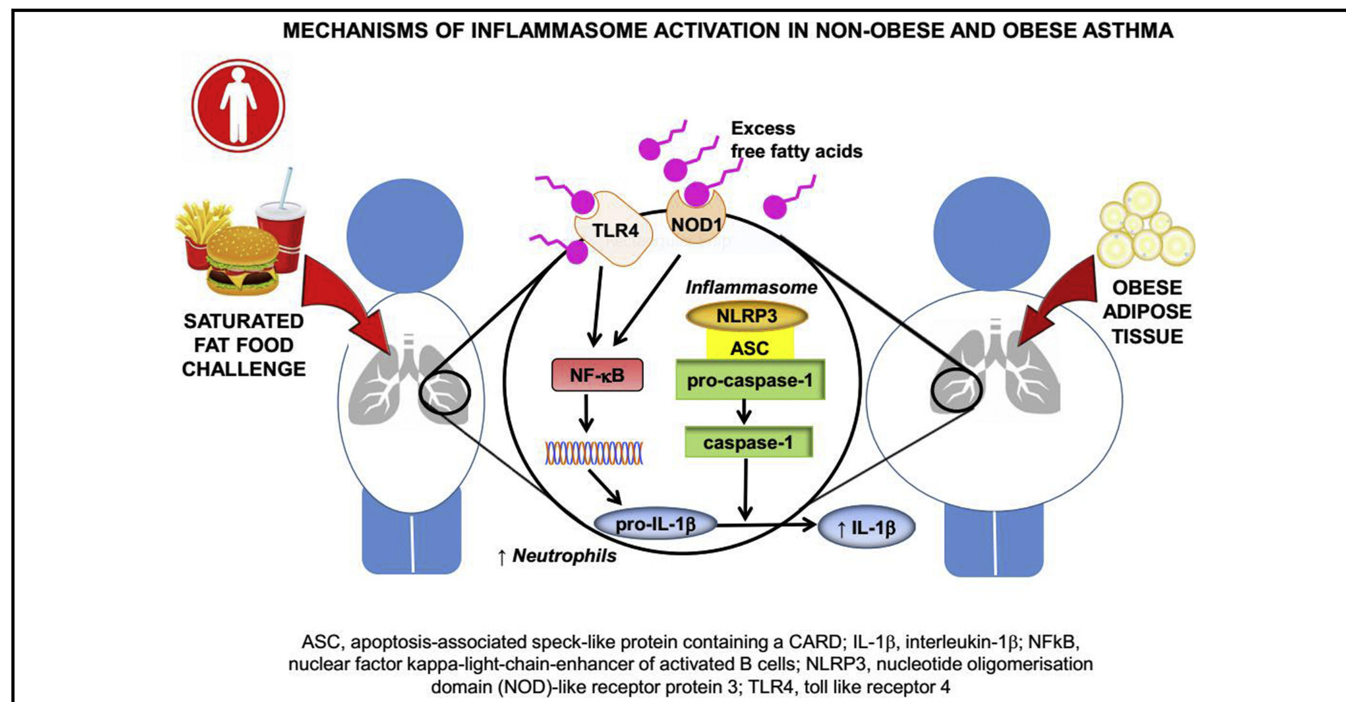
Saturated fatty acids, obesity, and the nucleotide oligomerization domain–like receptor protein 3 (NLRP3) inflammasome in asthmatic patients

Lisa G. Wood, PhD,[a] Qian Li, PhD,[a] Hayley A. Scott, PhD,[a] Sandra Rutting, MSc,[a,c] Bronwyn S. Berthon, PhD,[a] Peter G. Gibson, MBBS,[a,b] Philip M. Hansbro, PhD,[a] Evan Williams, BBSc,[a] Jay Horvat, PhD,[a] Jodie L. Simpson, PhD,[a] Paul Young, PhD,[c] Brian G. Oliver, PhD,[c] and Katherine J. Baines, PhD[a] *Newcastle and Sydney, Australia*

GRAPHICAL ABSTRACT



Background: Both obesity and high dietary fat intake activate the nucleotide oligomerization domain–like receptor protein 3 (NLRP3) inflammasome.

Objective: We aimed to examine NLRP3 inflammasome activity in the airways of obese asthmatic patients after macronutrient overload and in immune cells challenged by inflammasome triggers.

Methods: Study 1 was a cross-sectional observational study of nonobese (n = 51) and obese (n = 76) asthmatic adults. Study 2 was a randomized, crossover, acute feeding study in 23 asthmatic adults (n = 12 nonobese and n = 11 obese subjects). Subjects consumed 3 isocaloric meals on 3 separate occasions (ie, saturated fatty acid, n-6 polyunsaturated fatty acid, and carbohydrate) and were assessed at 0 and 4 hours. For Studies 1 and 2, airway inflammation was measured based on sputum differential cell counts, IL-1β protein levels (ELISA), and sputum cell gene expression (Nanostring nCounter). In Study 3 peripheral blood neutrophils and monocytes were isolated by using Ficoll density gradient and magnetic bead separation and incubated with or without palmitic acid, LPS, or TNF-α for 24 hours, and IL-1β release was measured (ELISA).

Results: In Study 1 *NLRP3* and nucleotide oligomerization domain 1 (*NOD1*) gene expression was upregulated, and sputum IL-1β protein levels were greater in obese versus nonobese

From [a]the Priority Research Centre for Healthy Lungs, University of Newcastle; [b]the Department of Respiratory and Sleep Medicine, John Hunter Hospital, Newcastle; and [c]the Woolcock Institute of Medical Research, Sydney.

The project was funded by the National Health and Medical Research Council (NHMRC) of Australia. P.G.G. is supported by an NHMRC Practitioner Fellowship, and P.M.H. is supported by an NHMRC Principal Research Fellowship and a Brawn Fellowship, Faculty of Health and Medicine, University of Newcastle.

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

Received for publication November 13, 2017; revised March 29, 2018; accepted for publication April 30, 2018.

Available online May 30, 2018.

Corresponding author: Lisa G. Wood, PhD, Centre for Healthy Lungs, Level 2, West Wing, HMRI Building, Kookaburra Crt, New Lambton Height, NSW, Australia, 2305. E-mail: lisa.wood@newcastle.edu.au.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/$36.00

© 2018 American Academy of Allergy, Asthma & Immunology

https://doi.org/10.1016/j.jaci.2018.04.037

asthmatic patients. In Study 2 the saturated fatty acid meal led to increases in sputum neutrophil percentages and sputum cell gene expression of Toll-like receptor 4 (*TLR4*) and *NLRP3* at 4 hours in nonobese asthmatic patients. In Study 3 neutrophils and monocytes released IL-1β when challenged with a combination of palmitic acid and LPS or TNF-α.
Conclusion: The NLRP3 inflammasome is a potential therapeutic target in asthmatic patients. Behavioral interventions that reduce fatty acid exposure, such as weight loss and dietary saturated fat restriction, warrant further exploration. (J Allergy Clin Immunol 2019;143:305-15.)

***Key words:** Fatty acids, saturated fat, obesity, inflammasome, airway inflammation, IL-1β, asthma*

Abbreviations used
BMI: Body mass index
FA: Fatty acid
IL-1R: IL-1 receptor
IL-1RAP: IL-1 receptor accessory protein
NF-κB: Nuclear factor kappa light chain enhancer of activated B cells
NLR: NOD-like receptor
NLRP3: Nucleotide oligomerization domain–like receptor protein 3
NOD: Nucleotide oligomerization domain
PUFA: Polyunsaturated fatty acid
SFA: Saturated fatty acid
TLR: Toll-like receptor

Asthma is a chronic inflammatory airways disease that affects 300 million persons worldwide, with prevalence rates of greater than 10% in many westernized countries.[1] Obesity rates are also alarmingly high, with more than 20% of adults in developed countries estimated to be obese.[2] Obesity increases asthma risk, and asthma in obese patients is recognized as a distinct clinical phenotype[3] that is difficult to manage and characterized by worse lung function[4] and symptoms[5] and reduced response to asthma pharmacotherapy, including glucocorticoids.[6-8] The unique inflammatory profile induced by excess adipose tissue in obesity likely contributes to this phenotype.[8]

In obese subjects adipocytes and adipose tissue–resident macrophages release proinflammatory mediators, such as TNF-α and IL-6, leading to chronic systemic inflammation. Integral to this process is activation of the nucleotide oligomerization domain–like receptor protein 3 (NLRP3).[9] NLRP3 can be activated by excess saturated fatty acids (SFAs), cholesterol, and cellular debris after adipocyte apoptosis, leading to assembly of the NLRP3 inflammasome, which recruits and activates caspase-1, leading to IL-1β secretion.[9] We[10] and others[11,12] have described increased airway neutrophilia in patients with obese asthma. Whether NLRP3 inflammasome activation contributes to neutrophil influx in obese patients with asthma is unknown.

Independent of the effects of excess adipose tissue, macronutrient loading, which is common in obese subjects, induces postprandial inflammation through mechanisms, such as direct activation of innate immune receptors and endoplasmic reticulum stress.[13] We have shown previously that a high-energy fast-food meal induces airway neutrophilia and upregulates sputum cell Toll-like receptor (*TLR*) 4 gene expression in asthmatic patients.[14] However, the possible role of NLRP3 inflammasome activity in postprandial airway inflammation has not been determined. Furthermore, the contribution of different macronutrients to postprandial airway inflammation has not been examined. Addressing these key questions will provide a better understanding of the nature of airway inflammation in obese patients with asthma, which will enable the development of more effective treatment strategies for this subgroup of asthmatic patients.

The aims of this study were to examine (1) the activity of the NLRP3 inflammasome in the airways of obese patients with asthma, (2) the effect of macronutrient (SFA, n-6 polyunsaturated fatty acid [PUFA], and carbohydrate) overload on inflammation and NLRP3 inflammasome activity in asthmatic airways, and (3) the effect of SFAs on NLRP3 inflammasome activity in specific immune cells (neutrophils and monocytes).

METHODS

Study 1: Obese versus nonobese asthmatic patients—Cross-sectional comparison of inflammatory pathways

A cross-sectional observational study was conducted in 127 adult asthmatic patients categorized as nonobese (body mass index [BMI], <30 kg/m^2; n = 51) or obese (BMI, ≥30 kg/m^2; n = 76). Data from a subset of these subjects have been reported previously.[14-16] Subjects fasted overnight, and asthma medications were withheld (short-acting bronchodilators, 6 hours; long-acting bronchodilators and inhaled corticosteroids, 24 hours). Blood was collected, and spirometry and sputum induction were performed during hypertonic saline challenge.[17]

Study 2: Effects of acute meal challenge on airways of asthmatic patients—SFAs and n-6 PUFAs versus carbohydrates

A randomized crossover trial was conducted in 23 adults (n = 12 nonobese and n = 11 obese adults) with stable asthma. Before each visit, subjects fasted overnight, and asthma medications were withheld (as above). At 0 hours, blood was collected, and sputum was induced (as above),[17] and then the study meal was consumed. At 4 hours, blood and induced sputum were collected again. On the following visits, subjects repeated these procedures with an alternate study meal. Meals were consumed in random order, with a minimum washout period of 7 days between visits.

Three different isocaloric meals were tested that were rich in either SFAs, n-6 PUFAs, or carbohydrates. At 0 hours, subjects consumed 200 g of potato. Subjects also consumed double cream and butter (SFA meal), safflower oil (n-6 PUFA meal), or glucose confectionary (carbohydrate meal). Meals were timed to ensure peak nutrient concentrations at 4 hours: 0 hours for the fatty acid (FA) meals and 2 hours for the carbohydrate meal. The nutrient composition of the meals is described in Table I. Studies 1 and 2 were approved by the Hunter New England and University of Newcastle Human Research Ethics Committees. Written informed consent was obtained from all subjects. Study 2 was prospectively registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12612000697886).

Procedures

Subject characterization. Subjects were recruited from ambulatory care clinics at John Hunter Hospital, Newcastle, Australia. Asthma was defined by clinical history and airway hyperresponsiveness to hypertonic saline (4.5%), which was defined as a 15% or greater decrease in FEV_1 from baseline. Stable asthma was defined as no exacerbation, respiratory tract infection, or oral corticosteroid use in the past 4 weeks. Skin prick allergy tests determined atopic status.

Blood collection and processing. Blood was collected into EDTA tubes, and full blood counts were performed with a Beckman Coulter LH series analyzer (Beckman Coulter, Brea, Calif) by Hunter Area

TABLE I. Nutrient composition of the isocaloric study meals: SFA, n-6 PUFA, and carbohydrate meals

	Carbohydrate meal	n-6 PUFA meal	SFA meal
Energy (kJ)	1850	1740	1840
Fat (g)	<1	47	48
Fat (% energy)	<1	100	>98
SFAs (g)	0	4	34
SFAs (% energy)	0	9	70
n-6 PUFA (g)	0	33	2
n-6 PUFA (% energy)	0	72	4
Carbohydrate (g)	100	0	<1
Carbohydrate (% energy)	>99	0	<1
Protein (g)	0	0	<1
Protein (% energy)	0	0	<1

Subjects consumed these meals after an overnight fast in addition to 200 g of mashed potato provided at time 0 hours (720 kJ, 0.2 g of fat, and 40 g of carbohydrate).

Pathology Service (Newcastle, Australia). In addition, plasma was separated by means of centrifugation (at 4°C for 10 minutes at 3000*g*) and stored at −80°C for FA analysis, as described previously,[18] by using gas chromatography with a 30 × 0.25–mm (DB-225) fused carbon-silica column coated with cyanopropylphenyl (J&W Scientific, Folsom, Calif) and flame ionization detector (Hewlett-Packard 6890 Series Gas Chromatograph with Chemstations software, version A.04.02; Hewlett-Packard, Palo Alto, Calif).

Sputum collection and processing. Lower respiratory tract sputum portions were selected and dispersed by using dithiothreitol, as previously described.[17] Total cell counts and viability were performed by using a hemocytometer, and cytospins were used for differential cell counts. Sputum supernatant concentrations of IL-1β were measured with ELISA DuoSet (R&D Systems, Minneapolis, Minn) and validated for use in sputum.[19] For gene expression analysis, 100 μL of selected sputum was added to Buffer RLT (Qiagen, Hilden, Germany) and stored at −80°C for RNA extraction.

Nanostring analysis. RNA was extracted from sputum with the RNeasy Mini Kit (Qiagen) and quantitated by using the Quant-iT RiboGreen RNA Quantitation Assay Kit (Molecular Probes, Life Technologies, Carlsbad, Calif). With the Nanostring nCounter Analysis System (Nanostring Technologies, Seattle, Wash), gene expression was analyzed by using a custom-designed code set containing 249 inflammation-related genes with 6 reference genes.

Study 3: *In vitro* investigation of the effects of SFAs on neutrophils and monocytes

Peripheral blood neutrophil and monocyte isolation. Peripheral blood was collected from healthy volunteers and neutrophils, and monocytes were isolated. Blood collected in citrate dextrose tubes was mixed with 10% dextran (MP Biomedicals, Santa Ana, Calif), and the top layer was overlayed on Ficoll Paque Plus density gradient medium (GE Healthcare, Little Chalfont, United Kingdom) and centrifuged (for 10 minutes at 2000*g*). Mononuclear cells at the interface of plasma and the Ficoll layer were collected, and monocytes were isolated with $CD14^+$ magnetic beads. Red blood cells were lysed, and neutrophils were positively selected by using $CD16^+$ magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany).[20] Neutrophils and monocytes were resuspended in RPMI 1640 containing 1% HEPES (Life Technologies, Mulgrave, Australia), 1% FBS, and 1% antibiotic-antimycotic and seeded (1×10^6 cells/mL).

Cell treatment. Cells were treated with palmitic acid (C16:0). Stock solutions of 0.5 mol/L C16:0 (Sigma-Aldrich, St Louis, Mo) were prepared in 100% ethanol and stored at −20°C. Working solutions of 10 mmol/L were generated by incubating C16:0 in FA-free BSA (5:1; Sigma Aldrich) at 65°C for 10 minutes and then at 39°C for 90 minutes with occasional vortexing. Neutrophils and monocytes were stimulated with 0 or 100 μmol/L palmitic acid and vehicle (EtOH/BSA/cell culture medium) with or without LPS (1 μg/mL; Sigma-Aldrich) or TNF-α (1 ng/mL; Sigma-Aldrich) and then incubated at 37°C with 5% CO_2 for 24 hours. Cell-free supernatants were stored at −80°C for measurement of IL-1β by means of ELISA (R&D Systems).

Statistical analysis

Analysis was conducted with Stata 11 software (StataCorp, College Station, Tex). Parametric and nonparametric data are presented as means ± SDs and medians (quartile 1-quartile 3), respectively. Comparisons were conducted by using the Student *t* test, Mann-Whitney *U* test, or Wilcoxon signed-rank test for quantitative data and the χ^2 or Fisher exact test for frequency data. Associations between variables were examined by using the Pearson correlation coefficient for parametric data and the Spearman rank correlation coefficient for nonparametric data. For the acute meal study (Study 2), changes within the intervention group compared with baseline were compared by using the Wilcoxon signed-rank test. Differences between intervention groups were compared by using general linear models, with atopy as a covariate. Nanostring data were analyzed by using nSolver Analysis Software v2.5 (Nanostring Technologies). Raw counts were normalized to 6 positive controls and the reference gene phosphoglycerate kinase 1 *(PGK1)* and log-transformed. Genes with a fold change of greater than 1.5 were assessed by using unpaired (Study 1) or paired (Study 2) *t* tests with the Benjamini-Hochberg adjustment for multiple comparisons (false discovery rate, 0.25). Search Tool for the Retrieval of Interacting Genes v10 (http://string-db.org)[21] was used to investigate pathway interactions between differentially expressed genes with medium confidence scores of greater than 0.4. In Study 1 Nanostring gene expression analysis was performed in a subset of obese (n = 11) and nonobese (n = 14) asthmatic patients. In Study 2 Nanostring analysis was performed in the nonobese subjects for whom paired sputum samples were available from both 0 and 4 hours after the SFA meal (n = 4).

RESULTS

Obese versus nonobese asthmatic patients: Cross-sectional comparison of inflammatory pathways

Subjects' characteristics are described in Table II. Obese asthmatic patients had higher plasma levels of total FAs, SFAs, monounsaturated FAs, C16:0, C18:0, and C20:4n-6 compared with nonobese asthmatic patients. Nanostring analysis of sputum cell gene expression in obese versus nonobese asthmatic patients identified 13 genes with a fold change of greater than 1.5 that were expressed differentially (Table III). The most significantly upregulated gene in obese asthmatic patients was *IL5*, followed by nucleotide oligomerization domain 1 *(NOD1)*. *IL5* expression was positively associated with *NLRP3* expression (r = 0.483, P = .015).

Because *NLRP3* gene expression was upregulated in obese asthmatic patients, we measured sputum IL-1β concentrations, a marker of NLRP3 activity. Sputum IL-1β concentrations were greater in obese versus nonobese asthmatic patients (Fig 1). Sputum cell *NLRP3* gene expression and sputum IL-1β protein concentrations correlated with BMI (Fig 2, *A* and *B*). Sputum cell *NLRP3* gene expression correlated with sputum IL-1β protein concentrations (Fig 2, *C*). *TLR4* gene expression correlated with BMI (r = 0.448, P = .025) and sputum neutrophil percentages (r = 0.435, P = .030). When analyzed by sex, in female subjects only (n = 14), *IL-1β* gene expression correlated with *NLRP3* gene expression (r = 0.877, P < .001), *TLR4* gene expression (r = 0.697, P = .007), and sputum neutrophil percentages (r = 0.775, P = .003) and inversely correlated with percent predicted FEV_1 (r = −0.649, P = .014) and percent predicted forced vital capacity (r = −0.613, P = .022).

Effect of acute meal challenge on asthmatic airways: SFAs and n-6 PUFAs versus carbohydrates

Subjects' characteristics are described in Table IV. At 4 hours after the n-6 PUFA meal challenge, plasma n-6 PUFA levels

TABLE II. Clinical characteristics, sputum cell counts, and plasma FA concentrations of obese and nonobese subjects in Study 1

	Nonobese subjects (n = 51)	Obese subjects (n = 76)	*P* value§
Clinical characteristics			
Age (y)	48 (16)	52 (14)	.231
Sex (M/F)	19/32	33/43	.488
BMI (kg/m^2)*	26.1 ± 2.7	35.6 ± 4.8	NA
Atopy, no. (%)	30 (70)	33 (73)	.711
FEV_1 (% predicted)*	79.4 ± 19.6	81.3 ± 18.8	.591
FVC (% predicted)*	94.1 ± 16.8	88.9 ± 16.1	.080
FEV_1/FVC ratio (%)*	67.7 ± 10.9	72.8 ± 9.6	.006
ICS dose (μg/d)‡	500 (0-1000)	500 (200-1000)	.355
SABA use, no. (%)	45 (88)	67 (88)	.989
LABA use, no. (%)	31 (61)	55 (72)	.173
ACQ (units)	1.0 (0.4-1.6)	1.0 (0.6-1.4)	.668
eNO (ppb)	18 (10-38)	20 (13-31)	.568
Sputum cell counts†			
Total cell count (10^6/mL)	3.1 (2.4-5.3)	2.1 (0.8-5.0)	.013
Neutrophils (%)	38.0 (24.0-54.8)	49.5 (24.5-64.3)	.305
Neutrophils (10^4/mL)	128.0 (53.0-301.0)	94.7 (24.4-259.2)	.177
Eosinophils (%)	1.5 (0.7-6.4)	2.0 (0.5-4.8)	.932
Eosinophils (10^4/mL)	6.7 (2.1-18.3)	5.1 (0.9-13.4)	.287
Macrophages (%)	52.8 (35.8-65.5)	43.5 (29.3-64.8)	.341
Macrophages (10^4/mL)	177.5 (114.1-235.3)	98.3 (34.1-202.9)	.001
Plasma fatty acids (mg/L)*			
C10:0	1.1 ± 0.2	1.7 ± 1.1	.208
C14:0	37.2 ± 3.2	39.3 ± 3.5	.374
C16:0	710.0 ± 39.4	807.8 ± 45.5	.042
C18:0	214.9 ± 11.5	245.3 ± 12.7	.014
C18:2 n-6	877.9 ± 30.8	940.3 ± 41.0	.274
C20:4 n-6	313.1 ± 14.8	357.1 ± 14.9	.022
SFA	1017.0 ± 54.9	1154.1 ± 63.3	.035
MUFA	887.1 ± 55.1	1042.4 ± 58.8	.006
PUFA	1571.2 ± 49.9	1701.4 ± 62.8	.107
Total FA	3475.3 ± 153.7	3897.9 ± 175.9	.029

ACQ, Asthma Control Questionnaire; *C10:0*, capric acid; *C14:0*, myristic acid; *C16:0*, palmitic acid; *C18:0*, stearic acid; *C18:2 n-6*, linoleic acid; *C20:4 n-6*, arachidonic acid; *eNO*, exhaled nitric oxide; *FVC*, forced vital capacity; *ICS*, inhaled corticosteroids; *MUFA*, monounsaturated fatty acids; *NA*, not applicable.
*Mean ± SD.
†Median (quartile 1–3).
‡Beclomethasone equivalents.
§Parametric data were analyzed by using the unpaired *t* test, and nonparametric data were analyzed by using the Mann-Whitney *U* test.

TABLE III. Sputum cell genes differentially expressed (>1.5 fold change) in obese (n = 11) versus nonobese (n = 14) asthmatic patients in Study 1

Gene symbol	Gene name	Fold change	*P* value (obese vs nonobese subjects)
IL5	Interleukin 5	5.4	.003
NOD1	Nucleotide-binding oligomerization domain containing 1	1.8	.015
TREM2	Triggering receptor expressed on myeloid cells 2	−2.4	.023
GNGT1	G protein subunit gamma transducin 1	3.1	.024
FASLG	Fas ligand	4.2	.025
IRF7	Interferon regulatory factor 7	1.5	.031
NLRP3	NLR family, pyrin domain containing 3	2.5	.035
IL9	Interleukin 9	3.1	.041
IL12B	Interleukin 12B	4.0	.046
TBXA2R	Thromboxane A2 receptor	1.8	.046
C1R	Complement component 1, r subcomponent	3.0	.048
TGFBR1	Transforming growth factor beta receptor 1	−1.5	.048
TGFB2	Transforming growth factor beta 2	2.2	.050

Data were assessed by using unpaired *t* tests with the Benjamini-Hochberg adjustment for multiple comparisons.

increased compared with baseline (total n-6 PUFA: +11.9%, $P = .005$; 18:2n-6: +16%, $P = .003$). At 4 hours after the SFA meal challenge, plasma SFA levels increased compared with baseline values (total SFA: +11.9%, $P = .005$; C16:0: +20.9%, $P = .001$; stearic acid: +19.7%, $P = .044$). After the carbohydrate meal, there were no changes in plasma FA concentrations.

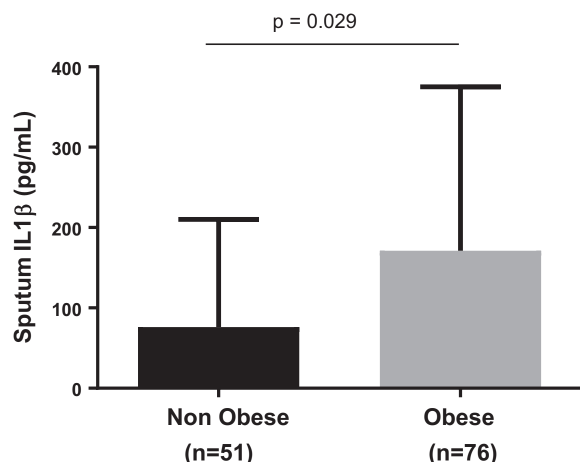


FIG 1. IL-1β protein concentration in sputum supernatants from nonobese (n = 51) versus obese (n = 76) asthmatic patients. Data were analyzed with the Mann-Whitney *U* test.

Changes in inflammatory cell counts after each intervention. Changes in blood and induced sputum leukocyte counts after each intervention are described in Table V. In blood total white cell, lymphocyte, and monocyte counts increased in each intervention group at 4 hours compared with baseline values. Blood neutrophil counts increased after the fat meals (SFA and n-6 PUFA) but not the carbohydrate meal. The increases in white cell and neutrophil counts were greater after the SFA meal than the carbohydrate meal. In induced sputum total cell and macrophage counts decreased at 4 hours versus baseline after both the carbohydrate and n-6 PUFA meals. An increase in eosinophil counts was evident in the n-6 PUFA group only.

When analyzed according to BMI category, in blood from both nonobese and obese asthmatic patients, total white cell and neutrophil counts were increased at 4 hours compared with baseline values after the fat meals (SFA and n-6 PUFA; Fig 3, *A*). In the nonobese group the change in white cell and neutrophil counts was greater after the fat meals than after the carbohydrate meal (Fig 3, *A*).

In sputum from nonobese subjects, neutrophil percentages increased after the SFA meal only (Fig 3, *B*). Total cell counts decreased after the carbohydrate and n-6 PUFA meals but not the SFA meal at 4 hours compared with baseline (Fig 3, *B*). In sputum from obese subjects, the only significant change was a decrease in total cell counts after the carbohydrate meal at 4 hours compared with baseline (Fig 3, *B*).

Effect of SFA on sputum cell gene expression in nonobese asthmatic patients. Nanostring analysis in sputum from nonobese subjects identified 9 genes with a fold change of greater than 1.5 that were differentially expressed at 4 hours after the SFA meal compared with baseline values (Table VI). From these genes, a main gene network containing 8 genes was identified (Fig 4).

In vitro investigation of effects of SFAs on neutrophils and monocytes

In both neutrophils and monocytes, *ex vivo* exposure to C16:0 alone did not induce IL-1β release. However, in both cell types the combination of C16:0 and LPS or TNF-α led to increased IL-1β release (Fig 5).

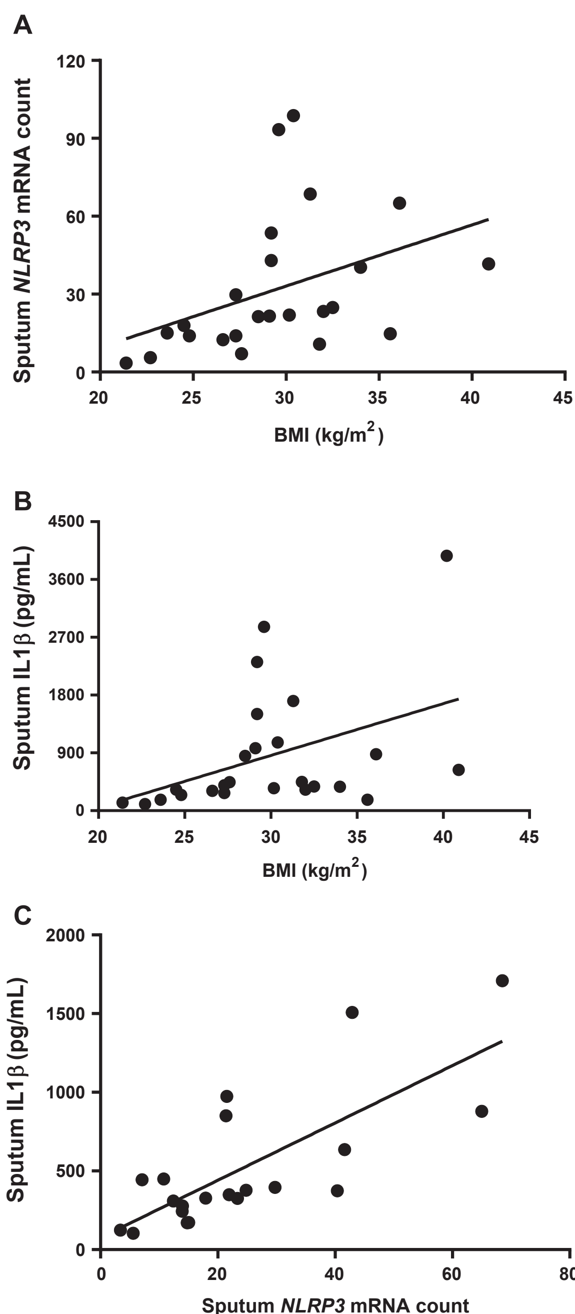


FIG 2. Associations between BMI and NLRP3 and IL-1β levels. **A,** Sputum cell NLRP3 gene expression and BMI (r = 0.585, P = .003). **B,** Sputum supernatant IL-1β concentration and BMI (r = 0.496, P = .012). **C,** Sputum cell NLRP3 gene expression and sputum supernatant IL-1β concentration (r = 0.679, P < .001). Associations were examined by using the Spearman rank correlation coefficient.

DISCUSSION

This study provides important new insight into airway NLRP3 inflammasome activity in asthmatic patients with a high BMI and after excess macronutrient intake. We have shown increased circulating FA levels, activation of the innate immune receptor *NOD1*, and increased *NLRP3* activity and IL-1β protein levels in obese asthmatic patients. We have also determined that SFAs induce postprandial airway inflammation with increased *TLR4*, *NLRP3*, and IL-1 pathway gene expression in nonobese

TABLE IV. Characteristics of asthmatic patients who completed the acute meal challenge study in Study 2 (visit 1, time = 0 hours)

	All samples	Nonobese subjects	Obese subjects	*P* value (obese vs nonobese subjects)*
No.	23	12	11	NA
Age (y)	46.0 (16.5)	41.2 (16.8)	51.3 (15.1)	.145
Sex (F/M)	16/7	7/5	9/2	.221
BMI (kg/m^2), mean ± SD	30.3 ± 4.7	26.7 ± 2.0	34.3 ± 3.5	NA
Atopy, no. (%)	13 (62)	8 (67)	5 (56)	.060
FEV_1 (%), mean ± SD	80.9 ± 19.6	79.6 ± 22.9	82.5 ± 16.3	.730
FVC (%), mean ± SD	87.9 ± 14.9	88.3 ± 15.7	87.4 ± 14.7	.887
FEV_1/FVC ratio (%), mean ± SD	73.4 ± 11.8	72.4 ± 14.4	74.6 ± 8.7	.665
ACQ (units), median (Q1-Q3)	0.7 (0.4-1.1)	0.8 (0.3-1.0)	0.7 (0.6-0.7)	.337
ICS use (μg/d),† median (Q1-Q3)	1000 (750-2000)	625 (0-1000)	1000 (0-1750)	.459
Sputum cell counts, median (Q1-Q3)				
Total cell count (×10^6/mL)	3.7 (1.7-5.5)	2.5 (1.4-6.3)	4.7 (2.3-5.6)	.472
Neutrophils (%)	24.8 (12.7-48.8)	23.5 (14.0-43.3)	25.3 (10.4-55.2)	.762
Eosinophils (%)	0.8 (0.5-2.4)	0.8 (0.4-2.7)	1.3 (0.4-2.6)	.593
Macrophages (%)	64.0 (44.1-78.4)	58.3 (48.6-71.4)	70.0 (39.8-80.7)	.650
Blood cell counts (× 10^9/L), median (Q1-Q3)				
Total white cells	5.9 (5.0-6.8)	5.2 (4.4-7.0)	6.0 (5.5-6.7)	.308
Neutrophils	3.4 (2.8-3.8)	3.3 (2.3-3.6)	3.4 (3.2-4.2)	.411
Lymphocytes	1.9 (1.5-2.1)	1.6 (1.3-2.0)	1.9 (1.5-2.2)	.339
Monocytes	0.4 (0.3-0.6)	0.4 (0.3-0.6)	0.4 (0.4-0.6)	.787
Eosinophils	0.1 (0.1-0.3)	0.2 (0.1-0.3)	0.1 (0.1-0.3)	.697

Kruskal-Wallis testing confirmed that there were no differences in baseline (time = 0 hours) cell counts for each of the study meals.
FVC, Forced vital capacity.
*Parametric data were analyzed by using the unpaired *t* test, and nonparametric data were analyzed by using the Mann-Whitney *U* test.
†Beclomethasone equivalents.

TABLE V. White blood cell and sputum supernatant counts of subjects at baseline and 4 hours after each isocaloric meal in Study 2

	Carbohydrate (n = 21)			n-6 PUFA (n = 22)			SFA (n = 21)			
Blood (× 10^9/L)	0 h	4 h	*P* value*	0 h	4 h	*P* value*	0 h	4 h	*P* value*	*P* value†
Total white cells	5.5 (5.0-6.3)	6.2 (5.4-7.4)	.010	5.8 (5-6.7)	6.6 (6.2-7.5)	.001	6.2 (5.4-6.8)	7.3 (6.7-8.4)	.001	.034‡
Neutrophils	3.2 (2.6-3.5)	3.6 (3.3-4.5)	.062	3.2 (2.8-3.6)	3.9 (3.6-4.4)	.001	3.9 (2.8-4.3)	4.3 (3.5-5.6)	.001	.036‡
Lymphocytes	1.7 (1.5-2.1)	1.9 (1.6-2.3)	.001	1.9 (1.5-2.1)	2.1 (1.7-2.5)	.001	1.9 (1.5-2.2)	2.1 (1.7-2.5)	.002	.476
Monocytes	0.4 (0.3-0.5)	0.5 (0.4-0.6)	.019	0.4 (0.3-0.5)	0.4 (0.4-0.5)	.040	0.4 (0.3-0.5)	0.5 (0.4-0.6)	.007	.347
Eosinophils	0.1 (0.1-0.3)	0.1 (0.1-0.3)	.620	0.2 (0.1-0.3)	0.2 (0.1-0.3)	.119	0.2 (0.1-0.3)	0.2 (0.1-0.2)	.565	.194
	Carbohydrate (n = 20)			**n-6 PUFA (n = 20)**			**SFA (n = 19)**			
Sputum	**0 h**	**4 h**	***P* value***	**0 h**	**4 h**	***P* value***	**0 h**	**4 h**	***P* value***	***P* value†**
Total cell count (× 10^6/L)	3.6 (2.6-6.4)	2.0 (1.2-3.7)	.003	2.6 (1.3-6.0)	2.1 (0.6-3.2)	.012	3.0 (1.5-4.6)	2.0 (1.4-3.5)	.382	.745
Neutrophils (%)	36.5 (16.6-56.4)	47.6 (11.8-59.8)	.266	34.9 (20.5-59.8)	28.1 (14.8-60.8)	1.000	33.0 (11.8-57.5)	44.9 (26.8-60.3)	.140	.530
Eosinophils (%)	1.1 (0.3-3.1)	1.4 (0.3-3.0)	.849	0.8 (0.6-2.3)	1.8 (0.4-2.8)	.029	1.0 (0.5-2.8)	1.3 (0.5-4.0)	.077	.180
Macrophages (%)	58.1 (41.3-77.6)	42.8 (31.7-64.0)	.049	54.3 (35.6-71.6)	50.0 (31.8-67.3)	.044	53.3 (38-8.8)	50.5 (23.5-57.5)	.056	.883
Lymphocytes (%)	0.9 (0.0-3.4)	1.0 (0.5-2.5)	.304	1.9 (0.5-2.8)	1.5 (0.63-5)	.238	1.3 (0.5-4.5)	0.63 (0.3-1.8)	.209	.208

Data are medians (quartile 1-quartile 3).
*Comparison of 4 hours versus 0 hours (analyzed by using the Wilcoxon signed-rank test).
†Comparison of change after each meal (analyzed by using repeated-measures ANOVA).
‡P < .05 for carbohydrate versus SFA (analyzed by using a general linear model with atopy as a covariate).

asthmatic patients. Using *ex vivo* models, we have confirmed that neutrophils and monocytes are a key source of NLRP3 inflammasome–driven postprandial inflammation. Hence our studies describe the independent yet consistent effects of both a high BMI and high saturated fat intake on NLRP3 inflammasome activation in asthmatic patients, with both contributing to airway inflammation.

We identified 13 genes that were expressed differentially in obese versus nonobese asthmatic patients. This included *NOD1* and *NLRP3*, which are both members of the NOD-like receptor (NLR) family and involved in activation of the NLRP3 inflammasome. The NLRP3 inflammasome integrates metabolic and inflammatory processes stimulated by various metabolites, including fatty acids, to induce production of IL-1β in a 2-step process.[22] In the first step, triggers, such as SFAs or TNF-α, bind to innate immune receptors, such as TLR4, NOD1, or TNF receptor 1/2, inducing nuclear factor kappa light chain enhancer of activated B cells (NF-κB) activity and production

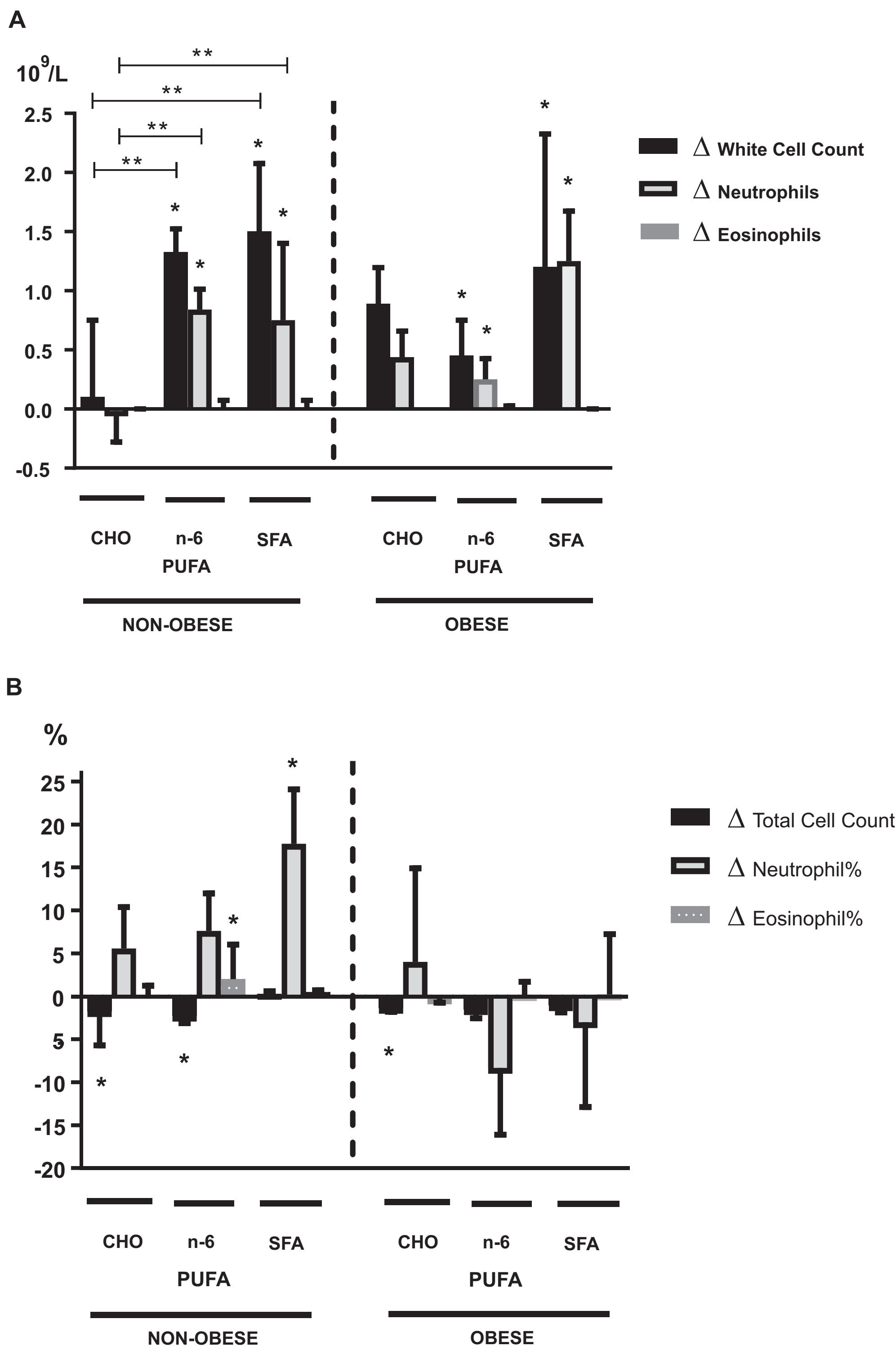


FIG 3. A, Changes in white blood cell counts after each isocaloric meal in nonobese and obese subjects. **B,** Changes in sputum cell counts after each isocaloric meal in nonobese and obese subjects. $^{*}P < .05$ for comparison of 4 hours versus baseline (analyzed by using the Wilcoxon signed-rank test) and $^{**}P < .05$ for between group comparison (analyzed by using repeated-measures ANOVA). *CHO*, Carbohydrates.

of pro–IL-1β. In the second step a stress signal, such as ATP or ceramides (which can be produced from SFAs[23]), triggers a signaling cascade that mediates cleavage of procaspase-1 to active caspase and then cleavage of pro–IL-1β to mature IL-1β.[22]

Our *ex vivo* experiments demonstrate the 2-stage process by which NLRP3 inflammasome activation occurs. Free SFA exposure (C16:0) alone did not stimulate inflammation. However, when C16:0 was combined with another stimuli known to be associated with the obese state (ie, LPS or TNF-α), IL-1β release was

TABLE VI. Sputum cell genes differentially expressed (>1.5 fold change) at 4 hours after consumption of an SFA meal by nonobese asthmatic patients (n = 4) in Study 2

Gene symbol	Gene name	Fold change	*P* value (4 h vs 0 h)
NLRP3	NLR family, pyrin domain containing 3	2.9	.003
TLR4	Toll-like receptor 4	1.5	.003
IL1RN	Interleukin 1 receptor antagonist	2.7	.007
PRKCB	Protein kinase C beta	2.0	.009
PTGS2	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	3.2	.010
TLR6	Toll-like receptor 6	1.5	.010
CXCL10	Chemokine (C-X-C motif) ligand 10	−2.1	.010
IL1RAP	Interleukin 1 receptor accessory protein	2.0	.025
CSF2	Colony stimulating factor 2 (granulocyte-macrophage)	2.4	.026

Data were analyzed by using paired *t* tests with the Benjamini-Hochberg adjustment for multiple comparisons.

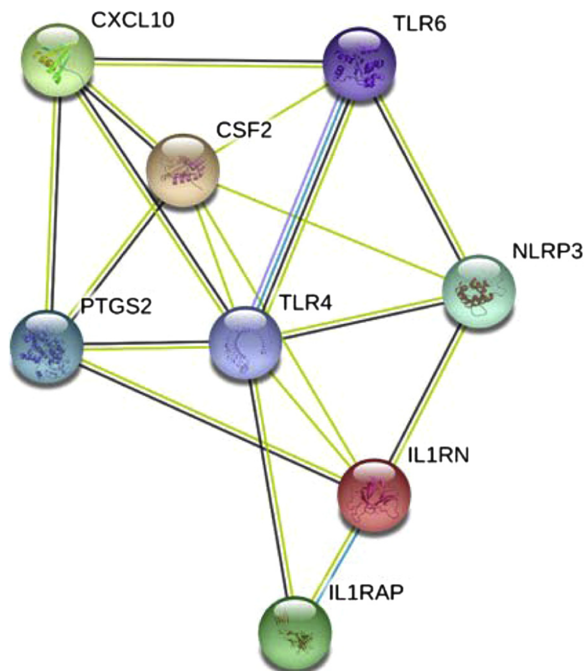


FIG 4. Sputum cell genes expressed differentially (>1.5 fold change) at 4 hours after consumption of a SFA meal by nonobese asthmatic patients interact in a network consisting of 8 genes. Data were assessed by using paired *t* tests with the Benjamini-Hochberg adjustment for multiple comparisons. Search Tool for the Retrieval of Interacting Genes, Search Tool for the Retrieval of Interacting Genes v10 was used to investigate pathway interactions between differentially expressed genes by using medium confidence scores of greater than 0.4. All nodes are colored, indicating they are first shell interactions. *Green lines* represent interactions determined by text mining, *black lines* represent interactions that are coexpressed, *blue lines* are known interactions from curated databases, and *purple lines* refer to protein homology.

induced from peripheral blood inflammatory cells. Indeed, both increased plasma FA levels and increased NOD1 and NLRP3 inflammasome expression in adipose tissue are reflective of the obese state.[24-28] It has been demonstrated that NLRP3 is also important in the airways, as in an allergic mouse model,[29] the absence of NLRP3 and IL-1β reduced the expression of IL-5, IL-13, and IL-3. Furthermore, in experimental models of severe steroid-resistant asthma, we have shown that NLRP3 and IL-1β responses are increased; that therapeutically targeting NLRP3 reduces IL-1β production, neutrophilic inflammation, and airways hyperresponsiveness; and that administration of IL-1β induces severe steroid-resistant neutrophilic asthma.[30] We also observed an association between NLRP3/IL-1β and neutrophil numbers and disease severity in asthmatic patients.[30]

Here we report increased *NLRP3* gene expression and increased protein levels of IL-1β in the airways of obese asthmatic patients, complementing a previous report of increased sputum IL-1β levels after ozone exposure in obese subjects.[31] Interestingly, in female subjects only, *IL-1β* gene expression correlated with *NLRP3* and *TLR4* gene expression and sputum neutrophil percentages, which suggests that our previous report of increased sputum neutrophil percentages in female obese asthmatic patients[10] might be a result of sex-specific NLRP3 activation. This may be clinically important because *IL-1β* expression was correlated inversely with lung function.

Upregulation of *IL5* expression in obese asthmatic patients and correlation between *IL5* and *NLRP3* levels suggest that the NLRP3 inflammasome can enhance T_H2 cytokine production. Airway eosinophilia is a hallmark of asthma, and IL-5 is the main cytokine associated with eosinophil development, mobilization, activation, redistribution, and survival.[32] However, in the current study sputum eosinophil percentages were not increased in obese asthmatic patients, which agrees with previous research from our group and others.[10,11,33] A recent study in obese mice reported lower eosinophil counts in airway lining fluid, despite higher IL-5 and eotaxin levels and higher eosinophil counts in the bone marrow and surrounding lung tissue.[34] Another recent clinical study showed that airway submucosal eosinophil numbers, but not sputum eosinophil numbers, were greater in obese subjects.[33] Collectively, these results suggest that obesity promotes eosinophil trafficking from bone marrow to the lung but inhibits transit into the airways. They also suggest that other factors, possibly eotaxins, might be involved in eosinophil responses in obese asthmatic patients. The clinical relevance of eosinophils in the mucosa versus lung tissue requires investigation.

Using acute meal challenges, we examined the postprandial effects of the macronutrients typically found in obesogenic diets: SFA, n-6 PUFA, and simple carbohydrates. We found that both types of high-fat meals led to increases in circulating blood neutrophil and leukocyte counts, which agrees with previous reports of increased blood neutrophils after a high-SFA meal[35,36] or high-fat mixed meal.[37] Our observation that dietary fat is more proinflammatory than carbohydrates is also supported by previous acute feeding studies, which have shown that although both fat and carbohydrate increase circulating neutrophil counts,[35] NF-κB activity, and TNF-α and IL-1β expression in mononuclear

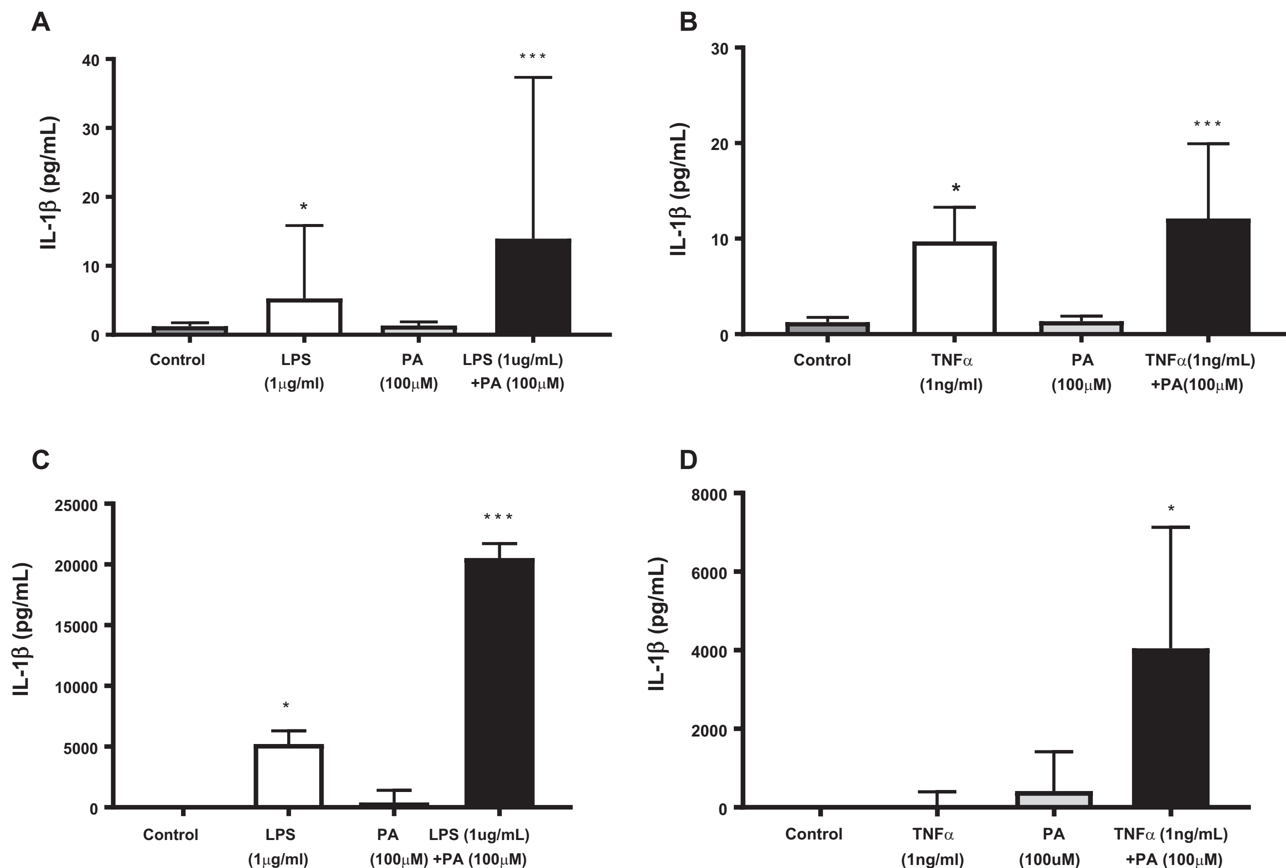


FIG 5. IL-1β release from neutrophils (**A** and **B**) and monocytes (**C** and **D**) collected from healthy control subjects after stimulation with LPS (Fig 5, *A* and *C*) and TNF-α (Fig 5, *B* and *D*) alone and in combination with palmitic acid (PA, C16:0). $*P < .05$ versus control subject and $**P < .001$ versus control subjects. Data were analyzed by using repeated-measures ANOVA.

cells,[38] dietary fat induces the greatest effect.[35,38] Increased migration of leukocytes occurs after a high-fat meal because cells exposed to excess free FAs are activated and subsequently release chemokines, such as CXCL8, and adhesion molecules, such as CD11a.[36] Indeed, neutrophils isolated from peripheral blood after an SFA meal display increased chemotaxis toward complement factor 5a, CXCL8, and *N*-formyl-methionyl-leucyl-phenylalanine.[36]

We observed an increase in airway inflammation (sputum neutrophil percentages) after the saturated fat meal in nonobese subjects only. We reported previously that neutrophilic airway inflammation was increased after a high-fat, high-energy mixed meal, which was high in saturated fat but also carbohydrates and n-6 PUFAs.[14] The current study has allowed us to determine which macronutrients activate inflammatory pathways in asthmatic airways. Here we have demonstrated that only saturated fat increases sputum neutrophil percentages, which is in agreement with the positive correlation we reported previously between the change in plasma SFA levels and the change in sputum neutrophil percentages after the high-fat mixed meal.[14] This also provides a potential explanation for previous observations that saturated fat intake is associated with increased asthma risk.[39,40]

Interestingly, there were no changes in airway inflammation in obese asthmatic patients, likely because of desensitization of obese asthmatic patients caused by chronic exposure to high circulating FA levels. Indeed, our data show that obese asthmatic patients have chronically increased circulating FA levels. This is likely to result from a combination of both excessive fat intake and metabolic abnormalities and impair the homeostatic mechanisms responsible for cellular uptake and storage of nutrients and maintenance of optimal circulating fatty acid levels.[41] They also had higher basal expression of *NLRP3* and increased IL-1β release. Hence it appears that obese asthmatic patients do not experience further postprandial upregulation of inflammation in response to a saturated fat-rich meal. Desensitization of inflammatory responses in asthmatic patients after chronic exposure to external stimuli, such as endotoxin, has been reported previously.[42,43]

There were 9 differentially expressed genes in the nonobese asthmatic patients after the SFA intervention. These included *NLRP3*, *TLR4*, IL-1 receptor antagonist (*IL-1RN*), and IL-1 receptor accessory protein (*IL-1RAP*), which also suggest NLRP3 inflammasome activation. *TLR4* was upregulated, demonstrating the first stage of inflammasome activation, because SFAs bind to TLR4, inducing NF-κB activity and inflammatory cytokine production.[14,44] This concurs with our previous postprandial study that showed increased gene expression of *TLR4* after a high-fat, high-energy mixed meal.[14] Here we also show upregulation of *TLR6* gene expression, which others have shown to form a

heterodimer with TLR2, which can be triggered by SFAs to activate an NF-κB–driven inflammatory cascade.[45] Furthermore, the IL-1 pathway was modified by the SFA meal in nonobese asthmatic patients, with transcripts of both *IL-1RN* and *IL-1RAP* being upregulated. *IL-RN* is the gene that encodes the IL-1 receptor antagonist protein, which functions as an anti-inflammatory cytokine by inhibiting the activities of IL-1α and IL-1β through interaction with the IL-1 receptor (IL-1R).[46] IL-1RAP enhances IL-1 activity, being a necessary component of the IL-1R complex that initiates signaling events resulting in activation of IL-1–responsive genes. Further exploration of these findings is warranted, as our previous studies have shown the importance of IL-1 pathway in inducing steroid-insensitive neutrophilic responses in mouse models[30] and predicting future exacerbations of airways disease.[47]

Our studies have several limitations. The studies presented in this article examine NLRP3 inflammasome activation in asthmatic airways in relation to obesity and saturated fat intake. Because airway NLRP3 inflammasome activity increases in nonasthmatic subjects with other exposures, such as ozone,[31] we cannot exclude the possibility that obesity and saturated fat would also activate the NLRP3 inflammasome in nonasthmatic airways. The clinical implications of such a finding are unknown, and this is an interesting area for further investigation. The cross-sectional analysis in Study 1 cannot establish cause and effect, and therefore our observations need to be explored further in longitudinal studies. Furthermore, the effect of comorbidities, such as obstructive sleep apnea, on chronic and postprandial inflammation are poorly understood and should be considered in future research.

The acute meal study gene expression analysis had a small sample size because paired sputum samples could not be collected from some subjects. This is difficult to address because the study design required 2 sputum inductions within 4 hours, which reduces the likelihood of obtaining a sufficient volume of mucus plugs on the second collection. Nonetheless, we had adequate samples to identify changes in airway cell numbers, which allowed us to focus our gene analysis.

Another limitation of this study is the absence of data on usual diets. This should be included in future studies to explore the effect of chronic nutrient intake on postprandial responses.

Finally, our studies examined gene expression in sputum samples, which are a heterogeneous mixture of inflammatory cells. We have addressed this limitation by using *ex vivo* experiments to confirm specific cell types that are activated by SFAs. Our *ex vivo* experiments also provide evidence of the tightly controlled 2-stage process by which inflammasome activation occurs. In both neutrophils and monocytes, we found that a combination of both free SFA (C16:0) and LPS/TNF-α were required to induce IL-1β release.

Our analyses provide insight into the nature of airway inflammation in asthmatic patients with a high BMI and in the postprandial phase. We have identified the NLRP3 inflammasome as being differentially regulated in obese asthmatic patients and highlighted several targets, including NOD1 and NLRP3, which could be furthered explored to develop improved therapies for managing obese asthma. Our study has also confirmed and extended our previous observation that a high-fat, high-energy meal induces neutrophilic airway inflammation. We have shown that SFAs are the responsible macronutrient and that the effect is confined to nonobese asthmatic subjects. We have also identified several genes involved in SFA-induced neutrophilic airway inflammation, including *TLR4/6* and *NLRP3*, which provide possible therapeutic targets for immunomodulation. This study provides translational outcomes, suggesting that both reversal of obesity in asthmatic patients and restriction of dietary saturated fat intake in nonobese asthmatic patients warrant further investigation as anti-inflammatory strategies.

We acknowledge the staff in Respiratory and Sleep Medicine, Hunter Medical Research Institute.

Clinical implications: Both obesity and saturated fat intake cause NLRP3 inflammasome–mediated airway inflammation in asthmatic patients. Hence weight loss and dietary fat restriction warrant further exploration as anti-inflammatory strategies in asthmatic patients.

REFERENCES

1. The Global Asthma Report 2014, Auckland, New Zealand: Global Asthma Network, 2014.
2. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014;384:766-81.
3. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med 2010;181:315-23.
4. Hakala K, Stenius-Aarniala B, Sovijarvi A. Effects of weight loss on peak flow variability, airways obstruction, and lung volumes in obese patients with asthma. Chest 2000;118:1315-21.
5. Sin DD, Jones RL, Man SF. Obesity is a risk factor for dyspnea but not for airflow obstruction. Arch Intern Med 2002;162:1477-81.
6. Boulet LP, Frannsen E. Influence of obesity on response to fluticasone with or without salmeterol in moderate asthma. Respir Med 2007;101:2240-7.
7. Peters-Golden M, Swern A, Bird SS, Hustad CM, Grant E, Edelman JM. Influence of body mass index on the response to asthma controller agents. Eur Respir J 2006; 27:495-503.
8. Hansbro PM, Kim RY, Starkey MR, Donovan C, Dua K, Mayall JR, et al. Mechanisms and treatments for severe, steroid-resistant allergic airway disease and asthma. Immunol Rev 2017;278:41-62.
9. Kim RY, Pinkerton JW, Gibson PG, Cooper MC, Horvat JC, Hansbro PM. Inflammasomes in respiratory diseases. Thorax 2015;70:1199-201.
10. Scott HA, Gibson PG, Garg ML, Wood LG. Airway inflammation is augmented by obesity and fatty acids in asthma. Eur Respir J 2011;38:594-602.
11. Telenga ED, Tideman SW, Kerstjens HAM, ten Hacken NHT, Timens W, Postma DS, et al. Obesity in asthma: more neutrophilic inflammation as a possible explanation for a reduced treatment response. Allergy 2012;67:1060-8.
12. Marjisse GS, Seys SF, Schelpe A-S, Dilissen E, Goeminne P, Dupont LJ, et al. Obese individuals with asthma preferentially have a high IL-5/IL-17A/IL-25 sputum inflammatory pattern. Am J Respir Crit Care Med 2014;15:1284-5.
13. Hotamisligil GS, Erbay E. Nutrient sensing and inflammation in metabolic diseases. Nat Rev Immunol 2008;8:923-34.
14. Wood LG, Garg ML, Gibson PG. A high-fat challenge increases airway inflammation and impairs bronchodilator recovery in asthma. J Allergy Clin Immunol 2011;127:1133-40.
15. Wood LG, Garg ML, Smart JM, Scott HA, Barker D, Gibson PG. Manipulating antioxidant intake in asthma: a randomized controlled trial. Am J Clin Nutr 2012;96:534-43.
16. Scott HA, Gibson PG, Garg ML, Pretto JJ, Morgan PJ, Callister R, et al. Dietary restriction and exercise improve airway inflammation and clinical outcomes in overweight and obese asthma: a randomized trial. Clin Exp Allergy 2013;43:36-49.
17. Gibson PG, Simpson JL, Saltos N. Heterogeneity of airway inflammation in persistent asthma—evidence of neutrophilic inflammation and increased sputum interleukin-8. Chest 2001;119:1329-36.
18. Wood LG, Fitzgerald DA, Lee AK, Garg ML. Improved antioxidant and fatty acid status of patients with cystic fibrosis after antioxidant supplementation is linked to improved lung function. Am J Clin Nutr 2003;77:150-9.

19. Simpson JL, Phipps S, Baines KJ, Oreo KM, Gunawardhana L, Gibson PG. Elevated expression of the NLRP3 inflammasome in neutrophilic asthma. Eur Respir J 2014;43:1067-76.
20. Baines KJ, Simpson JL, Bowden NA, Scott RJ, Gibson PG. Differential gene expression and cytokine production from neutrophils in asthma phenotypes. Eur Respir J 2010;35:522-31.
21. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 2015;43(Database issue):D447-52.
22. Ralston JC, Lyons CL, Kennedy EB, Kirwan AM, Roche HM. Fatty acids and NLRP3 inflammasome-mediated inflammation in metabolic tissues. Annu Rev Nutr 2017;37:77-102.
23. Glass CK, Olefsky JM. Inflammation and lipid signaling in the etiology of insulin resistance. Cell Metab 2012;15:635-45.
24. Zhou YJ, Liu C, Li CL, Song YL, Tang YS, Zhou H, et al. Increased NOD1, but not NOD2, activity in subcutaneous adipose tissue from patients with metabolic syndrome. Obesity (Silver Spring) 2015;23:1394-400.
25. Lappas M. NOD1 expression is increased in the adipose tissue of women with gestational diabetes. J Endocrinol 2014;222:99-112.
26. Chi W, Dao D, Lau TC, Henriksbo BD, Cavallari JF, Foley KP, et al. Bacterial peptidoglycan stimulates adipocyte lipolysis via NOD1. PLoS One 2014;9:e97675.
27. Rheinheimer J, de Souza BM, Cardoso NS, Bauer AC, Crispim D. Current role of the NLRP3 inflammasome on obesity and insulin resistance: a systematic review. Metabolism 2017;74:1-9.
28. Vandanmagsar B, Youm Y-H, Ravussin A, Galgani JE, Stadler K, Mynatt RL, et al. The NALP3/NLRP3 inflammasome instigates obesity-induced autoinflammation and insulin resistance. Nat Med 2011;17:179-88.
29. Besnard AG, Guillou N, Tschopp J, Erard F, Couillin I, Iwakura Y, et al. NLRP3 inflammasome is required in murine asthma in the absence of aluminum adjuvant. Allergy 2011;66:1047-57.
30. Kim RY, Pinkerton JW, Essilfie AT, Robertson AA, Baines KJ, Brown AC, et al. Role for NLRP3 inflammasome-mediated, IL-1beta-dependent responses in severe, steroid-resistant asthma. Am J Respir Crit Care Med 2017;196:283-97.
31. Todoric K, Zhou H, Zhang H, Mills K, Peden DB, Hernandez ML. Body mass index correlates with pollutant-induced interleukin-1beta in sputum and blood. Ann Allergy Asthma Immunol 2015;114:251-3.
32. Fahy JV. Eosinophilic and neutrophilic inflammation in asthma: insights from clinical studies. Proc Am Thorac Soc 2009;6:256-9.
33. Desai D, Newby C, Symon FA, Haldar P, Shah S, Gupta S, et al. Elevated sputum interleukin-5 and submucosal eosinophilia in obese individuals with severe asthma. Am J Respir Crit Care Med 2013;188:657-63.
34. Calixto MC, Lintomen L, Schenka A, Saad MJ, Zanesco A, Antunes E. Obesity enhances eosinophilic inflammation in a murine model of allergic asthma. Br J Pharmacol 2010;159:617-25.
35. van Oostrom AJ, Sijmonsma TP, Verseyden C, Jansen EH, de Koning EJ, Rabelink TJ, et al. Postprandial recruitment of neutrophils may contribute to endothelial dysfunction. J Lipid Res 2003;44:576-83.
36. van Oostrom AJHHM, Plokker HWM, van Asbeck BS, Rabelink TJ, van Kessel KPM, Jansen EHJM, et al. Effects of rosuvastatin on postprandial leukocytes in mildly hyperlipidemic patients with premature coronary sclerosis. Atherosclerosis 2006;185:331-9.
37. Kurti SP, Rosenkranz SK, Levitt M, Cull BJ, Teeman CS, Emerson SR, et al. Does moderate intensity exercise attenuate the postprandial lipemic and airway inflammatory response to a high-fat meal? BioMed Res Int 2015;2015:10.
38. Deopurkar R, Ghanim H, Friedman J, Abuaysheh S, Sia CL, Mohanty P, et al. Differential effects of cream, glucose, and orange juice on inflammation, endotoxin, and the expression of Toll-like receptor-4 and suppressor of cytokine signaling-3. Diabetes Care 2010;33:991-7.
39. Rodríguez-Rodríguez E, Perea JM, Jiménez AI, Rodríguez-Rodríguez P, López-Sobaler AM, Ortega RM. Fat intake and asthma in Spanish schoolchildren. Eur J Clin Nutr 2010;64:1065-71.
40. Huang S, Pan W. Dietary fats and asthma in teenagers: analyses of the first Nutrition and Health Survey in Taiwan (NAHSIT). Clin Exp Allergy 2001;31:1875-80.
41. Mook S, Halkes Cj C, Bilecen S, Cabezas MC. In vivo regulation of plasma free fatty acids in insulin resistance. Metabolism 2004;53:1197-201.
42. Nguyen LP, Omoluabi O, Parra S, Frieske JM, Clement C, Ammar-Aouchiche Z, et al. Chronic exposure to beta-blockers attenuates inflammation and mucin content in a murine asthma model. Am J Respir Cell Mol Biol 2008;38:256-62.
43. Randow F, Syrbe U, Meisel C, Krausch D, Zuckermann H, Platzer C, et al. Mechanism of endotoxin desensitization: involvement of interleukin 10 and transforming growth factor beta. J Exp Med 1995;181:1887-92.
44. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin HL, Flier JS. TLR4 links innate immunity and fatty acid–induced insulin resistance. J Clin Invest 2006;116:3015-25.
45. Stewart CR, Stuart LM, Wilkinson K, van Gils JM, Deng J, Halle A, et al. CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. Nat Immunol 2010;11:155-61.
46. Allan SM, Rothwell NJ. Cytokines and acute neurodegeneration. Nat Rev Neurosci 2001;2:734-44.
47. Baines KA, Fu JJ, McDonald VM, Gibson PG. Airway gene expression of IL-1 pathway mediators predicts exacerbation risk in obstructive airway disease. Int J Chron Obstruct Pulmon Dis 2017;12:541-50.