

Interestingly, even though significantly fewer IL-17 protein+ cells were seen in ILC3-injected xenografts compared with positive control grafts injected with IL2-activated PBMCs or T_H17/Tc17 cells ($P < .002$), ILC3 injection sufficed to induce psoriatic lesions (Fig 2, A and B).

As expected, the upregulation of T-cell-related dermal infiltrate markers (ie, CD4, CD8, and plasmacytoid dendritic cells) ($P < .005$)⁹ (Fig 1, J-M) was restricted to positive control grafts injected with IL2-activated PBMCs or T_H17 cells. These quantitative immunohistomorphometry data at the protein level were essentially confirmed at the mRNA level by extensive quantitative RT-PCR analyses for the read-out parameters listed above (for details, see text in this article's Online Repository at www.jacionline.org and Fig E3, D).

To ensure that dermal resident cells or residual CD3+ cells within the purified ILC3s were not involved in psoriasis induction, a group of grafted mice was sublethally irradiated before the injection of cultured cells or treated daily with anti-CD3+ cell (OKT3) antibodies for 30 days just after the cell injections. This demonstrated that neither irradiation nor injections of anti-CD3+ antibodies prevented the induction of the psoriatic lesions in human skin xenotransplants *in vivo* by autologous ILC3s (Fig 2, F-L). Furthermore, the psoriasis-inducing activity in human skin *in vivo* appeared to be T-cell-independent and was specific to ILC3, but not to ILC1 or ILC2 cells (Fig E4, A-D).

To our knowledge, the current study provides the first functional evidence that ILC3 cells are not only numerically increased in human psoriatic skin but can actually drive development of the human psoriatic phenotype and are sufficient to do so, even in the absence of T_H17 cells. This observation, combined with the fact that ILCs promote T-cell activation,^{E8} strongly supports the assumption that ILC3s and not T_H17 may play an initiating role in the pathogenesis of psoriasis¹ and thus the clinical utility of targeting ILC3 cells.

Professor Silvia Bulfone-Paus is gratefully acknowledged for helpful professional advice.

Aviad Keren, PhD^a
Avner Shemer, MD^a
Alex Ginzburg, MD^a
Yehuda Ullmann, MD^a
Adam G. Schrum, PhD^b
Ralf Paus, MD^c
Amos Gilhar, MD^a

From the ^aSkin Research Laboratory, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel; ^bDepartments of Molecular Microbiology and Immunology, Surgery, and Bioengineering, University of Missouri, Columbia, Columbia, Mo; and ^cthe Centre for Dermatology Research, University of Manchester and NIHR Manchester Biomedical Research Centre, Manchester, United Kingdom. E-mail: doritg2000@gmail.com.

This study was funded in part by Technion R&D Foundation Ltd (to A.G.). R.P. was supported by the NIHR Manchester Biomedical Research Centre.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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Available online March 2, 2018.
<https://doi.org/10.1016/j.jaci.2018.02.015>

Glutathione and arginine levels: Predictors for acetaminophen-associated asthma exacerbation?



To the Editor:

We recently demonstrated that as-needed use of acetaminophen (vs ibuprofen) was not associated with a higher incidence of asthma exacerbations or worse asthma control in young children with mild persistent asthma.¹ However, given prior epidemiological observations of more frequent acetaminophen use and greater asthma impairment in children,² it remains unclear whether there are subgroups of children with asthma at increased risk for poorer outcomes with acetaminophen exposure. The purported mechanism linking asthma and acetaminophen is a reduction in the antioxidant glutathione, which conjugates acetaminophen for excretion. While this process is crucial for detoxification, it also irreversibly consumes glutathione stores, resulting in increased and prolonged circulation of highly reactive intermediates (ie, "oxidant stress").

Abnormalities in glutathione and arginine metabolic pathways have been described in asthma, albeit in isolation,^{3,4} but each may potentially disrupt the balance of endogenous oxidation-reduction (redox) reactions and contribute to oxidant stress. Whereas glutathione is a tripeptide thiol antioxidant, arginine is a conditionally essential amino acid that functions as the obligate substrate for nitric oxide production through the actions of the nitric oxide synthase (NOS) enzymes. The paradigm uniting these mechanisms involves S-nitrosoglutathione, an endogenous airway smooth muscle relaxant that is decreased in the airways of patients with asthma. Low glutathione contributes to dysfunction of NOS enzymes, resulting in decreased coupling of nitric oxide to glutathione to form S-nitrosoglutathione, with increased reactive nitrogen species formation. In addition, low arginine bioavailability results in decreased substrate availability for NOS isoforms, resulting in less S-nitrosothiol formation and increased bronchoconstriction.

Inflammation simultaneously upregulates NOS and the arginine-consuming enzyme arginase, found to be elevated in children with asthma.⁴ Arginase metabolizes arginine away from nitric oxide production toward ornithine and downstream byproducts of proline and polyamines, which can contribute to airway

TABLE I. Plasma and erythrocyte glutathione, arginine, and ornithine concentrations according to baseline features

Variable	Grouping	N	Plasma ($\mu\text{mol/L}$)			Erythrocyte (mmol/g hemoglobin)		
			Glutathione	Arginine	Ornithine	Glutathione	Arginine	Ornithine
Age (mo)	12-24	36	1.4 ± 1.0	33.5 ± 14.0	29.0 ± 10.3	3.0 ± 0.9	0.07 ± 0.05	1.5 ± 0.6
	24-36	51	1.2 ± 0.8	38.3 ± 15.2	30.1 ± 9.9	2.6 ± 1.0	0.06 ± 0.04	1.6 ± 0.7
	36-48	77	1.3 ± 0.9	41.7 ± 19.3	36.2 ± 14.4	2.7 ± 1.2	0.06 ± 0.06	1.5 ± 0.7
	48-60	96	1.3 ± 0.7	43.3 ± 19.5	34.9 ± 14.0	2.6 ± 1.1	0.08 ± 0.08	1.7 ± 0.8
Sex	Female	102	1.2 ± 0.9	40.7 ± 18.6	34.4 ± 14.8	2.9 ± 1.1	0.07 ± 0.07	1.6 ± 0.7
	Male	158	1.3 ± 0.8	40.4 ± 18.0	33.0 ± 12.0	2.6 ± 1.1	0.07 ± 0.06	1.6 ± 0.7
Race	White	129	1.3 ± 0.9	39.8 ± 17.8	34.9 ± 13.2	2.7 ± 1.0	0.06 ± 0.04	1.6 ± 0.6
	Nonwhite	131	1.2 ± 0.8	41.2 ± 18.7	32.2 ± 13.1	2.7 ± 1.1	0.08 ± 0.08	1.6 ± 0.8
Tobacco exposure	No	167	1.3 ± 0.8	39.9 ± 18.8	33.8 ± 13.3	2.6 ± 1.0	0.06 ± 0.04	1.6 ± 0.7
	Yes	93	1.2 ± 0.9	41.6 ± 17.1	33.0 ± 13.1	2.8 ± 1.1	0.08 ± 0.08	1.6 ± 0.7
Wheezing episodes (past year)	0	6	1.4 ± 0.9	43.8 ± 13.2	36.3 ± 10.4	3.3 ± 1.0	0.08 ± 0.07	1.7 ± 0.6
	1-3	68	1.2 ± 0.7	43.6 ± 18.2	34.0 ± 12.3	2.3 ± 0.9	0.08 ± 0.08	1.6 ± 0.6
	4-6	107	1.2 ± 0.8	39.7 ± 18.8	33.8 ± 12.7	2.8 ± 1.1	0.06 ± 0.06	1.6 ± 0.7
	>6	79	1.5 ± 1.0	38.7 ± 17.6	32.6 ± 14.8	2.9 ± 1.1	0.07 ± 0.05	1.7 ± 0.8
Emergency department visits (past year)	0	38	1.2 ± 1.0	37.1 ± 19.3	33.9 ± 13.9	2.3 ± 1.0	0.07 ± 0.08	1.4 ± 0.6
	1-2	91	1.3 ± 0.7	42.0 ± 17.0	34.0 ± 14.0	2.7 ± 1.1	0.07 ± 0.06	1.6 ± 0.6
	3-4	67	1.3 ± 0.8	40.5 ± 20.2	34.1 ± 12.7	2.8 ± 1.1	0.07 ± 0.06	1.6 ± 0.6
	>4	64	1.3 ± 1.0	40.3 ± 17.2	32.0 ± 12.4	2.8 ± 1.0	0.06 ± 0.05	1.8 ± 0.9
Systemic corticosteroid courses (past year)	0	67	1.3 ± 0.8	41.9 ± 19.2	35.1 ± 14.1	2.6 ± 1.2	0.07 ± 0.08	1.5 ± 0.7
	1-2	138	1.3 ± 0.9	40.1 ± 17.1	33.2 ± 13.0	2.7 ± 1.0	0.06 ± 0.06	1.6 ± 0.6
Run-in asthma control days (% of days)*	≥ 3	54	1.2 ± 0.7	40.1 ± 19.9	32.4 ± 12.8	2.8 ± 1.1	0.07 ± 0.05	1.8 ± 0.8
	<70	37	1.4 ± 0.9	42.5 ± 22.6	38.4 ± 15.7	2.8 ± 1.1	0.05 ± 0.03	1.5 ± 0.5
	70-90	79	1.2 ± 0.8	38.8 ± 17.3	32.1 ± 12.7	2.7 ± 1.1	0.07 ± 0.05	1.5 ± 0.6
	>90	132	1.3 ± 0.8	40.7 ± 17.5	32.8 ± 12.4	2.7 ± 1.1	0.07 ± 0.07	1.7 ± 0.7
Blood eosinophils (per μL)	<200	97	1.2 ± 0.9	41.2 ± 17.6	33.8 ± 13.3	2.6 ± 1.0	0.08 ± 0.07	1.7 ± 0.8
	200-400	62	1.3 ± 0.9	39.9 ± 20.6	33.5 ± 15.2	2.7 ± 1.0	0.06 ± 0.05	1.5 ± 0.6
	>400	73	1.2 ± 0.8	40.5 ± 15.4	33.5 ± 12.6	2.8 ± 1.2	0.07 ± 0.06	1.6 ± 0.7
Serum IgE (kU/L)	<50	103	1.3 ± 0.8	42.1 ± 21.2	34.7 ± 14.0	2.7 ± 1.0	0.07 ± 0.07	1.7 ± 0.8
	50-150	78	1.2 ± 0.9	37.4 ± 14.3	32.9 ± 13.5	2.8 ± 1.1	0.07 ± 0.05	1.6 ± 0.6
	>150	77	1.2 ± 0.8	42.0 ± 17.1	32.8 ± 11.8	2.6 ± 1.1	0.07 ± 0.06	1.5 ± 0.6
Aeroallergen sensitization†	No	145	1.3 ± 0.8	40.5 ± 19.9	34.0 ± 13.9	2.6 ± 1.0	0.07 ± 0.06	1.6 ± 0.7
	Yes	113	1.3 ± 0.9	40.8 ± 15.7	33.0 ± 12.3	2.8 ± 1.2	0.07 ± 0.06	1.5 ± 0.6

Data represent the mean $\pm$ SD. Results are not statistically significant at $P < .05$.

*Defined as full calendar days without asthma symptoms, rescue medication use, or unscheduled health care visits.

†Defined as a specific IgE value of ≥ 0.35 kU/L.

remodeling and smooth muscle proliferation. Arginine/ornithine is a biomarker of arginase activity found to be a better reflection of arginine bioavailability than arginine alone.⁵

We therefore questioned whether systemic glutathione, arginine, and ornithine might be useful biomarkers for prediction of asthma exacerbation in children. We hypothesized that systemic glutathione levels would associate with measures of arginine bioavailability, and decreased glutathione and arginine availability would associate with more frequent asthma exacerbations in children treated with acetaminophen. We performed a secondary analysis of our aforementioned clinical trial in preschool children with mild persistent asthma (NCT01606319).¹ Details of that study are provided in this article's Online Repository at www.jacionline.org. Blood samples were obtained at randomization after a 2- to 8-week run-in with inhaled fluticasone or placebo; parents were also discouraged from administering over-the-counter acetaminophen or ibuprofen during the run-in period. Blood samples were analyzed for glutathione (reduced plus oxidized forms) and arginine and ornithine as previously described.⁶

Plasma and erythrocyte samples were available from 261 of 300 children; features of these children and baseline glutathione, arginine, and ornithine values are presented in Table E1 in this article's Online Repository at www.jacionline.org. Correlations between these values are shown in Fig E1 in this article's Online Repository at www.jacionline.org. Glutathione values were modestly positively correlated with ornithine in the plasma, whereas arginine and ornithine values were highly positively correlated in both the plasma and erythrocytes in raw analyses and after logarithmic transformation to account for outliers (Fig E1). Baseline glutathione, arginine, and ornithine values did not differ between children treated with fluticasone versus placebo during the run-in period (see Fig E2 in this article's Online Repository at www.jacionline.org) and were also not associated with demographic characteristics such as age group or features of allergic sensitization or asthma impairment (Table I). There were also no associations between baseline glutathione, arginine, and ornithine values and asthma exacerbations over the 48-week treatment period in univariate analyses (Fig 1) or

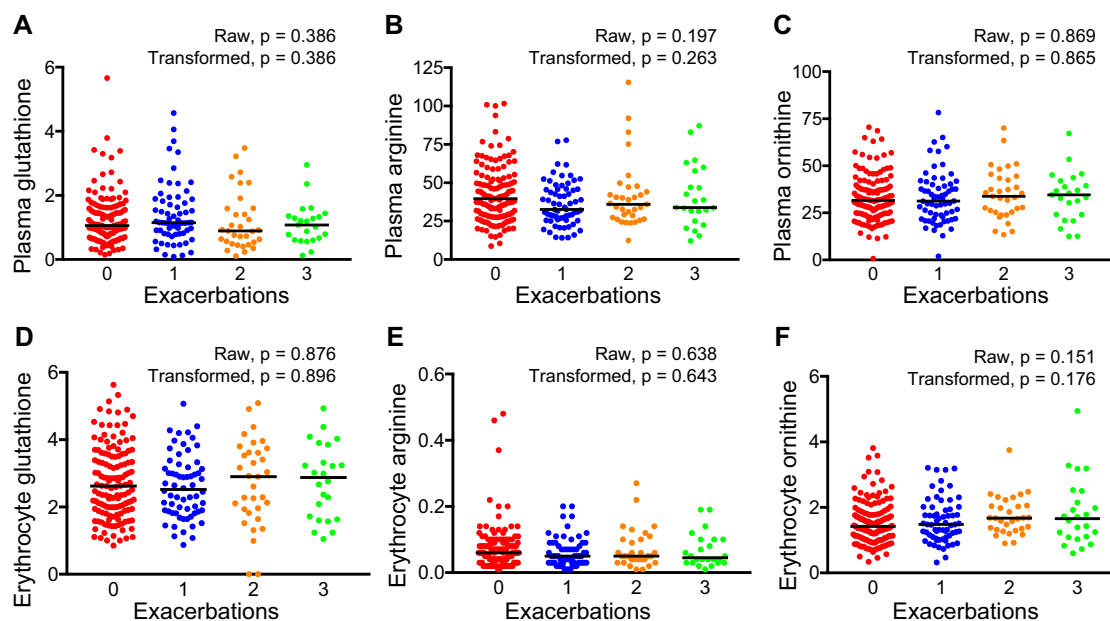


FIG 1. Baseline plasma (A) glutathione, (B) arginine, and (C) ornithine concentrations (μmol/L) and erythrocyte (D) glutathione, (E) arginine, and (F) ornithine concentrations (mmol/g hemoglobin), stratified by the number of exacerbations over 48 weeks. Circles represent individual participants; bars represent the median.

after adjustment for age group and acetaminophen versus ibuprofen treatment assignment (see Table E2 in this article's Online Repository at www.jacionline.org). Plasma and erythrocyte values, stratified by acetaminophen versus ibuprofen treatment assignment and the number of exacerbations, are provided in Tables E3 and E4 in this article's Online Repository at www.jacionline.org. Baseline levels of glutathione, arginine, and ornithine also failed to predict the time to first exacerbation in univariate analyses or after adjustment for as-needed acetaminophen versus ibuprofen treatment and asthma controller medication receipt (ie, daily inhaled fluticasone, as-needed inhaled fluticasone, or montelukast) before the exacerbation onset (see Fig E3 in this article's Online Repository at www.jacionline.org). Logarithmic transformation of the biomarkers did not yield different results in time to exacerbation analyses (data not shown).

In this secondary analysis, we failed to detect associations between baseline systemic measures of glutathione, arginine, and ornithine and asthma exacerbations in young children with mild persistent asthma. A strength was the prospective assessment of exacerbations in the context of highly supervised asthma controller therapy, thus reducing the potential confounding effects of asthma medication adherence or other factors that might contribute to poor asthma control. To our knowledge, this is also the first study to characterize plasma and erythrocyte concentrations of glutathione, arginine, and ornithine in young children with mild persistent asthma. Although variability in the concentrations was noted, the plasma levels are similar to those reported in children with asthma.^{4,7}

Nonetheless, there are limitations, including the lack of a healthy control group and the cross-sectional nature of the sample collection. We are therefore unable to comment on the longitudinal stability of the measures during health or acute exacerbations. Our focus on systemic samples is also a limitation because airway and systemic redox pools are not necessarily in equilibrium, particularly in cases of milder asthma in which inflammation is likely localized to the airways. Because this study evaluated young children with mild persistent asthma only, associations in older children or in children with more severe asthma remain unknown. Indeed, other reports have noted decreased expression of acetaminophen-metabolizing enzymes and glutathione in children with severe asthma after acetaminophen exposure.⁸ These previous reports do provide mechanistic feasibility to reports of asthma-related complications associated with frequent acetaminophen use. Likewise, arginine bioavailability has also been associated with airflow abnormalities in severe asthma⁹ and may play an important role during acute exacerbations.⁴

Our findings are not definitive, but we have provided some novel insight regarding glutathione and arginine bioavailability in young children with mild asthma. Further studies are still needed in heterogeneous populations to better understand temporal associations between these biomarkers, developmental maturation, and exacerbations of asthma in early childhood.

Claudia R. Morris, MD^a

David T. Mauger, PhD^b

Jung H. Suh, PhD^c

Wanda Phipatanakul, MD, MS^d

William J. Sheehan, MD^d

James N. Moy, MD^a
Ian M. Paul, MD, MSc^b
Stanley J. Szeffler, MD^c
Daniel J. Jackson, MD^d
Anne M. Fitzpatrick, PhD^e

on behalf of the National Institutes of Health/National Heart, Lung, and
Blood Institute AsthmaNet

From ^athe Department of Pediatrics, Emory University School of Medicine, Atlanta, Ga; ^bthe Department of Public Health Sciences, College of Medicine, Penn State University, Hershey, Pa; ^cChildren's Hospital Oakland Research Institute, Oakland, Calif; ^dthe Division of Allergy/Immunology, Boston Children's Hospital, Harvard Medical School, Boston, Mass; ^eStroger Hospital of Cook County, Department of Pediatrics, Rush University Medical Center, Chicago, Ill; ^fthe Department of Pediatrics, College of Medicine, Penn State University, Hershey, Pa; ^gChildren's Hospital Colorado, the Breathing Institute, and the University of Colorado School of Medicine, Aurora, Colo; and ^hPediatrics, Section of Allergy, Immunology and Rheumatology, University of Wisconsin School of Medicine and Public Health, Madison, Wis. E-mail: anne.fitzpatrick@emory.edu.

This study was supported by the National Institutes of Health (grant nos. HL098102, HL098096, HL098075, HL098090, HL098177, HL098098, HL098107, HL098112, HL098103, HL098115, TR001082, TR000439, TR000448, TR000454, K23AI104780, and K24AI106822).

Disclosure of potential conflict of interest: C. R. Morris has received royalties from a patent from Lifetrents/UCSF-Benioff Children's Hospital Oakland; and has consultant arrangements with Pfizer, Kantar Consulting, LEK Consulting, and Nestle. D. T. Mauger has received a grant from the National Institutes of Health and has received nonfinancial support from Merck and GlaxoSmithKline. J. N. Moy has received a grant from the National Institutes of Health/National Heart, Lung, and Blood Institute. I. M. Paul has received a grant from the National Institutes of Health/National Heart, Lung, and Blood Institute; and has received personal fees from Pfizer, Johnson & Johnson, Boehringer Ingelheim, and the Rocky Mountain Poison & Drug Center. S. J. Szeffler has received grants from the National Heart, Lung, and Blood Institute, AsthmaNet, and GlaxoSmithKline; has received compensation from Merck for participation in an advisory panel; has consulted for Boehringer Ingelheim; has participated in manuscript preparation and advisory boards for Genentech; has participated in advisory meetings for GlaxoSmithKline; has provided guidance in application of an inflammation monitoring device for Aerocrine; has participated in development of follow-up The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) II study for Novartis; has received payment for assistance in educating investigators regarding severe asthma in children and also for guidance in new product development from AstraZeneca; has received payment for advice on development of new medication from Daiichi Sankyo; has received payment for assistance in designing and monitoring a pediatric asthma study for a new medication from Roche; and has received payment for participation in an asthma advisory panel for a new medication from Teva. D. J. Jackson has received a grant from the National Heart, Lung, and Blood Institute and the National Institute of Allergy and Infectious Diseases and has received personal fees from Boehringer Ingelheim, Commense, GlaxoSmithKline, Merck, and Novartis. A. M. Fitzpatrick has received a grant from the National Institutes of Health. The rest of the authors declare that they have no relevant conflicts of interest.

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Available online March 5, 2018.
<https://doi.org/10.1016/j.jaci.2018.01.045>

A type III complement factor D deficiency: Structural insights for inhibition of the alternative pathway



To the Editor:

We investigated an alternative complement pathway (AP) deficiency in a patient with absent alternative pathway hemolytic activity but normal classical pathway hemolytic activity recovering from invasive meningococcal infection (for patient and sibling details, see [Patient details](#) in this article's Online Repository at www.jacionline.org). Serum reconstitution with proximal AP components suggested a factor D (FD) deficiency ([Fig 1, A](#)). Sanger sequencing of *CFD* identified a rare homozygous missense mutation (c.602G>C) in exon 4 in the patient (II-1) and sibling (II-2), resulting in an arginine to proline substitution (p. R176P) (see [Fig E1, A](#), and reference [E9](#) in this article's Online Repository at www.jacionline.org). This genotype cosegregated with an alternative pathway hemolytic activity-null phenotype, as the parents, both heterozygotes, had normal alternative pathway hemolytic activity ([Fig 1, B](#)). In contrast to previously confirmed FD deficiencies,¹⁻³ all members of the pedigree had normal levels of circulating FD, as corroborated by Western blot (see [Fig E1, B](#)). Meanwhile, identical circular dichroism spectra and melting curves of recombinant wild-type (WT) and R176P FD precluded gross changes in FD structure or stability, suggesting a functional deficiency ([Fig 1, C](#), and see [Fig E1, C](#)). We assessed the cleavage of C3b-bound factor B (FB) by recombinant WT and mutant FD (R176P, R176A, R176Q). WT FD could cleave C3b-bound FB to produce fragments Bb and Ba. Conversely, R176P FD demonstrated diminished *in vitro* catalytic activity at all concentrations and had negligible activity at physiological concentration (0.04 μmol/L) ([Fig 1, D](#), and see [Fig E1, D](#)). Reconstitution of FD-depleted serum with R176P FD also demonstrated impaired AP-mediated hemolysis (see [Fig E1, E](#)).

FD's serine protease activity depends on obligatory binding to the C3bB complex via 4 exosite loops (residues 132-135, 155-159, 173-176, 203-209). This leads to rearrangement of the self-inhibitory loop (199-202), allowing realignment of His41 and Asp89 with Ser183 to form the active catalytic triad (see [Fig E2, A and B](#), in this article's Online Repository at www.jacionline.org).^{4,5} Mutation R176P lies outside the active site, within one of the FB-binding exosite loops. We used molecular dynamics (MD) simulations to study how the R176P mutation affects the FD protein fold (see [Fig E2, C](#)). In mutant FD, we observed a rearrangement of the exosite loop 155-161 within 50 nanoseconds of simulation ([Fig 2, A](#)). This was unexpected because loop 155-161 was not in direct contact with residue 176. Average structures generated from the final 50 nanoseconds of simulation

ACKNOWLEDGMENTS

The National Heart, Lung, and Blood Institute's AsthmaNet acknowledges the following individuals for their contributions to the study:

- Allegheny Singer Research Institute, Allegheny General Hospital: Deborah Gentile, Erica Butler, Jennifer Maiolo, Sara Misplay, David Skoner, and Glennys Smith.
- Ann & Robert H. Lurie Children's Hospital: Jacqueline Pongratic, Rachel Robison, Iliana Flexas; Rajesh Kumar, MD, MS; Melanie Makhija; Nashmia Qamar; Christine Szychlinski, MS APN, CPNP-PC.
- Arizona Respiratory Center, University of Arizona: Fernando Martinez, Wayne Morgan, Cori Daines, Michael Daines, Valerie Bloss, Mark Brown, Katherine Chee, Sarah David, Clara S. Ehrman, Dima Ezmigna, Jamie Goodwin, Roni Grad, Anunya Hiranratta, Silvia Lopez, Andrea Paco, Janette Priefert, Natalie S. Provencio, Elizabeth Ryan, Monica Varela, Monica Vasquez, Rosemary Weese, and Jesus Wences.
- Benioff Children's Hospital, Oakland: Mindy Benson, Claudia Morris, Jung Suh, Jyothi Marbin, Jason Decker, Keonna Harrison, Dayna Long, Kelley Meade, Robert Mok, Cindy Nelson-Purdy, Dennis Ren, and Hollie Stessel.
- Boston Children's Hospital: Wanda Phipatanakul, William Sheehan, Jonathan Gaffin, Sachin Baxi, Lisa Bartnikas, Alisha Bouzaher, Christopher Burke, Matthew Cavanaugh, Julia Chen, Elizabeth Cunningham, Amparito Cunningham, James Friedlander, Enal Hindi, David Kantor, Lianne Kopel, Perdita Permaul, Deviko Rao, Melinda Rossi, Doris Schierembergg, Lynda Schneider, Jennifer Troung, Dale Umetsu, and Joseph Zhou.
- Brigham & Women's Hospital, Harvard Medical School: Elliot Israel.
- Children's Hospital of Colorado and the University of Colorado School of Medicine: Stanley Szefer.
- Children's Hospital of Pittsburgh of the University of Pittsburgh Medical Center: Fernando Holguin, Shean Aujla, John Broyles, Hey Chong, Patricia Dubin, Jonathan Finder, Todd Green, Lori Holt, Adam Kufen, Geoffrey Kurland, Rose Lanzo, David Nash, Julianne Parente, Catherine Smith, Jonathan Spahr, and Daniel Weiner.
- Emory University: Anne Fitzpatrick, Timothy Beaty, Shanae Wakefield Brown, Alice Bruce, Jennifer Dodds, Shaneka Douglas, Karen Freedle, Dawn Simon, and Denise Whitlock.
- National Jewish Health: Ronina Covar, Tod Olin, Melanie Gleason, Gayle Spears, D. Sundstrom, and Michael White.
- Nemours Children's Specialty Care, Jacksonville: Kathryn Blake, John Lima, Jenny Batalla, Michelle Littlefield, Burnese Rutledge, and Deanna Seymour.
- Nemours Children's Specialty Care, Orlando: Jason Lang, Shanae Brown, and Omar OquendoFlores.
- Pennsylvania State University: David Mauger, Ian Paul, Sue Boehmer, Loretta Doty, Beth Gern, Aimee Merchilinski, Jonathan Nelson, James Schmidt, Lindsay Texter, Angela Updegrave, Jennifer Zeller, and Ronald Zimmerman Jr.
- Rainbow Babies and Children's Hospital: James Chmiel, Ross Myers, Kristie Ross, Daniel Craven, Amy DiMarino, Meeghan Hart, Leigh Kerns, Laurie Logan, and Laura Veri.
- Rush University Medical Center: James Moy, Christopher Co-dispoti, Jun Fu, Grace Li, Diana Munoz-Mendoza, Shannon Stevens, Benjamin Thompson, and Samantha Zitzer.

- University of California-San Francisco: Michael Cabana, Stephen Lazarus, Ngoc Ly, Shannon Thyne, Jessica Bowman, Vicky Bowyer, Judy Gonzales-Vargas, Michelle McKean, Nancy Tran.
- University of Illinois, Chicago: Harsha Vardhan Kumar.
- University of New Mexico: Hengameh Raissy, Christina Batson, Lea Davies, Franceska Kelly, Esmeralda Morales, Abby Redway, and Mary Spicher.
- University of Wisconsin-Madison: Robert Lemanske, Daniel Jackson, Christine Sorkness, Lauren Kaminski, Megan Knutson, Shelly Olson, Sheila Turcsanyi, and Tanya Watson.
- Wake Forest University: Stephen Peters, Adam Gower, and Cheryl Wilmoth.
- Washington University School of Medicine: Leonard Bacharier, Avraham Beigelman, Wanda Caldwell, Courtney Dula, Alysa Ellis, Caroline Horner, Lila Kertz, Tina Norris, Katherine Rivera-Spoljaric, Oscar Rodriguez, and Robert Strunk.

METHODS

The Acetaminophen versus Ibuprofen in Children with Asthma (AVICA) trial was a multicenter, randomized, double-blind, parallel trial of as-needed acetaminophen versus ibuprofen for fever and pain in children aged 12 to 59 months with mild persistent asthma ([ClinicalTrials.gov](#) number, NCT01606319).^{E1} Asthma medications were controlled in a simultaneous, factorially linked, randomized, double-blind, double-dummy, triple-crossover trial ([ClinicalTrials.gov](#) number, NCT01606306).^{E2} After a 2- to 8-week run-in period, which varied according to symptom presentation and prior medication exposure, children entered the treatment portion of the study, where they were randomized to 3 sequential 16-week treatment periods with 1 of the following agents: (1) fluticasone propionate-HFA (Flovent-HFA 44 µg/actuation), 2 inhalations twice daily, (2) montelukast 4 mg granules (or chewable tablet) once daily at night, and (3) fluticasone propionate-HFA (Flovent-HFA 44 µg/actuation), 2 inhalations plus 2 inhalations of albuterol sulfate (90 µg/actuation) administered as needed for symptom relief. Inhaled medications were delivered with a valved holding chamber and face mask. There were no washouts between study treatments. Participants were randomized in 2 processes: one to determine the sequence of asthma controller therapy, and the other to determine which blinded antipyretic/analgesic medication was to be administered as needed during the course of the 48-week trial.

Acetaminophen suspension (160 mg per 5 mL; Little Fevers by Little Remedies [grape flavor], Medtech Products, Irvington, NY) and ibuprofen suspension (100 mg per 5 mL; Children's Advil [grape flavor], Pfizer Consumer Healthcare, New York, NY), which had similar taste and appearance, were placed in identical packaging for the 2 treatment groups. Parents or legal guardians were also provided with clear oral and written instructions for administering the medication according to the typical indicated use in home care as needed for pain, fever, or discomfort, with no more than 1 dose every 6 hours. The dosing strategy was in accordance with dosing guidelines of the American Academy of Pediatrics³ (15 mg/kg acetaminophen or 9.4 mg/kg ibuprofen every 6 hours as needed) and ensured that the volume of a single dose was the same for each medication (0.47 mL/kg per dose).

The Asthma Network (AsthmaNet) of the National Heart, Lung, and Blood Institute (NHLBI) funded the studies. The protocol was developed by the AsthmaNet Steering Committee and was approved by an NHLBI protocol review committee and data and safety monitoring board, as well as the institutional review board at each participating site. Parents or legal guardians provided written informed consent for all participants.

Trial participants

Inclusion criteria for study entry varied according to current asthma medication usage. To encourage recruitment and generalization of results, this

protocol enrolled inhaled corticosteroid (ICS) and leukotriene receptor antagonist (LTRA)-naïve children treated only with intermittent short-acting beta-agonists (SABAs) who required step-up therapy, as well as children currently treated with daily low-dose ICS, daily LTRA, or intermittent ICS or LTRA. Children not receiving an ICS or LTRA at any time over the preceding 6 months were eligible for inclusion if they had at least 1 of the following features: (1) daytime asthma symptoms more than 2 days per week (averaged over the preceding 4 weeks), (2) at least 1 nighttime awakening from asthma over the preceding 4 weeks, (3) 2 or more asthma exacerbations requiring systemic corticosteroids in the preceding 6 months, or (4) 4 or more wheezing episodes, each lasting 24 hours or more, in the preceding 12 months. Children receiving an ICS or LTRA at any time (ie, daily or intermittently) over the preceding 6 months were eligible for inclusion if they had at least 1 of the following features: (1) receiving ICS or LTRA for more than 3 months (or more than 90 days) out of the preceding 6 months (or 180 days), (2) daytime asthma symptoms more than 2 days per week (averaged over the preceding 4 weeks), (3) at least 1 nighttime awakening from asthma over the preceding 4 weeks, (4) 2 or more asthma exacerbations requiring systemic corticosteroids in the preceding 12 months, or (4) 4 or more wheezing episodes, each lasting 24 hours or more, in the preceding 12 months.

A child was considered ineligible for initial study entry if he or she had a history of allergic reaction to the study medications or any component of the study drugs, other chronic medical disorders that could interfere with drug metabolism or drug-related safety, comorbid wheezing disorders associated with wheezing such as premature birth before 35 weeks' gestation or airway anomalies, failure to thrive, or history of a near-fatal asthma exacerbation requiring assisted ventilation. During the run-in period, children were considered ineligible for randomization if they (1) displayed inadequate adherence to the study protocol (ie, <75% of electronic diaries completed), (2) experienced an asthma exacerbation requiring systemic corticosteroids, (3) had daily asthma symptoms if not taking asthma controller therapy, or (4) had asthma symptoms more than 2 days per week if taking asthma controller therapy.

Trial run-in period

During the run-in period, each child received 1 oral medication and 1 inhaled medication for daily use. Each participant also had access to an open-label short-acting bronchodilator (albuterol sulfate, 90 µg each, GlaxoSmithKline, Research Triangle Park, NC) and open-label prednisolone. Open-label, over-the-counter acetaminophen or ibuprofen products were discouraged during the run-in period. The run-in duration was based on whether or not the child was currently receiving step 2 therapy (ie, daily low-dose ICS or LTRA) according to national treatment guidelines^{E4} and whether or not the child qualified on the basis of exacerbation history. Children not on step 2 therapy during the 6 months before enrollment (including children who received ICS or LTRA intermittently) received placebo oral and inhaled therapy during the run-in. The run-in was completed in 2 weeks if the participant had a previous exacerbation requiring systemic corticosteroids. If the

participant did not have an exacerbation, the run-in period could be extended up to 8 weeks to elicit symptoms. Children who were receiving step 2 therapy received active ICS or active LTRA during the run-in period. If the participant had a previous exacerbation requiring systemic corticosteroids, the run-in was completed in 2 weeks. If not, the run-in lasted 4 weeks in total. Caregivers recorded symptoms, health care utilization, and medication use in electronic diaries each day at bedtime (Spirotel, Medical International Research, Rome, Italy). Children were ineligible for randomization if they (1) completed less than 75% of daily electronic diaries, (2) received systemic corticosteroids for an asthma exacerbation, (3) had daily asthma symptoms if not receiving active therapy, or (4) had asthma symptoms more than 2 days per week if receiving active therapy.

Statistical methods

Statistical analyses were performed with IBM SPSS software (version 24). Glutathione, arginine, and ornithine variables were analyzed as raw variables and also after logarithmic transformation to normalize highly skewed variables. Baseline features of the participants and differences in baseline glutathione, arginine, and ornithine values were assessed with chi-square tests and ANOVA. Post hoc tests were not performed given nonsignificant group differences. Pearson correlations were used to assess associations between baseline plasma, arginine, and ornithine measures. Linear regression was used to assess associations between baseline glutathione, arginine, and ornithine values and the number of exacerbations over 48 weeks of follow-up. Models were adjusted for age group (12-24 months, 24-36 months, 36-48 months, and 48-60 months) and as-needed acetaminophen versus ibuprofen use over 48 weeks. Cox regressions were used to assess associations between glutathione, arginine, and ornithine tertiles and the time to first exacerbation. Models were adjusted for as-needed acetaminophen versus ibuprofen treatment and asthma controller medication receipt (ie, daily inhaled fluticasone, as-needed inhaled fluticasone, or montelukast) before the exacerbation onset. A significance threshold of .05 was used for all statistical tests. *P* values were not corrected for multiple comparisons given the exploratory nature of this analysis.

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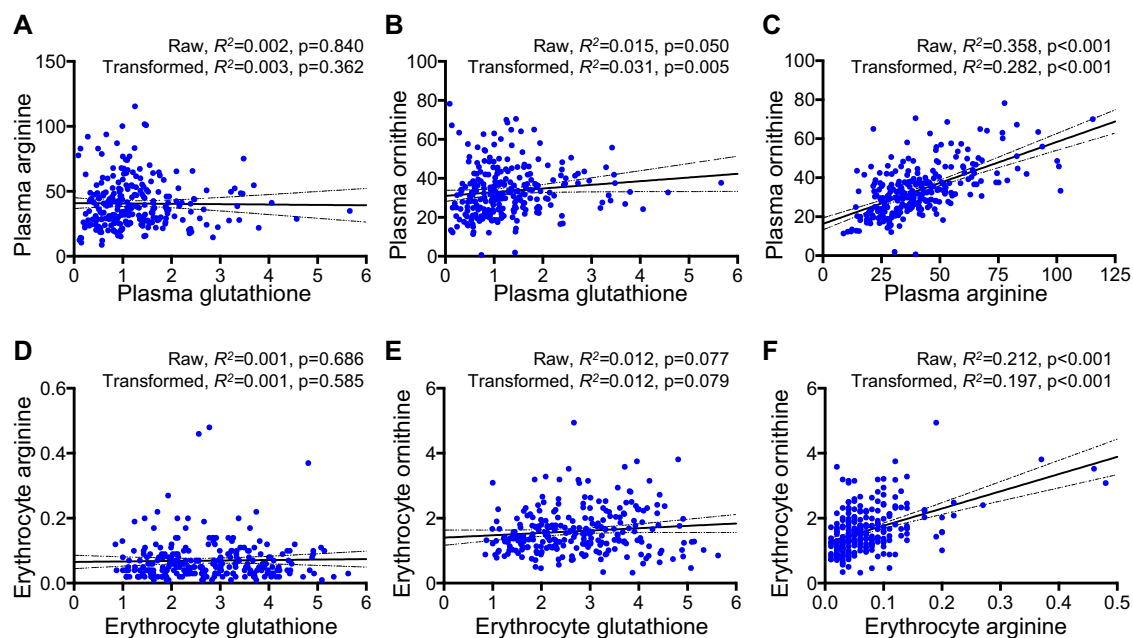


FIG E1. A-F, Associations between plasma and erythrocyte glutathione, arginine, and ornithine concentrations. Plasma and erythrocyte concentrations are expressed as $\mu\text{mol/L}$ and mmol/g hemoglobin, respectively. *Solid and dashed lines* represent the line of best fit and the 95% CI, respectively. R^2 values were obtained from Pearson correlation coefficients.

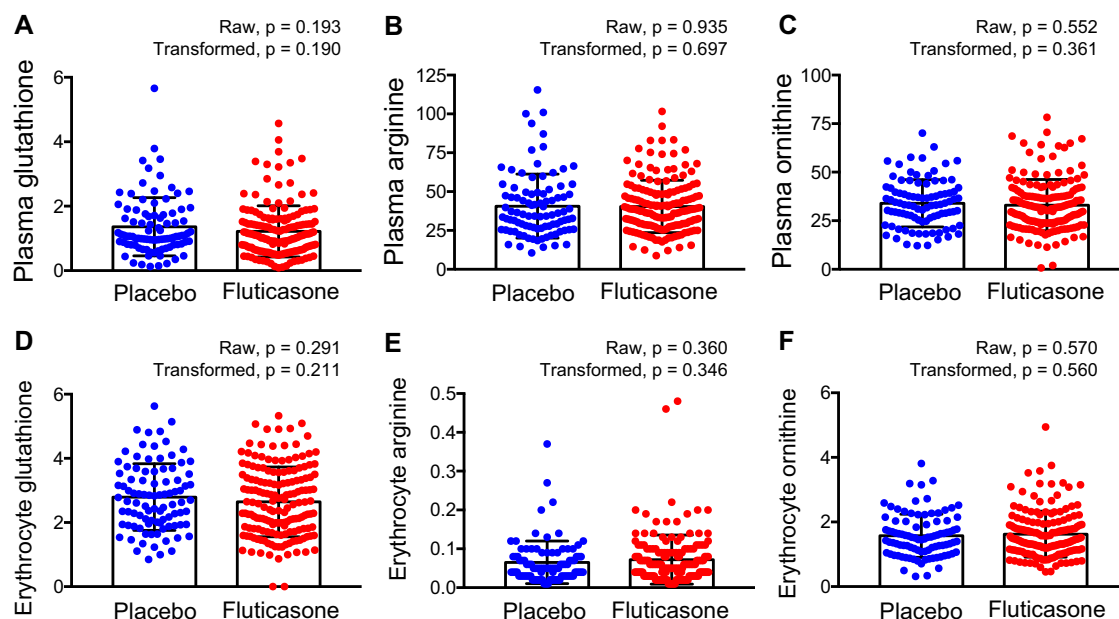


FIG E2. A-F, Plasma ($\mu\text{mol/L}$) and erythrocyte (mmol/g hemoglobin) glutathione, arginine, and ornithine stratified by placebo versus inhaled fluticasone receipt during the run-in period before sample collection. Dots represent individual participants; bars and whiskers represent the mean and SD, respectively.

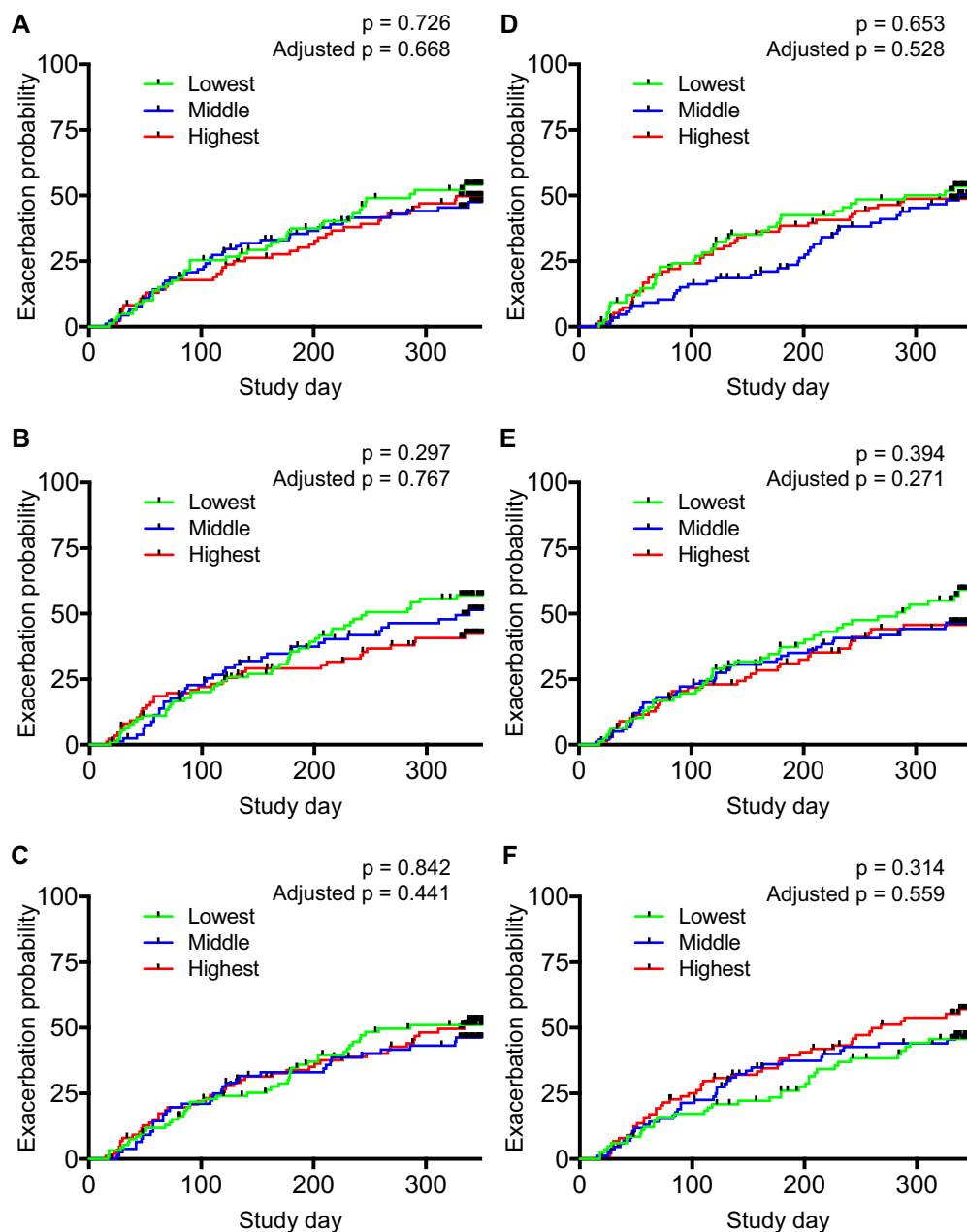


FIG E3. Time to first asthma exacerbation, stratified by tertile of baseline (A) plasma glutathione, (B) plasma arginine, (C) plasma ornithine, (D) erythrocyte glutathione, (E) erythrocyte arginine, and (F) erythrocyte ornithine. Black hash marks reflect censoring. Models were adjusted for age group (12-24, 24-36, 36-48, and 48-60 months) and as-needed acetaminophen versus ibuprofen treatment during the 48-week follow-up period.

TABLE E1. Features of the participants, as n (%) or mean $\pm$ SD

Feature	All trial participants (N = 300)	Participants with blood sample (N = 261)
Age at enrollment (mo)	39.9 $\pm$ 13.1	41.0 $\pm$ 12.9
Age at symptom onset (mo)	13.1 $\pm$ 10.2	12.9 $\pm$ 10.3
Males	179 (59.7)	158 (60.5)
White race	148 (49.3)	129 (49.4)
Tobacco smoke exposure	110 (36.7)	93 (35.6)
Wheezing episodes (past year)	5.9 $\pm$ 5.0	5.9 $\pm$ 5.1
Emergency department visits (past year)	3.0 $\pm$ 2.4	3.1 $\pm$ 2.4
Systemic corticosteroid courses (past year)	1.6 $\pm$ 1.4	1.6 $\pm$ 1.5
Run-in characteristics		
Fluticasone treatment	189 (63.0)	167 (64.0)
Asthma control days (% of days)*	85.5 $\pm$ 17.6	85.5 $\pm$ 17.4
Blood eosinophils (per μ L)	—	356.2 $\pm$ 307.2
Aeroallergen sensitization†	—	113 (43.3)
Plasma measures (μ M)		
Glutathione	—	1.3 $\pm$ 0.8
Arginine	—	40.5 $\pm$ 18.2
Ornithine	—	33.5 $\pm$ 13.2
Erythrocyte measures (mmol/g hemoglobin)		
Glutathione	—	2.7 $\pm$ 1.1
Arginine	—	0.07 $\pm$ 0.07
Ornithine	—	1.6 $\pm$ 0.7

Features of the participants with available blood samples were not significantly different from those of the larger sample.

*Defined as full calendar days without asthma symptoms, rescue medication use, or unscheduled health care visits.

†Defined as a specific IgE value of $\geq$ 0.35 kU/L.

TABLE E2. Linear regressions results with plasma ($\mu\text{mol/L}$) and erythrocyte (mmol/g hemoglobin) glutathione, arginine, and ornithine as predictors and the number of exacerbations over 48 weeks as an outcome variable

Model	Biomarker	Raw data			Transformed data		
		Unstandardized coefficient		P value	Unstandardized coefficient		P value
		B	SE		B	SE	
Model 1 (univariate)	Plasma glutathione	−0.043	0.074	.561	−0.152	0.188	.418
	Plasma arginine	−0.002	0.003	.498	−0.126	0.142	.377
	Plasma ornithine	0.003	0.005	.509	0.080	0.142	.573
	Erythrocyte glutathione	0.001	0.058	.987	−0.049	0.203	.808
	Erythrocyte arginine	−0.492	1.024	.631	−0.522	1.181	.659
	Erythrocyte ornithine	0.154	0.092	.097	0.418	0.249	.094
Model 2 (adjusted)*	Plasma glutathione	−0.045	0.074	.543	−0.149	0.187	.426
	Plasma arginine	−0.001	0.003	.678	−0.090	0.144	.532
	Plasma ornithine	0.004	0.005	.354	0.108	0.142	.449
	Erythrocyte glutathione	−0.007	0.058	.903	−0.082	0.203	.686
	Erythrocyte arginine	−0.352	1.023	.731	−0.368	1.179	.755
	Erythrocyte ornithine	0.159	0.092	.085	0.429	0.248	.084
Model 3 (adjusted)†	Plasma glutathione	−0.045	0.074	.542	−0.147	0.187	.433
	Plasma arginine	−0.001	0.003	.689	−0.091	0.144	.531
	Plasma ornithine	0.004	0.005	.372	0.103	0.143	.471
	Erythrocyte glutathione	−0.005	0.058	.931	−0.075	0.203	.714
	Erythrocyte arginine	−0.361	1.024	.724	−0.376	1.181	.751
	Erythrocyte ornithine	0.159	0.092	.085	0.426	0.248	.087

*Adjusted for age group (12-24 mo, 24-36 mo, 36-48 mo, and 48-60 mo).

†Adjusted for age group and as-needed acetaminophen vs ibuprofen treatment assignment over 48 weeks.

TABLE E3. Plasma glutathione, arginine, and ornithine concentrations ($\mu\text{mol/L}$) and asthma exacerbations over 48 weeks, stratified by acetaminophen vs ibuprofen treatment

Biomarker	Exacerbations	Acetaminophen			Ibuprofen		
		N	Median (25, 75 percentile)	Mean $\pm$ SD	N	Median (25, 75 percentile)	Mean $\pm$ SD
Glutathione	0	65	1.11 (0.75, 1.65)	1.32 $\pm$ 0.88	72	1.00 (0.69, 1.47)	1.19 $\pm$ 0.71
	1	35	1.10 (0.61, 1.44)	1.29 $\pm$ 1.03	30	1.42 (1.02, 2.08)	1.54 $\pm$ 0.82
	2	14	0.84 (0.46, 2.09)	1.22 $\pm$ 1.00	19	0.89 (0.56, 1.68)	1.15 $\pm$ 0.83
	3	14	1.05 (0.61, 1.36)	1.06 $\pm$ 0.54	10	1.20 (0.68, 1.47)	1.18 $\pm$ 0.79
Arginine	0	65	40.68 (31.66, 52.26)	42.78 $\pm$ 16.94	72	38.03 (27.55, 51.98)	41.76 $\pm$ 20.07
	1	35	33.95 (27.70, 45.54)	37.88 $\pm$ 15.38	30	32.33 (25.48, 45.83)	34.56 $\pm$ 12.98
	2	14	31.89 (23.87, 43.95)	41.30 $\pm$ 25.31	19	36.55 (28.01, 45.05)	41.30 $\pm$ 19.17
	3	14	33.79 (23.90, 43.61)	34.41 $\pm$ 13.55	10	57.70 (27.13, 73.85)	50.85 $\pm$ 25.57
Ornithine	0	65	33.02 (27.83, 38.95)	35.06 $\pm$ 12.01	72	29.52 (22.63, 38.38)	31.03 $\pm$ 12.33
	1	35	30.13 (21.67, 39.36)	32.87 $\pm$ 15.30	30	33.04 (24.65, 40.40)	33.69 $\pm$ 12.01
	2	14	34.62 (25.30, 42.91)	35.25 $\pm$ 13.86	19	33.24 (23.86, 44.76)	34.81 $\pm$ 13.21
	3	14	33.00 (22.17, 37.61)	30.65 $\pm$ 10.62	10	38.82 (22.57, 49.33)	38.80 $\pm$ 15.55

Differences were not significantly different.

TABLE E4. Erythrocyte glutathione, arginine, and ornithine concentrations (mmol/g hemoglobin) and asthma exacerbations over 48 weeks, stratified by acetaminophen vs ibuprofen treatment

Biomarker	Exacerbations	Acetaminophen			Ibuprofen		
		N	Median (25, 75 percentile)	Mean $\pm$ SD	N	Median (25, 75 percentile)	Mean $\pm$ SD
Glutathione	0	65	2.59 (1.59, 3.47)	2.64 $\pm$ 1.12	72	2.65 (1.99, 3.55)	2.80 $\pm$ 1.05
	1	35	2.74 (1.86, 3.18)	2.64 $\pm$ 0.98	30	2.37 (1.85, 3.20)	2.57 $\pm$ 0.91
	2	14	2.12 (1.58, 3.51)	2.27 $\pm$ 1.14	19	3.17 (2.28, 4.01)	3.05 $\pm$ 1.28
	3	14	3.23 (1.70, 3.91)	2.97 $\pm$ 1.13	10	2.48 (1.41, 3.01)	2.49 $\pm$ 1.03
Arginine	0	65	0.07 (0.03, 0.10)	0.08 $\pm$ 0.08	72	0.06 (0.04, 0.08)	0.07 $\pm$ 0.05
	1	35	0.05 (0.03, 0.08)	0.06 $\pm$ 0.05	30	0.05 (0.03, 0.08)	0.06 $\pm$ 0.04
	2	14	0.05 (0.03, 0.10)	0.07 $\pm$ 0.06	19	0.05 (0.04, 0.11)	0.08 $\pm$ 0.06
	3	14	0.03 (0.02, 0.10)	0.06 $\pm$ 0.05	10	0.07 (0.04, 0.12)	0.08 $\pm$ 0.05
Ornithine	0	65	1.42 (1.13, 1.83)	1.57 $\pm$ 0.58	72	1.41 (0.94, 1.82)	1.51 $\pm$ 0.71
	1	35	1.47 (1.09, 1.94)	1.59 $\pm$ 0.68	30	1.51 (1.08, 1.96)	1.59 $\pm$ 0.68
	2	14	1.48 (1.24, 2.14)	1.62 $\pm$ 0.52	19	1.68 (1.34, 2.11)	1.84 $\pm$ 0.62
	3	14	1.34 (1.08, 2.51)	1.70 $\pm$ 0.87	10	1.75 (1.24, 2.63)	2.03 $\pm$ 1.23

Differences were not significantly different.