High density lipoproteins and type 2 inflammatory biomarkers are negatively correlated in atopic asthmatics

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Abstract Blood eosinophil counts and serum periostin levels are biomarkers of type 2 inflammation. Although serum levels of HDL and apoA-I have been associated with less severe airflow obstruction in asthma, it is not known whether serum lipids or lipoprotein particles are correlated with type 2 inflammation in asthmatics. Here, we assessed whether serum lipids and lipoproteins correlated with blood eosinophil counts or serum periostin levels in 165 atopic asthmatics and 163 nonasthmatic subjects with and without atopy. Serum lipids and lipoproteins were quantified using standard laboratory assays and NMR spectroscopy. Absolute blood eosinophils were quantified by complete blood counts. Periostin levels were measured using the Elecsys® periostin assay. In atopic asthmatics, blood eosinophils negatively correlated with serum HDL cholesterol and total HDL particles measured by NMR spectroscopy (HDL NMR). Serum periostin levels negatively correlated with total HDL NMR. In contrast, blood eosinophil counts positively correlated with serum triglyceride levels. This study demonstrates for the first time that HDL particles were negatively correlated, whereas serum triglycerides were positively correlated, with blood eosinophils in atopic asthmatics. This supports the concept that serum levels of HDL and triglycerides may be linked to systemic type 2 inflammation in atopic asthma.—Barochia, A. V., E. M. Gordon, M. Kaler, R. A. Cuento, P. Theard, D. M. Figueroa, X. Yao, N. A. Weir, M. L. Sampson, M. Stylianou, D. F. Choy, C. T. J. Holweg, A. T. Remaley, and S. J. Levine. High density lipoproteins and type 2 inflammatory biomarkers are negatively correlated in atopic asthmatics. J. Lipid Res. 2017. 58: 1713–1721.

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A type 2 high asthma endotype has been identified that is characterized by airway and systemic eosinophilic inflammation (1–3). Biomarkers to define type 2 high asthma include blood eosinophil counts and serum periostin levels. Blood eosinophil counts correlate with disease severity in eosinophil-predominant asthma and predict responsiveness to treatment with anti-interleukin (IL)-5 monoclonal antibodies (4–17). Increased serum levels of periostin, which can be induced by IL-13 in airway epithelial cells (18–20), have also been used to identify type 2 high asthma for subgroup analysis in clinical trials of anti-IL-13 monoclonal antibody therapy (21–24).

There is an increasing recognition that apolipoprotein pathways may participate in the pathogenesis of lung disease (25). Both apoE and apoA-I mimetic peptides have been shown to suppress type 2 inflammation in murine models of allergen-induced airway disease (26–28). Endothelial lipase, which is a phospholipase that hydrolyzes phospholipids and promotes the catabolism of HDL particles, is expressed in the lung where it may modulate eosinophilic inflammation. Consistent with this, allergen-challenged endothelial lipase knockout mice have high serum HDL levels and decreased eosinophilic lung inflammation (29). Among obese adolescent asthmatics, serum HDL levels have been inversely correlated with monocyte activation and numbers of patrolling monocytes (30). We have also recently shown that serum levels of HDL cholesterol (HDL-C), large HDL particles measured by NMR spectroscopy (HDL NMR particles), and apoA-I are positively associated with forced expiratory volume in 1 s (FEV1) in atopic

Abbreviations: CRP, C-reactive protein; FEV1, forced expiratory volume in 1 s; HDL-C, HDL cholesterol; HDL NMR, HDL particles measured by NMR spectroscopy; ICS, inhaled corticosteroid; IL, interleukin; LDL-C, LDL cholesterol.

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asthmatics, whereas a negative association existed with LDL cholesterol (LDL-C), triglycerides, and apoB (31). This suggests that a link may exist between airflow obstruction and serum lipids and lipoproteins in atopic asthmatics. Here, we hypothesized that similar associations might exist between serum lipids or lipoproteins and the type 2 inflammatory markers, blood eosinophils, and serum periostin in asthmatic and nonasthmatic subjects.

MATERIALS AND METHODS

Study population

Informed consent was obtained from 163 healthy nonasthmatic subjects and 165 clinically stable atopic asthmatics for participation in institutional review board-approved protocols, 96-H-0100 and 15-H-0059, at the National Heart, Lung, and Blood Institute between 1999 and 2015. All subjects from these protocols who met criteria for the current study and had analyzable serum samples were included in the study. These subjects were current nonsmokers and had undergone chest radiography to exclude other lung diseases. Asthma was diagnosed according to National Heart, Lung, and Blood Institute guidelines (32) and severe asthma was defined using European Respiratory Society (ERS)/American Thoracic Society (ATS) guidelines (32,33). All asthmatics demonstrated either reversible airflow obstruction following inhalation of a short-acting β2-agonist or airway hyperreactivity to methacholine bronchoprovocation testing, as well as a history and physical examination consistent with asthma. All nonasthmatic subjects demonstrated absence of airway hyperreactivity by methacholine bronchoprovocation challenge testing. Reported spirometry values were those obtained prior to administration of a bronchodilator.

Atopy was defined by a positive clinical history and skin test reactivity to at least one allergen or a history of severe allergy or anaphylaxis. A negative clinical history of allergy and negative allergy skin testing defined nonatopic subjects. Allergy skin testing at the National Institutes of Health was performed using a MultiTest II® applicator (a generous gift from Lincoln Diagnostics, Decatur, IL) and an allergen panel that included cat dander, Dermatophagoides farinae, short ragweed, Timothy grass, Aspergillus fumigatus, and cockroach antigen (HollisterStier, Spokane, WA).

Lipid, apolipoprotein, eosinophil, and periostin assays

Blood and serum samples were collected in the nonfasting state. Serum samples were stored at −80°C and thawed on ice prior to analysis. Standard laboratory tests and serum lipid profiles were performed in the Clinical Laboratory Improvement Amendment-certified National Institutes of Health Clinical Research Center Clinical Laboratory. The absolute number of blood eosinophils (cells per microliter) was quantified through complete blood counts with differential using an automated hematology analyzer. Serum apoA-I and apoB levels were measured using a Dimension Vista® Intelligent Lab system (Siemens Healthcare, Erlangen, Germany). Serum was diluted 1:10,000 in PBS prior to quantification of apoE using a commercially available ELISA with a lower limit of detection of 0.02 ng/ml (MABTECH Inc., Cincin-

NMR spectroscopy was performed using a Varian clinical analyzer (LipoScience Inc., Raleigh, NC). Serum periostin levels were measured using the Elecsys® periostin assay (Roche Diagnostics, Penzberg, Germany) (34). We utilized NMR spectroscopy to quantify lipoprotein particle subsets based upon size, as this approach has been proposed to be similar or superior to the standard measurement of HDL-C for the assessment of cardiovascular risk (35,36). NMR spectroscopy is a method that analyzes distinct signals emitted from each lipoprotein particle class when exposed to electromagnetic pulses. A direct correlation exists between the NMR signal amplitude and the concentration of the lipoprotein particle, which can then be identified by comparison to a reference library of lipoproteins.

Statistics

The null hypothesis was that there were no significant correlations between blood eosinophil counts or serum periostin levels and serum lipids or lipoproteins (HDL-C, total and large HDL-NMR particles, LDL-C, triglycerides, apoA-I, apoB, and apoE) in atopic asthmatics. Statistical analyses were performed using SAS Enterprise Guide version 4.3 (SAS Institute Inc., Cary, NC). All statistical tests were two-tailed and performed at the 0.05 α level. Results are presented as means ± SDs and medians (and interquartile range). Nonsymmetric or skewed variables were transformed to natural logarithms prior to further analysis. One-way ANOVA with Bonferroni correction was used to assess raw differences between groups. Correlation analyses were performed using Pearson correlation. Multivariable regression analysis by fitting linear models adjusted for predefined variables [age, sex, race, BMI, and C-reactive protein (CRP) levels] were utilized to assess group differences in serum lipid or lipoprotein levels. While multiplicity adjustment was applied to between group comparisons as stated above, adjustment was not applied to global hypothesis testing. Multivariable regressions with these same variables were also performed to assess relationships between eosinophils or periostin and serum lipid levels. Lastly, sensitivity analyses were performed by excluding race from the multivariable models, as well as to assess the effect of lipid profile modifying medication or inhaled corticosteroid use.

RESULTS

The study cohort consisted of three groups; 165 atopic asthmatics, 79 atopic nonasthmatics, and 84 nonatopic nonasthmatics (Table 1). As compared with nonatopic nonasthmatics, asthmatic subjects were older and had higher BMI, absolute blood eosinophil counts (hereafter referred to as eosinophils), serum total IgE, and serum CRP levels. Asthmatics also had lower FEV₁ (percent predicted) and FEV₁/forced vital capacity ratio as compared with both nonatopic nonasthmatics and atopic nonasthmatics. Atopic nonasthmatics had similar characteristics as nonatopic nonasthmatics, except that atopic nonasthmatics had higher total IgE and eosinophils. Serum periostin levels (hereafter referred to as periostin) did not differ between the three groups. Asthmatics and nonatopic nonasthmatics had proportionally more women as compared with atopic nonasthmatics.

Unadjusted standard lipid profile results are presented in Table 2. Asthmatics had significantly higher total cholesterol as compared with both nonatopic and atopic nonasthmatics and significantly higher serum LDL-C as compared with atopic nonasthmatics. After adjusting for differences in age, sex, race, BMI, and serum CRP, the only significant difference was a higher mean serum HDL-C in asthmatics as compared with nonatopic nonasthmatics (Table 3). Because there were no significant differences between
of total HDL (total HDLNMR) and large HDL particles. Sinophils were negatively correlated with the concentration mediated by a subset of HDL particles. In asthmatics, eosinophils were negatively correlated with the concentration of total HDL-C, apoA-I, LDL-C, apoB, or apoE. In addition, there was no correlation between eosinophils and any of the lipid values. As shown in Table 5, similar analyses for periostin showed that the negative correlation with total HDL-NMR remained significant in asthmatics (P = 0.001).

A sensitivity analysis was performed excluding 17 subjects who might have been taking lipid profile-modifying medications. Eight asthmatic subjects, three atopic nonasthmatics, and six nonatopic nonasthmatics were either taking a lipid-modifying medication or had missing information regarding medication use (Table 1). When the potential effects of age, sex, race, BMI, and CRP were...
accounted for using multivariable regression, significant relationships between eosinophils and HDL-C ($P = 0.007$) and total and large HDL$_{\text{NMR}}$ particles ($P = 0.01$), as well as triglycerides ($P = 0.03$), persisted in asthmatics. No significant relationships were observed between eosinophils and any lipid parameter in nonasthmatics after exclusion of subjects taking lipid profile-modifying medications. Similar sensitivity analyses performed for periostin showed persistence of the negative correlation between periostin and total HDL$_{\text{NMR}}$ ($P = 0.001$) in asthmatics.

As the two groups were not significantly different regarding racial composition ($P = 0.24$), additional sensitivity analyses with multivariable regression, including all previously specified variables except for race, were performed. After excluding race from the model, eosinophils remained significantly negatively correlated with HDL-C, total HDL$_{\text{NMR}}$, and large HDL$_{\text{NMR}}$ ($P = 0.03$, 0.01, and 0.05, respectively) and positively correlated with triglycerides ($P = 0.007$), independent of age, sex, BMI, and CRP levels in asthmatics. Similarly, sensitivity analyses showed that periostin remained significantly negatively correlated with total HDL$_{\text{NMR}}$ in asthmatics ($P = 0.003$).

Because inhaled corticosteroids can potentially alter eosinophil counts and possibly HDL-C levels, we performed a post hoc subgroup analysis of asthmatics who were using inhaled corticosteroids (ICSs) and those who

### TABLE 3. Adjusted means for the standard clinical lipid profile from multivariable regression analysis

|                        | NN [mean (95% CI)] | n | AN [mean (95% CI)] | n | AA [mean (95% CI)] | n |
|------------------------|--------------------|---|--------------------|---|--------------------|---|
| Total cholesterol (mg/dl) | 178 (171, 186)    | 84 | 177 (169, 184)    | 79 | 186 (181, 192)    | 164 |
| Triglycerides (mg/dl)   | 117 (105, 130)    | 84 | 109 (98, 121)     | 79 | 112 (103, 121)    | 164 |
| LDL-C (mg/dl)           | 107 (99, 114)     | 83 | 103 (95, 110)     | 77 | 110 (104, 115)    | 163 |
| apoE (µg/ml)            | 23 (20, 26)       | 78 | 20 (17, 25)       | 68 | 24 (21, 26)       | 159 |
| HDL-C (mg/dl)           | 45 (42, 49)       | 84 | 48 (44, 51)       | 79 | 52 (49, 54)       | 164 |
| apoA-I (mg/dl)          | 161 (154, 168)    | 84 | 157 (151, 164)    | 79 | 164 (159, 169)    | 164 |
| apoB (mg/dl)            | 92 (87, 97)       | 84 | 88 (83, 92)       | 79 | 91 (88, 95)       | 164 |

Adjusted (least squares) means for clinical lipid profile parameters for the three groups, from multivariable regression using linear models incorporating age, BMI, race, sex, and CRP as covariates. NN, nonatopic nonasthmatics; AN, atopic nonasthmatics; AA, atopic asthmatics.

AA and AN are no different from NN with regard to all lipid panel parameters except HDL-C, which is significantly higher ($P < 0.05$) in the AA group compared with NN.

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**Fig. 1.** Blood eosinophils in asthmatics are positively correlated with serum triglyceride levels and negatively correlated with serum HDL-C. Correlations between absolute blood eosinophil counts (cells per microliter; log transformed) with serum levels of triglycerides (log transformed), HDL-C and apo A-I in nonasthmatics and asthmatics are shown. Pearson’s correlation coefficients and associated $P$ values are shown for significant relationships only.
HDL and inflammation are correlated in asthmatics

were not \( (n = 76 \text{ and } 89, \text{ respectively} \). Mean HDL-C level in those asthmatics using ICSs was 55.3 (±17.7) mg/dl and 53.0 (±17.3) mg/dl in those not using ICSs at the time of study \( (P = 0.4; \text{ one-way ANOVA}) \). Subjects who were using ICSs at the time of study had slightly higher mean eosinophil counts than those who were not \( (270 ± 3 \text{ eosinophils/μl vs. } 197 ± 2 \text{ eosinophils/μl, respectively; } P = 0.02) \). Similar to the overall group results, Pearson correlation between HDL-C and eosinophils showed a negative association between HDL-C and eosinophils in both of these subgroups \( (r = -0.199 \text{ and } r = -0.188, \text{ respectively; } P = 0.08 \text{ for both}) \). We did not separately assess the very small subgroup of asthmatics who were taking oral corticosteroids \( (n = 6) \) at the time of study. Only three asthmatic subjects were taking medications for the treatment of diabetes mellitus. Therefore, we did not perform any further subgroup analysis with regard to diabetics.

**DISCUSSION**

Here, we assessed whether correlations exist between serum levels of lipids, apolipoproteins, and lipoprotein particles with biomarkers of type 2 inflammation. We report that serum levels of HDL-C are negatively correlated,
whereas serum triglycerides are positively correlated, with blood eosinophil counts in atopic asthmatics, independent of age, sex, race, BMI, and serum CRP levels. Furthermore, the negative association with blood eosinophil counts was mediated by large HDL\textsubscript{NMR} particles. In addition, serum periostin was inversely associated with the concentration of total HDL\textsubscript{NMR} particles in asthmatics.

HDL mediates reverse cholesterol transport, which effluxes phospholipids and cholesterol out of cells, to facilitate its atheroprotective effects (37). In addition, HDL has anti-oxidant, anti-thrombotic, and anti-inflammatory functions. In particular, HDL-mediated cholesterol efflux can decrease cellular cholesterol content and thereby reduce receptor localization to lipid raft microdomains, which attenuates cytokine receptor signaling, as well as the ability of antigen-presenting dendritic cells to activate T cell responses (38–41). We hypothesize that these anti-inflammatory properties of HDL may contribute to its atheroprotective effects (37). In addition, HDL facilitates its atheroprotective effects (37).

Similar to the associations of HDL and triglycerides with lower and higher cardiovascular risk, respectively, we now show that HDL particles are negatively associated, whereas triglycerides are positively associated, with type 2 inflammation in asthma (42, 43). Although qualitatively similar correlations were initially observed in nonasthmatics, these relationships became nonsignificant after adjusting for age, sex, race, BMI, and CRP levels. While the correlation coefficients for the significant relationships between eosinophils and lipoproteins and triglycerides are not large, these findings nevertheless suggest that serum lipids and lipoproteins are associated with systemic type 2 inflammation in asthma. This level of correlation could be seen to be consistent with the biology of systemic type 2 inflammation, which is very complex and regulated by numerous factors, just two of which may include HDL and triglycerides (1).

That these relationships could be biologically relevant in asthma is suggested by the robustness of the results in asthmatics even after adjusting for multiple potential confounders, including the use of lipid profile modifying medications or inhaled corticosteroids. In addition, these results are consistent with the associations we previously described between FEV\textsubscript{1} and serum lipids and lipoproteins in asthmatics (31).

Several prior studies have reported contrasting findings regarding the association between lipids and eosinophils or periostin in general populations. For example, similar to our analysis, the prospective LifeLines Cohort Study of 13,301 subjects from the Netherlands found that higher eosinophil counts were associated with lower serum HDL levels, as well as higher serum levels of triglycerides, total cholesterol, and LDL (44). Similarly, a multiple regression analysis of 822 Korean subjects with type 2 diabetes mellitus found that serum HDL-C levels were negatively correlated with blood eosinophil counts (45). However, a study of 3,282 Taiwanese subjects found no significant correlations.

| Lipid/Lipoprotein Fractions     | Nonasthmatics |            |            | P    |            |            | P    |
|---------------------------------|---------------|------------|------------|------|------------|------------|------|
| Total cholesterol (mg/dl)       |               |           |            |      |            |            |      |
| Triglycerides (mg/dl)           |               | 2.35      | (5.00, 7.90)| 0.53 |            |            |      |
| LDL-C (mg/dl)                   |               |            |            |      |            |            |      |
| apoE (μg/ml)                    |               | (0.11, 0.11)| 0.49 |            |            |            |      |
| HDL-C (mg/dl)                   |               | 2.65      | (2.85, 2.95)| 0.43 |            |            |      |
| apoA-I (mg/dl)                  |               | 3.73      | (3.91, 3.97)| 0.23 |            |            |      |
| apoB (mg/dl)                    |               | 1.24      | (1.26, 1.38)| 0.33 |            |            |      |
| HDL particles (μmol/l)          |               | 0.54      | (0.87, 1.95)| 0.45 |            |            |      |
| Total HDL\textsubscript{NMR}    |               | 0.17      | (0.98, 0.64)| 0.68 |            |            |      |
| Large HDL\textsubscript{NMR}    |               |           |            |      |            |            |      |

Intercepts and estimates for NMR lipid parameters from multivariable regression, using linear models incorporating BMI, log CRP, age, sex, and race as covariates, to test for significant associations between serum periostin levels and lipid or lipoprotein parameters. P values for significant associations are shown in bold italicized text.

| Lipid/Lipoprotein Fractions     | Nonasthmatics |            |            | P    |            |            | P    |
|---------------------------------|---------------|------------|------------|------|------------|------------|------|
| Total cholesterol (mg/dl)       |               | 0.12      | (0.27, 0.51)| 0.53 |            |            |      |
| Triglycerides (mg/dl)           |               | 0.003     | (0.003, 0.01)| 0.27 |            |            |      |
| LDL-C (mg/dl)                   |               | 0.04      | (0.32, 0.39)| 0.84 |            |            |      |
| apoE (μg/ml)                    |               | 0.001     | (0.001, 0.01)| 0.88 |            |            |      |
| HDL-C (mg/dl)                   |               | 0.01      | (0.17, 0.19)| 0.90 |            |            |      |
| apoA-I (mg/dl)                  |               | 0.12      | (0.45, 0.29)| 0.46 |            |            |      |
| apoB (mg/dl)                    |               | 0.01      | (0.26, 0.24)| 0.94 |            |            |      |
| HDL particles (μmol/l)          |               |           |            |      |            |            |      |
| Total HDL\textsubscript{NMR}    |               | (0.27, 0.51)| 0.53 |            |            |            |      |
| Large HDL\textsubscript{NMR}    |               | (0.27, 0.51)| 0.53 |            |            |            |      |

Intercepts and estimates for NMR lipid parameters from multivariable regression, using linear models incorporating BMI, log CRP, age, sex, and race as covariates, to test for significant associations between serum periostin levels and lipid or lipoprotein parameters. P values for significant associations are shown in bold italicized text.
between eosinophils and lipid levels using multivariable regression that included age, gender, BMI, and smoking status (46). Plasma periostin levels have also been associated with plasma triglycerides, TNF-α levels, and insulin resistance in 161 Chinese subjects with obesity and type 2 diabetes mellitus (47).

There have been a number of studies and reviews that have examined the efficacy of statins in asthma, as these cholesterol-lowering agents have anti-inflammatory and anti-oxidant effects and are a mainstay of risk reduction in cardiovascular disease (48–50). However, statins have not been demonstrated to be consistently effective for the treatment of asthma, which may reflect a variety of factors that have been reviewed elsewhere (48). We have found that serum cholesterol levels are not associated with blood eosinophil counts, serum periostin levels, or FEV1, which suggests that modifying cholesterol levels alone may not be sufficient to manage asthma control. HDL particles are comprised of at least 85 different proteins, which raises the hypothesis that HDL-associated proteins, rather than its cholesterol content, may be relevant for the associations that we have shown with biomarkers of type 2 inflammation and airflow obstruction (51).

It is important to clarify several points regarding our study. First, although asthmatics had both higher adjusted HDL-C levels as well as higher eosinophil counts, there is a negative correlation between these two variables. This apparent discrepancy is not an inconsistency; simply comparing group means does not allow examination of the relationship between HDL-C and eosinophils at the level of the individual subject. Consequently, we performed correlation analyses precisely to be able to determine whether these variables could be linked at the level of each individual subject. Second, lipids and lipoprotein levels were measured from samples collected in the nonfasting state. Most lipid parameters, other than triglycerides, collected in the nonfasting state have been shown to be similar to fasting levels, and also predictive of cardiovascular outcomes such as myocardial infarction, stroke and death. Nonfasting measurements of serum lipids have now become standard practice except in special circumstances such as severe hypertriglyceridemia (52). Only four subjects from our entire cohort had triglyceride levels that exceeded 400 mg/dl, so we believe that it is unlikely that the nonfasting lipid measurements impacted our findings. Third, apoA-I is the major protein component of HDL, facilitating reverse cholesterol transport (53). Although we previously found a positive association between serum apoA-I levels and FEV1 in atopic asthmatics, in the current study no association was found between serum apoA-I levels and blood eosinophil counts or serum periostin levels (31). This raises the possibility that protein or lipid components of HDL other than apoA-I may be the link between HDL and biomarkers of type 2 inflammation. Fourth, although we did not find a difference in standard lipid values between the nonatopic and atopic nonasthmatics, we cannot exclude the possibility that our sample size was too small to detect a significant difference between these two groups. Lastly, it is important to emphasize that the associations that we report between biomarkers of type 2 inflammation and lipids or lipoproteins in atopic asthmatics do not establish causality. Thus, for example, we cannot infer based on our data that type 2 inflammation results in lower serum HDL levels; or the reverse, that higher serum HDL levels result in lower eosinophil counts.

Prior studies have assessed differences in lipid profiles between asthmatics and normal healthy individuals, as well as the relationships between serum lipids and asthma, with divergent results. Potential explanations for these heterogeneous findings include differences in the assays used, the demographic characteristics and cultural and genetic backgrounds of the populations studied, and definitions of asthma used in these studies (31, 54). Here, we took care to characterize the clinical phenotype of asthmatics versus nonasthmatics, but we acknowledge that other factors, such as the metabolic syndrome, could potentially be modifying the observed relationships in our study. Additionally, this was a cross-sectional study and these relationships may change over time. Thus, we have initiated the collection of longitudinal data to address this concern.

In conclusion, to the best of our knowledge, this is the first report of an association between biomarkers of type 2 inflammation and serum HDL and triglycerides in human atopic asthma. These results extend our previous finding that showed a positive correlation in atopic asthmatics between FEV1 and serum HDL-C, whereas FEV1 and triglycerides were negatively correlated (31). Collectively, these results show that serum levels of HDL and triglycerides are correlated with both systemic type 2 inflammation and airflow obstruction in atopic asthma. Our findings provide new insights regarding potential mechanisms that may modulate type 2 inflammation in allergic asthma. If, indeed, these results are confirmed by additional studies, they will support further investigations to determine how lipids or lipoproteins might modulate disease pathogenesis in allergic asthma, and shed light on potential new targets for therapy.

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