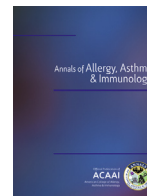




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Review

Airway nitric oxide and psychological processes in asthma and health: a review

Thomas Ritz, PhD; and Ana F. Trueba, MA

Southern Methodist University, Dallas, Texas

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ABSTRACT

Objective: The fraction of exhaled nitric oxide (FeNO) has been widely used as a marker of airway inflammation in asthma in recent years. However, NO serves multiple functions throughout the organism, and various influences on FeNO levels beyond inflammation have been documented. Emerging literature indicates that psychological processes are systematically linked to FeNO.

Data Sources: Academic Search Complete, PubMed, PsychArticles, and PsychInfo databases.

Study Selection: Relevant studies were identified using keywords *exhaled nitric oxide* paired with *psychological stress*, *stress psychology*, *emotion*, *major depression*, *anxiety*, or *psychopathology*. Studies measuring FeNO during naturalistic observation of emotion and stress, laboratory stress and emotion-induction protocols, and correlational designs using psychological questionnaires were included.

Results: Acute stress, anxiety, and negative affect have been repeatedly linked with higher FeNO levels, whereas more prolonged states of stress, in particular depression, have been associated with lower FeNO levels. The literature on FeNO is paralleled by research on NO in the cardiovascular and central nervous systems, which also shows systematic associations with psychosocial variables. Potential mechanisms of association include stimulation of NO release from different cells, including the epithelia and macrophages, through noradrenaline, interferon- γ , or vascular endothelial growth factor, changes in oxidative stress or arginase levels, or facilitation of diffusion by mechanical factors.

Conclusion: Psychosocial factors may need to be considered in the interpretation of longitudinal FeNO changes in monitoring and management of patients with asthma. The distinction between constitutive and inducible sources of NO will be essential for future research.

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Introduction

Nitric oxide (NO) is a gaseous molecule with a wide variety of biological functions. It is secreted by the epithelium as part of the innate immune defense against pathogens, acts as vasodilator in its endothelium-derived form, and serves as a neurotransmitter in the peripheral and central nervous systems.¹ In addition, multiple immune cells may secrete larger quantities of NO in inflammatory states of the airways. In recent years, the fraction of exhaled NO (FeNO) has been used increasingly as a marker of allergic inflammatory activity in asthma.² Measurement of this very small gas fraction in exhaled air, by chemiluminescence or electrochemical techniques, has become common in research. This measurement has proved to be reliable, and guidelines have been issued for its standardization.³ The utility of FeNO assessments for clinical use also has been explored, such as in diagnosis of airway disease and medication treatment of asthma, although findings have remained mixed.^{2,4}

Elevated levels of FeNO in asthma have traditionally been thought to be strongly linked to the mobilization of eosinophils and

other immune cells in the airways, and corticosteroid treatment has been shown to lower FeNO levels in asthma.² However, the function of elevated NO levels in the airways has been debated, and the interpretation of findings has been complicated by some beneficial immune and airway functions of NO.⁵ Research has a long history of demonstrating influences of psychological factors on pulmonary function and airway immunology.^{6–8} Recent studies have suggested that NO levels also are systematically associated with psychological states and psychopathology,^{9,10} but findings have remained equivocal, probably owing to variations across compartments and differences in underlying NO synthase (NOS) activity. Three types of NOS are typically distinguished: inducible (iNOS), which is typically associated with inflammatory processes; neuronal (nNOS); and endothelial (eNOS). The latter 2 are categorized as constitutive NOS (cNOS). Activity of cNOS is often associated with a range of beneficial effects in the cardiovascular and respiratory systems and in central nervous system (CNS) structures.^{11,12} In healthy individuals, cNOS is thought to contribute to FeNO levels.¹³

In this article, the authors review the literature on associations of airway NO with psychosocial factors, potential underlying mechanisms of such associations, and consequences for research and clinical practice. The literature search was conducted using the

Reprints: Thomas Ritz, PhD, Department of Psychology, Southern Methodist University, PO Box 750442, Dallas, TX 75275-0442; E-mail: tritz@smu.edu.

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Academic Search Complete, PubMed, PsychArticles, and PsychInfo databases with keywords *exhaled nitric oxide* paired with *psychological stress*, *stress psychology*, *emotion*, *major depression*, *anxiety*, or *psychopathology*. In addition, the authors include the latest work from their laboratory on FeNO measurements and psychological processes.

Airway NO and Psychological Stress

The systematic exploration of FeNO changes associated with psychological stress in humans has typically relied on standardized laboratory stress induction or observations of real-life stress situations. In addition, associations between FeNO levels and stress questionnaire assessments have been studied. Table 1 presents an overview of these studies.^{14–22} Participants with and without asthma were included. One of the first studies used a final academic examination paradigm in students, with 1 measurement during the examination period and 1 reference measurement in a low-stress period during the academic term.¹⁴ No significant change was observed in participants with allergic asthma, despite changes in parameters of systemic T-helper cell activity (greater bias toward T-helper cell type 2 activity during stress). In contrast, FeNO decreased significantly in healthy controls during the examination period, a finding that was not particularly highlighted in the article. The latter finding was replicated in a more recent study.¹⁵ An observational study with 2 FeNO assessments approximately 3 months apart found that increases in daily hassles during this period were related to decreases in FeNO in participants with asthma but not in control participants.¹⁶ However, another recent study with longitudinal monitoring of FeNO in 20 patients with asthma found that higher self-report of daily hassles predicted elevated FeNO levels on the 2 days after the self-report assessment.¹⁷

With regard to acute stress, 2 laboratory studies found evidence for increases in FeNO after laboratory stressors, such as free speech or conflict interview: one study for adult participants with asthma and healthy adult participants⁷ and another study only for children with asthma and low compared with high socioeconomic status.¹⁸ FeNO increases owing to a speech stressor also were stronger for participants with asthma and controls who scored higher on a questionnaire measuring perceived stress in the previous 2 weeks.¹⁹ A dependency on background stress levels also may be the explanation for the findings of the study in children with asthma,¹⁸ if higher socioeconomic status can serve as a proxy for life stress levels. As an exception to the acute stress findings, an earlier study observed a FeNO decrease during pentagastrin-induced panic attacks in healthy volunteers,²⁰ but these decreases were most likely secondary to the associated increases in minute ventilation.

Thus, acute increases in FeNO have been observed consistently in response to short-term stressors. In contrast, more prolonged

stress over days or weeks may be linked to decreases in FeNO levels, although increases have also been observed. Acute stress-induced increases of NO production or release also may be elevated during periods of sustained stress.

Airway NO, Negative Affect, Anxiety, and Depression

One of the more consistent findings across studies is that questionnaire measurements of negative affect and anxious mood are significantly associated with higher FeNO levels and stronger reactivity (increases in FeNO) to stress induction (Table 1). This has been observed from a cross-sectional perspective with the Positive and Negative Affect Schedule measuring current negative affect^{15,19} and the Hospital Anxiety and Depression Scale measuring anxious mood in the past week.^{19,22} From a longitudinal perspective, the authors' team recently completed an FeNO home monitoring study of patients with asthma that showed a positive association between negative mood diary ratings and FeNO for the concurrent association (ratings and FeNO from the same "day 0") and the association between FeNO on day 0 and the average of negative mood calculated from day 0 and the previous day (day –1).²¹ On an individual-item basis, the diary rating of "tense/anxious" alone accounted for most of the significant associations.

In contrast, higher depressive mood, as measured by the Hospital Anxiety and Depression Scale, was associated with lower FeNO levels in a group of healthy participants across low-stress midterm assessments and a stressful final examination period.¹⁵ In addition, FeNO increases from acute laboratory stress were smaller in healthy and asthmatic individuals with greater depressive mood.¹⁹ Recently, the authors replicated the negative association between depressive mood and FeNO in another sample of patients with asthma using 4 measurements of FeNO across a period of several months. However, the association was found to be positive for healthy controls in this analysis.²² Indirectly relevant to these associations is the finding in the same study that social support was associated positively with FeNO, although only in patients with asthma. In another study, FeNO increases also were observed for 8 weeks between and within sessions in a group of participants receiving training in relaxation techniques.²³

In summary, there is substantial evidence that negative affect and anxiety are associated positively with FeNO in asthma and health; associations with depression are more inconsistent and have sometimes shown a negative direction.

Psychological Processes and NO in Other Compartments

Substantial literature has focused on the role of NO in the CNS. The role of NO at various CNS sites is likely to be complex, because enhancement of excitatory and inhibitory activities has been observed.^{1,24} NO also is involved in synaptic plasticity, neuroprotection, long-term potentiation, and long-term depression.

Table 1

Association of nitric oxide levels and reactivity with measurements of stress, negative affect, anxiety, and depression in humans

Study	Groups (n)	Acute stress	Prolonged stress	Acute negative affect	Anxiety	Depression
Lara et al, ²⁰ 2003	H (17)	H↓	—	—	—	—
Höglund et al, ¹⁴ 2006	A (16), H (19)	—	H↓	—	—	—
Kullowatz et al, ¹⁶ 2008	A (46), H (26)	—	A↓	A↑	—	—
Chen et al, ¹⁸ 2010	A (23), H (38)	A low SES↑	—	—	—	—
Ritz et al, ¹⁰ 2011	A (20), H (19)	A, H↑	—	NS	—	—
Ritz et al, ¹⁹ 2012	A (39), H (41) ^a	A, H↑	A, H Δ↑	A, H↑	A, H Δ↑	A, H Δ↓
Ritz et al, ^{17,21} 2013, in press	A (20)	—	A↑	A↑	A↑	A↑
Ritz et al, ²² 2013b	A (52), H (22) ^b	—	—	—	H↑	A↓, H↑
Trueba et al, ¹⁵ 2013	H (41)	—	H↓	H↑	—	H↓

Abbreviations: —, not measured; A, asthma; H, healthy; NS, results not significant; SES, socioeconomic status.

^aIncludes subjects of Ritz et al.¹⁹

^bIncludes participants of Kullowatz et al¹⁶ but uses different questionnaire measurements and 2 more time points.

In animal models, chronic stress has been shown to increase iNOS and nNOS activity in the neocortex and hippocampus, and inhibition of NOS activity has been shown to produce antinociceptive effects²⁵ or antidepressive effects.^{26–28} Similarly, injecting a NOS inhibitor into the medial amygdala has been reported to produce anxiolytic effects in rats.²⁹ However, both excess and shortage of hippocampal nNOS have been linked to depressive-like behaviors.³⁰ Concentration-dependent differential effects of NO have been observed in other brain areas, such as in the nucleus tractus solitarius, where NO enhances or dampens baroreflex action by affecting glutamatergic or γ -aminobutyric acidergic transmission.²⁴ In the dorsal raphe nucleus, low doses of NOS inhibitors have been found to foster exploratory behavior in rats but higher doses have been found to inhibit it,³¹ which provides another example for the dualities observed in the neuroregulation by NO and its inhibitors (see also for pain regulation³²). Moreover, high concentrations of CNS iNOS are neurotoxic, promoting neuroinflammation, neurodegeneration, and apoptosis.³³ These findings from animal models provide further insight to the intimate link between NO and behavioral factors. However, despite the success of animal models in the exploration of asthma pathophysiology and psychological phenomena, such as learning and behavioral dysfunction, caution should be exercised in extrapolating to humans.

Cardiovascular physiology has focused on eNOS activity for its multiple beneficial effects, including vasodilation, inhibition of platelet aggregation and adhesion, and prevention of thrombosis and atherosclerosis. Converging studies in humans and animals have shown increases in eNOS activity under laboratory stress and decreases in eNOS activity during acute and chronic stresses through increases in endothelin production by endothelial cells or effects of glucocorticoids and proinflammatory cytokines on these cells.³⁴ Patients with major depression also have been found to have to lower levels of platelet NOS activity and NO metabolites in plasma,³⁵ and antidepressants (selective serotonin reuptake inhibitors) have been found to have a normalizing effect on plasma NO levels.⁹ The clinical importance of decreased NO mobilization in depression remains unclear, given that an alternative vasodilatory pathway (by hydrogen peroxide, as demonstrated in a mouse model) appears to be in place, which at least in part compensates this deficit.³⁶

Thus, findings on NO and psychological processes in the CNS and cardiovascular system show some parallels with findings on airway NO, such as stress-induced increases in NO in various CNS structures and the vasculature or low endothelial NO levels related to depression. However, the complexity of the roles of NO in these compartments certainly rivals that of airway NO; therefore, findings need to be compared with great caution. At the minimum, they demonstrate that an association of NO with psychological processes is reflected in multiple organ systems.

Potential Mechanism Underlying Associations between NO and Psychological Factors

Different pathways could account for observable increases in FeNO in the reviewed studies. FeNO assessments are not selectively sensitive to the different NOS isoforms, thus complicating the interpretation of findings. The literature is inconsistent regarding the relative contributions of iNOS and cNOS to FeNO in individuals with healthy airways.^{13,37–39} Aminoguanidine, a selective iNOS inhibitor, did not affect FeNO in healthy individuals in an earlier study.¹³ However, moderately high correlations between epithelial iNOS mRNA and FeNO have been observed in healthy children.³⁹ Nevertheless, associations that are equally present in health and asthma conditions do not provide strong support for a modification of iNOS activity that is linked to an allergic pathway. This may be the case in the observed associations between FeNO and acute

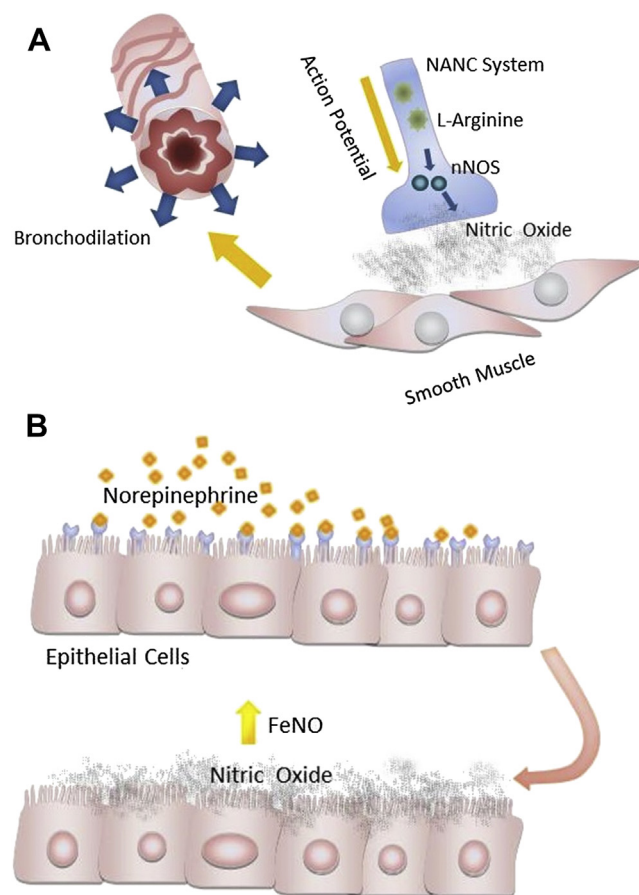


Figure 1. Potential pathways linking psychological stress with changes in fraction of exhaled nitric oxide (FeNO). (A) NO is released from the non-adrenergic–non-cholinergic (NANC) system, which has bronchodilation effects and leads to an increase in FeNO. (B) Norepinephrine release stimulates NO from epithelial cells lining the airways. This results in an increase in FeNO. (C) The presence of stress or antigens increases the production of interferon- γ (IFN- γ) from T-helper cells, which enhances the expression of inducible NO synthase (iNOS) in macrophages and epithelia, thus resulting in an increased production of NO and the elevation of FeNO. (D) The sympathetic nervous system releases peripheral corticotropin-releasing hormone (CRH), which stimulates the production of vascular endothelial growth factor (VEGF) in mast cells. VEGF enhances the production of NO by increasing the expression of iNOS and endothelial NOS (eNOS). (E) L-arginine is the substrate of constitutive NOS (cNOS) and iNOS. These enzymes use L-arginine to produce NO (ultimately FeNO). L-arginine also is the substrate of arginase, which results in a competition between NOS and arginase for L-arginine. Therefore, increases in arginase, as shown for individuals with depression, decrease the available L-arginine that can be used to produce NO. In epithelial and other cell types, increases in reactive oxygen species (ROS; as shown in psychological stress) could contribute to a decrease in NO. ROS scavenge NO, thus lowering NO levels. Therefore, the increase in ROS and arginase results in a decrease in FeNO. (F) Catecholamines released from the adrenal medulla and neurotransmitters from the NANC system foment bronchodilation by relaxing the bronchial smooth muscles. This facilitates the diffusion of NO from epithelial cells, which ultimately results in an increase in FeNO. nNOS, neuronal nitric oxide synthase; Th1, T-helper type 1 cell.

stress, acute negative affect, and anxiety (Table 1). However, not all studies have shown comparable associations in healthy individuals and those with asthma.^{16,18,22} The relatively small amount of change and the brief time frame of FeNO elevations in acute stress studies^{10,18,19} also supports the notion that effects are linked to a constitutive mechanism that does not depend on gene transcription. In addition, eNOS may be less likely to contribute to FeNO owing to its distance from the airway lumen, which may not allow it to diffuse into the airspace in sufficient quantities.³⁷ This would leave nNOS as a potential source of FeNO changes under psychological challenges. NO serves as a neurotransmitter for the

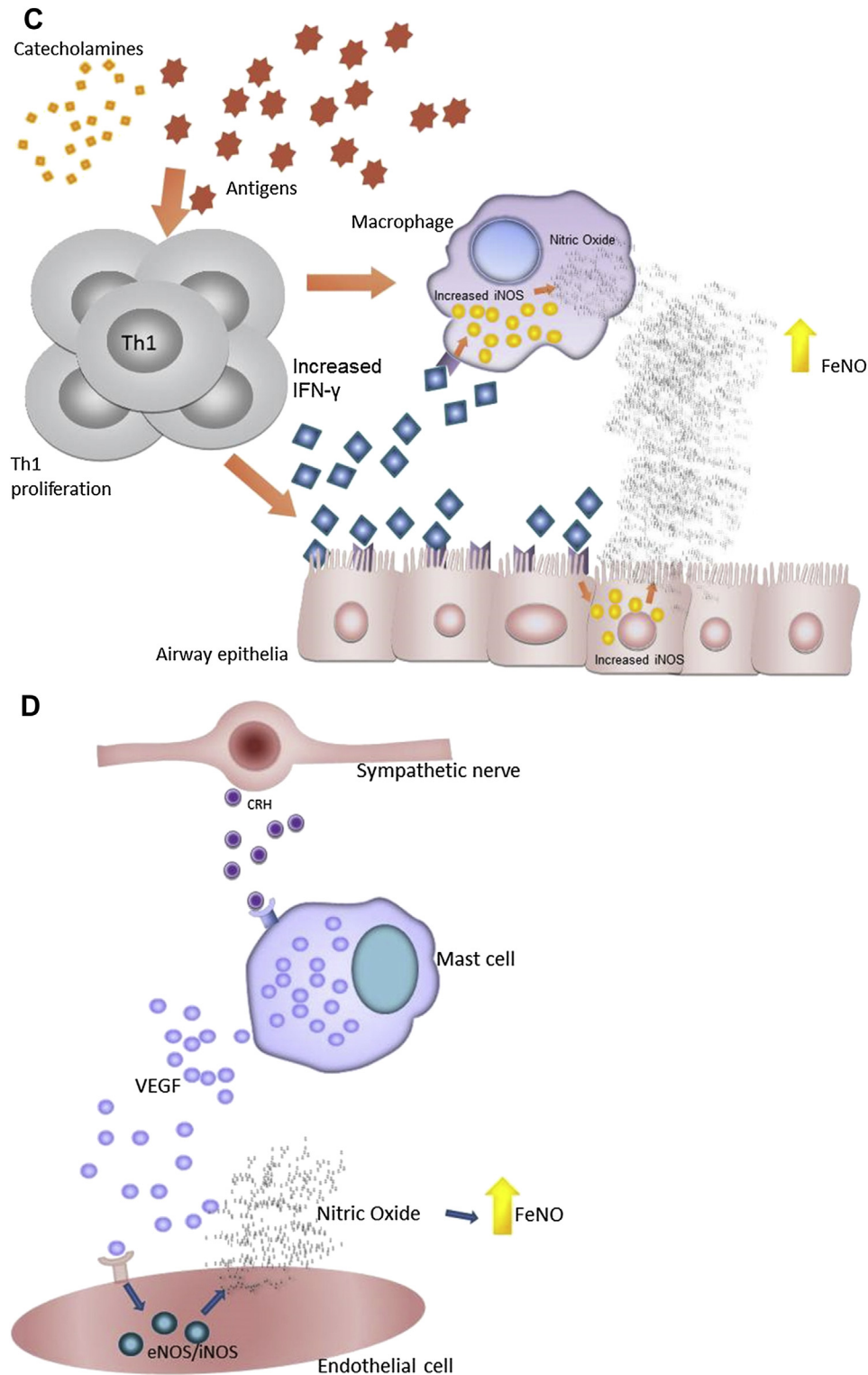


Figure 1. (continued).

bronchodilation pathway of the non-adrenergic–non-cholinergic system, and its activity is compatible with longitudinal data showing an association between increases in FeNO and bronchodilation²¹ (Fig 1A). The relative distance of eNOS activity to the airway lumen can be raised as argument against the importance of this source.³⁷ Direct secretion of some form of constitutive NO from epithelial cells by norepinephrine action also might take place, as

demonstrated for the urinary bladder⁴⁰ (Fig 1B). Stress or antigens also stimulate interferon- γ from T cells,^{41,42} which has been postulated to tonically stimulate iNOS activity underlying FeNO from healthy airways³⁷ (Fig 1C). Other pathways could involve stimulation of NO through stress-induced mast cell release of vascular endothelial growth factor and subsequent stimulation of iNOS and eNOS^{43,44} (Fig 1D). Decreases in NO observed in

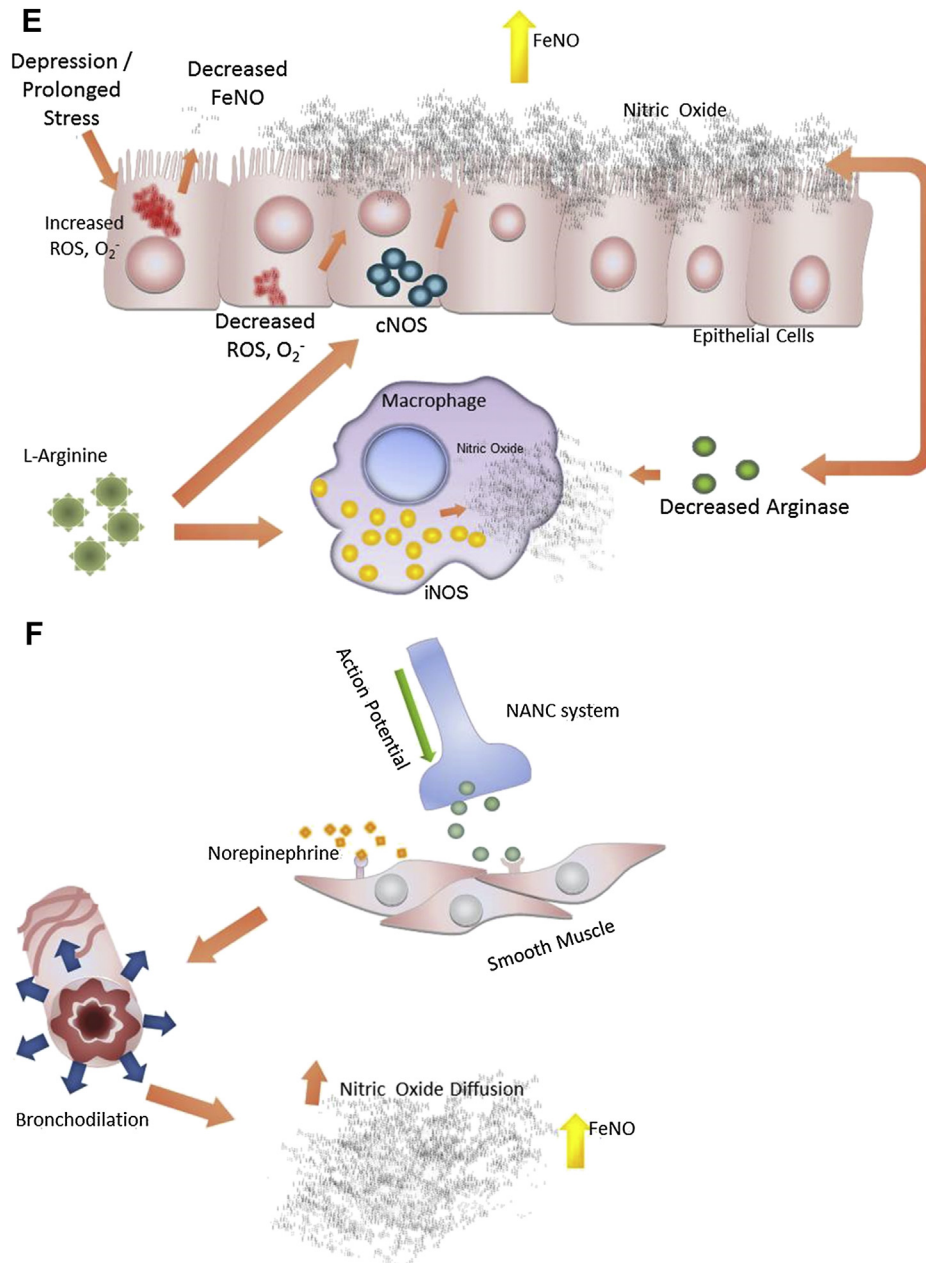


Figure 1. (continued).

depression and prolonged or chronic stress could involve mechanisms that compete with the L-arginine–NOS–NO pathway (Fig 1E). Because measurable NO also is determined by the extent to which NO is consumed by reactive oxygen species,⁴⁵ increases in oxidative stress by psychological stress or depression^{46–48} could decrease measurable FeNO levels; however, iNOS activity may be increased at the same time, as demonstrated in a guinea pig model of repeated forced swim tests.⁴⁶ Alternatively, arginase may interfere with NO production by competing for L-arginine (Fig 1E). Indeed, higher arginase levels in depression may serve as an explanation for NO deficiencies observed in patients with depression.^{49,50} Elevated arginase levels also have been linked to asthma pathophysiology.⁵¹ A mechanical link also has been considered, in that stress-induced bronchodilation could enhance NO diffusion from epithelial cells⁵² (Fig 1F).

Beyond these direct pathways linking psychological factors with FeNO, some potential indirect influences through demographics,

health status, health behaviors, and environmental factors should be considered. These indirect influences have been examined to varying degrees in studies of FeNO associations with psychological factors listed in Table 1. Guidelines⁵³ have particularly stressed the control of smoking, dietary factors (nitrate and nitrite-rich foods), and exercise or strenuous physical activity for 1 to 2 hours before FeNO measurements, which has been documented for most studies. Controlling such influences is important, particularly when systematic associations with psychological states can be suspected. For example, nutrition and exercise levels can change systematically during periods of greater life stress¹⁴ and thus mediate associations between stress and FeNO. Respiratory infections also can elevate FeNO levels, but sufficient control was achieved by all studies through the exclusion of respiratory diseases other than asthma and additional monitoring of respiratory infection symptoms.¹⁵ Control of medications, in particular anti-inflammatory agents, was reported by almost all studies, although washout for

systemic corticosteroids ranged from 2 weeks¹⁸ to 3 months.^{10,16,17,19,21,22} Additional control for, or washout of, anti-asthmatic medication also was reported by most studies including individuals with asthma. Other factors potentially influencing FeNO were addressed by only a minority of studies, such as menstrual cycle influences,^{17,20,21} ambient aeroallergen levels, and air pollution.^{16,20,21} However, it should be noted that if statistical associations of FeNO with such factors were reported, they remained mostly nonsignificant or inconsistent across these studies. Attention to ventilatory adjustments that accompany psychological processes is also recommendable. This was particularly important in the induction of acute laboratory stress, where 2 studies had included ventilation measurements^{10,20} and 1 of them found an influence of stress-induced ventilation levels on FeNO.²⁰ Exclusion of medical conditions other than asthma has been reported inconsistently across studies, and attention to conditions such as metabolic syndrome⁵⁴ or gastric reflux-induced airway acidity⁵⁵ is needed in future research. Associations of FeNO with psychological factors have not been systematically studied for disease states other than asthma.

Perspectives on Research and Consequences for Clinical Practice

Although there is substantial evidence that psychological factors are associated with altered FeNO levels, the interpretation of these findings is difficult given the uncertainties about the sources of NO in FeNO. The literature provides good arguments for a constitutive source of NO as the basis of most findings, but blockade studies with NOS inhibitors, such as the selective iNOS antagonist aminoguanidine,^{13,38} exploration of NOS polymorphisms,⁵⁶ or mRNA analysis,³⁹ provide more certainty. From a functional standpoint, upregulation of NOS activity in acute stress may make sense as a protective response against challenges that threaten the integrity of the organism, by facilitating blood flow to skeletal muscles, dilating airways and limiting their responsiveness to constricting agents, and mounting a first line of defense against pathogens. Stress hormones, such as catecholamines, most likely play a key role in such adaptations. It is also reasonable to expect that the NO change in more prolonged or chronic stress could follow different dynamics, which might be determined more by competing processes such as oxidative stress or arginase levels.^{48,50} Differential adaptations to acute vs chronic stress have been demonstrated in the immune,^{57,58} cardiovascular,⁵⁹ and respiratory^{60,61} systems. Ideally, large-scale studies that combine laboratory stress and emotion induction with longitudinal observations of naturalistic acute and chronic stress states, anxiety, and depression using large samples of patients that vary in respiratory disease and psychiatric status would be needed to shed more light on such dynamics. Research also will need to explore further the differential pathways of airway NO response linked to various forms of psychological processes. One of the recommended expansions of this research would be to include estimates of NO consumption by airway oxidative stress.⁴⁴ The fact that stress modulates the late-phase component of the allergic cascade by enhancing airway eosinophil infiltration⁶² also would lead to the expectation of an upregulation of iNOS activity in the airways. This effect would add another layer of FeNO modulation that may exist independently from the effects of cNOS activity and would need to be disentangled from it. Thus, well-designed future studies would include these 2 perspectives: the modulation of basal FeNO and FeNO under allergen-induced early- and late-phase responses by stress states.

The use of FeNO assessments in clinical practice as the sole indicator of airway inflammatory status may be complicated by psychological influences. Although stress effects on FeNO appear to be relatively small on average, with changes in the range of a

few parts per billion, much larger changes per unit of mood change are observed in individual patients. For example, the authors observed patients who showed FeNO increases of approximately 5 ppb associated with a 1-unit increase on a negative mood scale that ranges from 0 to 10.²¹ Such changes could add to the uncertainty of the value of FeNO assessments for adjustment of medication treatment in individual patients. Findings have remained equivocal regarding the added benefit of monitoring FeNO for this purpose.⁴

Thus, in addition to a host of lifestyle variables that can confound FeNO levels,³ exploration of the psychosocial situation of the patient appears advisable and may help explain unexpected findings in the interpretation of FeNO. Therefore, screening for life events, anxiety, and depression should take on a mandatory role in the diagnosis and management of patients with asthma. Should future research solidify findings on cNOS activity decreases in chronic or prolonged states of stress and depression, the development of therapeutic measures to restore NO needed for a range of beneficial physiological functions, including bronchodilation and bronchoprotection, may be indicated.

Summary and Conclusion

An evolving literature suggests significant associations of FeNO with psychosocial variables. Acute stress, anxiety, and negative mood have been linked consistently to FeNO increases, whereas depression and prolonged or chronic stress present a more mixed picture, with some findings suggesting decreases in FeNO. Although multiple pathways can be conceived that link stress, anxiety, and depression with FeNO, much remains speculative at this point. Evidence from comparisons with healthy individuals and from the temporal dynamics of FeNO changes under stress make an involvement of a constitutive source of NO changes more plausible. The study of the relation between psychological processes and FeNO also may add substantially to the discussion on the role of elevated FeNO levels in asthma and the nature and function of airway NO in general. In conclusion, there is still much to be learned about the sources and dynamics of FeNO in asthma and health.

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