

## **SUPPLEMENTARY INFORMATION**

### **Older Age and Obesity are Associated with Increased Airway Closure in Response to Methacholine in Patients with Asthma**

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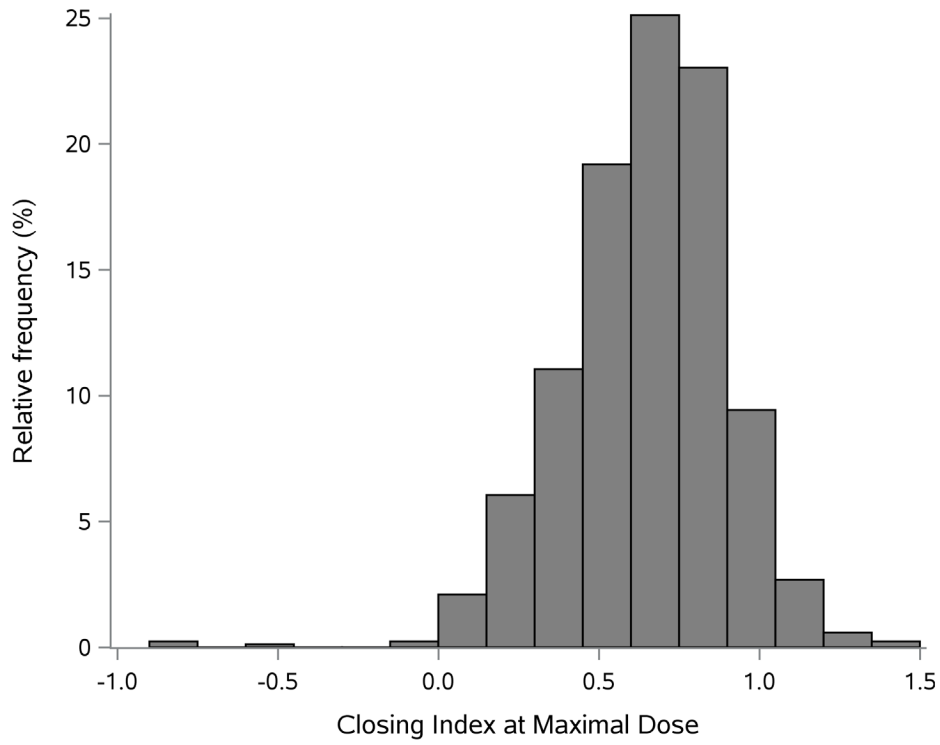
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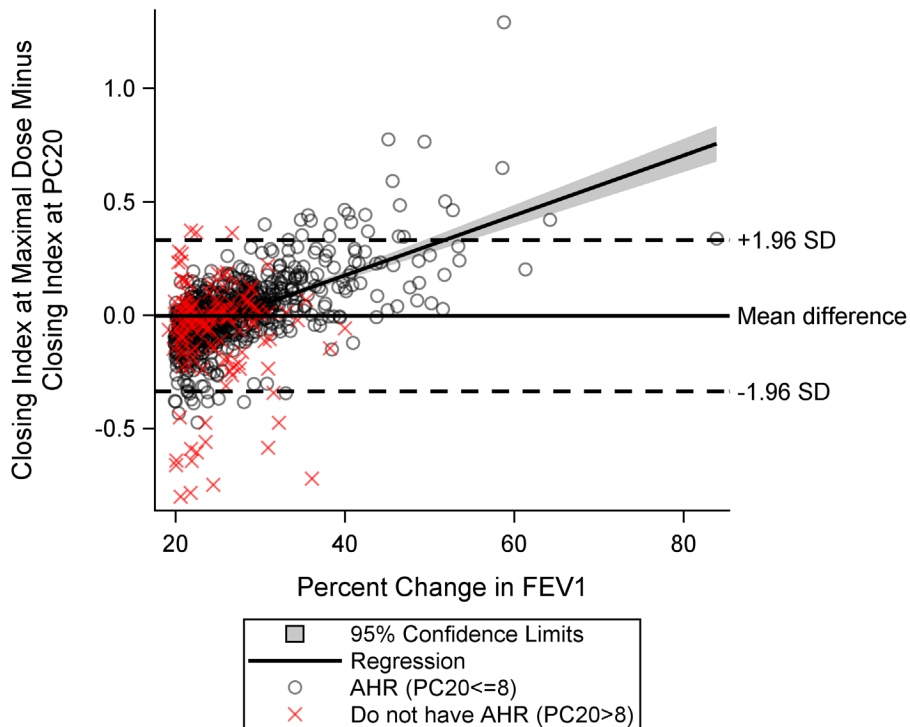
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**Figure S1** – Distribution of Closing Index Among Participants with Airways

Hyperresponsiveness ( $PC_{20} \leq 8$  mg/ml).



**Figure S2** - Difference between Closing Index Calculated Using the Maximal Dose vs. the PC20 Dose Relative to the Percent Change in FEV1 at the Maximal Dose of Methacholine.



The diagonal, solid line represents the regression line and the shaded regions represent the 95 % confidence interval for the regression. The coefficient estimate for the regression = 0.0129 (0.0115, 0.0143). The horizontal, dashed lines represent the upper and lower bounds of the 95% confidence interval (+/- 1.96 SD) for the raw data compared to a mean difference of zero. Circles denote participants with airways hyperresponsiveness (AHR), defined as PC20 < 8mg/ml. X's denote participants without AHR.

**Table S1-** Number of Participants in Each Phase of Each Study

Study (Years of Study)	SARA 2004-07	SARCA 2007-11	STAN 2010-13	MECIS 2009-10	CPAP 2012-14
Total Participants	402	306	388	126	194
Tested for PC20*	183	233	314	120	194
Achieved PC20	175	214	259	102	194
PC20 $\leq$ 8 mg/ml	135	175	223	90	181

\*Having a positive PC20 was not a requirement of all studies, as some inclusion criteria required either a positive PC20 or a positive bronchodilator response.

SARA (Study of Acid Reflux and Asthma) (S1)

n = 402 adults, poorly controlled asthma, randomized to protein pump inhibitor (PPI) vs. placebo. ClinicalTrials.gov Identifier: NCT00069823

SARCA (Study of Acid Reflux in Childhood Asthma) (S2)

n = 306 children, poorly controlled asthma, randomized to PPI vs. placebo. ClinicalTrials.gov Identifier: NCT00442013

STAN (Study of Asthma and Nasal Steroids) (S3)

n = 388 children and adults, poorly controlled asthma, randomized to nasal inhaled corticosteroids vs. placebo. ClinicalTrials.gov Identifier: NCT01118312

MeCIS (Methacholine Bronchoprovocation – Influence of High-Potency Inhaled Corticosteroids in Asthma) (S4, S5)

n = 126 adults, physician-diagnosis of stable asthma vs. healthy controls  
n = 26 asthmatics with PC20  $\leq$  8 mg/ml, on same ICS dose with 2 or 3 repeat PC20 at 4 week intervals. ClinicalTrials.gov Identifier: NCT00705341

CPAP (Effect of Positive Airway Pressure on Reducing Airway Reactivity in Patients with Asthma) (S6)

n = 194 adults, stable asthma, inclusion by PC20  $\leq$  8 mg/ml, randomized to continuous positive airway pressure (CPAP) vs. placebo (sham). ClinicalTrials.gov Identifier: NCT01629823

**Table S2 - Demographics and Baseline Pulmonary Function of Study Participants with Airways Hyperresponsiveness (PC20 ≤ 8)**

Characteristic	Total (N= 860)	CPAP† (N= 194)	MeCIS† (N= 96)	SARA† (N= 147)	SARCA† (N= 186)	STAN† (N= 237)	P-Value*
Age, Median(Q1,Q3)	25 (14, 40)	31 (20, 41)	41 (27, 51)	36 (26, 46)	12 (9, 14)	22 (12, 37)	<.001
Male, N(%)	377 (44%)	81 (42%)	35 (36%)	40 (27%)	116 (62%)	105 (44%)	<.001
Race, N(%)							
White	387 (45%)	85 (44%)	54 (56%)	79 (54%)	66 (35%)	103 (43%)	0.002
Black	321 (37%)	62 (32%)	32 (33%)	54 (37%)	86 (46%)	87 (37%)	
Hispanic	115 (13%)	36 (19%)	8 (8%)	11 (7%)	23 (12%)	37 (16%)	
Other	37 (4%)	11 (6%)	2 (2%)	3 (2%)	11 (6%)	10 (4%)	
Age Asthma Onset, Median (Q1,Q3)	5 (2, 13)	8 (3, 15)	12 (4, 31)	8 (3, 23)	2 (1, 5)	5 (1, 12)	<.001
BMI for adults ≥20, Median (Q1,Q3)	28 (25, 33)	26 (23, 30)	28 (25, 34)	30 (25, 35)		30 (25, 36)	<.001
BMI percentage for children <20, Median (Q1,Q3)	84 (52, 96)	68 (42, 90)	61 (41, 93)	97 (86, 99)	85 (58, 96)	87 (57, 97)	0.014
Obese**, N(%)	307 (36%)	41 (21%)	38 (40%)	69 (47%)	59 (32%)	99 (42%)	<.001
On ICS/LABA, N(%)	400 (47%)	54 (28%)	53 (55%)	111 (76%)	108 (58%)	74 (31%)	<.001
Questionnaires, Median (Q1,Q3)							
Asthma Control Questionnaire	1.6 (1.0, 2.1)		1.0 (0.6, 1.6)	1.9 (1.6, 2.4)			<.001
ACT Score	19 (16, 22)	22 (20, 23)			18 (16, 21)	16 (13, 18)	<.001
ACT ≤19	284 (61%)	46 (24%)			57 (64%)	181 (100%)	<.001
Child ACT score	18 (15, 20)				19 (16, 22)	17 (13, 18)	<.001
CACT ≤19	105 (70%)				49 (53%)	56 (100%)	<.001
ASUI	0.83 (0.69, 0.92)	0.92 (0.84, 0.98)		0.77 (0.63, 0.86)	0.83 (0.72, 0.89)	0.75 (0.60, 0.87)	<.001
Spirometry, Median(Q1,Q3)							
% Pred FEV <sub>1</sub> , Pre-BD	90 (82, 99)	91 (83, 98)	85 (78, 93)	87 (80, 96)	94 (86, 103)	89 (81, 102)	<.001
% Pred FVC, Pre-BD	99 (90, 107)	101 (91, 108)	96 (87, 105)	94 (87, 103)	101 (92, 111)	100 (91, 109)	<.001

Characteristic	Total (N= 860)	CPAP† (N= 194)	MeCIS† (N= 96)	SARA† (N= 147)	SARCA† (N= 186)	STAN† (N= 237)	P-Value*
FEV FVC ratio, Pre-BD	0.77 (0.72, 0.82)	0.76 (0.71, 0.81)	0.74 (0.69, 0.80)	0.77 (0.72, 0.81)	0.81 (0.76, 0.86)	0.77 (0.72, 0.81)	<.001
% Change FEV1 from MC, last challenge	25 (22, 29)	24 (22, 28)	23 (21, 27)	28 (23, 33)	25 (23, 29)	24 (22, 29)	<.001
% Change FVC from MC, last challenge	17 (12, 22)	17 (12, 22)	17 (11, 21)	22 (16, 26)	16 (12, 22)	15 (10, 20)	<.001
% Change FEV from MC, at PC20	20 (20, 20)	20 (20, 20)	20 (20, 20)	20 (20, 20)	20 (20, 20)	20 (20, 20)	0.110
% Change FVC from MC, at PC20	13 (10, 16)	13 (10, 16)	14 (10, 17)	15 (11, 17)	13 (9, 15)	13 (9, 16)	0.003
Other respiratory measures, Median(Q1,Q3)							
PC20	0.75 (0.25, 2.27)	0.75 (0.24, 1.90)	1.02 (0.33, 3.32)	0.76 (0.29, 2.30)	0.82 (0.28, 2.61)	0.59 (0.18, 2.03)	0.026
Closing Index at last challenge	0.67 (0.49, 0.81)	0.68 (0.51, 0.82)	0.69 (0.49, 0.83)	0.74 (0.62, 0.88)	0.62 (0.48, 0.79)	0.62 (0.43, 0.78)	<.001
Closing Index at PC20	0.66 (0.49, 0.82)	0.66 (0.48, 0.80)	0.68 (0.52, 0.83)	0.74 (0.56, 0.87)	0.63 (0.46, 0.77)	0.63 (0.46, 0.80)	0.003

P-values are based upon Chi-squared and Kruskal-Wallis tests for categorical and continuous characteristics, respectively

† acronyms for clinical studies included: CPAP = Effect of Positive Airway Pressure on Reducing Airway Reactivity in Patients with Asthma; MeCIS = Methacholine Bronchoprovocation – Influence of High-Potency Inhaled Corticosteroids in Asthma; SARA = Study of Acid Reflux and Asthma; SARCA = Study of Acid Reflux in Childhood Asthma; STAN = Study of Asthma and Nasal Steroids

\*\*obesity defined as BMI > 30 kg/m<sup>2</sup> in adults (≥ 20 years old), and BMI > 95<sup>th</sup> percentile in children (< 20 years old)

#### Abbreviations:

ACT = Asthma Control Test (Low scores indicate better health), ASUI = Asthma Symptom Utility Index (High scores indicate better health), FEV1 = forced expiratory volume in one second, FVC = forced vital capacity, MC = methacholine challenge, PC20 = provocative concentration causing a 20% fall in FEV1

#### Missing Data:

-ACT: was not done in MeCIS, or SARA, or children in SARCA or STAN (Total N=464)

-cACT was only done in children in SARCA & STAN (Total N=149)

-ASUI: not done in MeCIS, missing one subject from SARCA (Total N=763)

\*P-values are based upon Chi-squared and Kruskal-Wallis tests for categorical and continuous characteristics, respectively

↑ High scores indicate better health

↓ Low scores indicate better health

Table S3 – Results of Univariate Regression  
Modeling of Closing Index for Participants with  
Airways Hyperresponsiveness

<i>Characteristic</i>	<i>Regression Coefficient (95% CI)</i>	<i>P-Value</i>
Age (10 year intervals)	0.032 (0.022, 0.043)	<.001
Male (vs. Female)	-0.048 (-0.082, -0.015)	0.005
Black (vs. White)	-0.017 (-0.052, 0.018)	0.334
Hispanic (vs. White)	-0.027 (-0.077, 0.023)	0.288
Other race (vs. White)	0.059 (-0.024, 0.143)	0.163
Age of Asthma Onset	0.003 (0.002, 0.005)	<.001
BMI for adults 20 years or older	0.005 (0.002, 0.008)	<.001
BMI percentile for children <20 years	0.001 (0, 0.002)	0.006
Obese (vs. not)	0.076 (0.041, 0.111)	<.001
On ICS/LABA (vs. not)	0.035 (0.001, 0.069)	0.042
Asthma Control Questionnaire	0.015 (-0.024, 0.053)	0.459
Asthma Control Test (ACT) score	0.002 (-0.004, 0.008)	0.502
ACT score ≤ 19 (vs. 20 or more)	-0.017 (-0.064, 0.03)	0.483
Child ACT score	0.006 (-0.003, 0.014)	0.195
Child ACT score ≤ 19 (vs. 20 or more)	-0.075 (-0.16, 0.01)	0.084
Adult or Child ACT score ≤ 19 (vs. 20 or more)	-0.03 (-0.071, 0.012)	0.160
Asthma Symptom Utility Index (ASUI)	0.042 (-0.062, 0.145)	0.429
% Pred FEV <sub>1</sub> , Pre-BD	-0.001 (-0.003, 0)	0.016
% Pred FVC, Pre-BD	-0.003 (-0.004, -0.002)	<.001
FEV <sub>1</sub> /FVC ratio, Pre-BD	0.092 (-0.126, 0.31)	0.407
PC20	0.006 (0.001, 0.011)	0.014
Study, Ref.=STAN		
CPAP	0.012 (-0.028, 0.053)	0.551
MeCIS	0.001 (-0.053, 0.055)	0.974
SARA	0.105 (0.061, 0.15)	<.001
SARCA	-0.02 (-0.061, 0.022)	0.350

Abbreviations as for Table S2

Table S4 – Results of Multivariable Regression Modeling of Closing Index for Participants with Airways Hyperresponsiveness

<i>Characteristic</i>	<i>Estimate (95% CI)</i>	<i>P-Value</i>
Age (10 year units)	0.032 (0.018, 0.045)	<.001
Male (vs. female)	-0.019 (-0.053, 0.015)	0.274
Black (vs. white)	-0.016 (-0.053, 0.02)	0.379
Hispanic (vs. white)	-0.002 (-0.053, 0.05)	0.954
Other race (vs. white)	0.068 (-0.014, 0.15)	0.105
Obese (vs. not)	0.062 (0.027, 0.097)	<.001
Study, Ref.=STAN		
CPAP	0.053 (0.006, 0.101)	0.027
McCIS	0.011 (-0.048, 0.071)	0.709
SARA	0.1 (0.048, 0.152)	<.001
SARCA	0.091 (0.041, 0.142)	<.001

Table S5 – Covariates Removed from Multivariable Regression Modeling of Closing Index for All Participants

<i>Characteristic</i>	<i>Estimate (95% CI)</i>	<i>P-Value</i>
Age onset	0.001 (-0.001, 0.002)	0.347
On ICS/LABA (vs. not)	0.019 (-0.014, 0.053)	0.260

Table S6 – Covariates Removed from Multivariable Regression Modeling of Closing Index for Participants with Airways Hyperresponsiveness (Table S4)

<i>Characteristic</i>	<i>Estimate (95% CI)</i>	<i>P-Value</i>
Age onset	0.001 (-0.001, 0.003)	0.271
On ICS/LABA (vs. not)	0.012 (-0.024, 0.047)	0.509



Table S7 – Results of Multivariable Regression Modeling of Closing Index for All Child Participants, N=172

<i>Characteristic</i>	<i>Estimate (95% CI)</i>	<i>P-Value</i>
Male (vs. Female)	0.005 (-0.07, 0.08)	0.900
Black (vs. White)	0.03 (-0.05, 0.11)	0.449
Hispanic (vs. White)	0.04 (-0.07, 0.15)	0.480
Other race (vs. White)	0.1 (-0.04, 0.24)	0.169
Obese (vs. not)	0.07 (0, 0.15)	0.062
Child ACT score <= 19 (vs. 20 or more)	-0.08 (-0.16, -0.01)	0.034

Table S8 – Results of Multivariable Regression Modeling of Closing Index for Child Participants with Airways Hyperresponsiveness, N=149

<i>Characteristic</i>	<i>Estimate (95% CI)</i>	<i>P-Value</i>
Male (vs. Female)	-0.004 (-0.08, 0.08)	0.925
Black (vs. White)	0.03 (-0.06, 0.12)	0.517
Hispanic (vs. White)	0.04 (-0.07, 0.16)	0.492
Other race (vs. White)	0.15 (-0.01, 0.32)	0.074
Obese (vs. not)	0.07 (-0.01, 0.15)	0.094
Child ACT score <= 19 (vs. 20 or more)	-0.08 (-0.17, 0)	0.059

Table S9 – Results of Multivariable Negative Binomial Regression Modeling of EPACS for Participants with Airways Hyperresponsiveness who had Diary Card Data, N=791

<i>Characteristic</i>	<i>Risk Ratio (95% CI)</i>	<i>P-Value</i>
Closing Index	1.01 (0.64, 1.58)	0.977
Age (10 year units)	0.93 (0.85, 1.02)	0.118
Male (vs. Female)	0.73 (0.58, 0.92)	0.007
Black (vs. White)	1.44 (1.14, 1.82)	0.002
Hispanic (vs. White)	0.84 (0.59, 1.2)	0.338
Other race (vs. White)	1.53 (0.9, 2.6)	0.118
Obese (vs. not)	0.96 (0.76, 1.21)	0.723
Study, Ref.=STAN		
CPAP	0.65 (0.46, 0.92)	0.015
McCIS	0.53 (0.3, 0.96)	0.035

<i>Characteristic</i>	<i>Risk Ratio (95% CI)</i>	<i>P-Value</i>
SARA	1.18 (0.86, 1.61)	0.308
SARCA	1.29 (0.95, 1.76)	0.108

### **Supplementary References**

- S1. Mastronade J, Anthonisen N, Castro M, Holbrook J, Leone F, Teague W, Wise R. Efficacy of esomeprazole for treatment of poorly controlled asthma. *N Engl J Med* 2009; 360: 1487-1499.
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