Cysteine oxidation impairs systemic glucocorticoid responsiveness in children with difficult-to-treat asthma

ONLINE REPOSITORY

Susan T. Stephenson, Ph.D.[1]

Lou Ann S. Brown, Ph.D.[1,2]

My N. Helms, Ph.D.[1,2]

Hongyan Qu, M.S.[1]

Sheena D. Brown, Ph.D.[1]

Milton R. Brown, Ph.D.[1,2]

Anne M. Fitzpatrick, Ph.D.[1,2]

[1] Emory University Department of Pediatrics, Atlanta, GA

[2] Children's Healthcare of Atlanta Center for Cystic Fibrosis and Airways Disease Research

This study was funded by RO1 NR012021

and was supported in part by the National Center for Advancing Translational Sciences of the National Institutes of Health, award number UL1 TR000454.

Table E1. Associations between tertiles of plasma cysteine/cystine redox potentials[1] and clinical features in participants with difficult-to-treat asthma.

Asthma feature	Adjusted OR[2] (95% CI)	p-value
Middle tertile (-61.30 to -30.40 mV)		
High-dose inhaled glucocorticoids (previous 6 months)	1.43 (0.46, 4.42)	0.535
Daily short-acting beta-agonists (previous 3 months)	2.26 (0.69, 7.38)	0.177
Asthma Control Questionnaire Score >1.5[3]	1.81 (0.46, 7.05)	0.394
≥ 3 oral corticosteroid bursts (previous year)	1.46 (0.49, 4.38)	0.498
Emergency department visit (previous year)	1.72 (0.55, 5.43)	0.353
Hospitalization for asthma (ever)	1.27 (0.27, 5.96)	0.761
Intubation for asthma (ever)	2.11 (0.53, 8.32)	0.288
Exhaled nitric oxide >35 ppb	0.88 (0.28, 2.749)	0.822
FEV_1/FVC <80% of predicted value	0.79 (0.17, 3.74)	0.768
Highest tertile (-30.41 to 54 mV)		
High-dose inhaled glucocorticoids (previous 6 months)	3.52 (1.05, 11.79)	0.041
Daily short-acting beta-agonists (previous 3 months)	3.87 (1.19, 12.57)	0.024
Asthma Control Questionnaire Score >1.5[3]	4.13 (1.10, 15.46)	0.035
≥ 3 oral corticosteroid bursts (previous year)	1.04 (0.34, 3.15)	0.951
Emergency department visit (previous year)	9.29 (2.31, 37.35)	0.002
Hospitalization for asthma (ever)	2.17 (0.45, 10.37)	0.333
Intubation for asthma (ever)	4.55 (1.25, 16.60)	0.022
Exhaled nitric oxide >35 ppb	1.60 (0.53, 4.80)	0.405
FEV_1/FVC <80% of predicted value	1.98 (0.51, 7.69)	0.324

[1] The lowest tertile of the plasma cysteine/cystine redox potential (-99 to -61.29 mV) served as the reference group. A more positive redox potential reflects greater oxidation of the sample.

[2] Analyses were adjusted for race, sex, and BMI percentile.

[3] Scores on the Asthma Control Questionnaire range from 0 to 6, with lower scores indicating better asthma control.

Table E2. Features of the asthmatic participants at the baseline visit. Data represent the median (IQR) or the number of participants (%).

Feature	Did not receive triamcinolone N = 42	Received triamcinolone[1] N = 57	p-value
Age (years)	12 (10, 15)	12 (10, 15)	0.773
Sex			
Male	22 (52)	44 (77)	0.010
Female	20 (48)	13 (23)	
Race			
White	14 (33)	2 (4)	< 0.001
Black	26 (62)	43 (75)	
Asian	1 (2)	0	
More than one race	1 (2)	12 (21)	
Body mass index			
< 85th percentile (normal weight)	17 (41)	26 (46)	0.660
85th to 95th percentile (overweight)	13 (21)	13 (23)	
≥ 95th percentile (obese)	12 (29)	18 (32)	
Asthma Medications			
Inhaled corticosteroids	28 (67)	57 (100)	< 0.001
Montelukast	14 (25)	43 (75)	0.009
Self-reported allergic rhinitis	36 (86)	55 (97)	0.052
Asthma Control Questionnaire Score[2]	0.36 (0.14, 0.61)	1.29 (0.57, 2.14)	< 0.001
Pediatric Asthma Quality of Life Questionnaire total score[3]	6.36 (5.71, 6.85)	5.30 (4.28, 6.00)	< 0.001
Serum IgE (kU/L)[4]	44 (20, 254)	365 (126, 646)	< 0.001
Exhaled nitric oxide (ppb)[4]	23 (12, 43)	28 (19, 56)	0.121
Baseline lung function			
FVC (% predicted)	107 (103, 117)	100 (92, 114)	0.182
FEV_1 (% predicted)	97 (88, 107)	90 (75, 101)	0.044
FEV_1/FVC	0.81 (0.77, 0.84)	0.77 (0.69, 0.82)	0.060
FEV_1/FVC (% predicted)	92 (88, 96)	90 (80, 94)	0.124
FEF_{25-75} (% predicted)	79 (67, 91)	67 (49, 77)	0.118

Post-bronchodilator lung function			
FVC (% predicted)	110 (101, 118)	109 (99, 122)	0.930
FEV_1 (% predicted)	108 (93, 114)	101 (93, 115)	0.770
FEV_1/FVC	0.87 (0.82, 0.88)	0.83 (0.77, 0.87)	0.339
FEV_1/FVC (% predicted)	98 (93, 102)	96 (90, 100)	0.726
FEF_{25-75} (% predicted)	103 (87, 112)	90 (73, 107)	0.421

[1] Triamcinolone was administered at the completion of the baseline characterization visit to participants with symptomatic asthma despite moderate-to-high dose inhaled corticosteroid therapy.

[2] Scores on the Asthma Control Questionnaire range from 0 to 6, with lower scores indicating better asthma control.

[3] Scores on the Pediatric Asthma Quality of Life Questionnaire range from 0 to 7, with higher scores indicating better quality of life.

[4] Data were logarithmically transformed prior to statistical analyses.

FIGURE LEGENDS

Figure E1. Flowchart of participant enrollment and inclusion in the study.

Figure E2. Plasma (A) cysteine and (B) cystine concentrations and (C) the cysteine/cystine redox potential (E_h) in healthy control children, children with non-severe asthma and children with severe asthma. Cysteine and cystine data are presented as the mean ± SEM and the cysteine/cystine E_h boxplots are shown with minimum and maximum values. Dots represent individual participants. Control: n = 15, non-severe asthma: n = 43, severe asthma: n = 56 for each panel. *$p < 0.05$, **$p < 0.01$

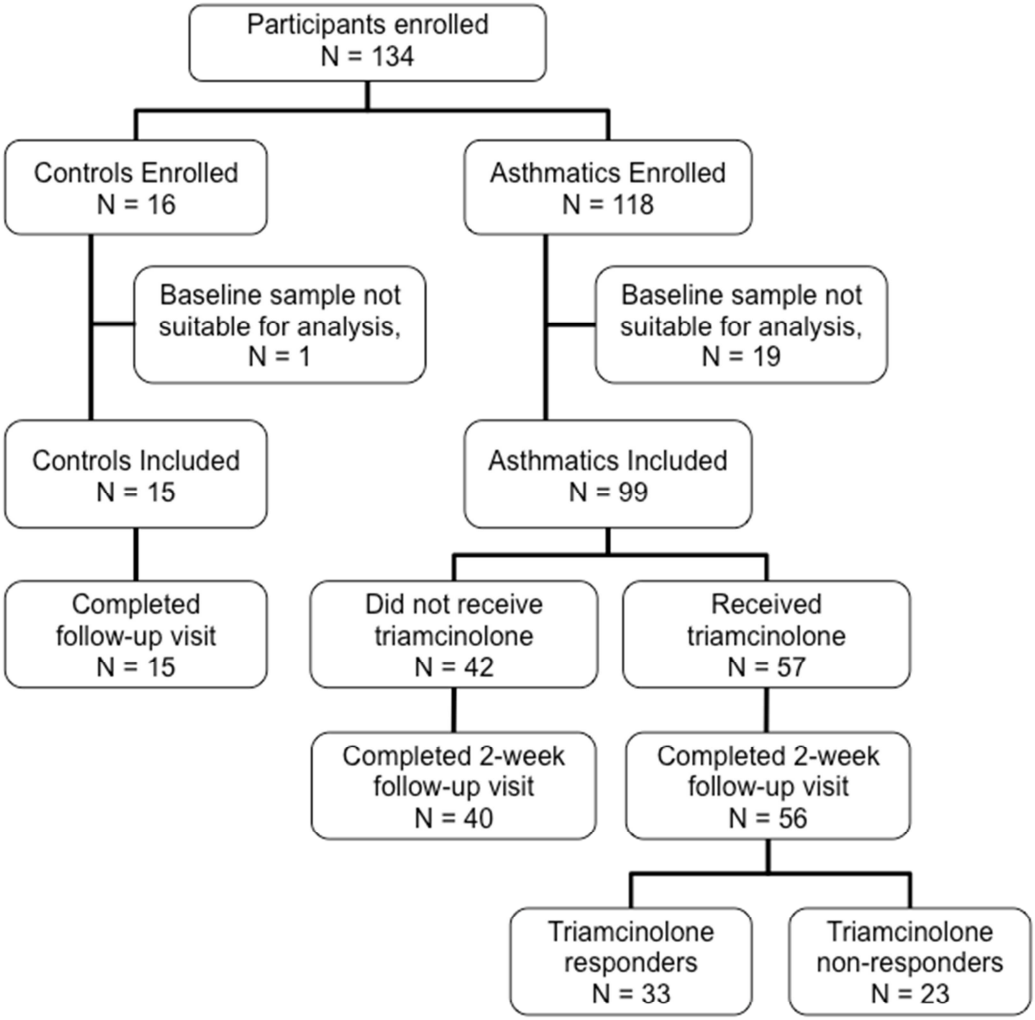
Figure E3. Peripheral blood mononuclear cell (A) glutathione disulfide (GSSG) concentrations, (B) reactive oxygen species (ROS) generation, and (C) *CCL3* and (D) *CXCL1* mRNA gene expression in healthy control children, children with non-severe asthma and children with severe asthma. GSSG and ROS data are presented as the mean ± SEM and *CCL3* and *CXCL1* mRNA data are presented as boxplots with minimum and maximum values. Dots represent individual values. *$p < 0.05$, **$p < 0.01$

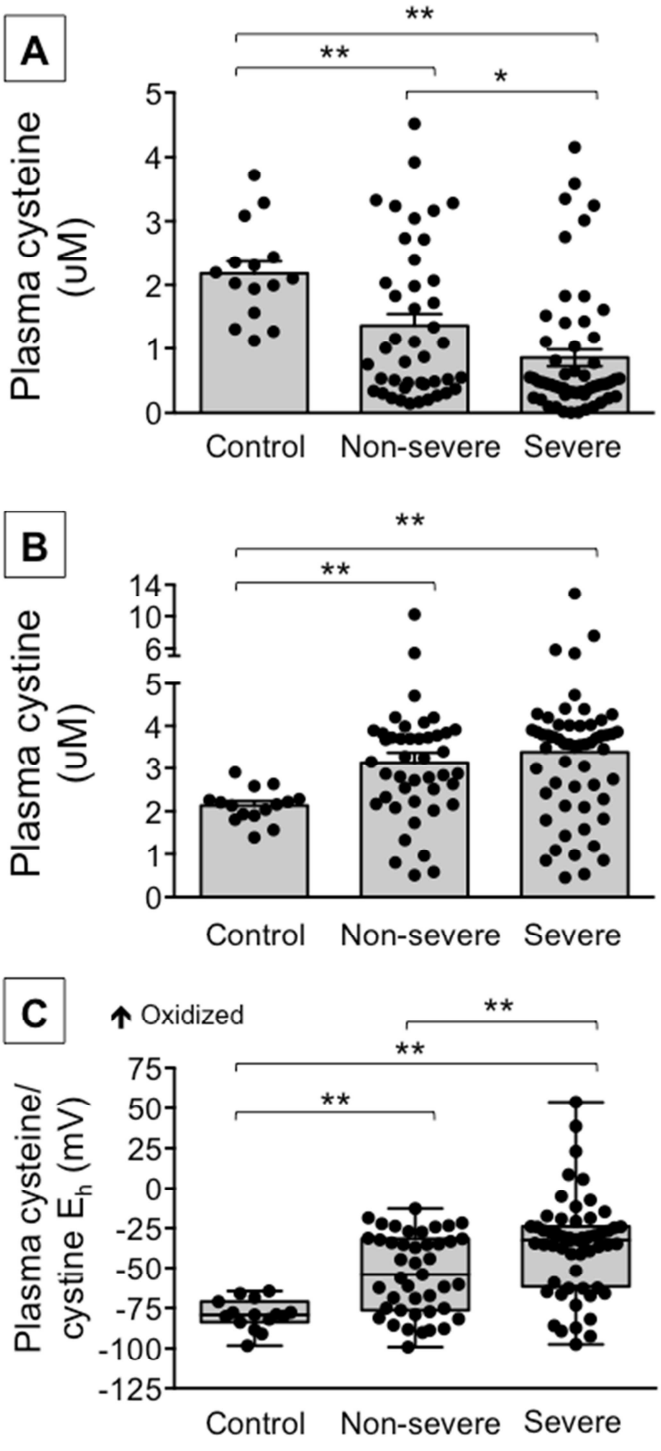
Figure E4. Stability of the plasma cysteine/cystine redox potential (E_h) between the baseline and two-week follow-up visit in (A) healthy control children and (B) children with asthma who did not receive triamcinolone. Differences between the baseline and 2-week follow-up visits were not statistically significant.

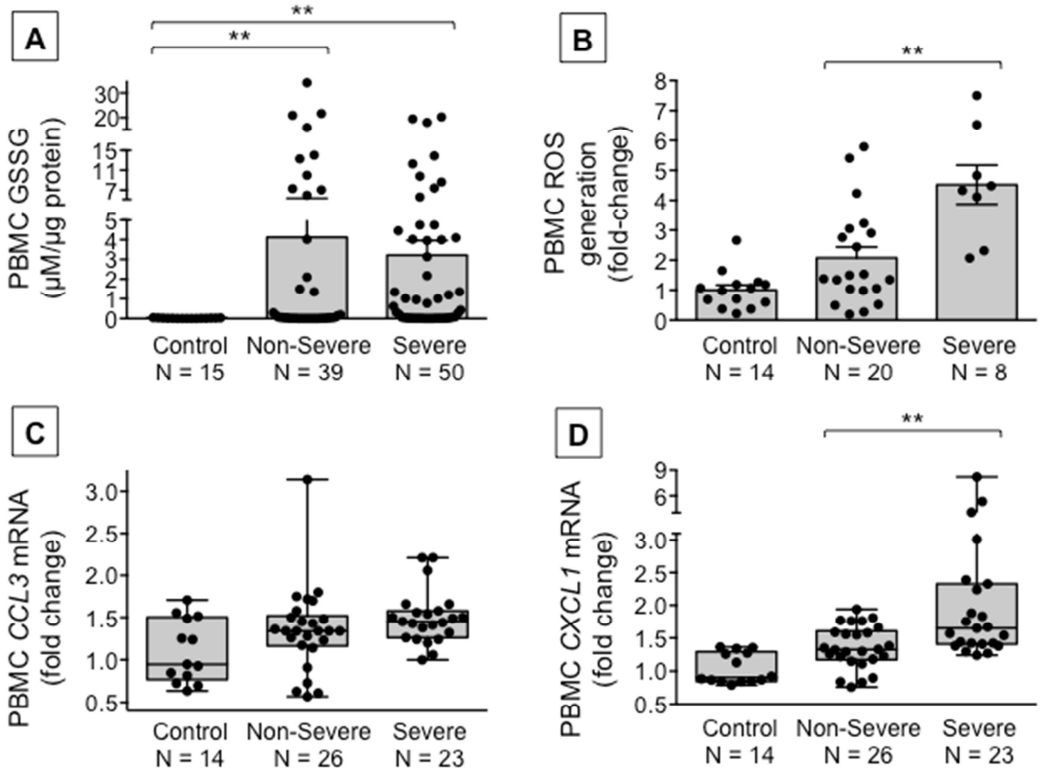
Figure E5. Peripheral blood mononuclear cell (PBMC) mRNA expression of (A) *CCL3* and (B) *CXCL1* in healthy control children, children with asthma who did and did not respond to triamcinolone, and children with asthma who did not receive triamcinolone. Boxplots depict fold-change values relative to controls are shown with minimum and maximum values. Dots represent individual participants. *$p < 0.05$, **$p < 0.01$

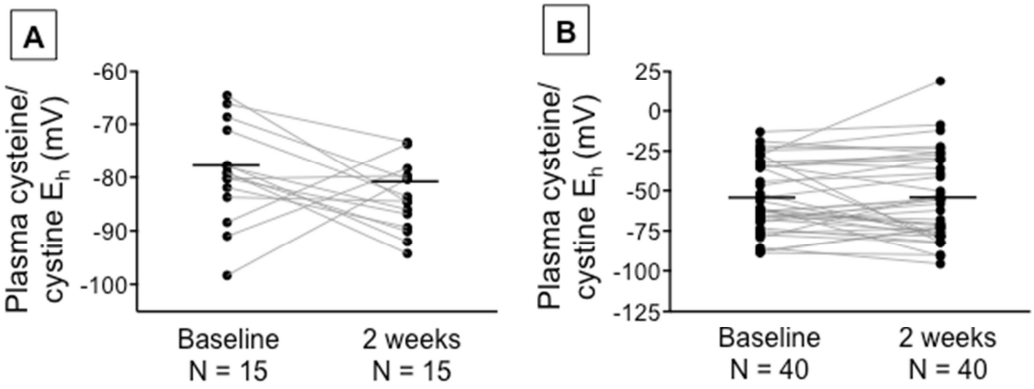
Figure E6. Extracellular cysteine/cystine redox potentials (E_h), intracellular glutathione disulfide (GSSG) concentrations, and intracellular reactive oxygen species (ROS) generation in THP-1 monocytes (A-C, respectively) and human primary PBMCs (D-E, respectively) after exposure to extracellular oxidizing versus reduced conditions. Horizontal lines in panels A and D represent the median. Other data are shown as the mean ± SEM.

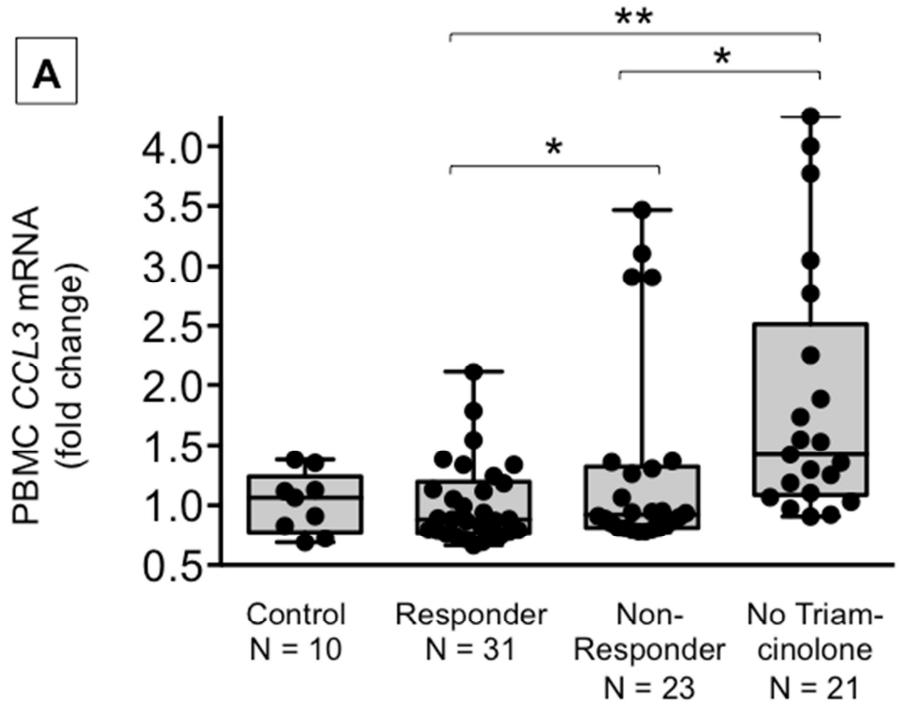
Figure E7. Glucocorticoid receptor (GR) protein sulfhydryl (-SH) groups available for binding in (A) THP-1 monocytes and (B) human primary PBMCs after exposure to extracellular oxidizing versus reduced conditions, and (C) healthy control children and children with asthma. Data are shown as the mean ± SEM and are normalized to total GR protein. Representative images from participants are provided in panel (D).

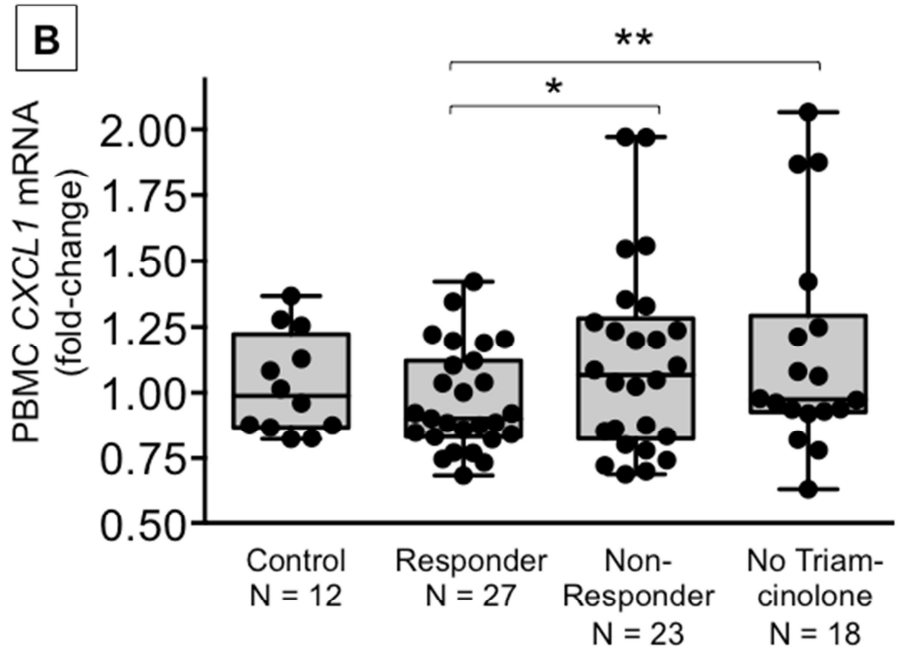

Participants enrolled
N = 134
Controls Enrolled
N = 16
Asthmatics Enrolled
N = 118
Baseline sample not suitable for analysis, N = 1
Baseline sample not suitable for analysis, N = 19
Controls Included
N = 15
Asthmatics Included
N = 99
Completed follow-up visit
N = 15
Did not receive triamcinolone
N = 42
Received triamcinolone
N = 57
Completed 2-week follow-up visit
N = 40
Completed 2-week follow-up visit
N = 56
Triamcinolone responders
N = 33
Triamcinolone non-responders
N = 23

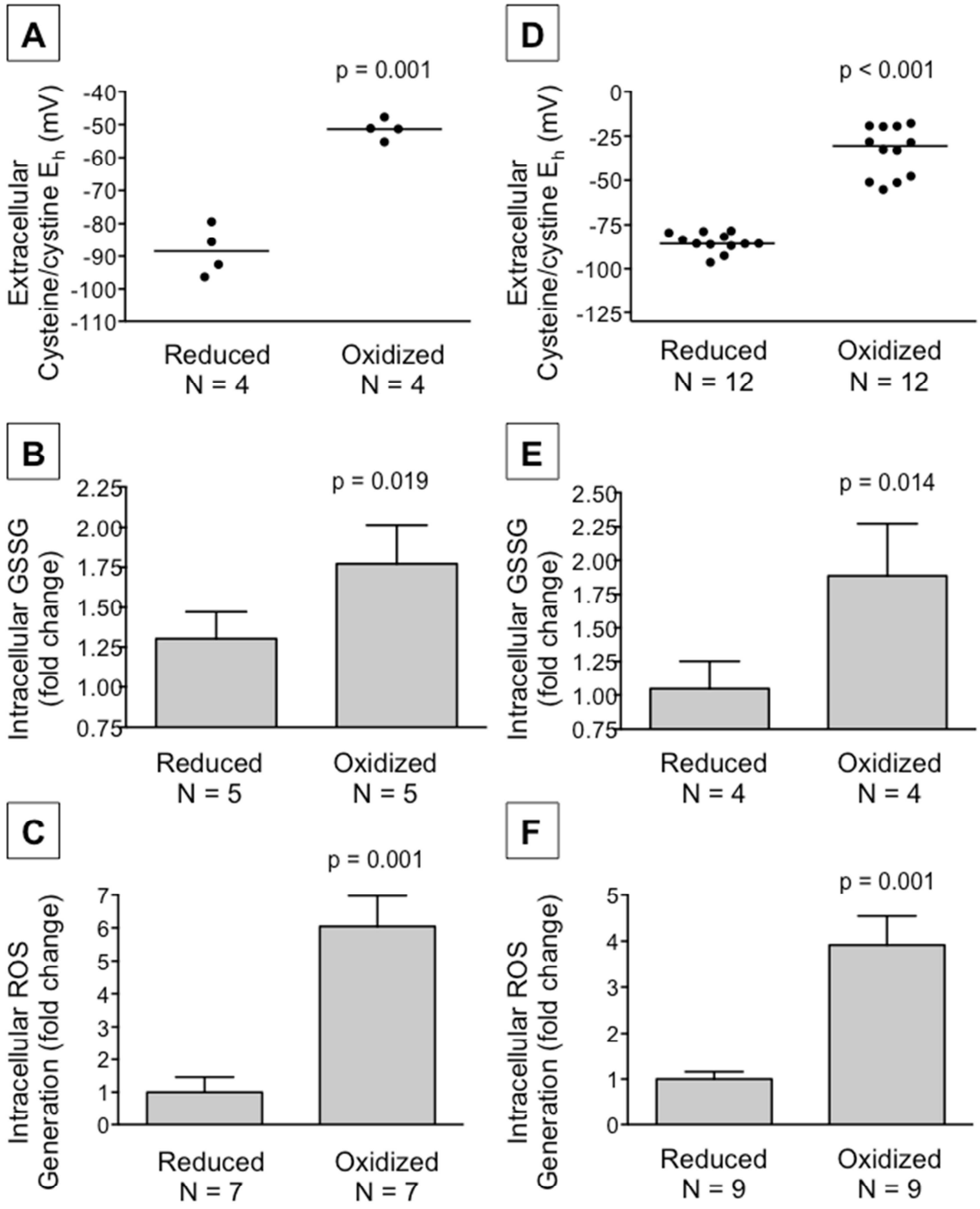

A
Plasma cysteine (uM)
**
**
*
5
4
3
2
1
0
Control
Non-severe
Severe
B
Plasma cystine (uM)
**
**
14
10
6
5
4
3
2
1
0
Control
Non-severe
Severe
C
Oxidized
Plasma cysteine/ cystine E_h (mV)
**
**
**
75
50
25
0
-25
-50
-75
-100
-125
Control
Non-severe
Severe

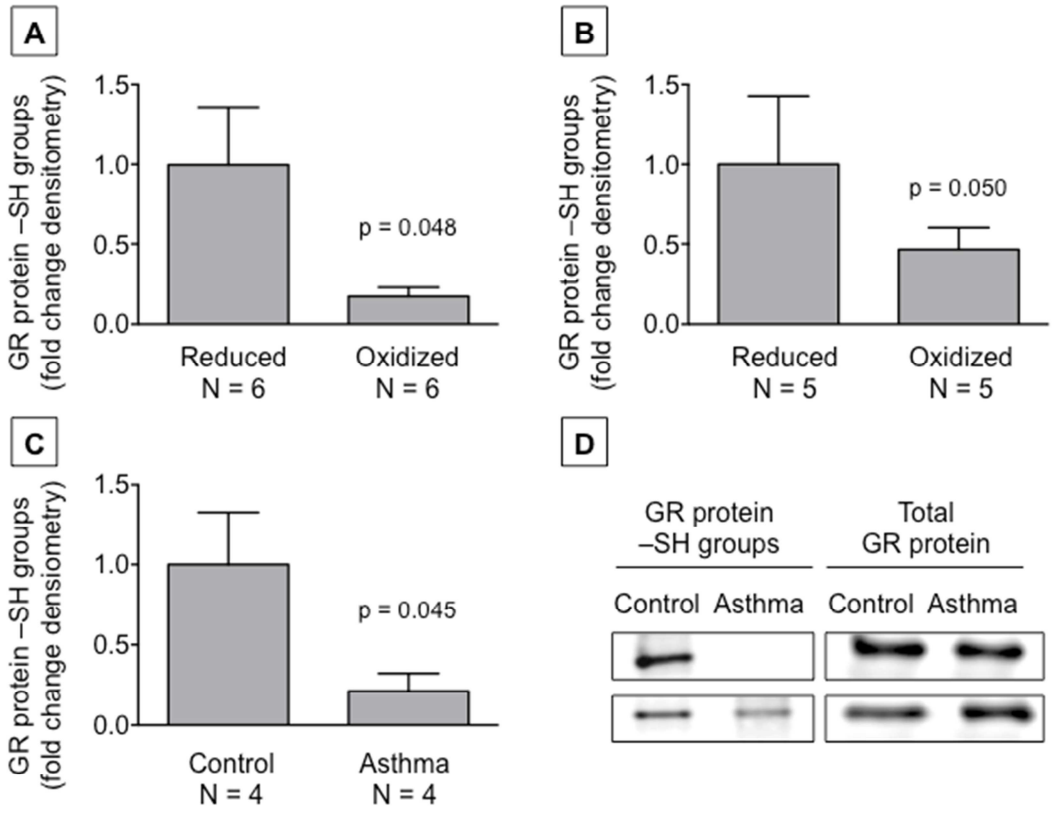

A
**
**
PBMC GSSG (µM/µg protein)
30
20
15
11
7
5
4
3
2
1
0
Control N = 15
Non-Severe N = 39
Severe N = 50
B
**
PBMC ROS generation (fold-change)
8
7
6
5
4
3
2
1
0
Control N = 14
Non-Severe N = 20
Severe N = 8
C
PBMC CCL3 mRNA (fold change)
3.0
2.5
2.0
1.5
1.0
0.5
Control N = 14
Non-Severe N = 26
Severe N = 23
D
**
PBMC CXCL1 mRNA (fold change)
9
6
3.0
2.5
2.0
1.5
1.0
0.5
Control N = 14
Non-Severe N = 26
Severe N = 23


A
Plasma cysteine/
cystine E_h (mV)
-60
-70
-80
-90
-100
Baseline
N = 15
2 weeks
N = 15
B
Plasma cysteine/
cystine E_h (mV)
25
0
-25
-50
-75
-100
-125
Baseline
N = 40
2 weeks
N = 40


A
PBMC CCL3 mRNA
(fold change)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
**
*
*
Control
N = 10
Responder
N = 31
Non-
Responder
N = 23
No Triam-
cinolone
N = 21


B
PBMC CXCL1 mRNA
(fold-change)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
**
*
Control
N = 12
Responder
N = 27
Non-
Responder
N = 23
No Triam-
cinolone
N = 18


A
p = 0.001
Extracellular Cysteine/cystine E_h (mV)
-40
-50
-60
-70
-80
-90
-100
-110
Reduced N = 4
Oxidized N = 4
D
p < 0.001
Extracellular Cysteine/cystine E_h (mV)
0
-25
-50
-75
-100
-125
Reduced N = 12
Oxidized N = 12
B
p = 0.019
Intracellular GSSG (fold change)
2.25
2.00
1.75
1.50
1.25
1.00
0.75
Reduced N = 5
Oxidized N = 5
E
p = 0.014
Intracellular GSSG (fold change)
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
Reduced N = 4
Oxidized N = 4
C
p = 0.001
Intracellular ROS Generation (fold change)
7
6
5
4
3
2
1
0
Reduced N = 7
Oxidized N = 7
F
p = 0.001
Intracellular ROS Generation (fold change)
5
4
3
2
1
0
Reduced N = 9
Oxidized N = 9


A
GR protein –SH groups
(fold change densitometry)
1.5
1.0
0.5
0.0
p = 0.048
Reduced
N = 6
Oxidized
N = 6
B
GR protein –SH groups
(fold change densitometry)
1.5
1.0
0.5
0.0
p = 0.050
Reduced
N = 5
Oxidized
N = 5
C
GR protein –SH groups
(fold change densiometry)
1.5
1.0
0.5
0.0
p = 0.045
Control
N = 4
Asthma
N = 4
D
GR protein
–SH groups
Total
GR protein
Control
Asthma
Control
Asthma