A high-fat challenge increases airway inflammation and impairs bronchodilator recovery in asthma

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Background: Dietary fat activates systemic innate immune responses, but the effect on airway responses is unknown.
Objective: To examine effects of a high-fat versus low-fat meal on systemic and airway inflammation in asthma.
Methods: Nonobese subjects with asthma were randomized to consume a high-fat (n = 19; 48% [49 g] fat) or low-fat (n = 18; 15% [3 g] fat) meal. Fourteen obese patients with asthma and 21 healthy controls also consumed a high-fat meal. Another group of patients with asthma consumed a high-trans (n = 5; 5.2 g trans fat) or nontrans (n = 5, <0.3 g trans fat) fatty acid meal. Lung function was measured at baseline (prebronchodilator) and 2, 3, and 4 hours after bronchodilator. Airway inflammation was assessed by using induced sputum cell counts and Toll-like receptor 4 mRNA expression by real-time PCR. Systemic inflammation was measured by ELISA quantification of plasma TNF-α, high-sensitivity C-reactive protein, and IL-6 concentrations.
Results: In patients with asthma, at 4 hours postmeal, increases in sputum % neutrophils and Toll-like receptor 4 mRNA expression were higher and increases in FEV_1/forced vital capacity (FVC) were lower in the high-fat versus low-fat groups. Changes in plasma fatty acids correlated with changes in sputum % neutrophils and were negatively associated with changes in % FEV_1, % FVC, and FEV_1/FVC. After the high-trans fatty acid meal, sputum % neutrophils were significantly higher than after the nontrans meal.
Conclusion: A high-fat meal augments neutrophilic airway inflammation, with the effect dependent on the type of fat consumed. A high-fat meal also suppresses bronchodilator recovery in asthma. Modifying dietary fat intake may be useful in asthma. (J Allergy Clin Immunol 2011;127:1133-40.)

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Fatty acids are an essential dietary component, providing the key energy source *in vivo*. However, metabolism of a high-fat meal results in transient production of proinflammatory mediators, with damaging effects occurring when fats are consumed in excess. Fatty acids initiate innate immune responses via mechanisms such as Toll-like receptor (TLR) activation. Recent evidence demonstrates that TLR4 is activated by dietary fatty acids such as lauric, palmitic, and oleic acids.[1-3] *In vivo* experiments have demonstrated that within 1 hour of consumption of a high-fat meal, innate immune responses are stimulated, with increased TLR2, TLR4,[4] and nuclear factor-κB (NF-κB) activity[5,6] and increased release of proinflammatory cytokines such as TNF-α[7] and IL-6.[7,8] Chronic consumption of excess fatty acids can induce a lipotoxic state,[9] leading to adverse outcomes such as vascular events,[10,11] insulin resistance,[12] or circulating inflammatory cell recruitment.[13]

The innate immune system contributes to airway inflammation in asthma.[14] Inflammation in asthma is heterogeneous. Although allergen-induced T_H2 immune activation leads to airway eosinophilia, asthma can also involve neutrophilic inflammation that is associated with innate immune dysfunction. Increased sputum IL-8, neutrophil influx, and increased gene expression for TLR2 and TLR4 are observed.[14] Airway neutrophils appear to be clinically important, because most severe forms of asthma[15] involve elevated neutrophils, neutrophil counts increase with asthma severity,[16] and sputum neutrophils negatively correlate with lung function and airflow obstruction in asthma.[17] Thus, stimuli that activate innate immune responses, leading to increased airway neutrophilia, may be relevant to asthma development and progression.

Several studies have examined the role of dietary fat in asthma. A high fat intake and plasma triglyceride levels have been associated with airway hyperresponsiveness,[18] asthma risk,[19] and adult-onset wheeze.[20] Other studies report that fat quality affects asthma outcomes. Trans fatty acids,[21] margarine (a trans fatty acid source),[22,23] and n-6 polyunsaturated fatty acids[24] have been associated with increased asthma risk. Conversely, milk fat[25,26] and n-3 polyunsaturated fatty acids or fish[27,28] have been associated with decreased asthma risk in some but not all[24] studies. Thus, epidemiologic associations link the amount and type of dietary fat consumption and asthma risk; however, the underlying mechanism of this association remains unclear.

We hypothesized that a high fat intake in asthma activates the innate immune response, causing an increase in systemic and

Abbreviations used
ACQ: Asthma Control Questionnaire
AHF: Asthma high-fat
AHF-NonO: Asthma high-fat/high energy—nonobese
AHF-O: Asthma high-fat/high energy—obese
ALF: Asthma low-fat/low-energy
BMI: Body mass index
CRP: C-reactive protein
DRS: Dose response slope
eNO: Exhaled nitric oxide
FVC: Forced vital capacity
HCHF: Healthy controls high-fat
MUFA: Monounsaturated fatty acid
NF-κB: Nuclear factor-κB
PUFA: Polyunsaturated fatty acid
SFA: Saturated fatty acid
TE: Total energy
TLR: Toll-like receptor

airway inflammation and worsening clinical outcomes. The aim of this study was to examine the effect of a high-fat versus a low-fat meal on inflammation and clinical outcomes in asthma. We also aimed to determine whether the proinflammatory effects of a high-fat meal were modified by obesity or fat quality.

METHODS

Study participants

Nonobese (body mass index [BMI] < 30 kg/m^2; n = 37) and obese (BMI ≥ 30 kg/m^2; n = 14) subjects older than 18 years with stable asthma were recruited. Before the study, subjects fasted for 12 hours and withheld short-acting and long-acting β-agonist medications. Exclusion criteria were current smoking (smoked within past 6 months), abnormal electrocardiogram at screening, pregnancy, diabetes mellitus, cardiac arrhythmia, angina, congestive heart failure, renal or hepatic failure, and systemic diseases. Asthma stability was defined as no exacerbation, respiratory tract infection, or oral corticosteroids in the past 4 weeks. Asthma control was assessed by using the Asthma Control Questionnaire (ACQ).[29] Clinical asthma pattern was determined according to previous Global Initiative for Asthma recommendations.[30] Nonobese healthy controls (n = 21) were also recruited who had no respiratory symptoms, had never had a doctor's diagnosis of asthma, had normal lung function without airway hyperresponsiveness, and were steroid-naive. Atopy was determined by using skin allergen testing. Atopy was defined as an immediate reaction (wheal ≥ 3 mm) to 1 or more of 19 common aeroallergens, carried out by using a 1:10 wt/vol dilution of *Aspergillus fumigatus*, *Aspergillus* mix, *Penicillium*, *Alternaria tenuis*, *Dermatophagoides pteronyssinus, Dermatophagoides farinae,* cockroach, peanut, hazelnut, wild oats, plantain, timothy, bent grass, blue grass, rye grass, cat hair, dog hair, feather mix, and horse dander, with histamine and saline as control (Bayer Australia Ltd, Pymble, Australia). All subjects were nonsmokers. Written informed consent was obtained from all subjects, and the study was approved by the Hunter New England Human Research Ethics Committee.

Food challenge

Nonobese subjects with asthma were randomized to consume a high-fat/high-energy (AHF-NonO; n = 19) or low-fat/low-energy (ALF; n = 18) meal. Group assignment was determined by computer-generated random allocation, derived by an independent statistician. Initial treatment allocation was concealed from clinical staff, who allocated the next available number as participants entered the trial. Twenty-one nonobese healthy controls (HCHF) and 14 obese patients with asthma (AHF-O) also consumed a high-fat/high-energy meal. The high-fat meal contained total energy (TE) of 3846 kJ and consisted of 79 g (33% of TE) carbohydrate, 39 g (18% of TE) protein, and 48 g (49% of TE) total fat, including 20.5 g (21% of TE) saturated fat. The low-fat meal contained total energy (TE) of 806 kJ and consisted of 32 g (64% of TE) carbohydrate, 10 g (21% of TE) protein, and 3.4 g (15% of TE) total fat, including 2.2 g (10% of TE) saturated fat. Neither subjects nor investigators were blind to the treatments. However, primary outcome assessments (induced sputum markers) were conducted blind to both subjects and investigators. At baseline (time = 0), prebronchodilator lung function was measured. A hypertonic saline challenge was conducted, and at the conclusion of the challenge, 200 μg salbutamol was administered to all subjects. The study meal was consumed within 15 minutes, and then lung function was monitored at 2, 3, and 4 hours, with no additional administration of salbutamol. Blood was collected at 0, 2, 3, and 4 hours, and induced sputum was collected at 0 and 4 hours. Exhaled nitric oxide (eNO) was also measured at 0 and 4 hours (NIOX, Aerocrine AB, Solna, Sweden).

TABLE I. Plasma fatty acid concentrations (baseline and changes at 4 hours)

FAs (mg/L)	Challenge type	0 h	Δ 4 h
SFAs	High fat	950 ± 32	112 ± 20†*
	Low fat	804 ± 75	24 ± 19
MUFAs	High fat	835 ± 36	138 ± 23†*
	Low fat	733 ± 67	47 ± 23†
PUFAs	High fat	1632 ± 50	77 ± 22†
	Low fat	2158 ± 707	25 ± 20
Total FAs	High fat	3416 ± 109	327 ± 62†*
	Low fat	2869 ± 253	96 ± 58

FA, Fatty acid.
Data are normally distributed and presented as means ± SEMs, with groups compared by using the Student *t* test.
*$P < .05$ compared with low fat.
†$P < .05$, 4 hours compared with baseline.

Trans fatty acid meal challenge

Another group of subjects with asthma were assigned, via computer-generated random sequence, to receive a high-trans (n = 5) or nontrans (n=5) fatty acid meal. The meals were equivalent in energy, total fat, and saturated fat content, with each containing approximately 2700 kJ, with 35% energy from fat and 9% energy from saturated fat. The trans fatty acid content of the meals was 5.2 g versus <0.3 g, in trans versus nontrans meals, respectively. Sputum was induced at 0 and 4 hours.

Hypertonic saline challenge

Spirometry (Minato Autospiro AS-600; Minato Medical Science, Osaka, Japan) and combined bronchial provocation and sputum induction with hypertonic saline (4.5%) were performed as previously described.[31] After baseline spirometry and expectoration instruction, hypertonic saline was administered for increasing periods (30 seconds, 1 minute, 2 minutes, 4 minutes, 4 minutes, 4 minutes) via an ultrasonic nebulizer (ULTRA-NEB Model 2000; De Vilbiss Healthcare Inc, Somerset, Pa) with a Hans Rudolph 2-way valve (Hans Rudolph Inc, Shawnee, Kan). Spirometry was measured 1 minute after each dose, and participants were asked to expectorate after each dose of hypertonic saline. Salbutamol 200 μg was administered via pressurized inhaler and valved holding chamber if FEV_1 dropped below 15% of baseline. These subjects were considered to be airway hyperresponsive. In subjects for whom FEV_1 did not decrease by 15% from baseline, salbutamol 200 μg was administered at the maximum nebulization time of 15 minutes. Spirometry was performed according to American Thoracic Society standards, with the largest forced vital capacity (FVC) and FEV_1 measured from a series of at least 3 usable forced expiratory curves.[3]

Sputum processing and analysis

Induced sputum was collected, and mucus plugs were separated from the salivary portion of the sample. Mucus plugs were dispersed with dithiothreitol,

TABLE II. Subject characteristics at baseline

Clinical characteristic	HCHF	ALF	AHF-NonO	AHF-O
n	21	18	19	14
Age (y)*	49.6 ± 4.6	41.7 ± 3.2	50.9 ± 4.3	56.5 ± 4.3
Sex (M/F)	9/12	7/11	9/10	4/10
BMI (kg/m^2)*	24.0 ± 0.7	24.5 ± 0.8	24.7 ± 0.8	35.9 ± 1.4§
Atopy (yes/no)	9/12‖	15/3	16/3	10/4
ACQ*	NA	0.7 ± 0.5	0.7 ± 0.6	0.9 ± 0.4
Asthma pattern (intermittent/mild/moderate/severe persistent)	NA	5/4/5/4	8/1/9/1	3/4/3/4
Maintenance ICS (μg beclomethasone equivalents/d)†	NA	325 (0-575)	400 (100-800)	250 (0-500)
PD_{15} (mL)*‡	NA	0.64 ± 0.45	0.70 ± 0.47	0.67 ± 0.66
AHR (yes/no)	0/21	11/7	13/6	6/8
DRS (% fall FEV_1/mL saline)†	0.2 (0.0-0.4)‖	1.8 (0.5-6.1)	1.6 (0.6-4.1)	0.9 (0.2-1.8)

AHR, Airway hyperresponsive; *F,* female; *ICS,* inhaled corticosteroid; *M,* male; *NA,* not applicable.
*Data are normally distributed and presented as means ± SEMs, with group comparisons performed by using the ANOVA test, with Bonferroni *post hoc* testing.
†Data are nonparametric, presented as median (quartile 1-quartile 3), with group comparisons performed by using the Kruskal-Wallis test, with Dunn *post hoc* testing.
‡PD_{15}, Provocation dose resulting in 15% fall in baseline FEV_1, expressed as geometric mean ± log SD.
§$P < .05$ versus HCHF, AHF-NonO, and ALF.
‖$P < .05$ versus AHF-NonO, AHF-O, and ALF.

and a total and differential cell count of leukocytes was performed, as previously described.[31] For *TLR4* gene expression analysis, 100 μL selected sputum was added to Buffer RLT (Qiagen, Hilden, Germany) and stored at –80°C. RNA was extracted and quantified and gene expression of *TLR4* evaluated in a duplex reaction by using the ABI 7500 Real Time PCR system (Applied Biosystems, Mulgrave, Victoria, Australia) as previously described.[14] The expression of *TLR4* was calculated relative to a positive calibrator and normalized to the endogenous control.

Blood processing and analysis

Blood was collected in EDTA tubes and centrifuged at 4°C, 3000*g*, for 10 minutes, and plasma was stored at –80°C. Plasma fatty acid concentrations were analyzed by using gas chromatography as previously described.[32] Commercial ELISAs were used to measure IL-6 and TNF-α (R&D Systems, Minneapolis, Minn) and high-sensitivity C-reactive protein (CRP; MP-Biomedicals, Solon, Ohio).

Statistical analysis

Results were analyzed by using Minitab version 15 for Windows (Minitab Inc, State College, Pa). Statistical comparisons between groups at each time point were performed by using 1-way ANOVA followed by Bonferroni *post hoc* testing for parametric data or Kruskal-Wallis followed by Dunn *post hoc* testing for nonparametric data. An additional analysis was performed comparing the combined asthma high-fat groups (AHF) with the ALF group. The means ± SEMs are reported for normally distributed data; for nonparametric data the medians (quartiles 1-3) are reported. Associations between variables were examined by using the Pearson correlation coefficient for normally distributed data and the Spearman rank correlation coefficient for nonparametric data. Significance was accepted if $P < 05$. Some of the results included in this article have been published previously in abstract form.[33]

RESULTS

Compared with baseline, there was a significant increase in plasma levels of total fatty acids, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFA) at 4 hours after the high-fat challenge (Table I). The high-fat challenge led to a significantly greater increase in plasma levels of total fatty acids, SFAs, and MUFAs than the low-fat challenge (Table I).

There were no significant differences in age or sex of each of the groups (Table II). There was no difference in ACQ, asthma pattern, maintenance inhaled corticosteroid dose, dose response slope (DRS), or provocation dose resulting in 15% fall in baseline FEV_1 (pd15) across the asthmatic groups. As expected, the median DRS was lower and there was a lower proportion of atopic subjects in the HCHF compared with the asthma groups (Table II).

Administration of salbutamol in the ALF group led to a significant improvement in % predicted FEV_1 at 2 and 3 hours compared with baseline (prebronchodilator % predicted FEV_1; Table III; Fig 1, *A*). In the AHF-NonO group, there was a smaller improvement in % predicted FEV_1 at 2 and 3 hours, and in the AHF-O group there was a small improvement in % predicted FEV_1 at 2 hours only (Table III). At 3 hours, the improvement in % predicted FEV_1 was significantly less in the AHF-O group than in the ALF group (Table III). A similar pattern occurred with FEV_1/FVC. In the ALF group, administration of salbutamol led to an improvement in FEV_1/FVC compared with baseline (prebronchodilator FEV_1/FVC) at 2 hours, and this was maintained at 3 and 4 hours (Table III; Fig 1, *B*). In the AHF-NonO and AHF-O groups, there were small improvements in FEV_1/FVC at 2 hours only. At 4 hours, the increase in FEV_1/FVC was significantly higher in the ALF compared with the AHF-NonO, AHF-O, and HCHF groups (Table III).

At baseline, sputum % neutrophils were similar across all groups, whereas % eosinophils were higher in the asthma groups compared with the HCHF group (Table IV). At 4 hours postmeal, the fold increase in *TLR4* mRNA expression in the AHF groups was significantly higher than in the ALF group, in which it did not increase compared with baseline (Fig 2, *A*). Also at 4 hours after the food challenge, there was a significant increase in sputum % neutrophils compared with baseline in each of the high-fat groups. The increase in sputum % neutrophils in the AHF groups was significantly different from the ALF group, in which it did not increase compared with baseline (Fig 2, *B*). There were no diet-related changes in eNO or sputum % eosinophils.

At baseline, concentrations of IL-6 and CRP were higher in the AHF-O group compared with the HCHF and ALF groups (Table V). There were small increases in plasma IL-6 compared with baseline in the AHF-O group at 2, 3, and 4 hours and in

TABLE III. Lung function (baseline and changes at 2, 3, and 4 hours)

Lung function variable	Diet group	0 h	*P* value	Δ 2 h§	*P* value	Δ 3 h§	*P* value	Δ 4 h§	*P* value
FEV_1,	HCHF	102.1 ± 2.4	.001	2.5 ± 0.7	.021	0.4 ± 1.1*	.004	–0.1 ± 0.5	.207
% predicted	ALF	82.9 ± 4.2†		6.6 ± 1.7‡		6.2 ± 1.6‡		3.8 ± 1.5	
	AHF-NonO	84.2 ± 3.5†		6.5 ± 1.2‡		3.8 ± 0.9‡		1.5 ± 1.5	
	AHF-O	85.6 ± 6.2†		2.9 ± 1.0‡		0.5 ± 1.2*		1.3 ± 1.7	
FVC, % predicted	HCHF	106.5 ± 2.4	.109	–0.9 ± 1.08	.086	–1.0 ± 0.8	.075	–0.9 ± 0.6	.410
% predicted	ALF	98.8 ± 3.2		4.1 ± 1.9‡		2.8 ± 1.5		1.3 ± 1.2	
	AHF-NonO	101.2 ± 2.8		1.8 ± 1.5		1.2 ± 0.7‡		–0.4 ± 1.3	
	AHF-O	95.3 ± 5.0		1.0 ± 1.2		–0.4 ± 1.5		0.3 ± 0.8	
FEV_1/FVC %	HCHF	78.7 ± 1.3	.002	2.3 ± 0.4	.444	1.3 ± 0.6	.059	0.7 ± 0.3*	.002
	ALF	69.9 ± 2.5†		3.3 ± 0.7‡		3.5 ± 0.8‡		3.8 ± 1.0‡	
	AHF-NonO	67.7 ± 2.1†		2.6 ± 0.5‡		2.2 ± 0.7		1.1 ± 0.6*	
	AHF-O	72.2 ± 2.7		2.0 ± 0.5‡		0.9 ± 0.7		0.1 ± 0.7*	

Data are normally distributed, presented as means ± SEMs, with group comparisons performed by using the ANOVA test, with Bonferroni *post hoc* testing.
*$P < .05$ versus ALF.
†$P < .05$ versus HCHF.
‡$P < .05$ compared with baseline.
§ Δ 2 h, Δ 3 h, and Δ 4 h is the change in % predicted values at each time point—for example, Δ 2 h for % predicted FEV_1 is (% predicted FEV_1 at 2 h – % predicted FEV_1 at 0 h).

the HCHF group at 4 hours. There was also an increase in plasma CRP compared with baseline in the AHF-O group at 2 hours, and this was significantly different from the HCHF group (Table V). No differences in key outcomes were identified in subjects with and without atopy, although the number of subjects in these comparisons was small and the study was not powered to detect such differences.

There were several significant correlations between the increases in plasma fatty acids and changes in lung function and systemic and airway inflammation (Table VI). The increase in total fatty acids and PUFAs at 4 hours was associated with an increase in TNF-α at 3 hours. The increase in total fatty acids, SFAs, and MUFAs at 4 hours was also associated with the increase in sputum % neutrophils at 4 hours. Furthermore, the increase in total fatty acids, SFAs, and MUFAs at 4 hours was inversely associated with the change in % FEV_1 at 4 hours, and the increases in total fatty acids, SFAs, MUFAs, and PUFAs at 4 hours were inversely associated with % FVC and FEV_1/FVC at 4 hours (Table VI).

In the trans fatty acid meal challenge, the change in sputum % neutrophils was significantly different after the trans compared with the nontrans meals (mean ± SEM, 29.4% ± 6.7% vs 1.7% ± 9.8%; $P = .048$). Consumption of the trans fatty acid meal led to a significant increase in sputum % neutrophils compared with baseline, whereas there was no change in sputum % neutrophils compared with baseline after the nontrans meal.

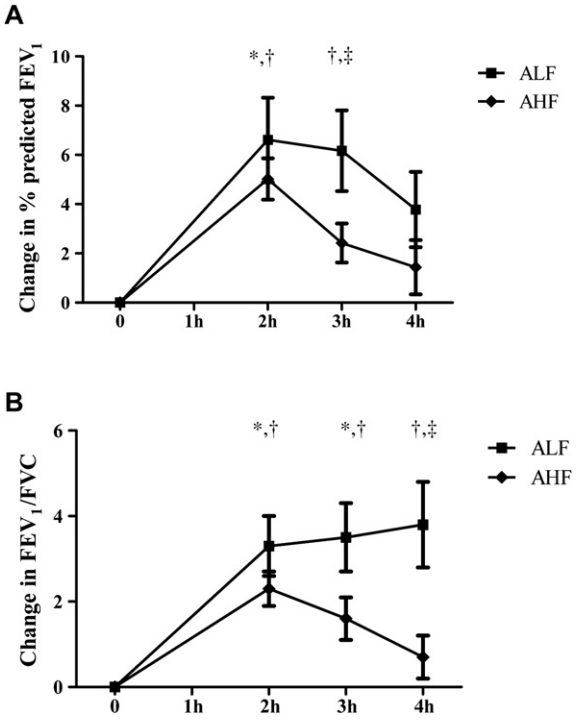

FIG 1. In subjects with asthma, changes in lung function monitored over 4 hours after administration of bronchodilator and consumption of the high fat (AHF) and low fat (ALF) food challenges. **A,** Change in % predicted FEV_1 (% predicted FEV_1 at 2, 3, and 4 hours – % predicted FEV_1 at 0 hour). **B,** Change in FEV_1/FVC (FEV_1/FVC at 2, 3, and 4 hours – FEV_1/FVC at 0 hour). *$P < .05$, AHF versus baseline; †$P < .05$, ALF versus baseline; ‡$P < .05$, AHF versus ALF.

DISCUSSION

This is the first study to investigate the direct effect of dietary fat on systemic and airway inflammation in asthma. We have demonstrated that in patients with asthma, consumption of a single high-fat meal leads to increased circulating fatty acid levels, and this corresponds with an increase in induced sputum % neutrophils and *TLR4* mRNA expression in sputum cells. This fat-induced inflammation corresponds with attenuation of airway bronchodilator recovery, which correlates with the increase in plasma fatty acid concentrations.

The increase in airway neutrophilia that we observed after the high-fat meal provides the first evidence that airway inflammation can be modified by dietary fat consumption. Various studies have used an acute fat challenge model to investigate the systemic inflammatory effects of dietary fat[34] in nonasthmatic groups. Although there is some heterogeneity in the data, evidence of a fat challenge activating the innate immune responses includes upregulation of TLR2, TLR4,[4] and NF-κB[5,6] activity; an increase in proinflammatory cytokines, including TNF-α,[7] IL-6,[7,8] and CRP[5]; and an increase in circulating neutrophils, IL-8, and hydroperoxides associated with impaired endothelial

TABLE IV. Airway inflammation (baseline and changes at 4 hours)

Inflammatory marker	Diet	0 h	*P* value	Δ 4 h	*P* value
Total cell count ($\times 10^6$/L)§	HCHF	2.6 (1.8, 6.3)	.790	–1.5 (–3.0, 0.0)†	.379
	ALF	3.9 (2.2, 6.3)		–2.0 (–3.2, –0.7)†	
	AHF-NonO	4.5 (3.4, 6.1)		–1.9 (–3.4, –0.9)†	
	AHF-O	4.0 (1.0, 5.9)		–0.5 (–3.3, 2.1)	
% Neutrophils‡	HCHF	37.0 ± 5.4	.978	15.3 ± 6.2†	.044
	ALF	38.7 ± 5.4		–2.7 ± 7.1	
	AHF-NonO	38.3 ± 6.2		11.9 ± 3.1†	
	AHF-O	40.9 ± 6.7		20.9 ± 5.8†	
% Eosinophils§	HCHF	0.0 (0.0, 0.5)*	.001	0.6 (–2.5, 7.0)	.550
	ALF	0.8 (0.3, 11.5)		–0.1 (–0.9, 3.1)	
	AHF-NonO	3.6 (0.4, 9.3)		0.0 (–3.8, 1.6)	
	AHF-O	2.1 (0.3, 5.2)		0.0 (0.0, 0.6)	
eNO (ppb)§	HCHF	17 (10, 30)	.073	–1 (–3, 1)	.135
	ALF	18 (15, 71)		–4 (–8, 0)†	
	AHF-NonO	30 (19, 43)		1 (–6, 3)	
	AHF-O	18 (9, 32)		–3 (–4, 1)	

	Diet	0 h	*P* value	Fold increase (4 h)	*P* value
TLR4 mRNA expression‡	HCHF	0.044 ± 0.015	.051	3.2 ± 0.8†	.044
	ALF	0.045 ± 0.005		1.1 ± 0.2	
	AHF-NonO	0.051 ± 0.012		2.9 ± 0.9	
	AHF-O	0.197 ± 0.117		1.5 ± 0.3	

*$P < .05$ versus ALF, AHF-NonO, and AHF-O.
†$P < .05$ compared with baseline.
‡Data are normally distributed and presented as means ± SEMs, with group comparisons performed by using the ANOVA test, with Bonferroni *post hoc* testing.
§Data are nonparametric, presented as median (quartile 1, quartile 3), with group comparisons performed by using the Kruskal-Wallis test, with Dunn *post hoc* testing.

function.[13] This study is the first to use the acute challenge model to investigate effects of dietary fat on airway inflammation in asthma.

We have also shown that in patients with asthma, dietary fat increases TLR4 mRNA expression in sputum cells. Although our data support the hypothesis that TLR4 activation has a central role in fat-induced inflammation, other receptors are also likely to be involved. A study by Zhao et al[35] demonstrated that nucleotide-binding oligomerization domain-containing proteins recognize saturated fatty acids in intestinal epithelial cells, resulting in modulation of NF-κB and IL-8 gene expression. The relevance of innate immune receptors in the airways is an important area for future research.

We did not see any differences in eNO levels after the high-fat meal in our study. eNO is a marker of eosinophilic disease rather than a general marker of airway inflammation in asthma.[1] Because the inflammatory changes that we observed involved increases in airway neutrophils, with no changes in sputum eosinophils, it is not surprising that eNO levels were unchanged. Another study using an acute fat challenge in healthy controls has recently reported a small increase in eNO production postmeal.[36] Although the increase was statistically significant, the size of the increase was very small (mean, 3.6 ppb) and is unlikely to be clinically significant. Atopy is also associated with eosinophilic, rather than neutrophilic, infiltration into the airways. Hence, it might be expected that subjects with nonatopic disease may be more susceptible to fat-induced inflammation. There were no differences in our key outcomes in subjects with and without atopy. However, the number of subjects included in these comparisons is small, and the study is not powered to detect such differences. This would be an interesting avenue for future research.

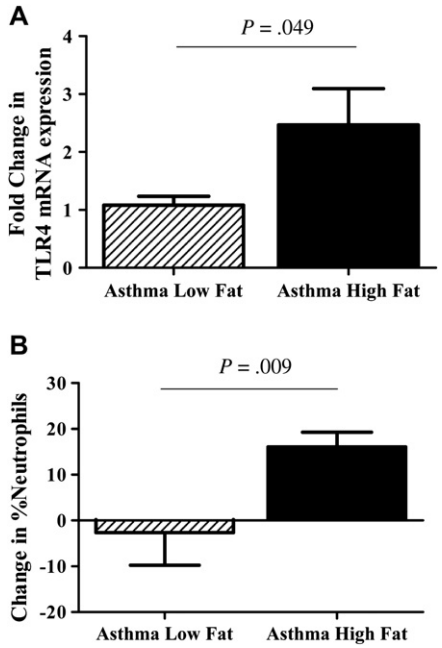

FIG 2. In subjects with asthma, changes in innate immune activity at 4 hours after administration of bronchodilator and consumption of the high fat (AHF) and low fat (ALF) food challenges. **A,** Fold change in induced sputum cell TLR4 mRNA expression. **B,** Change in induced sputum percent neutrophils.

We also observed that the high-fat meal impaired bronchodilator recovery in asthma by attenuating the increase in % predicted FEV_1 and FEV_1/FVC in the AHF versus the ALF groups. Our study design required subjects to withhold

TABLE V. Systemic inflammation (baseline and changes at 2, 3, and 4 hours)

Inflammatory marker	Diet group	0 h	*P* value	Δ 2 h	*P* value	Δ 3 h	*P* value	Δ 4 h	*P* value
IL-6 (pg/mL)	HCHF	0.8 (0.7, 1.2)	.001	0.0 (–0.3, 0.3)	.118	0.2 (–0.3, 0.9)	.119	0.3 (–0.1, 0.7)‡	.035
	ALF	1.2 (0.7, 1.6)		0.0 (–0.2, 0.3)		0.0 (–0.1, 0.4)		0.1 (–0.1, 0.4)	
	AHF-NonO	1.3 (1.1, 2.4)†		0.0 (–0.3, 0.3)		0.1 (–0.2, 0.8)		0.0 (–0.2, 0.6)	
	AHF-O	2.0 (1.6, 2.8)*†		0.2 (0.1, 1.2)‡		0.4 (0.2, 1.4)‡		0.8 (0.3, 1.2)‡	
TNF-α (pg/mL)	HCHF	1.4 (1.4, 2.1)	.578	0.0 (–0.2, 0.1)	.287	–0.1 (–0.4, 0.0)‡	.625	–0.1 (–0.3, 0.0)‡	.955
	ALF	1.6 (1.2, 2.3)		–0.2 (–0.5, 0.1)		–0.3 (–0.6, 0.1)‡		0.0 (–0.4, 0.2)	
	AHF-NonO	1.4 (0.9, 1.9)		–0.2 (–0.4, 0.0)‡		–0.2 (–0.4, 0.1)		–0.1 (–0.4, 0.2)	
	AHF-O	1.2 (0.9, 2.1)		–0.1 (–0.3, 0.2)		–0.1 (–0.3, 0.1)		0.0 (–0.4, 0.2)	
CRP (mg/mL)	HCHF	1.6 (0.8, 2,8)	.001	–0.1 (–0.3, 0.1)	.038	0.0 (–0.3, 0.1)	.631	–0.3 (0.1, 0.2)	.904
	ALF	1.3 (0.4, 3.4)		0.0 (–0.2, 0.1)		–0.1 (–0.2, 0.3)		0.1 (–0.1, 0.2)	
	AHF-NonO	2.6 (0.9, 8.9)		0.0 (–0.3, 0.3)		–0.2 (–0.8, 0.1)		0.0 (–0.5, 0.1)	
	AHF-O	7.0 (4.3, 13.0)*†		0.8 (–0.1, 1.5)†		0.0 (–1.1, 0.8)		0.3 (–0.5, 1.8)	

Data are nonparametric, presented as median (quartile 1, quartile 3), with group comparisons performed by using the Kruskal-Wallis test, with Dunn *post hoc* testing.
*$P < .05$ versus ALF.
†$P < .05$ versus HCHF.
‡$P < .05$ compared with baseline.

short-acting and long-acting β-agonists before the study. At baseline, subjects then underwent a hypertonic saline challenge, followed by the administration of salbutamol. In the subsequent 3-hour period, subjects in the ALF group had a mean improvement in % predicted FEV_1 of 6.2 compared with prebronchodilator % predicted FEV_1. However, for subjects in the AHF group, the recovery after bronchodilator administration was attenuated in both magnitude and duration, and only a small increase in % predicted FEV_1 was observed. Our data suggest that the attenuation of FEV_1 recovery in response to β-agonist was worse in the obese subjects with asthma. It is unclear why this difference would exist, because the inflammatory response to fat was similar in obese and nonobese subjects with asthma. However, it has previously been demonstrated that obesity leads to a worsening of asthma control and reduced responsiveness to treatment.[37] This observation requires further investigation.

The role of dietary fat in this differential bronchodilator recovery is reinforced by the consistent inverse correlations between change in lung function and change in plasma fatty acid levels. The effect of dietary fat on the response to a bronchodilator delivered by aerosol has not been reported previously. Airway inflammatory responses may be contributing to suppressed lung function, because sputum neutrophils have previously been shown to correlate negatively with lung function (% FEV_1) and airflow obstruction in asthma.[17] Fatty acids may also modify bronchodilator recovery by other mechanisms. A study of transdermal delivery of bronchodilators found that longer-chain fatty acids are more easily incorporated into cell membranes; however, their greater lipophilicity reduces membrane permeability.[38] In our study, the high fat load was dominated by longer-chain fatty acids (C16, C18, and C20), which may have reduced penetration of bronchodilator into the airway epithelium. Alternatively, it is possible that these exogenous fatty acids modulate the sympathetic nervous system, acting as β_2-adrenergic receptor antagonists, thereby inhibiting the bronchodilator effect. The mechanisms involved need further investigation, because the observation that a high-fat meal attenuates bronchodilator recovery has very important implications for asthma management.

There were few significant differences in systemic inflammatory markers in our study. Small increases in plasma IL-6 and CRP were observed in the AHF-O group. In the subjects with asthma, there was also a correlation between the increases in plasma fatty acid levels and TNF-α at 3 hours. The systemic inflammatory mediators that we evaluated, including IL-6,[39] CRP,[40] and TNF-α,[39] have previously been shown to be elevated after a high-fat meal. Although there were small increases in these mediators in our study, we observed a much stronger inflammatory response in the airways. Thus, it appears that the results we have observed are not simply the result of overspill of systemic inflammatory mediators into the airways.

The differential responses that we observed as a result of the trans versus nontrans fatty acid meals demonstrate the different innate immune responses that occur in response to fatty acids of different structures. It has previously been reported that induction of NF-κB activity,[41] CRP levels,[42] and TLR activity vary according to fat type,[43,44] with SFA having the most potent inflammatory actions. We chose to examine the effect of trans fatty acids on airway inflammation because they have been shown to have systemic proinflammatory effects.[45] Trans fatty acids exist primarily because of hydrogenation of fats in food manufacturing processes. Because of negative health outcomes associated with trans fatty acids, they are being eliminated from the food supply.

TABLE VI. Correlations among change (Δ) in plasma fatty acids, systemic and airway inflammatory markers, and lung function in nonobese subjects with asthma

	Outcome				
Predictor	Δ TNF-α (3 h)	Δ % N (4 h)	Δ FEV_1 (4 h)	Δ FVC % (4 h)	Δ FEV_1/FVC (4 h)
Δ SFA (4 h)					
r	0.349	0.451	–0.443	–0.532	–0.383
P	.064	.031	.010	.001	.028
Δ MUFA (4 h)					
r	0.333	0.512	–0.407	–0.472	–0.389
P	.078	.013	.019	.006	.025
Δ PUFA (4 h)					
r	0.479	0.382	–0.371	–0.332	–0.455
P	.009	.072	.034	.059	.008
Δ Total FA (4 h)					
r	0.394	0.490	–0.421	–0.464	–0.420
P	.034	.018	.007	.007	.015

FA, Fatty acid; *N,* neutrophils.

This study provides further evidence of the proinflammatory effects of trans fatty acids and demonstrates that the inflammatory effects of dietary fat on airway inflammation vary with fat quality. This is also an important area for future research.

The nutritional composition of the high-fat meal was mixed, containing fats of different quality and degree of saturation, as well as other macronutrients, all of which may have influenced the inflammatory response. This meal was chosen because it represents a dietary pattern that is typically associated with westernized diets and has been demonstrated to increase asthma risk.[46,47] Nonetheless, our data suggests that the dietary fat was important to the effects that we observed, because gene expression of *TLR4*, which is activated by nutritional fatty acids, was increased after the high-fat meal. In addition, the high-fat meal led to significant increases in plasma fatty acids compared with baseline, which were correlated with the increases in airway inflammation and suppression of lung function. Thus we can conclude that the increase in circulating fatty acids levels have contributed to the effects that we have observed. A limitation of the study is that the data presented are the result of a single meal. Further research is needed to confirm whether chronic consumption of high-fat foods would have similar sustained effects. Nonetheless, we provide evidence for a mechanism that may be contributing to the recent epidemiologic reports of an association between fast food and asthma risk.[46,47]

In summary, this study demonstrates that a high-fat/high-energy meal augments airway inflammation in asthma, resulting in an increased proportion of sputum neutrophils and increased *TLR4* mRNA expression in sputum cells, suggesting activation of the innate immune response by fatty acids in the airways. This fat-induced inflammation is also associated with a suppression of the airway response to bronchodilator, with the extent of this suppression correlating with the increase in fatty acid concentrations. We conclude that dietary fat leads to airway inflammation in asthma, and worse clinical outcomes. Strategies aimed at modifying dietary fat intake may be useful in reducing airway neutrophilia, which is known to have clinical significance in asthma.

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Clinical implications: Dietary fat leads to airway inflammation in asthma and impairs bronchodilator recovery. This suggests that strategies aimed at modifying dietary fat intake may be important in asthma management.

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