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Cortisol awakening response and fractional exhaled nitric oxide in asthma

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Running Title: Cortisol awakening response in asthma

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To the Editor:

A small body of research indicates that hypothalamic-pituitary-adrenal (HPA) axis activity and subsequent endogenous cortisol production is involved in allergic disease, including asthma. The most consistent findings observe reduced basal cortisol levels in asthma [1,2,3,4]. Authors have purported that low levels of cortisol, an anti-inflammatory steroid hormone with diurnal fluctuations, may be a risk factor for asthma development and allergic symptom exacerbation [1]. If systemic cortisol production is reduced in asthma, the airways may not benefit from cortisol's anti-inflammatory properties, which could be one of

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many biological processes contributing to prolonged airway inflammation observed in asthma. Indeed, during the morning cortisol peak individuals with asthma have reduced allergic responses compared to allergic responses induced during the afternoon cortisol nadir [5]. Furthermore, prescription corticosteroids as the synthetic form of cortisol are the first line of anti-inflammatory treatment for asthma and allergic disease [6]. As there is currently no cure for asthma, increased understanding of endocrine-immune interactions could provide valuable insight into both treatment targets and the etiology of this chronic inflammatory disease.

Fractional exhaled nitric oxide (FE_{NO}) is increasingly used as an indicator of airway inflammation in allergic asthma and corticosteroid treatment has been found to lower FE_{NO} levels [6]. However, FE_{NO} associations with endogenous glucocorticoid levels are less well studied. Some observations in the context of psychological stress suggest an inverse relationship between FE_{NO} and systemic cortisol. Individuals with higher basal levels of cortisol demonstrate less of an increase in FE_{NO} following laboratory induction of social evaluative stress, [4] and greater cortisol increases during sustained stress (academic exams) are associated with greater FE_{NO} reductions [7]. These findings suggest that endogenous cortisol may provide an anti-inflammatory buffer in the airways during periods of stress. To the best of our knowledge, no study to date has examined the cortisol awakening response (CAR) in patients with asthma compared to controls. CAR measures the typically steep rise in cortisol from awakening to 30-45 minutes post-awakening, which could inform about morning mobilization and a critical time period of protection against allergic exacerbation in the circadian regulation [5]. One presumed function of the CAR is to adjust cortisol availability depending on anticipated demand for the upcoming day, and deviations from the typical response pattern uniquely predict various physical and psychiatric disorders [8]. We therefore compared the CAR of individuals with and without asthma and examined

associations with FE_{NO} . We expected lower cortisol levels and CAR in asthma, and for these to be associated with higher FE_{NO} .

Individuals with and without a physician's diagnosis of asthma were enrolled in a larger study of neurocognitive and endocrine function (for detailed methods, see [9]). Participants provided saliva samples as a non-invasive measure of convenience by cotton swab (*Salivette*, Sarstedt, Germany) at bedtime, awakening, and thirty minutes after awakening. The two morning time points (thirty minutes after awakening – awakening) were used to calculate CAR. Participants returned their samples on a second assessment day, where they provided measurements of FE_{NO} (*Niox Mino* electrochemical analyzer, Aerocrine Systems, Sweden) and lung function by spirometry (% of predicted forced expiratory volume in the first second, FEV_1 ; *AM2*, Jaeger/Toennies, Höchberg, Germany). Participants with asthma completed the Asthma Control Questionnaire (ACQ) and Asthma Control Test (ACT). Current or recent smokers (past year) or taking oral or individuals taking injected corticosteroid medication were excluded, as systemic application likely influences HPA axis by negative feedback, dampening endogenous cortisol levels and influencing diurnal pattern [8]. As inhaled corticosteroids (ICS) can have a similar influence, current prescription of ICS was recorded for use as covariate in analyses for participants with asthma. No individuals were under the acute influence of changes in sleep pattern (e.g. self-report of jet lag from any recent travel, shift work requiring overnight working hours, final exams), which can additionally impact the diurnal cortisol pattern. The study was approved by The University of Texas Southwestern Medical Center (STU 082011-038) and Southern Methodist University (2015-007-RITT) Institutional Review Boards and conforms to the standards of the Declaration of Helsinki.

Samples were frozen and *Salivettes* were subsequently spun at 1600xg for 3 minutes to obtain saliva. Standards and quality controls (QCs) were spiked with phosphate-buffered

saline. 100 microliters of samples, standards, and QCs were precipitated with 200ul of crash containing: methanol, formic acid (0.15%, final 0.1%) and D4 Cortisol (*Sigma-Aldrich*, final 10ng). Tubes were incubated at room temperature for 10 minutes and centrifuged at 13,200 rpm for 5 minutes at 4 °C. Aliquots of ~250 microliters supernatant were analyzed by liquid chromatography-mass spectrometry. Values from three analytical runs were averaged, with an average CV% 5.98. Eight samples (7.6% of total) with values $\leq .025$ were identified as below the detection limit and recorded as .025ng/ml.

Due to data skewness, generalized linear models with a robust estimator, gamma distribution, and log link function were used to test differences in CAR between groups (asthma vs. control) and between participants using ICS versus no ICS in the asthma group. Non-parametric Spearman partial-correlations were used to examine relations among FeNO and cortisol variables, controlling for medication use (ICS versus no ICS). Ancillary analyses examined relations among CAR and asthma control, again controlling for medication use. T tests were used to compare groups across all variables, and FeNO was log transformed for all analyses due to skewness. Cohen's d was used as a measure of effect size. IBM SPSS Statistics (Version 24) was used for analyses.

Out of 40 participants enrolled, 35 participants (17 asthma; 18 controls) completed the samples as instructed. There were no significant group differences on demographics, body mass index, lung function (% of predicted FEV₁), or self-report stress, depression, or anxiety (Table 1). Individuals with asthma ranged from intermittent to moderate persistent presentation of disease (Table 1), reported few sleep disturbances due to evening asthma exacerbation, and had, on average, higher levels of lnFE_{NO}, ($t(31) = 2.40$, $p = .028$, $d = .86$) and lower CAR ($\chi^2 = 6.83$, $p = .009$, $d = .51$) compared to non-asthmatic controls. Among asthma participants, there was no significant difference in CAR between those prescribed and not prescribed ICS ($\chi^2 = .20$, $p = .655$). For all participants, those with lower evening salivary

cortisol levels had higher $\ln\text{FE}_{\text{NO}}$ the following day, controlling for ICS ($r(33) = -.49$, $p = .004$). In asthma participants, this remained significant ($r(14) = -.58$, $p = .023$) but was not significant in controls ($r(17) = -.26$, $p = .312$). CAR was neither associated with $\ln\text{FE}_{\text{NO}}$ in asthma participants, controlling for ICS ($r(13) = -.01$, $p = .984$), nor in controls ($r(17) = .08$, $p = .764$).

Ancillary analyses revealed that, controlling for ICS, CAR was correlated at trend level with two measures of asthma control, scored in opposite directions, ACQ ($r(14) = -.455$, $p = .094$), and ACT ($r(14) = .463$, $p = .071$). These findings indicate that participants with asthma who had a larger CAR tended to have better asthma control.

Our findings suggest that young adults with asthma have a blunted CAR, consistent with a small literature observing lower total cortisol levels throughout the day in both children and adults with asthma [2, 3, 10]. These findings support the idea of an overall dampening of HPA axis activity in asthma. Whereas we could not establish a link with CAR and FE_{NO} , findings were suggestive of a role of CAR for asthma control.

Evening salivary cortisol levels were associated with FE_{NO} , where those with lower cortisol levels had stronger airway inflammation the following day. It is possible that these findings emanate from a less robust influence of the anti-inflammatory properties of cortisol. This interpretation is partly consistent with previous findings observing lower cortisol levels associated with larger FE_{NO} increase in response to stress [4,7]. Stress and strong emotions can trigger both asthma exacerbations and acute elevations in FE_{NO} [7]. To the extent that asthma exacerbations are accompanied by elevated airway inflammation, the present findings build upon earlier suggestions that blunted neuroendocrine activity may be a risk factor for allergic or asthma exacerbation [1] and highlight airway inflammation as a potential mechanism.

Limitations include smaller sample size, salivary cortisol sampling which, compared to plasma, provides only a window into systemic cortisol production, and a sampling schedule that may not have identified the exact CAR peak [8]. Capturing three morning time points, with an additional time point at 45 minutes post awakening, would have better assessed CAR. Furthermore, differences in circadian phase relationships between endogenous cortisol secretion and sleep timing might contribute to the observed group differences in CAR. Although patients in this sample did not report a high incidence of sleep interruptions due to asthma symptoms, nor were they diagnosed with comorbid disorders (e.g. obstructive sleep apnea, rhinosinusitis), which could contribute to sleep interruptions in asthma [11], the influence of sleep disturbance cannot be ruled out with our study design. Multiple biological processes can additionally contribute to FE_{NO} , and FE_{NO} levels cannot be interpreted purely as an indicator of airway inflammation. Nonetheless, to the best of our knowledge this is the first study to observe a blunted CAR in participants with asthma. Future research should examine possible group differences in timing of the CAR peak, measure and control for habitual sleep wake times and ambient light, which can all influence circadian rhythm. Equally important will be studies that replicate these findings in larger samples, and examine associations among CAR, asthma control, and airway inflammation through prospective study design. Incorporating criteria of circadian precision medicine when exploring interactions between multiple biological systems, by aligning all participants to the same ‘internal time’ and optimize the timing of asthma therapies may provide additional clarity to future results [12]. The present analyses controlled for ICS use as a binary variable; however, continued assessment of corticosteroid treatment, including dosages, will be essential for future study [10].

The present findings indicate that in young adults with asthma, there may be an overall dampening of HPA axis activity with lower basal samples linked to higher subsequent airway inflammation – even while controlling for ICS use. The findings also suggest a potential beneficial influence of higher CAR on dampening asthma control, a major clinical end-point in the management of this chronic disease.

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Table 1. Demographics, physical characteristics, and self-report

	Asthma n=17			Healthy Controls n=18			p
	Mean	SD	Min-Max	Mean	SD	Min - Max	
Age (years)	26.0	9.4	18 – 44	24.89	9.05	18 – 45	.724
Gender (male)	41%			50%			.613
Education (years)	14.9	2.5	12 – 23	14.67	2.05	12 – 19	.704
BMI (kg/m ²)	24.1	4.0	17.7 – 32.3	26.55	5.53	18.5 – 37.7	.141
FEV ₁ (% predicted)	95.4	8.78	77.0 – 179.0	100.3	13.3	80.0 – 130.0	.366
FE _{NO} (ppb)	52.06	55.00	7 – 197	20.41	13.83	8 – 51	.028*
ACT (5-25)	20.29	3.73	12 – 25	N/A			.434
ACT-PM awakening (1-5)	4.18	1.19	2 – 5				
ACQ (0-6)	0.97	0.60	0.14 – 2.14	N/A			
ACQ-PM awakening (0-6)	0.59	1.06	0 – 3				
ICS (yes/no) (%)	35.3			N/A			
Asthma severity ^a (%)							
Intermittent	29.4			N/A			
Mild (Persistent)	47.1			N/A			
Moderate (Persistent)	23.5			N/A			
Severe (Persistent)	0.0			N/A			
Race (%)							
White/Caucasian	70			50			.226
Ethnicity (%)							
Hispanic	7			14			.493

Abbreviations: BMI, body mass index; FEV₁, forced expiratory volume in 1 second; ACT, asthma control test, lower scores indicate poorer control with scores ≤19 indicating poorly controlled asthma; ACT-PM awakening, single item from ACT of how frequently asthma symptoms woke participant up at night, 1= all of the time, 5=none of the time; ACQ, asthma control questionnaire, higher scores indicate poorer control; ACQ-PM awakening, single item from ACQ of how frequently participant was woken by their asthma during the night, 0=never, 6=unable to sleep because of asthma; ICS, inhaled corticosteroid currently prescribed. Note, independent samples (two-tailed) t-test found no statistically significant difference between groups on any variables at the p<.05 level, other than FE_{NO}. Possible ranges of questionnaire scores are provided in parentheses after each measure.

^aAsthma severity was calculated for participants on short-term medication and for those on maintenance medication whose asthma was well controlled according to NIH/NAEPP (2007) severity and medication step therapy guidelines. Two individuals who were on maintenance medication and did not have well-controlled asthma according to the ACT, were therefore excluded from severity classification.

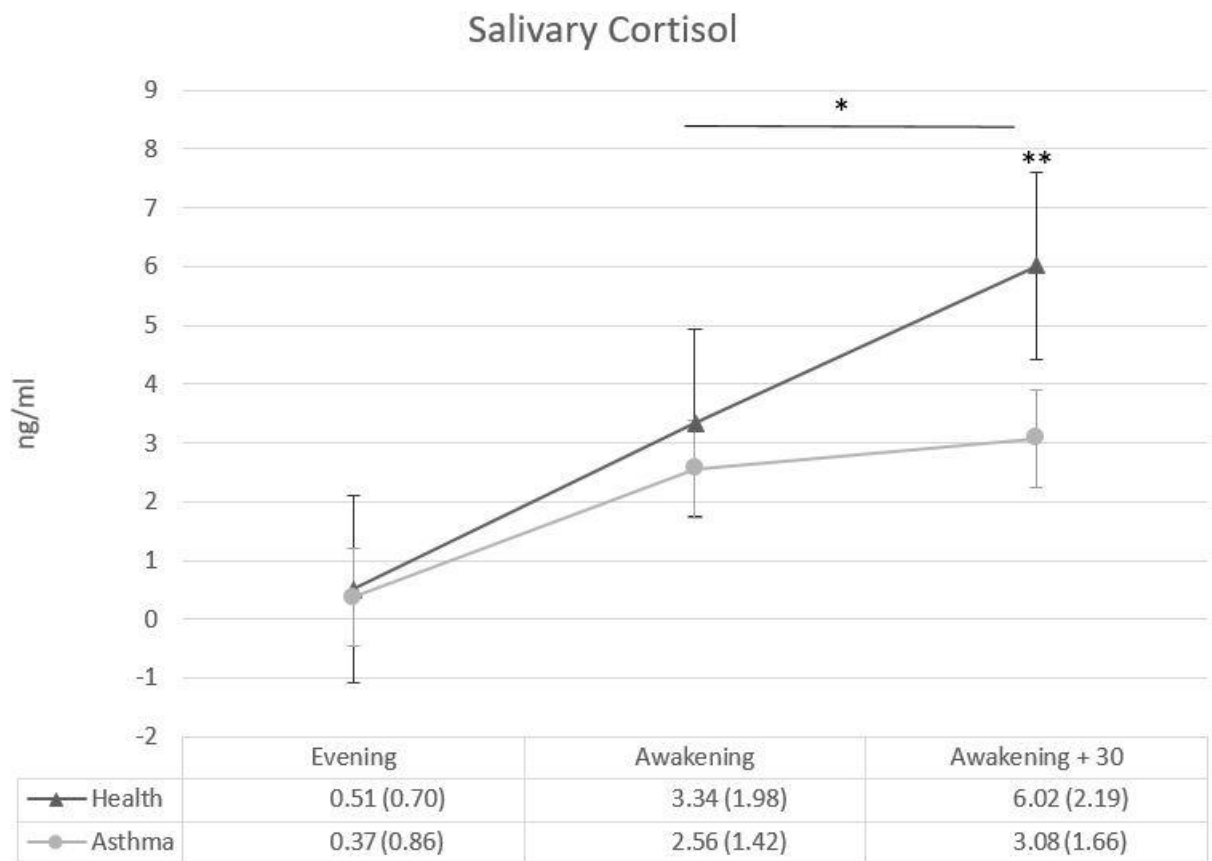


Figure 1. Salivary cortisol (ng/ml) evening and awakening response in asthma compared to healthy controls. Mean values displayed, standard deviation in parentheses, error bars indicate standard error. *indicates significant difference between awakening and awakening +30 minutes at $p < .05$, ** indicates significant difference between asthma and healthy participants at $p < .001$.