ORIGINAL ARTICLE

Serum Apolipoprotein A-I and Large High-Density Lipoprotein Particles Are Positively Correlated with FEV_1 in Atopic Asthma

Amisha V. Barochia[1,2], Maryann Kaler[1,2], Rosemarie A. Cuento[1,2], Elizabeth M. Gordon[1,2], Nargues A. Weir[1,2], Maureen Sampson[3], Joseph R. Fontana[2], Sandra MacDonald[2], Joel Moss[2], Vincent Manganiello[2], Alan T. Remaley[2], and Stewart J. Levine[1,2]

[1]Laboratory of Asthma and Lung Inflammation, NHLBI, [2]Cardiovascular and Pulmonary Branch, NHLBI, and [3]National Institutes of Health Clinical Center, National Institutes of Health, Bethesda, Maryland

Abstract

Rationale: Although lipids, apolipoproteins, and lipoprotein particles are important modulators of inflammation, varying relationships exist between these parameters and asthma.

Objectives: To determine whether serum lipids and apolipoproteins correlate with the severity of airflow obstruction in subjects with atopy and asthma.

Methods: Serum samples were obtained from 154 atopic and nonatopic subjects without asthma, and 159 subjects with atopy and asthma. Serum lipid and lipoprotein levels were quantified using standard diagnostic assays and nuclear magnetic resonance (NMR) spectroscopy. Airflow obstruction was assessed by FEV_1% predicted.

Measurements and Main Results: Serum lipid levels correlated with FEV_1 only in the subjects with atopy and asthma. Serum levels of high-density lipoprotein (HDL) cholesterol and apolipoprotein A-I (apoA-I) were positively correlated with FEV_1 in subjects with atopy and asthma, whereas a negative correlation existed between FEV_1 and serum levels of triglycerides, low-density lipoprotein (LDL) cholesterol, apolipoprotein B (apoB), and the apoB/apoA-I ratio. NMR spectroscopy identified a positive correlation between FEV_1 and HDL_{NMR} particle size, as well as the concentrations of large HDL_{NMR} particles and total IDL_{NMR} (intermediate-density lipoprotein) particles in subjects with atopy and asthma. In contrast, LDL_{NMR} particle size and concentrations of LDL_{NMR} and $VLDL_{NMR}$ (very-low-density lipoprotein) particles were negatively correlated with FEV_1 in subjects with atopy and asthma.

Conclusions: In subjects with atopy and asthma, serum levels of apoA-I and large HDL_{NMR} particles are positively correlated with FEV_1, whereas serum triglycerides, LDL cholesterol, and apoB are associated with more severe airflow obstruction. These results may facilitate future studies to assess whether apoA-I and large HDL_{NMR} particles can reduce airflow obstruction and disease severity in asthma.

Keywords: asthma; airflow obstruction; lipids; lipoprotein particles; apolipoproteins

Lipoprotein metabolism has been extensively investigated based on the central role of cholesterol and triglycerides (TGs) in the pathogenesis of vascular inflammation and atherosclerosis (1). Cholesterol is primarily carried to cells by low-density lipoprotein (LDL) particles, which interact with LDL receptors (LDLR) to internalize their lipid cargo by receptor-mediated endocytosis (1). In contrast, high-density lipoprotein (HDL) particles are formed in the liver and intestine via the interaction of lipid-poor apolipoprotein A-I (apoA-I) with the cholesterol-phospholipid transporter, ABCA1 (ATP-binding cassette, subfamily A, member 1), followed by the acquisition of additional lipids and apolipoproteins (2, 3). Epidemiologic

(Received in original form November 7, 2014; accepted in final form February 15, 2015)

Supported by the Division of Intramural Research, NHLBI, National Institutes of Health.

Author Contributions: A.V.B., A.T.R., and S.J.L. conceived the study. A.V.B., M.K., R.A.C., N.A.W., J.R.F., and S.M. collected the data. A.V.B., E.M.G., and M.S. performed the assays. A.V.B., N.A.W., J.R.F., J.M., V.M., A.T.R., and S.J.L. analyzed the data. A.V.B. and S.J.L. wrote the manuscript. All authors provided critical review of the manuscript and approved of its contents and submission.

Correspondence and requests for reprints should be addressed to Amisha V. Barochia, M.B. B.S., Laboratory of Asthma and Lung Inflammation, Cardiovascular and Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, 10 Center Drive, CRC Room 6-3152, Bethesda, MD 20892. E-mail: barochiaav@nhlbi.nih.gov

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 191, Iss 9, pp 990–1000, May 1, 2015
Copyright © 2015 by the American Thoracic Society
Originally Published in Press as DOI: 10.1164/rccm.201411-1990OC on February 18, 2015
Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the Subject: Apolipoproteins have increasingly been recognized to modulate lung development and homeostasis, as well as adaptive immune responses and host defense. Multiple prior studies, however, have reported heterogeneous results regarding whether serum apolipoprotein and lipid levels are altered in subjects with asthma compared with subjects without asthma.

What This Study Adds to the Field: This study provides new data that serum apolipoprotein and lipid levels correlate with the severity of airflow obstruction in atopic asthma. A positive correlation exists between the FEV_1% predicted and serum levels of high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A-I (apoA-I), whereas there is a negative correlation between the FEV_1% predicted and serum levels of triglycerides, low-density lipoprotein cholesterol, apolipoprotein B (apoB), and the apoB/apoA-I ratio. Furthermore, our findings, using nuclear magnetic resonance (NMR) spectroscopy, suggest that the positive correlation between FEV_1% predicted and HDL-C or apoA-I in atopic asthma may be largely mediated by the subfraction of large HDL_{NMR} particles. These findings may serve as the basis for future studies to assess whether apoA-I and large HDL_{NMR} particles can reduce airflow obstruction and disease severity in asthma.

studies have shown that increases in serum levels of LDL cholesterol (LDL-C) are associated with an increased risk of myocardial infarction, whereas HDL cholesterol (HDL-C) is inversely associated with the risk of coronary artery disease (1, 3).

Apolipoproteins, which are known to modulate normal lung development and homeostasis, as well as adaptive immune responses and host defense in the lung (4–12), have also been shown to participate in the pathogenesis of experimental murine asthma. For example, apoA-I levels are reduced in bronchoalveolar fluid from ovalbumin (OVA)-challenged mice and this finding has similarly been demonstrated in human subjects with asthma (13, 14). Furthermore, OVA-challenged apoA-$I^{-/-}$ mice have significant increases in bronchoalveolar fluid neutrophils that are primarily mediated by enhanced granulocyte colony-stimulating factor expression (13). Moreover, administration of apoA-I mimetic peptides attenuates airway inflammation, airway hyperreactivity, and mucous cell metaplasia in house dust mite– and OVA-induced models of experimental asthma (15, 16). Similarly, administration of holo–apoA-I to house dust mite–challenged mice attenuates airway inflammation and airway hyperreactivity, promotes the recovery of disrupted epithelial tight junction proteins, and increases the production of lipoxin A4 (14). Also, OVA-challenged transgenic mice that express human ABCA1 under the control of the Tie2 promoter have reduced OVA-induced bronchoalveolar fluid neutrophils and decreased granulocyte colony-stimulating factor production by vascular endothelial cells and alveolar macrophages (17).

Collectively, these studies support the concept that the apoA-I/ABCA1 pathway has a protective effect in experimental murine models of allergic asthma. Additionally, the ABCG1 transporter has also been shown to regulate asthma pathogenesis because OVA-challenged *Abcg1*$^{-/-}$ mice have reduced Th2-mediated adaptive immunity, but increased IL-17–dependent airway neutrophils (18). Lastly, apoE, which is expressed by alveolar macrophages, has also been shown to negatively regulate airway hyperreactivity and mucous cell metaplasia in experimental house dust mite–induced asthma by interacting with LDLRs expressed by ciliated airway epithelial cells (19).

Although these experimental studies in mostly animal models suggest a role for apolipoprotein pathways in the pathogenesis of asthma, prior clinical studies have found heterogeneous results regarding the relationship between serum lipid levels and asthma (20). However, a recent cross-sectional analysis of a large pediatric cohort found a statistically significant association between asthma prevalence and higher serum TG levels, and higher rates of insulin resistance, independent of body mass. This suggested that metabolic abnormalities, such as hypertriglyceridemia and insulin resistance, may influence asthma prevalence (21). Here, we sought to assess whether serum lipids, apolipoproteins, and lipoprotein particles, as quantified using standard diagnostic assays, correlate with the severity of airflow obstruction, as measured by the FEV_1% predicted, in a cohort comprised of nonatopic subjects without asthma, atopic subjects without asthma, and atopic subjects with asthma. Furthermore, we used nuclear magnetic resonance (NMR) spectroscopy to assess whether the size and concentration

Table 1. Clinical Characteristics of the Study Groups

	Nonatopic Nonasthmatic	Atopic Nonasthmatic	Atopic Asthmatic
Number of subjects	80	74	159
Age	31 ± 12.9	33.4 ± 12.4	37.5 ± 14.4*
Sex, female/male	56/24	35/39	100/59
Race, white/black/other	53/16/11	48/14/12	99/39/21
Body mass index, kg/m^2	25.5 ± 5.2	25.8 ± 4.8	28.2 ± 6.7*
FEV_1% predicted	110 ± 14.9	109 ± 12.6	86 ± 21.1*
FEV_1/FVC	85 ± 5.5%	84 ± 5.6%	71 ± 13.1%*
Immunoglobulin E, IU/ml†	22 (8.5–48)	80 (35–234)*	222 (103.5–472.5)*
Absolute eosinophil count, per μl†	100 (70–144)	131 (82–194)*	232 (143–375)*
C-reactive protein, mg/L†	0.9 (0.4–2.3)	0.8 (0.4–2.3)	1.4 (0.6–4.9)*
No. of subjects on lipid-modifying medication	4	1	9

Values shown are means ± SD, except as noted in the footnote.
*$P \leq 0.05$ as compared with the nonatopic nonasthmatic group; one-way analysis of variance.
†Denotes median (lower quartile–upper quartile).

of specific subgroups of lipoprotein particles correlate with FEV_1.

Methods

Study Population

Healthy subjects without asthma with and without atopy and clinically stable subjects with atopy and asthma were recruited to participate in institutional review board–approved protocols (96-H-0100 and 13-H-0059) at the NHLBI between 1999 and 2014, after providing written informed consent. The diagnosis of asthma was established according to NHLBI guidelines (22), and severe asthma was defined using European Respiratory Society/American Thoracic Society guidelines (23). All subjects with asthma demonstrated either reversible airflow obstruction following administration of an inhaled short-acting β_2-agonist or, alternatively, airway hyperreactivity in response to methacholine bronchoprovocation testing. Subjects without asthma had a history and physical examination that was negative for asthma, and absence of airway hyperreactivity as documented by a negative methacholine bronchoprovocation challenge. Atopy was defined by a positive clinical history and skin test reactivity to at least one of four common aeroallergens, or a history of severe allergy or anaphylaxis. Subjects without atopy had a negative clinical history of allergy and negative skin test reactivity to the four aeroallergens.

Statistics

Statistical analyses were performed using SAS Enterprise Guide Version 4.3 (SAS Institute Inc., Cary, NC). Differences between study groups and control subjects were examined using a one-way analysis of variance (Dunnett test), whereas correlation analyses were performed using Pearson correlation. Type 1 error was controlled for at the level of comparison with alpha set at 0.05. Additional details regarding the methods and statistical analyses are provided in the online supplement.

Results

Table 1 presents the baseline clinical characteristics of the three subject groups: (*1*) subjects with no atopy or asthma (NN), (*2*) subjects with atopy and no asthma (AN), and (*3*) subjects with atopy and asthma (AA). A total of 130 (82%) subjects within the AA group had mild or moderate asthma, whereas 29 (18%) had severe disease. As compared with the NN control group, the AA but not the AN group were older, had higher body mass index and C-reactive protein levels, but lower FEV_1. The AA group, but not the AN group, also had a lower FEV_1/FVC ratio, indicative of airflow obstruction, as compared with the NN group, whereas both the AN and AA groups had higher eosinophil counts and IgE levels than the NN group, consistent with the presence of atopy.

The standard clinical lipid profiles of the three groups were compared using one-way analysis of variance, and no significant differences were found between the AN group and NN control subjects for any of the lipid or apolipoprotein levels, whereas in the AA group, only serum levels of total cholesterol (TC) were significantly higher than the NN group (Table 2). To account for differences in age, sex, race and ethnicity, body mass index, and C-reactive protein levels among the groups that may have affected the clinical lipid profiles, a multivariate regression analysis using linear models was performed. These variables were found to be significant in the multivariate analysis and

Table 2. Standard Clinical Lipid Profiles of the Study Population Presented as Unadjusted Means

	Nonatopic Nonasthmatic	Atopic Nonasthmatic	Atopic Asthmatic
Number of subjects	80	74	159
Total cholesterol, mg/dl	176.7 ± 32.9	173.2 + 37.3	191.4 ± 35.7*
Triglycerides, mg/dl†	105.5 (70.5–141)	97.5 (72–165)	108 (76–161)
LDL-C, mg/dl	103.3 ± 31	98.7 ± 29.1	112.3 ± 32
apoE, μg/ml†	20.0 (14.0–32.2)	19.6 (12.0–28.6)	23.2 (14.7–32.5)
HDL-C, mg/dl	49.6 ± 16.7	47.6 + 16.7	53.8 ± 17.1
apoA-I, mg/dl	169.6 ± 30.3	158.3 ± 31.8	170.4 ± 34.4
apoB, mg/dl	87.2 ± 22.3	84.7 ± 23.8	91.6 ± 24.2
apoB/apoA-I ratio	0.54 ± 0.19	0.56 ± 0.19	0.56 ± 0.19

Definition of abbreviations: apo = apolipoprotein; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol.
Values shown are means ± SD, except as noted in the footnote.
*$P \leq 0.05$, as compared with the nonatopic nonasthmatic group, one-way analysis of variance.
†Denotes median (lower quartile–upper quartile).

Table 3. Adjusted (Least Squares) Means for Standard Clinical Lipid Profile Parameters from Multivariate Linear Regression Models Incorporating Age, Body Mass Index, Race/Ethnicity, Sex, and C-Reactive Protein

	Nonatopic Nonasthmatic	Atopic Nonasthmatic	Atopic Asthmatic
Total cholesterol, mg/dl	180.6 (172.7–188.4)	176.8 (169.0–184.6)	187.6 (181.8–193.3)
Triglycerides, mg/dl	118.6 (106.5–132.1)	113.4 (101.9–126.2)	111.4 (102.9–120.6)
LDL-C, mg/dl	108.8 (101.5–116.0)	103.0 (95.8–110.2)	111.1 (105.9–116.4)
apoE, μg/ml	22.7 (19.8–26.1)	19.4 (16.8–22.4)	22.7 (20.4–25.2)
HDL-C, mg/dl	45.2 (41.6–48.9)	46.5 (42.8–50.1)	51.4 (48.7–54.0)*
apoA-I, mg/dl	162.6 (155.7–169.4)	157.4 (150.6–164.2)	164.7 (159.7–169.7)
apoB, mg/dl	92.7 (87.5–98.0)	88.0 (82.8–93.3)	91.5 (87.6–95.3)
apoB/apoA-I ratio	0.60 (0.6–0.6)	0.6 (0.5–0.6)	0.6 (0.6–0.6)

Definition of abbreviations: apo = apolipoprotein; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol.
Values shown are means (95% confidence intervals).
*$P \leq 0.05$, as compared with the nonatopic nonasthmatic group, multivariate regression analysis (linear models).

were included in the linear models. After accounting for these potential confounding variables, there again were no statistically significant differences between the AN and NN control groups, whereas the AA group had significantly higher HDL-C levels as compared with the NN control group. Adjusted (least squares means) values are provided in Table 3.

Next, a Pearson correlation analysis was performed to determine if there were significant correlations between serum lipids and lipoproteins and the severity of airflow obstruction, as measured by the FEV_1. Interestingly, as shown in Figures 1 and 2, correlations between components of the standard clinical lipid profiles and the severity of airway obstruction were found only in the AA group. In particular, FEV_1 was found to be negatively associated with serum TG, LDL, and apoB levels, as well as the ratio of apoB/apoA-I. In contrast, FEV_1 was positively associated with HDL-C and apoA-I levels. Serum levels of TC or apoE, however, were not found to correlate with FEV_1 in either of the groups.

Having shown that serum HDL and apoA-I are positively correlated with FEV_1 in the AA group alone, we next performed a more detailed examination of lipoprotein subfractions, using NMR spectroscopy to determine which particle subfractions could be mediating this effect. Similar to the correlations with the standard lipid profiles, only in the AA group were the NMR lipoprotein subfractions correlated with lung function. In particular, there was a positive correlation between FEV_1 and HDL_{NMR} size (Figure 3), the concentration of large HDL_{NMR} particles (Figure 4), and the concentration of total intermediate-density lipoprotein (IDL_{NMR}) particles (Figure 5). A negative correlation was found between FEV_1 and LDL_{NMR} particle size (Figure 3), and the concentration of total LDL_{NMR} particles (Figure 5) and total very-low-density lipoprotein ($VLDL_{NMR}$) particles (Figure 6). In addition, the concentrations of large and small LDL_{NMR} particles (Figure 5), and the concentrations of large and small $VLDL_{NMR}$ particles (Figure 6) were negatively correlated with FEV_1. We also performed linear regression analyses to assess whether serum TG concentrations, which can modify the composition of HDL and LDL particles (24), could have influenced the correlations between FEV_1 and HDL_{NMR} size or LDL_{NMR} size. As shown in Table 4, the significant associations between FEV_1 and HDL_{NMR} size and LDL_{NMR} size persisted regardless of serum TG concentrations.

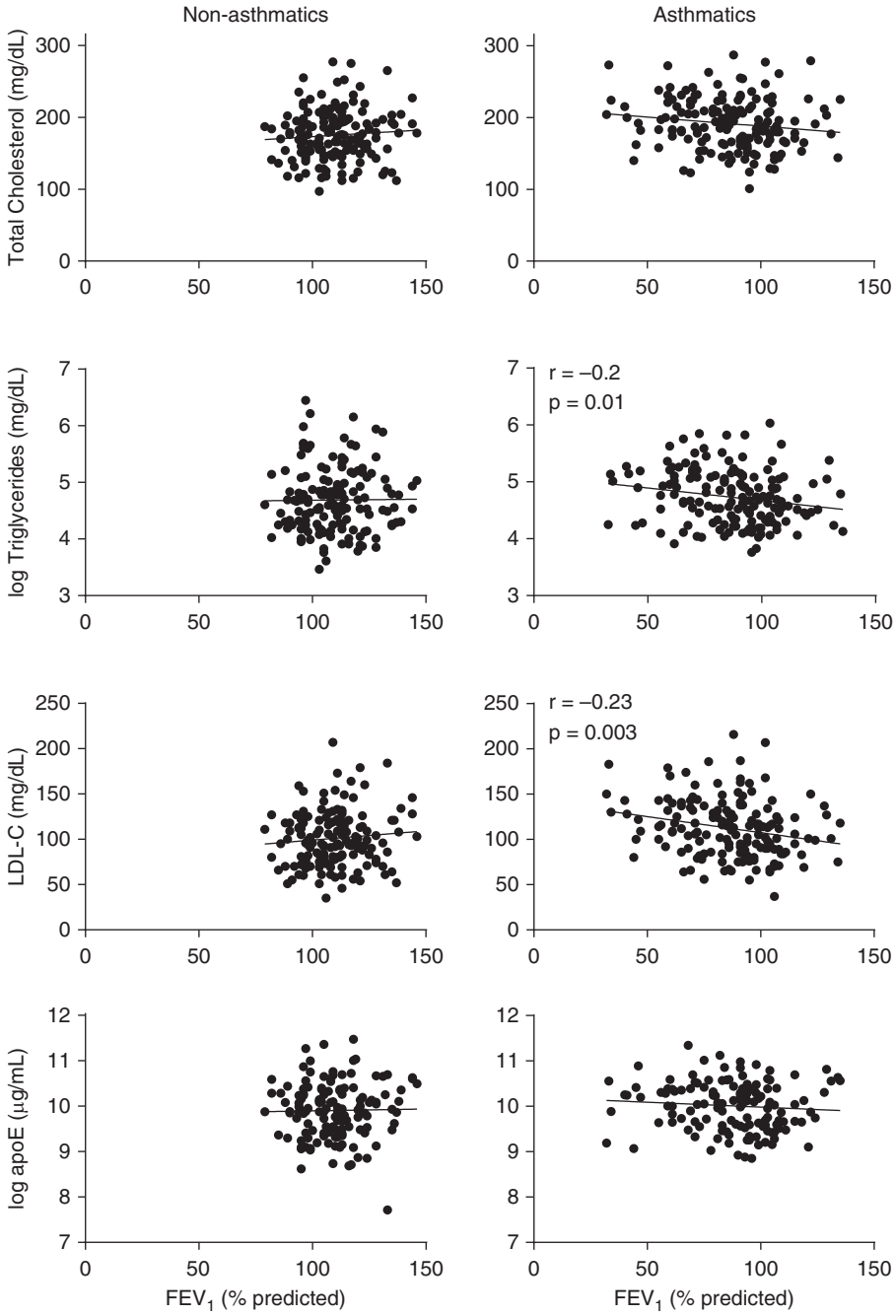


Figure 1. The FEV_1 in subjects with atopy and asthma is inversely correlated with serum triglyceride and low-density lipoprotein cholesterol (LDL-C) levels. Correlations between FEV_1% predicted and serum levels of total cholesterol, triglycerides, LDL-C, and log apolipoprotein E (apoE) levels in subjects without asthma and subjects with atopy and asthma are shown. Pearson correlation coefficients and associated *P* values are shown for significant relationships only.

We then divided the subjects with asthma into those with mild to moderate asthma and those with severe asthma, to assess whether the correlations between serum lipids and FEV_1 persisted when subjects with asthma were grouped by

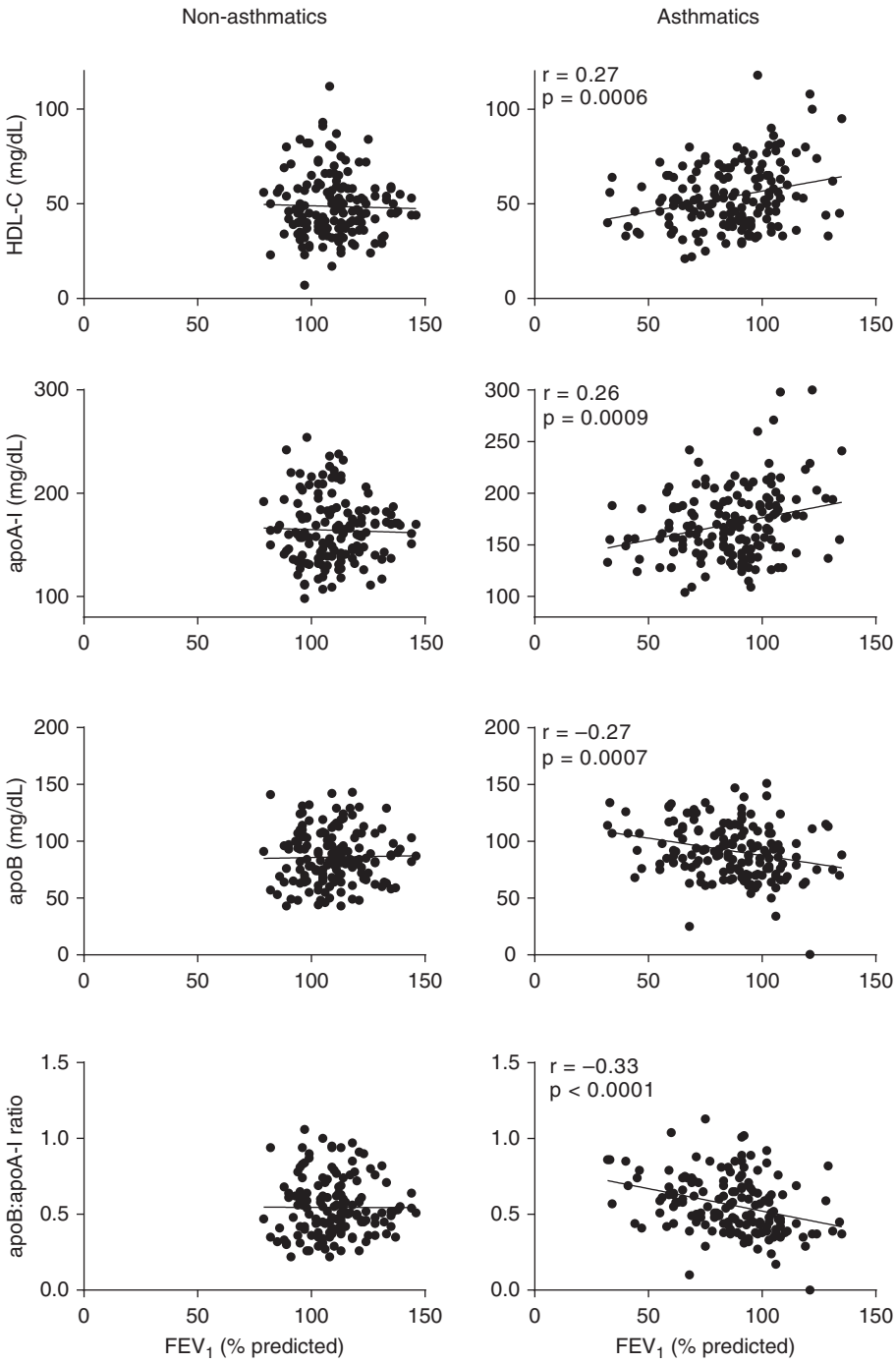


Figure 2. The FEV_1 in subjects with atopy and asthma is positively correlated with serum high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A-I (apoA-I) levels, but inversely correlated with serum levels of apolipoprotein B (apoB) and the apoB/A-I ratio. Correlations between FEV_1% predicted and serum levels of HDL-C, apoA-I, and apoB, and apoB/A-I ratio in subjects without asthma and subjects with atopy and asthma are shown. Pearson correlation coefficients and associated *P* values are shown for significant relationships only.

disease severity. Because of the small number of subjects in the severe asthma group, only the mild to moderate asthma group was analyzed, which showed that many of the statistically significant correlations with FEV_1 persisted in this subgroup (*see* Figures E1 and E2 in the online supplement).

Lastly, a sensitivity analysis was performed to determine whether use of medications that could alter the serum lipid profile affected the correlation between FEV_1 and serum lipid and lipoprotein levels. After excluding the 14 subjects (four in the NN, one in the AN, and nine in the AA group) who were known to be taking a statin, niacin, gemfibrozil, cholesterol absorption inhibitor, or another lipid profile–altering agent, correlation analyses using the Pearson method were repeated. Again, no significant associations between FEV_1 and any of the serum lipids were found in the group with no asthma. In the AA group, all of the previously described significant relationships persisted when only those subjects who were not taking any lipid profile–altering medications were analyzed. In addition, a significant negative association was now found between medium $VLDL_{NMR}$ particles and FEV_1 in the AA group (Pearson, $r = -0.19$; $P = 0.046$).

Discussion

In this study, we show that serum levels of apoA-I and HDL-C are positively correlated with FEV_1 in subjects with atopy and asthma, whereas an inverse correlation was found between FEV_1 and serum levels of TG, LDL-C, apoB, and the apoB/apoA-I ratio. Furthermore, NMR spectroscopy identified a positive correlation between FEV_1 and both HDL_{NMR} particle size and the concentration of large HDL_{NMR} particles. A positive correlation was also found between the FEV_1 and the concentration of total IDL_{NMR} particles in subjects with atopy and asthma. In contrast, LDL_{NMR} size and the concentration of large and small LDL_{NMR} particles, and the concentration of large and small $VLDL_{NMR}$ particles, were negatively correlated with FEV_1 in subjects with atopy and asthma. Thus, these findings support the hypothesis that pathways involving serum lipids and specific lipoprotein particles may influence the severity of airflow obstruction in atopic asthma, with apoA-I and HDL-C potentially having a protective effect, whereas TG, LDL-C, and apoB are associated with more severe airflow obstruction.

A typical HDL particle is comprised of two to five molecules of apoA-I and approximately 100 molecules of

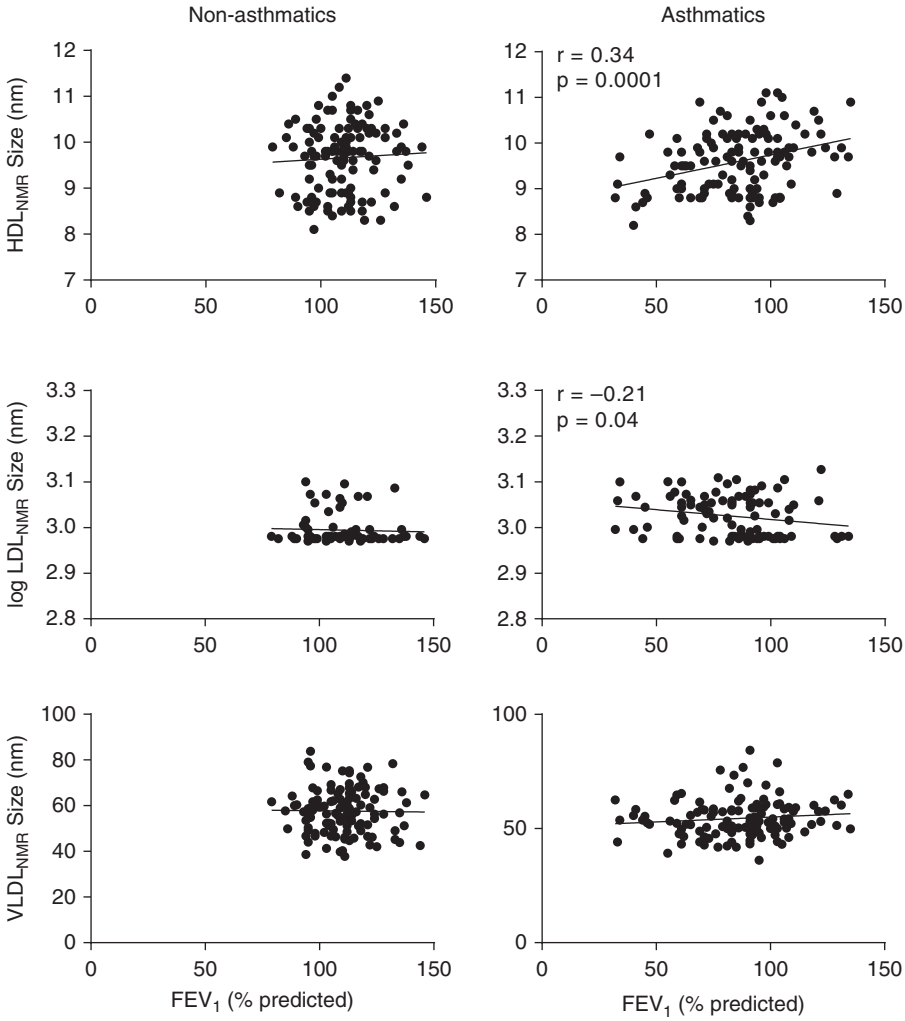


Figure 3. The FEV_1 in subjects with atopy and asthma is positively correlated with the size of HDL_{NMR} particles and negatively correlated with the size of LDL_{NMR} particles. Correlations between FEV_1% predicted and lipoprotein particle sizes as measured by NMR spectroscopy in subjects without asthma and subjects with atopy and asthma are shown. Pearson correlation coefficients and associated *P* values are shown for significant relationships only. HDL = high-density lipoprotein; LDL = low-density lipoprotein; NMR = nuclear magnetic resonance; VLDL = very-low-density lipoprotein.

phosphatidylcholine, which form an amphipathic shell around a hydrophobic core of cholesterol esters and TGs (2). The composition of HDL is heterogeneous, containing varied amounts of different lipids, proteins, and microRNAs (2, 25). Both apoA-I and HDL mediate a wide variety of atheroprotective functions, such as acting as an acceptor for cholesterol that is transported out of lipid-laden macrophages in the vascular wall by the reverse cholesterol transport pathway (26). Other protective functions of HDL involve its antiinflammatory, antioxidative, antithrombotic, and antifibrotic properties, as well as its ability to down-regulate adhesion molecule expression and bind endotoxins (2, 27). HDL-C has long been identified in population-based studies as being inversely associated with the risk of coronary heart disease and death (2, 3, 28). However, not all HDL is cardioprotective, because HDL from some patients with coronary artery disease, diabetes, or chronic renal disease may be dysfunctional with reduced antiinflammatory and endothelial repair functions (2). In addition, apoA-I in human atheromas may also become dysfunctional because of myeloperoxidase-dependent oxidation with resultant loss of cholesterol acceptor activity and increase in proinflammatory activity (29). Thus, a more detailed characterization of HDL besides its cholesterol content (HDL-C) may be essential in defining its relationship to airflow obstruction and asthma.

Lipoprotein particles are typically classified according to size and density, with HDL particles that contain apoA-I being the smallest and most dense (1). LDL, IDL,

Table 4. Intercepts and Parameter Estimates of HDL, LDL, and VLDL Particle Sizes from Linear Regression with FEV_1 Incorporating Log Serum Triglyceride Levels for Subjects with and without Asthma

	Nonasthmatic			Atopic Asthmatic		
Variable	**Intercept**	**Parameter Estimate ± SE**	***P* Value**	**Intercept**	**Parameter Estimate ± SE**	***P* Value**
HDL_{NMR} size	92.46	1.295 ± 1.96	0.51	7.79	10.003 ± 3.05	0.001*
Log LDL_{NMR} size	164.27	−19.067 ± 48.16	0.69	554.93	−138.574 ± 50.71	0.008*
$VLDL_{NMR}$ size	117.63	−0.009 ± 0.13	0.95	122.71	0.584 ± 0.25	0.02*

Definition of abbreviations: HDL = high-density lipoprotein; LDL = low-density lipoprotein; NMR = nuclear magnetic resonance; VLDL = very-low-density lipoprotein.
*Statistically significant relationship between lipid particle size and FEV_1 after adjusting for log serum triglyceride levels.

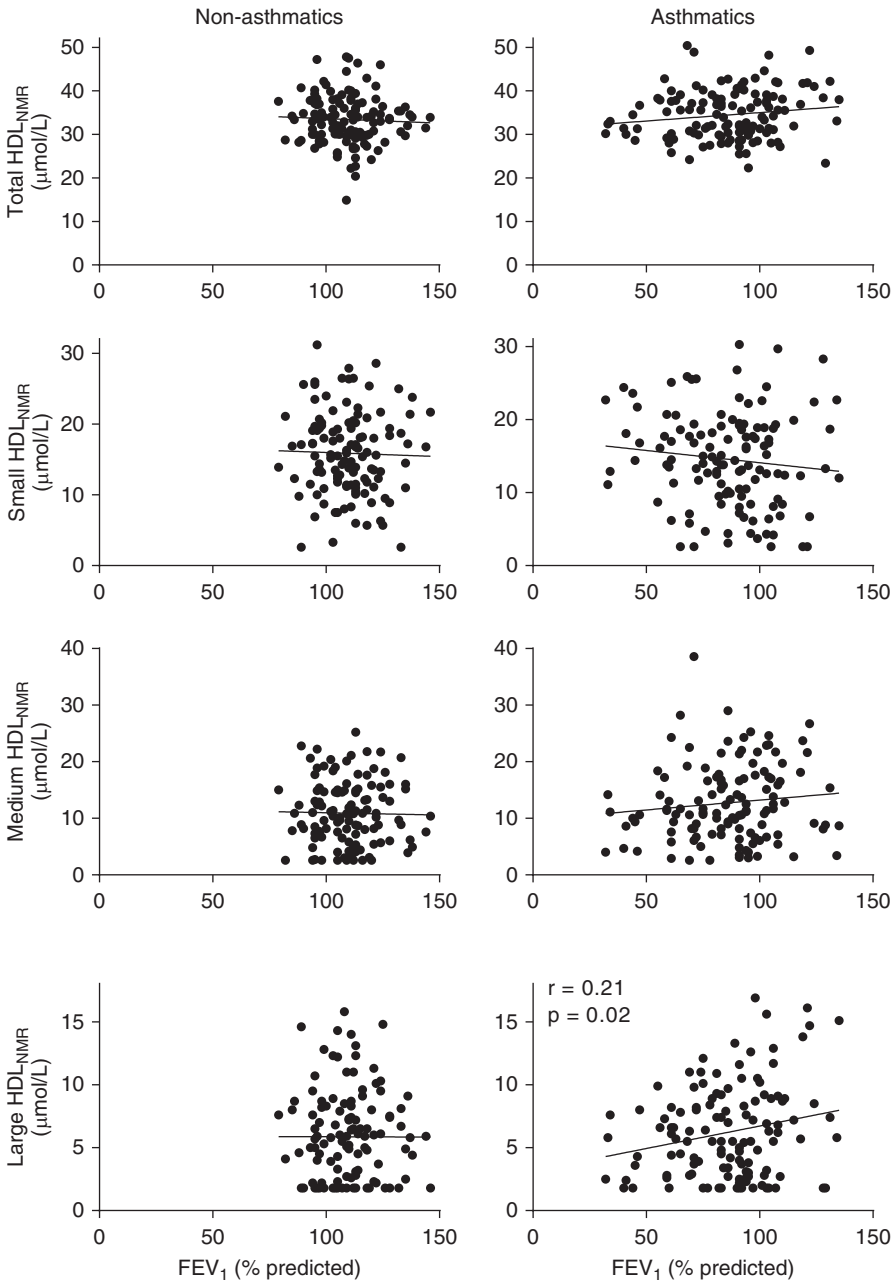


Figure 4. The FEV_1 in subjects with atopy and asthma is positively correlated with the concentration of large HDL_{NMR} particles. Correlations between FEV_1% predicted and HDL_{NMR} particle subfractions as measured by NMR spectroscopy in subjects without asthma and subjects with atopy and asthma are shown. Pearson correlation coefficients and associated *P* values are shown for significant relationships only. HDL = high-density lipoprotein; NMR = nuclear magnetic resonance.

VLDL, chylomicron remnants, and chylomicrons, which are all progressively larger and less dense than HDL, all contain apoB and are largely viewed as being proatherogenic (1). Each of these major classes of lipoproteins is further composed of subgroups of heterogeneous particles of varying size and composition. By measuring the NMR proton signal from the terminal methyl groups on lipids, one can determine not only the amount of each type of lipoprotein particle present but also their size because of characteristic differences in the chemical shift associated with different-sized lipoprotein particles (30). This allows NMR-derived concentrations to be quantified for each of the different-sized lipoprotein subfractions.

Recent studies have suggested that NMR measurements of LDL-P and HDL-P particle numbers (which we have referred to as the total concentration of LDL_{NMR} and HDL_{NMR} particles) may have advantages for the prediction of cardiovascular events over the traditional measurement of LDL-C or HDL-C (31). However, it is important to note that although NMR measurements seem to be strongly correlated with HDL number and size, the absolute accuracy of this approach for the true abundance of HDL particles in plasma has not been clearly established, and that lipoprotein particle size measured by NMR spectroscopy and other laboratory methods has produced different results (32).

Here, we demonstrated that HDL particle size (HDL_{NMR} size) and the concentration of large HDL_{NMR} particles, as well as the concentration of total IDL_{NMR} particles, were positively correlated with FEV_1 in atopic subjects with asthma. These results extend our findings from the standard lipid profile analysis regarding the positive correlation between serum HDL-C and FEV_1, and also demonstrate a similar correlation with serum IDL_{NMR}. Furthermore, these results suggest that any possible protective effect of HDL-C on airflow obstruction in atopic asthma may be specifically mediated by large HDL_{NMR} particles. Interestingly, in a prior study of healthy women, only the number of large HDL_{NMR} particles was associated with a reduction in risk of cardiovascular disease (33). Our results also show that in contrast to other apoB-containing lipoprotein particles, such as $VLDL_{NMR}$ and LDL_{NMR}, the concentration of total IDL_{NMR} particles is positively, rather than negatively, correlated with FEV_1 in subjects with atopy and asthma. We hypothesize that, as compared with $VLDL_{NMR}$ and LDL_{NMR} particles, IDL_{NMR} particles may possess distinct lipid or protein constituents that may mediate the positive correlation with FEV_1.

We also demonstrated a negative correlation between FEV_1 and LDL_{NMR} size, and with the concentration of total, and large and small subfractions of LDL_{NMR} particles. A negative correlation was also demonstrated between FEV_1

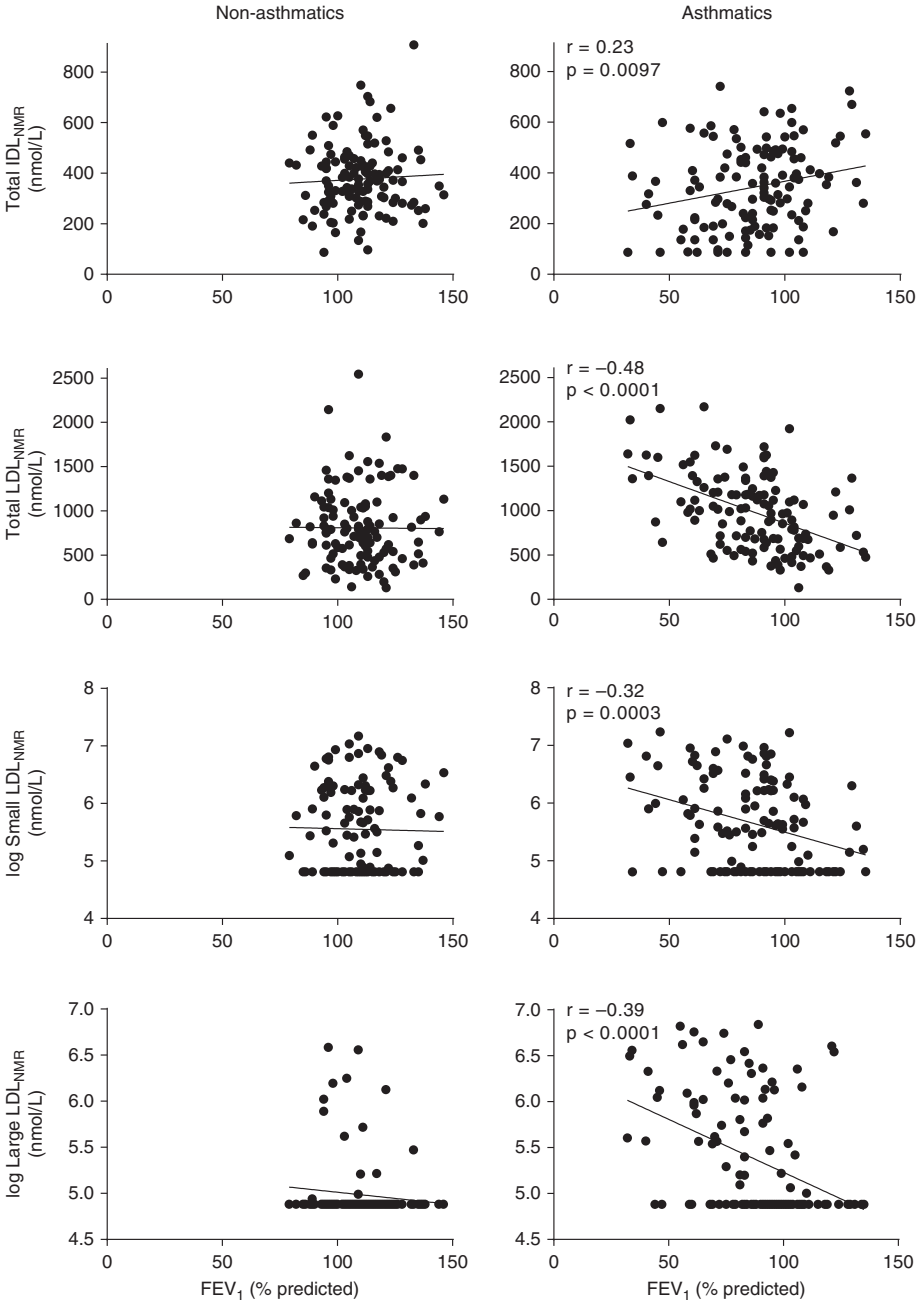


Figure 5. The FEV_1 in subjects with atopy and asthma is positively correlated with the concentration of IDL_{NMR} particles and inversely correlated with the concentration of LDL_{NMR} particles. Correlations between FEV_1% predicted and the concentration of IDL_{NMR} particles and LDL_{NMR} particle subfractions as measured by NMR spectroscopy in subjects without asthma and subjects with atopy and asthma are shown. Pearson correlation coefficients and associated *P* values are shown for significant relationships only. IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; NMR = nuclear magnetic resonance.

and the concentration of total, and large and small subfractions of $VLDL_{NMR}$ particles. These results similarly confirm and extend our findings from the standard lipid profile analysis regarding a negative correlation between serum LDL-C or apoB levels and FEV_1 in atopic asthma. In contrast to HDL-C, raised levels of LDL-C and, more recently, TGs have been linked with increased risk of cardiovascular disease (1, 34). LDL particles and their associated apoB molecules are the primary carriers of cholesterol to tissues where the cholesterol cargo is internalized into cells via LDLR (1, 35). Plasma TGs are a marker for remnant cholesterol, which is the cholesterol content of all TG-enriched lipoproteins, including chylomicron remnants, VLDL, and IDL (34). The role of LDL-C in cardiovascular disease is well established, because multiple randomized trials have shown that statin-mediated reductions in LDL-C significantly reduce the risk of coronary events (36). Additionally, data from epidemiologic and genetic studies support a role for raised serum TG levels, and remnant cholesterol or TG-rich lipoproteins as a cause of cardiovascular disease (34). Consistent with the deleterious effects of LDL-C, apoB, and TGs in vascular cells, our results show that serum levels of LDL-C, apoB, apoB/apoA-I ratio, and TG are negatively correlated with FEV_1 in atopic asthma.

A correlation between serum lipid levels and airflow obstruction has not previously been reported in subjects with asthma. However, an association between FEV_1 and lipid profiles has been shown in an analysis of 14,135 subjects without respiratory disease who participated in the Third National Health and Nutrition Examination Survey (37). In this study, serum levels of HDL-C and apoA-I were positively correlated with FEV_1, whereas LDL-C, apoB, and apoB/apoA-I ratio were negatively correlated. Serum TG levels were not reported. The Third National Health and Nutrition Examination Survey findings are similar to our results where we found that a positive correlation existed between serum levels of HDL-C or apoA-I with FEV_1 and a negative correlation between FEV_1 and serum levels of LDL-C, apoB, or TG in subjects with atopy and asthma. Although we did not find an association between FEV_1 and any of the lipid parameters in our subjects without asthma, we cannot exclude the possibility that our sample size for this group may have been too small to identify an association.

It is also important to note that samples from subjects with and without asthma in our study were collected in the nonfasting state. It has previously been demonstrated that, except for serum TGs, most other lipid and lipoprotein parameters, such as apoA-I, apoB, ratio of apoB/apoA-I, and non–HDL-C, do not significantly change with

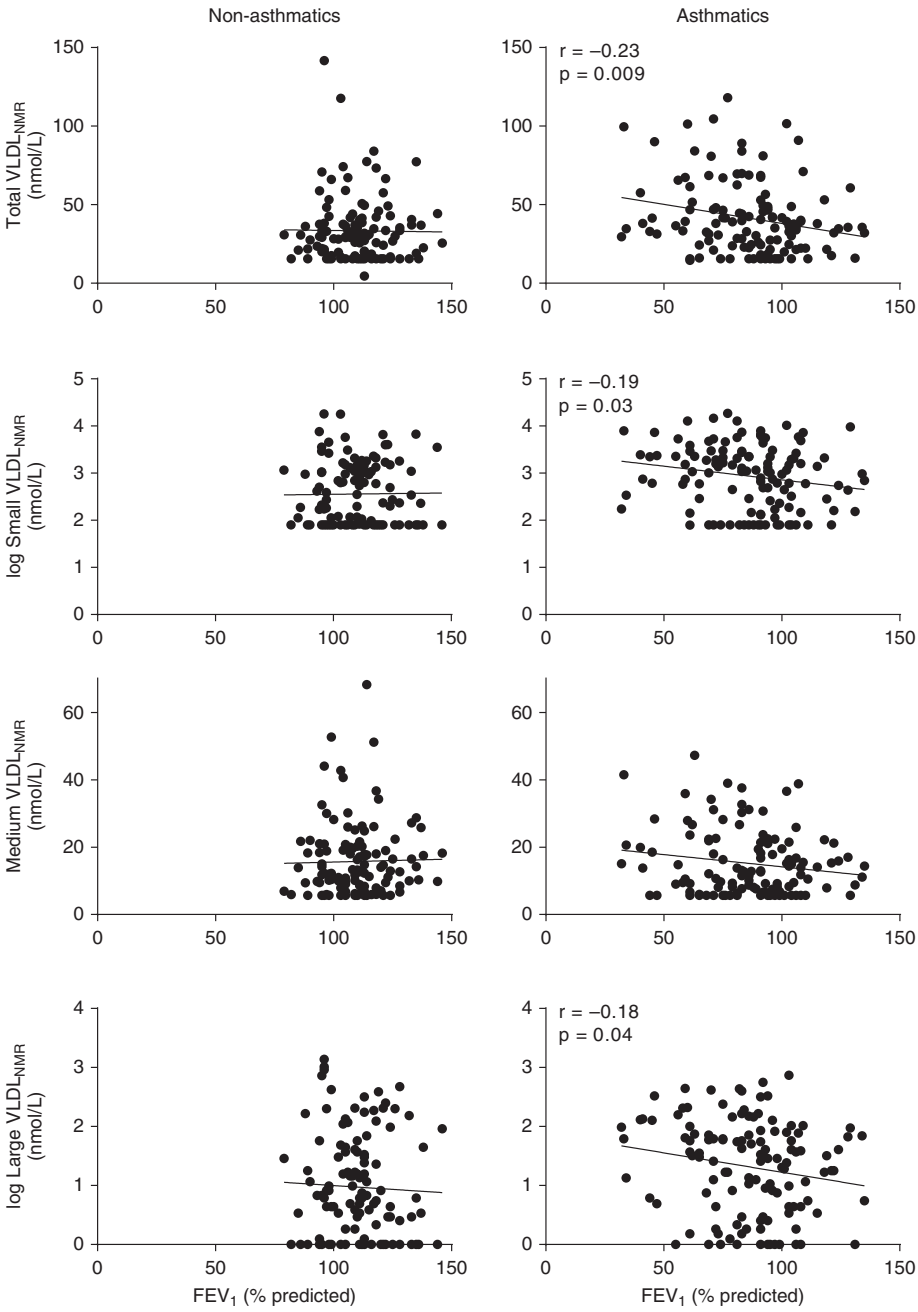


Figure 6. The FEV_1 in subjects with atopy and asthma is inversely correlated with the concentration of $VLDL_{NMR}$ particles. Correlations between FEV_1% predicted and the concentration of $VLDL_{NMR}$ particle subfractions as measured by NMR spectroscopy in subjects without asthma and subjects with atopy and asthma are shown. Pearson correlation coefficients and associated *P* values are shown for significant relationships only. NMR = nuclear magnetic resonance; VLDL = very-low-density lipoprotein.

fasting status (38). Nonfasting lipid profiles, however, are predictive of an increased risk of cardiovascular events, including myocardial infarction, ischemic heart disease, ischemic stroke, and death (38–40). Additionally, nonfasting TG levels are considered superior to fasting TG levels for the prediction of cardiovascular events (1, 41). Based on the advantages of nonfasting lipid levels, the measurement of nonfasting serum lipids has now become a standard practice for cardiovascular risk screening, particularly in pediatric populations (34, 42).

Our study also found that serum HDL-C levels were increased in the AA group as compared with the NN group, whereas there were no differences in LDL-C, TC, TG, or apoB between NN and either the AN or AA groups. Although prior studies have also assessed the relationship between serum HDL-C and asthma, the results have been heterogeneous with serum HDL-C levels either being increased, decreased, or unchanged in subjects with asthma (20, 43–50). Similar to our findings, prior studies have also not found a change in serum LDL-C in subjects with asthma, whereas TC was either decreased or unchanged and TG levels were either increased or unchanged. Potential explanations for these disparate results could include the various definitions of asthma used in the studies, differences in lipid and lipoprotein assays, differences in age and genetic backgrounds, and cultural and environmental influences (20). Thus, these cumulative data have only clearly established that LDL-C levels do not differ between subjects with and without asthma.

Furthermore, although the adjusted mean HDL-C was higher and the FEV_1 was lower in subjects with asthma than control subjects in our study, we do not believe this is inconsistent with a positive correlation between FEV_1 and HDL-C. The overall group mean for each parameter may not be reflective of the relationship between the FEV_1 and lipid levels for each individual subject. Therefore, we propose that in addition to assessing whether serum lipid levels differ between subjects with and without asthma, it is also important to determine whether serum lipids levels modify airflow obstruction as a measure of disease severity in asthma on an individual basis.

Although our study has notable strengths, it also has several limitations. One strength of our study was the definitive establishment of asthmatic versus nonasthmatic clinical phenotypes using objective criteria, thus minimizing confounding interpretations caused by subject misclassification. Second, this is the first study describing a correlation between the severity of airflow obstruction and serum lipid profiles in subjects with atopy and asthma. Third, our study is the first to use NMR spectroscopy to analyze serum lipoprotein particle subfractions in asthma, and correlate them with the severity of airflow obstruction. However,

similar to many previously published studies, we could not account for differences in diet or exercise levels among study groups.

We acknowledge that other metabolic factors, such as the metabolic syndrome and obesity, could potentially be modifying the association between FEV_1 and serum lipids and lipoproteins. Additionally, although the relationships between FEV_1 and HDL_{NMR} size or LDL_{NMR} size remained significant even after adjusting for serum TG concentrations in our study, we cannot exclude the possibility that these relationships may be altered in individuals with more severe hypertriglyceridemia, because only 18 of our subjects with asthma had high levels of serum TGs (200–499 mg/dl) and none had very high serum TGs (>500 mg/dl). Furthermore, our study is a cross-sectional analysis which does not describe how serum lipids in subjects with asthma may vary over time. To address this, we are currently conducting a longitudinal study of subjects with asthma to assess whether the associations reported in this study persist over time.

Although our cohort was very well characterized regarding disease phenotype and severity, our relatively small sample size can also be considered a shortcoming. Therefore, additional studies involving larger cohorts of subjects will be important to confirm and extend the findings from our study, which is the first investigation using NMR spectroscopy as a novel technique to identify a correlation between serum apolipoprotein particle size and the severity of airflow obstruction in atopic asthma. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank Drs. Mario Stylianou and Junfeng Sun for their careful review of the manuscript.

References

1. Ridker PM. LDL cholesterol: controversies and future therapeutic directions. *Lancet* 2014;384:607–617.
2. Lüscher TF, Landmesser U, von Eckardstein A, Fogelman AM. High-density lipoprotein: vascular protective effects, dysfunction, and potential as therapeutic target. *Circ Res* 2014;114:171–182.
3. Rader DJ, Hovingh GK. HDL and cardiovascular disease. *Lancet* 2014; 384:618–625.
4. Draper DW, Madenspacher JH, Dixon D, King DH, Remaley AT, Fessler MB. ATP-binding cassette transporter G1 deficiency dysregulates host defense in the lung. *Am J Respir Crit Care Med* 2010;182: 404–412.
5. Goldklang M, Golovatch P, Zelonina T, Trischler J, Rabinowitz D, Lemaître V, D'Armiento J. Activation of the TLR4 signaling pathway and abnormal cholesterol efflux lead to emphysema in ApoE-deficient mice. *Am J Physiol Lung Cell Mol Physiol* 2012;302:L1200–L1208.
6. Gowdy KM, Fessler MB. Emerging roles for cholesterol and lipoproteins in lung disease. *Pulm Pharmacol Ther* 2013;26:430–437.
7. Martens GW, Arikan MC, Lee J, Ren F, Vallerskog T, Kornfeld H. Hypercholesterolemia impairs immunity to tuberculosis. *Infect Immun* 2008;76:3464–3472.
8. Massaro D, Massaro GD. Developmental alveologenesis: new roles for ApoE and LDL receptor. *Pediatr Res* 2011;70:458–461.
9. Samokhin AO, Bühling F, Theissig F, Brömme D. ApoE-deficient mice on cholate-containing high-fat diet reveal a pathology similar to lung sarcoidosis. *Am J Pathol* 2010;176:1148–1156.
10. Thomassen MJ, Barna BP, Malur AG, Bonfield TL, Farver CF, Malur A, Dalrymple H, Kavuru MS, Febbraio M. ABCG1 is deficient in alveolar macrophages of GM-CSF knockout mice and patients with pulmonary alveolar proteinosis. *J Lipid Res* 2007;48:2762–2768.
11. Wang W, Xu H, Shi Y, Nandedkar S, Zhang H, Gao H, Feroah T, Weihrauch D, Schulte ML, Jones DW, *et al*. Genetic deletion of apolipoprotein A-I increases airway hyperresponsiveness, inflammation, and collagen deposition in the lung. *J Lipid Res* 2010; 51:2560–2570.
12. Yamashita CM, Fessler MB, Vasanthamohan L, Lac J, Madenspacher J, McCaig L, Yao L, Wang L, Puntorieri V, Mehta S, *et al*. Apolipoprotein E-deficient mice are susceptible to the development of acute lung injury. *Respiration* 2014;87:416–427.
13. Dai C, Yao X, Keeran KJ, Zywicke GJ, Qu X, Yu ZX, Dagur PK, McCoy JP, Remaley AT, Levine SJ. Apolipoprotein A-I attenuates ovalbumin-induced neutrophilic airway inflammation via a granulocyte colony-stimulating factor-dependent mechanism. *Am J Respir Cell Mol Biol* 2012;47:186–195.
14. Park SW, Lee EH, Lee EJ, Kim HJ, Bae DJ, Han S, Kim D, Jang AS, Uh ST, Kim YH, *et al*. Apolipoprotein A1 potentiates lipoxin A4 synthesis and recovery of allergen-induced disrupted tight junctions in the airway epithelium. *Clin Exp Allergy* 2013;43:914–927.
15. Nandedkar SD, Weihrauch D, Xu H, Shi Y, Feroah T, Hutchins W, Rickaby DA, Duzgunes N, Hillery CA, Konduri KS, *et al*. D-4F, an apoA-1 mimetic, decreases airway hyperresponsiveness, inflammation, and oxidative stress in a murine model of asthma. *J Lipid Res* 2011;52:499–508.
16. Yao X, Dai C, Fredriksson K, Dagur PK, McCoy JP, Qu X, Yu ZX, Keeran KJ, Zywicke GJ, Amar MJ, *et al*. 5A, an apolipoprotein A-I mimetic peptide, attenuates the induction of house dust mite-induced asthma. *J Immunol* 2011;186:576–583.
17. Dai C, Yao X, Vaisman B, Brenner T, Meyer KS, Gao M, Keeran KJ, Nugent GZ, Qu X, Yu ZX, *et al*. The ABCA1 transporter attenuates ovalbumin-induced neutrophilic airway inflammation. *Am J Respir Cell Mol Biol* 2014;51:626–636.
18. Draper DW, Gowdy KM, Madenspacher JH, Wilson RH, Whitehead GS, Nakano H, Pandiri AR, Foley JF, Remaley AT, Cook DN, *et al*. ATP binding cassette transporter G1 deletion induces IL-17-dependent dysregulation of pulmonary adaptive immunity. *J Immunol* 2012;188: 5327–5336.
19. Yao X, Fredriksson K, Yu ZX, Xu X, Raghavachari N, Keeran KJ, Zywicke GJ, Kwak M, Amar MJ, Remaley AT, *et al*. Apolipoprotein E negatively regulates house dust mite-induced asthma via a low-density lipoprotein receptor-mediated pathway. *Am J Respir Crit Care Med* 2010;182:1228–1238.
20. Fessler MB. Next stop for HDL: the lung. *Clin Exp Allergy* 2012;42: 340–342.
21. Cottrell L, Neal WA, Ice C, Perez MK, Piedimonte G. Metabolic abnormalities in children with asthma. *Am J Respir Crit Care Med* 2011;183:441–448.
22. National Asthma Education and Prevention Program (National Heart Lung and Blood Institute). Third Expert Panel on the Management of Asthma. Guidelines for the diagnosis and management of asthma: full report 2007. Bethesda, MD: U.S. Department of Health and Human Services, National Institutes of Health, National Heart, Lung, and Blood Institute; 2010.
23. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, Adcock IM, Bateman ED, Bel EH, Bleecker ER, *et al*. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014;43:343–373.
24. Deckelbaum RJ, Granot E, Oschry Y, Rose L, Eisenberg S. Plasma triglyceride determines structure-composition in low and high density lipoproteins. *Arteriosclerosis* 1984;4:225–231.
25. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat Cell Biol* 2011;13: 423–433.
26. Rosenson RS, Brewer HB Jr, Davidson WS, Fayad ZA, Fuster V, Goldstein J, Hellerstein M, Jiang XC, Phillips MC, Rader DJ, *et al*. Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport. *Circulation* 2012;125: 1905–1919.

27. Navab M, Reddy ST, Van Lenten BJ, Fogelman AM. HDL and cardiovascular disease: atherogenic and atheroprotective mechanisms. *Nat Rev Cardiol* 2011;8:222–232.
28. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. *Am J Med* 1977;62:707–714.
29. Huang Y, DiDonato JA, Levison BS, Schmitt D, Li L, Wu Y, Buffa J, Kim T, Gerstenecker GS, Gu X, *et al*. An abundant dysfunctional apolipoprotein A1 in human atheroma. *Nat Med* 2014;20: 193–203.
30. Rosenson RS, Brewer HB Jr, Chapman MJ, Fazio S, Hussain MM, Kontush A, Krauss RM, Otvos JD, Remaley AT, Schaefer EJ. HDL measures, particle heterogeneity, proposed nomenclature, and relation to atherosclerotic cardiovascular events. *Clin Chem* 2011;57: 392–410.
31. deGoma EM, Rader DJ. High-density lipoprotein particle number: a better measure to quantify high-density lipoprotein? *J Am Coll Cardiol* 2012;60:517–520.
32. Davidson WS. HDL-C vs HDL-P: how changing one letter could make a difference in understanding the role of high-density lipoprotein in disease. *Clin Chem* 2014;60:e1–e3.
33. Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation* 2009;119: 931–939.
34. Nordestgaard BG, Varbo A. Triglycerides and cardiovascular disease. *Lancet* 2014;384:626–635.
35. Goldstein JL, Brown MS. The LDL receptor. *Arterioscler Thromb Vasc Biol* 2009;29:431–438.
36. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, Peto R, Barnes EH, Keech A, Simes J, *et al*.; Cholesterol Treatment Trialists' (CTT) Collaboration. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet* 2010; 376:1670–1681.
37. Cirillo DJ, Agrawal Y, Cassano PA. Lipids and pulmonary function in the Third National Health and Nutrition Examination Survey. *Am J Epidemiol* 2002;155:842–848.
38. Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. *Circulation* 2008; 118:2047–2056.
39. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* 2007;298:299–308.
40. Freiberg JJ, Tybjaerg-Hansen A, Jensen JS, Nordestgaard BG. Nonfasting triglycerides and risk of ischemic stroke in the general population. *JAMA* 2008;300:2142–2152.
41. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA* 2007;298:309–316.
42. Mitchell F. Screening: No need to fast before lipid level tests. *Nat Rev Endocrinol* 2013;9:65.
43. Enright PL, Ward BJ, Tracy RP, Lasser EC; The Cardiovascular Health Study Research Group. Asthma and its association with cardiovascular disease in the elderly. *J Asthma* 1996;33:45–53.
44. Erel F, Gulec M, Kartal O, Caliskaner Z, Ozturk S, Yaman H, Kurt Y, Gocgeldic E, Ors F, Karaayvaz M. Serum leptin levels and lipid profiles in patients with allergic rhinitis and mild asthma. *Allergol Immunopathol (Madr)* 2007;35:232–238.
45. Fenger RV, Gonzalez-Quintela A, Linneberg A, Husemoen LL, Thuesen BH, Aadahl M, Vidal C, Skaaby T, Sainz JC, Calvo E. The relationship of serum triglycerides, serum HDL, and obesity to the risk of wheezing in 85,555 adults. *Respir Med* 2013;107:816–824.
46. Fessler MB, Massing MW, Spruell B, Jaramillo R, Draper DW, Madenspacher JH, Arbes SJ, Calatroni A, Zeldin DC. Novel relationship of serum cholesterol with asthma and wheeze in the United States. *J Allergy Clin Immunol* 2009;124:967–974 e961–915.
47. Picado C, Deulofeu R, Lleonart R, Agustí M, Casals E, Quintó L, Mullol J. Lipid and protein metabolism in asthma. Effects of diet and corticosteroid therapy. *Allergy* 1999;54:569–575.
48. Schäfer T, Ruhdorfer S, Weigl L, Wessner D, Heinrich J, Döring A, Wichmann HE, Ring J. Intake of unsaturated fatty acids and HDL cholesterol levels are associated with manifestations of atopy in adults. *Clin Exp Allergy* 2003;33:1360–1367.
49. Shenoi A, Kumar L, Sarihyan S, Gangully NK. High density lipoprotein cholesterol and total cholesterol in children with asthma and allergic rhinitis. *Acta Paediatr* 1992;81:150–152.
50. Yiallouros PK, Savva SC, Kolokotroni O, Behbod B, Zeniou M, Economou M, Chadjigeorgiou C, Kourides YA, Tornaritis MJ, Lamnisos D, *et al*. Low serum high-density lipoprotein cholesterol in childhood is associated with adolescent asthma. *Clin Exp Allergy* 2012;42:423–432.

Online Supplementary Data

Serum Apolipoprotein A-I and Large HDL Particles are Positively Correlated with FEV_1 in Atopic Asthma

Amisha V. Barochia, Maryann Kaler, Rosemarie A. Cuento, Elizabeth M. Mushaben, Nargues A. Weir, Maureen Sampson, Joseph R. Fontana, Sandra MacDonald, Joel Moss, Vincent Manganiello, Alan T. Remaley, and Stewart J. Levine

I. Supplemental Methods:

Study population. None of the study subjects were current smokers. All subjects underwent a chest radiograph to exclude the presence of other lung diseases. Skin testing was performed using cat dander, *Dermatophagoides farinae*, short ragweed and Timothy Grass (HollisterStier, Spokane, WA) with a Multi-Test II® applicator, which was a generous gift from Lincoln Diagnostics, Decatur, IL.

Lipid and apolipoprotein assays. Prospectively collected non-fasting serum samples that had been stored at -80°C were thawed on ice and standard clinical lipid profiles were measured in the CLIA-certified NIH Clinical Research Center Clinical Laboratory. NMR spectroscopy was performed using a Vantera Clinical Analyzer (LipoScience Inc., Raleigh, NC). Serum apoA-I and apoB levels were measured using a Dimension Vista® Intelligent Lab System (Siemens Healthcare, Erlangen, Germany). ApoE levels in serum samples that had been diluted 1:10,000 in PBS were quantified using a human apoE ELISA (MABTECH Inc., Cincinnati, OH) with a lower limit of detection of 0.02 ng/mL.

Statistics: Non-normally distributed parameters (eosinophil counts, immunoglobulin E, apoE, triglyceride and C-reactive protein [CRP] levels) were log transformed prior to further analysis, which resulted in normally distributed data. Summary statistics (means ± standard deviations, or medians, with upper and lower quartiles) were calculated. Differences between groups were examined using one-way ANOVA, with the non-atopic, non-asthmatic group serving as control against which the other two groups were

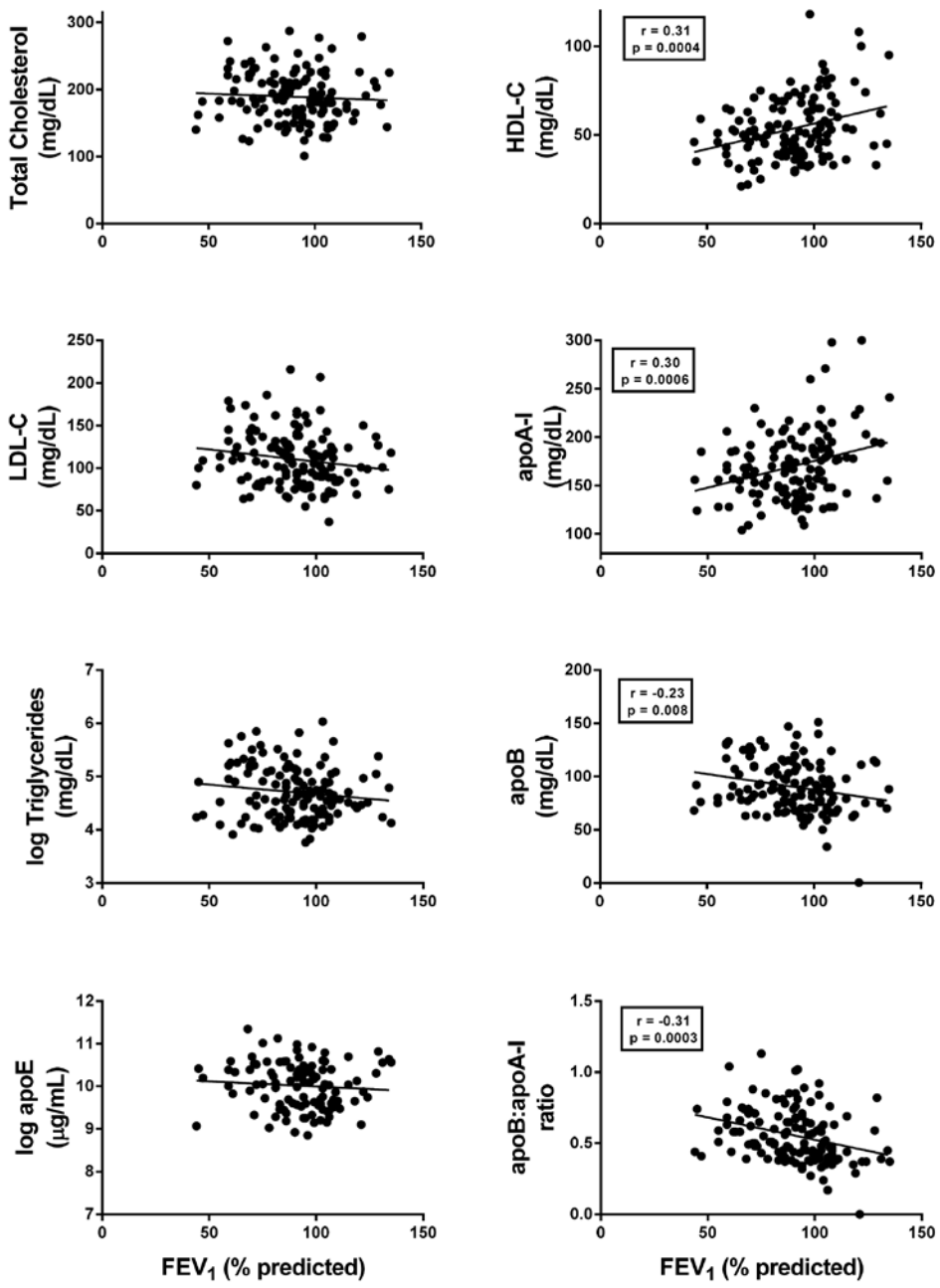
compared, using Dunnett's test to limit the number of multiple comparisons. We controlled for type 1 error at the level of the comparison by setting alpha at 0.05. In order to adjust for potential confounding factors, which were defined *a priori,* a multivariate regression analysis by fitting linear models, using age, body mass index [BMI], gender, race/ethnicity and CRP levels, was performed to determine whether groups differed significantly after accounting for these.

Correlation analyses between the % predicted forced expiratory volume in 1 second (FEV_1) and lipid profile components were performed using Pearson correlation to determine if there were significant associations between lung function and lipid profiles in each group. As the atopic non-asthmatic and non-atopic non-asthmatic groups did not differ significantly based on the preceding analyses, these groups were combined into a single non-asthmatic group for the correlation analyses. To ensure an unbiased approach, all parameters within the NMR panel were analyzed. Furthermore, since this was the first study to assess the correlation between FEV_1 and serum lipid profiles using NMR spectroscopy in atopic asthma, we considered these analyses to be exploratory in nature and therefore did not adjust for multiple comparisons. As LDL and HDL particle sizes can be influenced by triglyceride levels(1), the association between lipid particle sizes and FEV_1 was further examined by linear regression incorporating serum triglyceride levels to explore potential interactions.

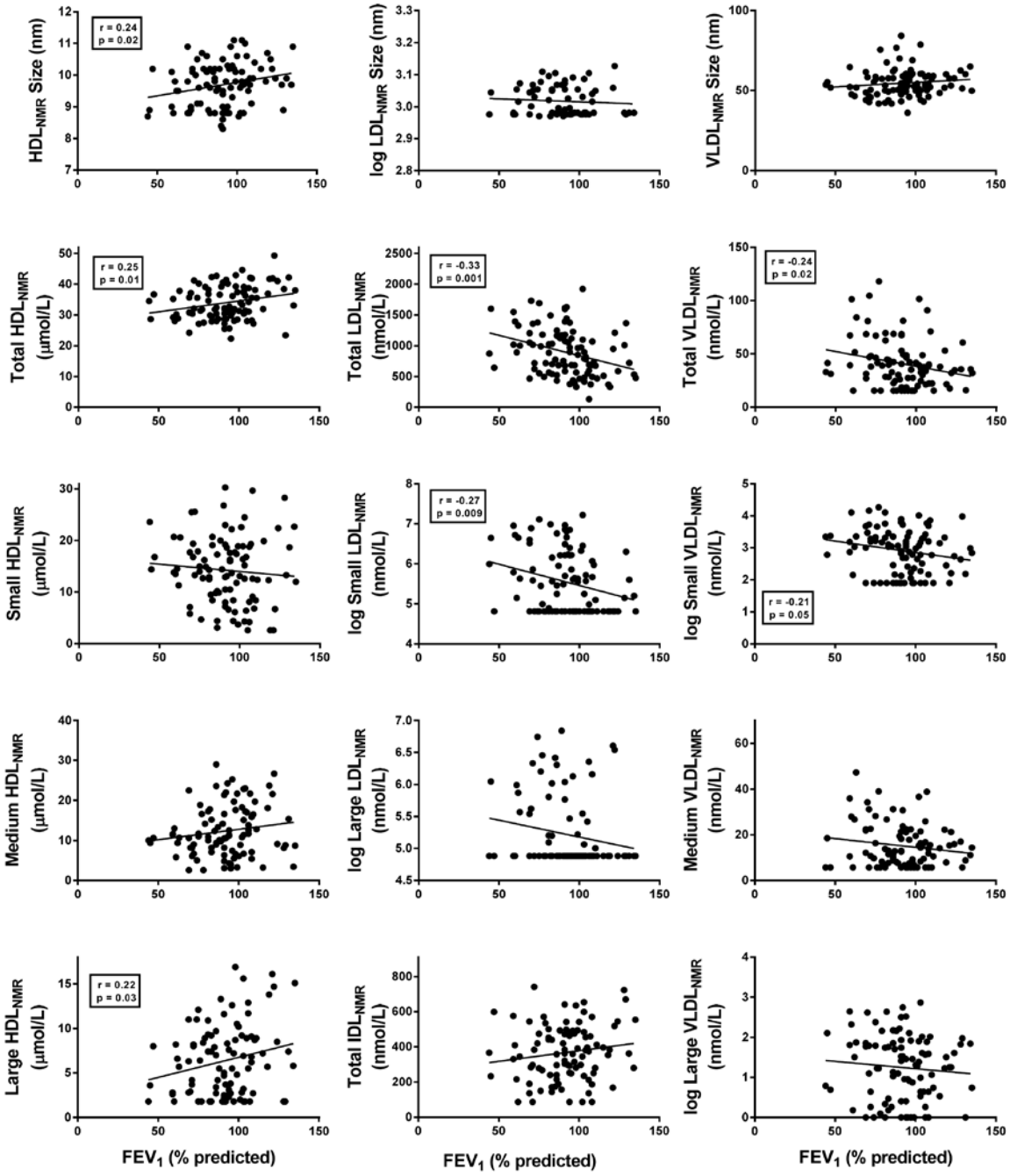
Reference

1. Deckelbaum RJ, Granot E, Oschry Y, Rose L, Eisenberg S. Plasma triglyceride determines structure-composition in low and high density lipoproteins. *Arteriosclerosis* 1984;4:225-231.

II. Supplemental Figures



Supplemental Figure 1. The FEV_1 in mild to moderate atopic asthmatics is positively correlated with serum HDL-C and apoA-I levels, but inversely correlated with serum levels of apoB and the apoB:apoA-I ratio. Correlations between FEV_1 (% predicted) and serum levels of lipids and apolipoproteins in the group of mild to moderate atopic asthmatics (n = 130) are shown. Pearson's correlation coefficients and associated p values are shown for significant relationships only.



Supplemental Figure 2. The FEV_1 in mild to moderate atopic asthmatics is positively correlated with HDL_{NMR} size and the concentration of total and large HDL_{NMR} particles. Correlations between FEV_1 (% predicted) and apolipoprotein particles, as measured by NMR spectroscopy, in mild to moderate atopic asthmatics (n = 130) are shown. Pearson's correlation coefficients and associated p values are shown for significant relationships only.