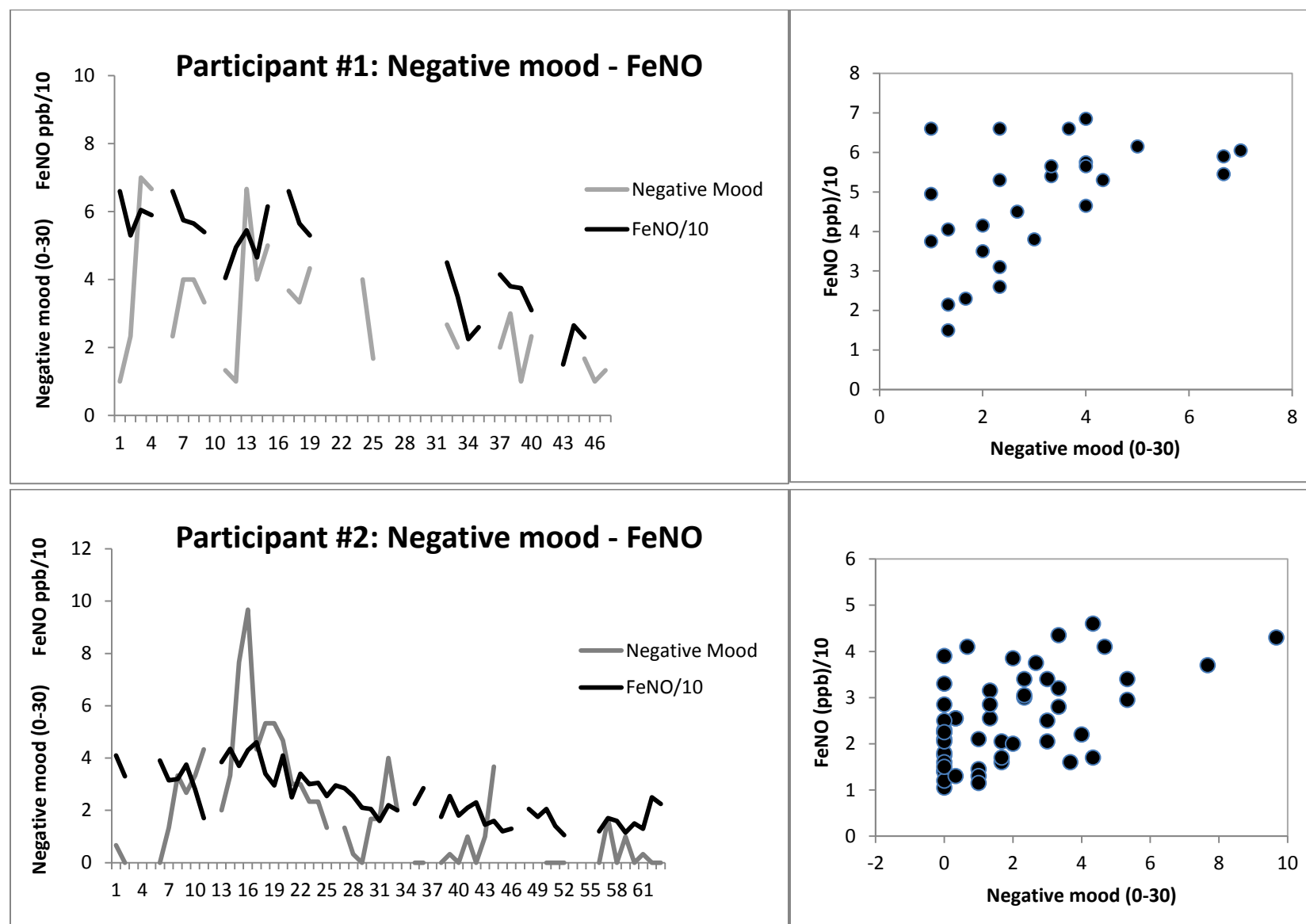
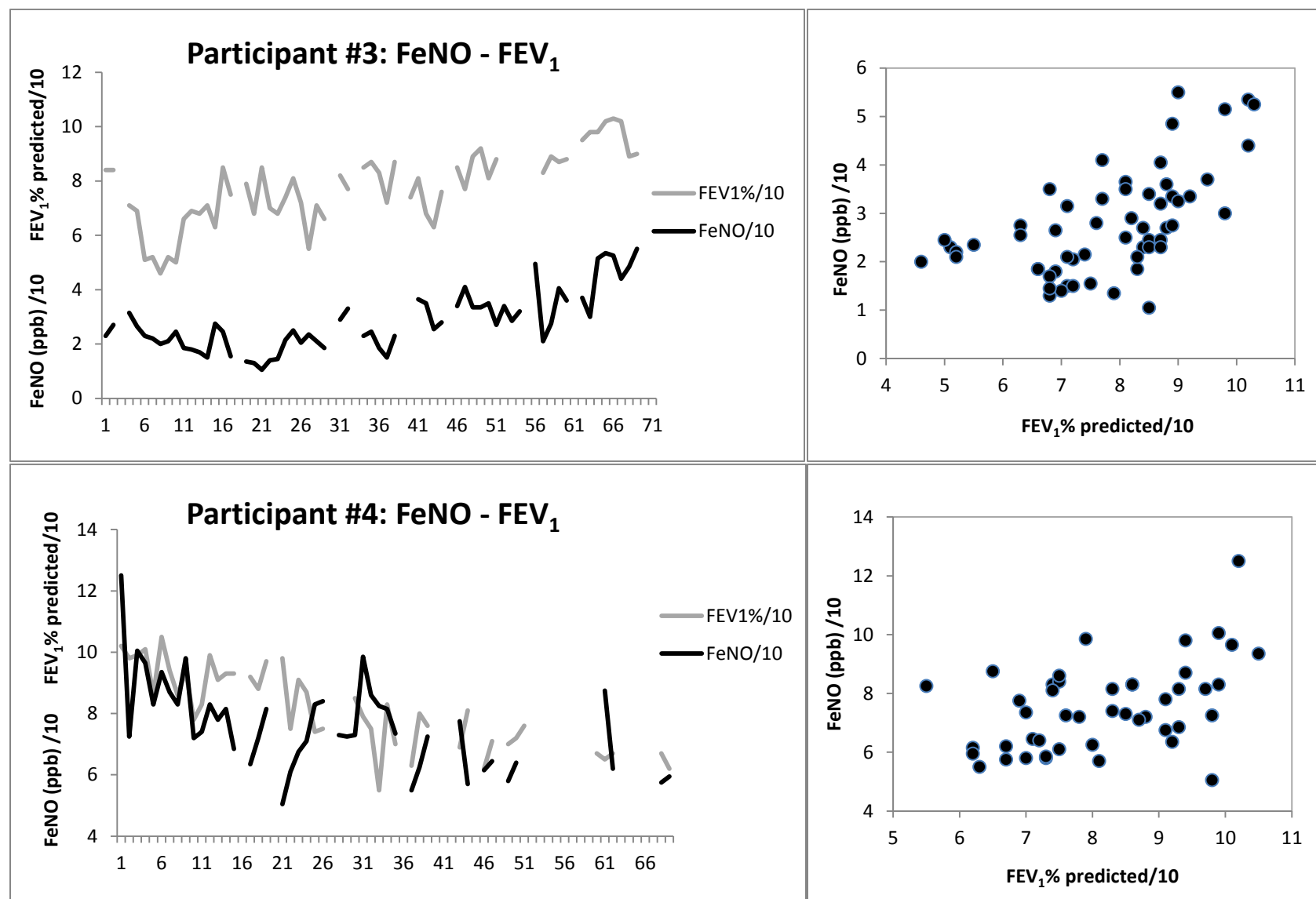
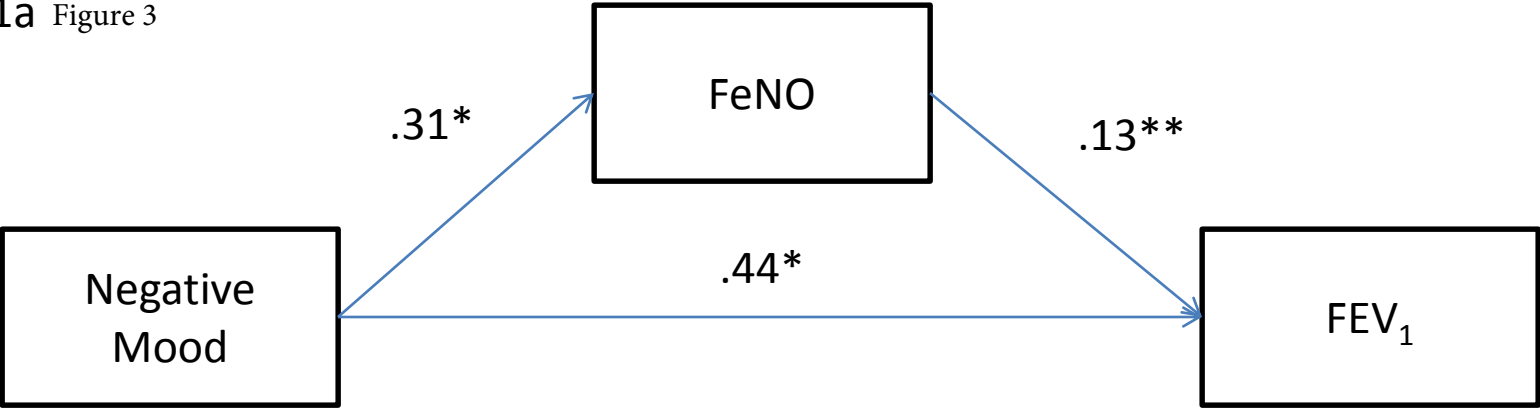


Figure 2



1a Figure 3



1b

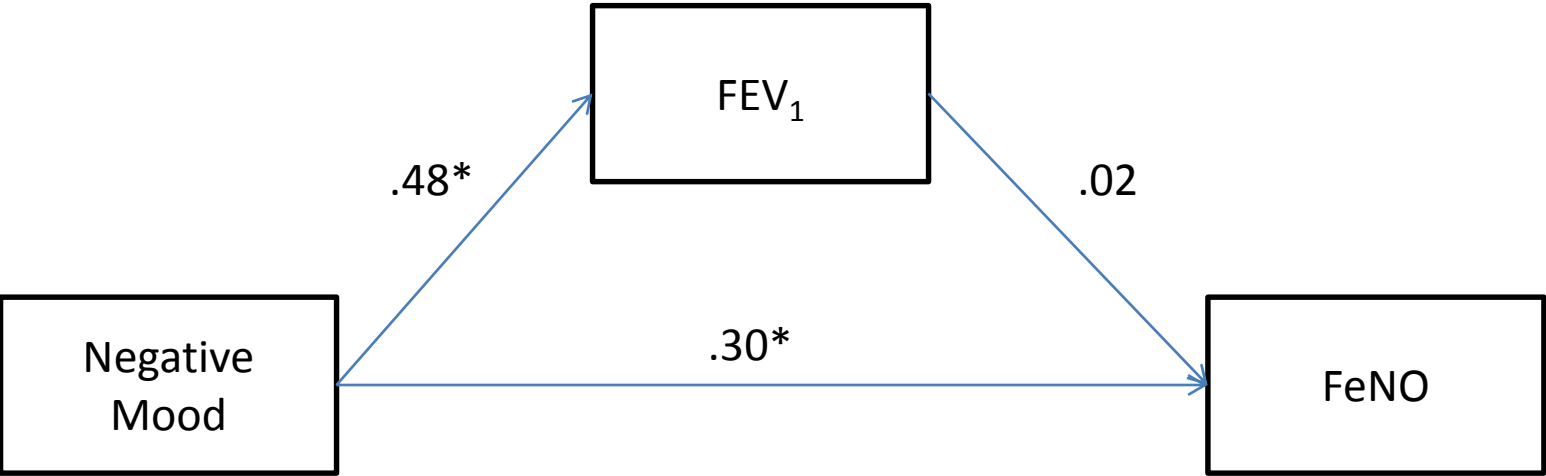


Table 1. Demographic, biometric and psychological variables at screening for asthma patients participating in FeNO home-monitoring (*N*=20)

Age, years; <i>M</i> ± <i>SD</i> (range)	29.4±11.4 (18-51)
Body Mass Index; <i>M</i> ± <i>SD</i> (range)	23.9±4.6 (19.5-40.2)
Sex, women (%)	95.0
Race, 100% Caucasian (%)	72.0
Ethnicity, Non-Hispanic (%)	90.0
Marital status, single (%)	66.7
Education, at least some college (%)	61.1
Age asthma onset; <i>M</i> ± <i>SD</i> (range)	14.2±12.5 (0-46)
Family history of asthma, positive (%)	65.0
Seasonal variations in asthma, incl. winter (%)	90.0
Allergens as triggers (%)	85.0
Night time symptoms, at least some (%)	45.0
Physician visits, asthma past year; <i>M</i> ± <i>SD</i> (range)	2.2±3.1 (0-14)
Emergency asthma treatments, life, <i>M</i> ± <i>SD</i> (range)	2.6±3.9 (0-15)
Asthma well-controlled, %	50.0
not well-controlled, %	50.0
FEV ₁ (% of predicted); <i>M</i> ± <i>SD</i> (range)	99.8±13.8 (71.9-120.6)
FeNO (ppb); <i>M</i> ± <i>S</i> (range)	31.5±25.2 (7-106)
Anxiety (HADS-A); <i>M</i> ± <i>SD</i> (range)	8.2±4.5 (1-19)
Depression (HADS-D; <i>M</i> ± <i>SD</i>)	3.2±3.8 (1-13)

Table 2. Means, standard deviations, minima and maxima for exhaled nitric oxide, lung function, and diary assessments across the home monitoring period

	<u>M</u>	<u>SD</u>	Minimum	Maximum
FeNO (ppb)	24.3	20.0	7.2	75.2
FEV ₁ (l)	2.61	0.44	1.88	3.56
PEF (l/min)	397.0	67.2	297.1	592.0
Negative mood (0-30)	5.00	3.21	0.49	10.75
Tense/anxious (0-10)	2.38	1.42	0.08	5.05
Depressed (0-10)	1.23	1.29	0.00	3.73
Frustrated/angry (0-10)	1.38	1.09	0.03	3.19

Table 3. Associations between FeNO and negative mood on concurrent or prior days in multilevel modeling analyses

Negative mood	Relation with FeNO
Lag 0 (concurrent)	$b=.31, t(809)=2.45, p=.014$
mean of Lag 0 and Lag -1 (prior day)	$b=.70, t(742)=2.86, p=.004$
mean Lag-0, Lag -1, and Lag -2	$b=.59, t(578)=1.65, p=.099$
Lag -1	$b=.04, t(708)=0.85, p=.398$
Lag -2	$b=.01, t(18)=0.17, p=.872$

Online Supplement**Daily Life Negative Mood and Exhaled Nitric Oxide in Asthma**

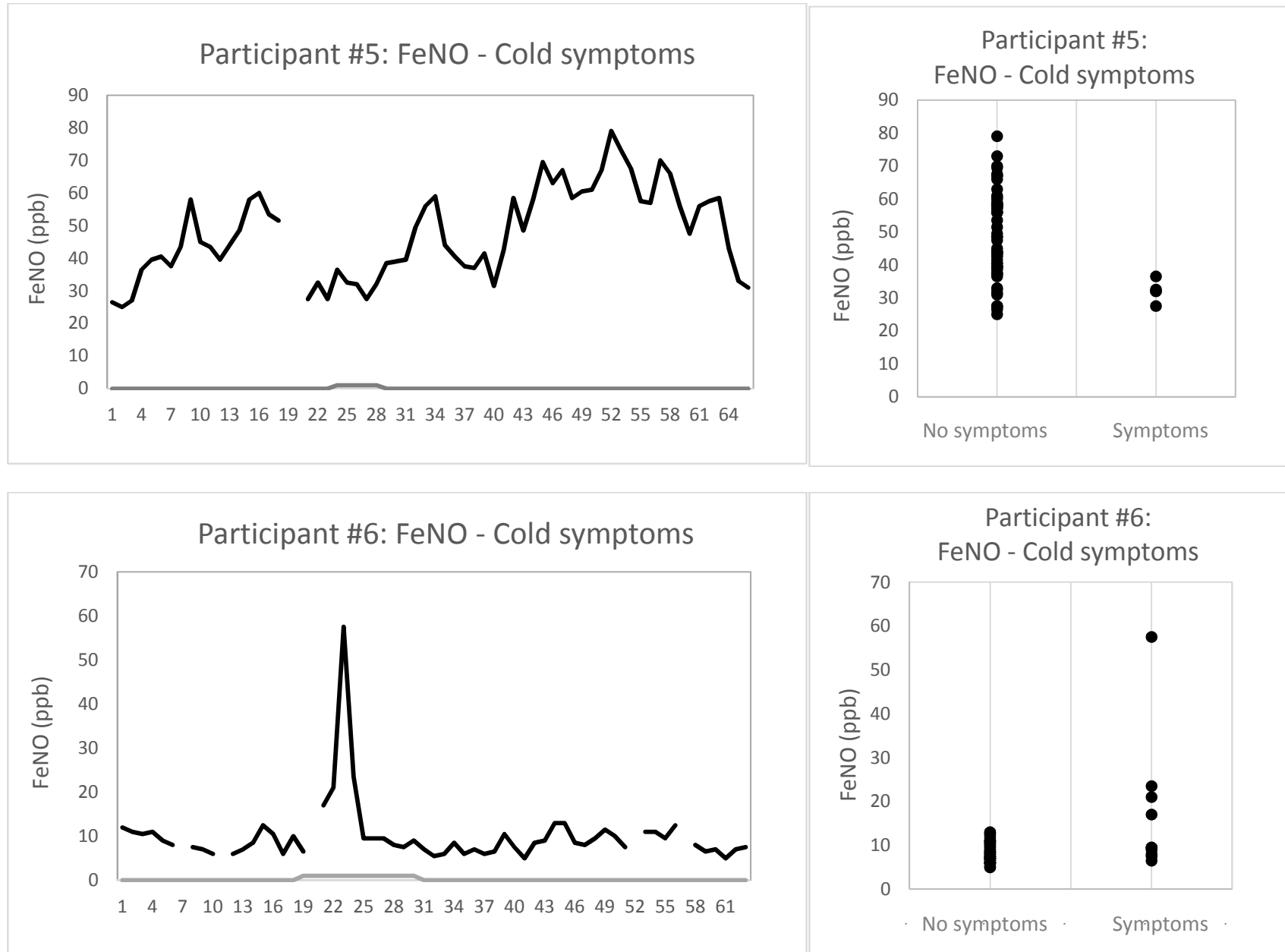
Thomas Ritz, Antje Kullowatz, Michelle N. Bill, & David Rosenfield

Southern Methodist University, Dallas, TX, USA

Online Supplement Figure 1. Two participants with the strongest within-individual associations between cold symptoms and FeNO, $r(62)=-.34$, $p=.006$, and $r(59)=.42$, $p=.001$, for Participant # 5 and 6, respectively; note that significance tests are only for rough guidance, due to the uncertainty about true dfs for associations within single individuals. Presence of cold symptoms is shown as level increase of the line close to the x-axis.

Online Supplement Figure 2. Two participants with the strongest within-individual associations between maintenance medication change and FeNO: $r(33)=.66$, $p<.001$, and $r(62)=-.37$, $p=.003$, for Participant # 7 and 5, respectively; note that significance tests are only for rough guidance, due to the uncertainty about true dfs for associations within single individuals. Medication level step-up or step-down is shown as level change of the line close to the x-axis.

Online Supplement Figure 1



Online Supplement Figure 2

