

Letter to the Editor

Opposing effects of nasal epithelial NAD(P)H dehydrogenase quinone 1 and heme oxygenase 1 expression on upper and lower airway symptoms in adolescents with asthma

To the Editor:

Oxidative stress plays an important role in asthma.¹ Nuclear factor (erythroid-derived 2)-like 2 (NRF2), a basic leucine zipper transcription factor, appears to be critical in this context because it regulates the expression of important antioxidant genes.² As such, small molecule activators, such as sulforaphane, are being pursued as therapeutic agents in asthma.³ NRF2 target genes, including NAD(P)H dehydrogenase quinone 1 (NQO1) and heme oxygenase 1 (HO-1), are routinely assessed as markers of NRF2 activity in both laboratory and clinical settings with an underlying assumption that greater expression of these NRF2 target genes is a marker of a more robust antioxidant response, which should protect against airway inflammation and asthma symptoms.

To test the hypothesis that greater HO-1 and NQO1 gene expression is associated with less airway inflammation, better lung function, and fewer asthma symptoms, we examined relationships between expression of these NRF2 target genes and clinical features of asthma in nonsmoking Baltimore City adolescents (14–17 years; $n = 22$) with moderate to severe asthma (detailed [Methods](#) available in this article's Online Repository at www.jacionline.org).

Study participants were predominantly black (85%) and male (55%). Eighty-two percent had public health insurance. Ninety percent of participants had at least 1 positive skin test. NQO1 and HO-1 mRNA levels in nasal epithelium were inversely correlated (Spearman's correlation [r_s] = -0.43 ; $P = .04$; [Fig 1, A](#)). Higher NQO1 expression was associated with more days of exercise-related symptoms ($r_s = 0.44$; $P = .04$) and slowed activity because of asthma ($r_s = 0.57$; $P = .006$; [Fig 1, D and E](#)). NQO1 expression was not associated with days of asthma symptoms ($r_s = -0.24$; $P = .27$). In contrast, higher HO-1 expression was associated with fewer days of slowed activity because of asthma ($r_s = -0.45$; $P = .03$; [Fig 1, C](#)) and asthma symptoms ($r_s = -0.43$; $P = .05$). Higher HO-1 expression was also associated with fewer days of exercise-induced symptoms ($r_s = -0.27$; $P = .21$; [Fig 1, B](#)), but this finding was not statistically significant. NQO1 expression was positively correlated with days of cough without a cold ($r_s = 0.48$; $P = .03$), whereas HO-1 expression was negatively correlated with this symptom variable ($r_s = -0.56$; $P = .01$). NQO1 expression was also higher among subjects with rhinoconjunctivitis (see this article's [Fig E1](#) in the Online Repository at www.jacionline.org), resulting in greater odds of rhinoconjunctivitis symptoms (odds ratio [95% CI], 1.32 [1.02–1.70]), whereas higher HO-1 expression was associated with lower nonsignificant odds of rhinoconjunctivitis symptoms (odds ratio [95% CI], 0.36 [0.12–1.08]). Overall, higher NQO1 expression was positively associated with upper and lower respiratory symptoms, whereas higher HO-1 expression was inversely associated upper and lower respiratory symptoms.

Relationships between NQO1 and HO-1 expression and rescue medication use and percent change in FEV₁ with bronchodilator were consistent with the relationships observed with upper and lower respiratory symptoms. For example, although not statistically significant, there were trends toward a negative correlation between HO-1 expression and response to bronchodilator and a

positive correlation between NQO1 expression and response to bronchodilator (see this article's [Table E1](#) in the Online Repository at www.jacionline.org). No relationships were observed with either fractional exhaled nitric oxide or FEV₁/forced vital capacity.

These results demonstrate a consistent inverse relationship between HO-1 expression and a variety of upper and lower airway clinical outcomes. Our findings support the observation that greater HO-1 expression may mitigate asthma symptoms. This is supported by previous investigations that found higher HO-1 expression in airway epithelial cells to be protective against asthma via regulatory T cells, IL-10, and membrane-bound TGF-1 production.^{4–6} In contrast, higher NQO1 expression was positively associated with upper and lower airways symptoms, suggesting that induction of NQO1 expression may not be accompanied by an improvement in upper or lower airway symptoms and that simultaneous induction of both HO-1 and NQO1 expression may result in limited clinical effects. These findings may have important implications for the use of NQO1 and HO-1 gene expression as biomarkers in the development of oxidative stress-targeted therapy. Specifically, induction of NQO1 expression as a marker of potential clinical efficacy should be viewed with some skepticism because our findings highlight the challenge of predicting clinical effects from *in vitro* and biomarker studies of putative therapeutic agents. Furthermore, the inverse correlation between HO-1 and NQO1 expression indicates that there are likely NRF2-independent regulatory mechanisms that significantly influence the expression of both or at least 1 of these genes.⁷ In fact, recent data demonstrate posttranscriptional regulation of HO-1 microRNA by miRNA and stabilization in a mitogen-activated protein kinase-dependent manner.^{8,9} The activity of these regulatory mechanisms may influence the ability of NRF2 inducers to upregulate antioxidant defense genes and could provide an explanation for the inverse correlation between HO-1 and NQO1 expression in our study population. Ultimately, though, human trials are needed to understand the clinical effects of nonspecific augmentation of the oxidative stress response.

There are limitations of our study that should be considered. It is possible that nasal corticosteroid use could have biased our results. However, of the 22 participants, only 2 reported using nasal corticosteroids, making this an unlikely source of bias. The study population was small, urban, and predominantly black, so whether our findings are applicable to other populations is unknown. In addition, the study was cross-sectional, so associations between gene expression and clinical outcomes could be examined, but how HO-1 and NQO1 expression change over time within an individual is not clear. Nevertheless, the inverse correlation between expression of these 2 antioxidant genes in airway epithelial cells and their opposing relationships to upper and lower respiratory symptoms is striking and serves to highlight potential pitfalls in our understanding of the clinical impact of pathways that control oxidative stress in asthma.

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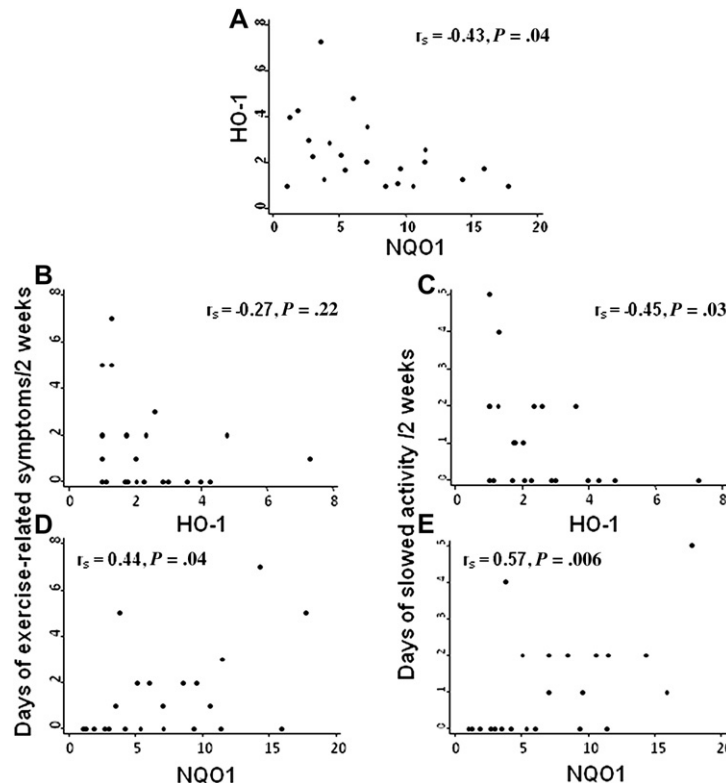


FIG 1. Scatterplots of gene expression and asthma symptoms. **A**, NQO1 and HO-1 mRNA levels were inversely and significantly correlated in this population. HO-1 mRNA levels were inversely correlated with days of exercise-related symptoms (**B**) and slowed activity (**C**). NQO1 mRNA levels were positively correlated with days of exercise-related symptoms (**D**) and slowed activity (**E**).

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METHODS

Clinical outcome measurements

Nonsmoking Baltimore City adolescents (14-17 years; $n = 22$) with moderate to severe asthma were drawn from a larger cohort. Participants underwent spirometry, exhaled nitric oxide measurement (fractional exhaled nitric oxide; Niox MINO; Aerocrine, Uppsala, Sweden), and allergy skin testing to a panel of 14 aeroallergens (net wheal diameter ≥ 3 mm was considered positive). A questionnaire that included asthma symptom questions that have been used extensively in multicenter inner-city asthma studies and validated rhinoconjunctivitis questions from the International Study of Asthma and Allergies in Childhood survey captured upper and lower respiratory symptoms.^{E1-E3} Participants were considered to have rhinoconjunctivitis if they reported nasal symptoms accompanied by itchy, watery eyes in the past 12 months.

Nasal epithelial RNA

Nasal epithelial cells were collected by using a Rhinoprobe curette and immediately lysed in 100 μ L lysis solution provided with the RNAqueous-micro kit (Ambion, Austin, Tex). Lysed nasal samples were stored at -80°C before RNA isolation. Briefly, 50 μ L 100% ethanol was added to 100 μ L lysate and vortexed thoroughly. Lysate/ethanol mixture was loaded onto a microfilter cartilage assembly (column) and centrifuged for 30 seconds at 13,000g. The filter column was washed once with 180 μ L wash I and twice with 180 μ L wash II. Finally, the filter column was spun at 13,000g without adding any wash buffer. RNA was eluted in 20 μ L (10 μ L $\times$ 2) elution buffer

and quantified by using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Rockford, Ill).

cDNA synthesis and real-time PCR

RNA samples were reverse-transcribed by using a high-capacity cDNA reverse-transcription kit following the manufacturer's instructions. A 50-ng cDNA template was used for each real-time reaction with ABI 7000. Mastermix, primers, and probes for real-time analysis were purchased from Applied Biosystems. Primer and probes used for this study are inventoried by Applied Biosystems, span the exon junction, and do not detect genomic DNA. The gene expression assay identification numbers for actin, NQO1, and HO-1 are Hs99999903_m1, Hs00168547_m1, and Hs00157965_m1, respectively. The sequences for these primers and probes are proprietary of Applied Biosystems.

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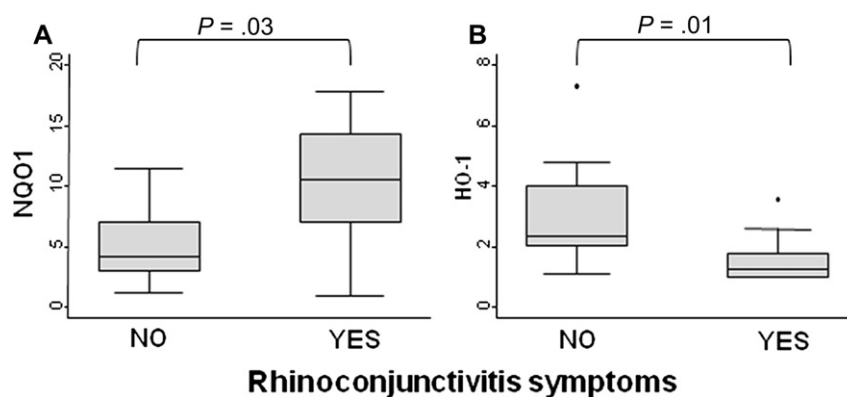


FIG E1. Boxplots of NQO1 (A) and HO-1 (B) mRNA levels by rhinoconjunctivitis symptoms. Yes means the participant had rhinoconjunctivitis, and no means an absence of rhinoconjunctivitis symptoms.

TABLE E1. Spearman correlations for relationships between NQO1 and HO-1 mRNA expression and FENO, lung function, and short-acting β -agonist use

Asthma Characteristics	NQO1		HO-1	
	r_s	<i>P</i> value	r_s	<i>P</i> value
FENO (ppb)	0.24	.32	−0.02	.92
FEV ₁ /FVC	0.08	.74	−0.13	.58
FEV ₁ , % change after short-acting β -agonist	0.32	.14	−0.31	.16
Short-acting β -agonist use, d/2 wk	0.08	.71	−0.30	.17

FENO, Fractional exhaled nitric oxide; FVC, forced vital capacity.

Spearman correlation coefficients and corresponding *P* values are presented.