

Allergic rhinitis is associated with complex alterations in high-density lipoprotein composition and function

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ABSTRACT

Despite strong evidence that high-density lipoproteins (HDLs) modulate the immune response, the role of HDL in allergies is still poorly understood. Many patients with allergic rhinitis (AR) develop a late-phase response, characterized by infiltration of monocytes and eosinophils into the nasal submucosa. Functional impairment of HDL in AR-patients may insufficiently suppress inflammation and cell infiltration, but the effect of AR on the composition and function of HDL is not understood. We used apolipoprotein (apo) B-depleted serum as well as isolated HDL from AR-patients (n = 43) and non-allergic healthy controls (n = 20) for detailed compositional and functional characterization of HDL. Both AR-HDL and apoB-depleted serum of AR-patients showed decreased anti-oxidative capacity and impaired ability to suppress monocyte nuclear factor-κB expression and pro-inflammatory cytokine secretion, such as interleukin (IL)-4, IL-6, IL-8, tumor necrosis factor alpha and IL-1 beta. Sera of AR-patients showed decreased paraoxonase and cholesteryl-ester transfer protein activities, increased lipoprotein-associated phospholipase A2 activity, while lecithin-cholesterol acyltransferase activity and cholesterol efflux capacity were not altered. Surprisingly, apoB-depleted serum and HDL from AR-patients showed an increased ability to suppress eosinophil effector responses upon eotaxin-2/CCL24 stimulation. Mass spectrometry and biochemical analyses showed reduced levels of apoA-I and phosphatidylcholine, but increased levels of apoA-II, triglycerides and lyso-phosphatidylcholine in AR-HDL. The changes in AR-HDL composition were associated with altered functional properties. In conclusion, AR alters HDL composition linked to decreased anti-oxidative and anti-inflammatory properties but improves the ability of HDL to suppress eosinophil effector responses.

1. Introduction

High-density lipoproteins (HDLs) are conserved and present in most

species, suggesting an important biological role from an evolutionary standpoint. HDL particles are structurally and metabolically heterogeneous, due to the persistent remodeling by lipolytic enzymes and

Abbreviations: apo, apolipoprotein; AR, allergic rhinitis; CETP, cholesteryl ester transfer protein; HDL, high-density lipoprotein; IgE, immunoglobulin E; IL, interleukin; LCAT, lecithin-cholesterol acyltransferase; LDL, low-density lipoprotein; LPC, lyso-phosphatidylcholine; Lp-PLA2, lipoprotein-associated phospholipase A2; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; PAF, platelet-activating factor; PC, phosphatidylcholine; PON, paraoxonase; sIgE, specific immunoglobulin E; TNF, tumor necrosis factor

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apolipoprotein (apo) exchange with other circulating lipoproteins and tissues [1]. HDL contains several apolipoproteins, the two major proteins being apoA-I and apoA-II, and a large number of less abundant proteins including apoC, apoE, serum amyloid A (SAA) and some enzymes such as lecithin-cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), paraoxonase (PON) and lipoprotein-associated phospholipase A2 (Lp-PLA2), also known as platelet-activating factor (PAF) acetylhydrolase [2–6]. HDL particles are involved in a number of physiological processes. The most studied and, conceivably, best understood function of HDL is its ability to promote the transport of excess cholesterol from peripheral tissues to the liver for excretion, a mechanism thought to confer protection against atherosclerotic cardiovascular disease [7]. In addition, HDL-associated apolipoproteins, lipids and enzymes modulate endothelial function [8] and have potent anti-inflammatory [9] and anti-oxidative properties [1,10]. Besides its well-known anti-atherogenic properties, HDL takes part in regulating proliferation of hematopoietic stem cells in the bone marrow [11]. HDL is involved in protection against certain parasitic infections, as certain apolipoproteins found in HDL confer human resistance towards infection with *Trypanosoma brucei brucei* [12]. Furthermore, HDL-associated apolipoproteins and lipids as well as HDL-associated enzymes are thought to regulate immune responses, given that HDL suppresses immune cell activation [13], including chemotaxis and shape change of eosinophils [14]. Biological activities of HDL may change and new evidence suggests that in some pathological conditions, such as coronary artery disease [15], psoriasis [16], chronic kidney disease [17], rheumatoid arthritis [18] and atopic dermatitis [14], HDL may lose its protective properties [19].

AR is an immunoglobulin E (IgE) mediated disease that affects approximately one in every four individuals in westernized countries and is characterized by nasal itching, sneezing, watery discharge and congestion [20]. Upon the initial sensitization process, several common aeroallergens cleave tight junctions in the airway epithelium and activate epithelial cells. Dendritic cells process the antigen and present it to T cells, which along with a plethora of cytokines and chemokines lead to activation of B cells for the production of specific IgE (sIgE) [21]. Upon re-exposure of sensitized individuals, the allergen binds to IgE on mast cells, which release preformed and newly synthesized mediators, such as histamine, platelet activating factor, interleukins, prostaglandins and tumor necrosis factor (TNF) alpha (TNF- α), and proteases, such as tryptase and chymase [22]. This step comprises the early-phase response, which is accompanied by early rhinitis symptoms. Approximately half of the AR-patients develop a late-phase response, which is characterized by stimulation, production, maturation and subsequent infiltration of inflammatory cells, such as monocytes, basophils and eosinophils, towards the nasal submucosa. Release of mediators by inflammatory cells enhances and sustains the inflammation, leading to more persistent symptoms [22–25]. A significant increase in the presence of activated eosinophils has been reported in patients with seasonal AR after allergen challenge [26].

In spite of the strong evidence that HDL modulates immune responses, the role of HDL in allergy remains poorly understood and no evidence is available on HDL function in AR. Functional impairment of HDL may inadequately suppress inflammation and effector responses of immune cells in AR-patients.

In the present study, we hypothesized that HDL from subjects with AR displays an altered protein and lipid composition associated with altered functional properties. We used mass spectrometry, biochemical analyses and various cell-based assays to assess alterations in function of apoB-depleted serum and isolated HDL from AR-patients. We observed decreased anti-oxidative and anti-inflammatory properties of apoB-depleted serum and HDL from AR-patients, but on the other hand an improved ability to suppress eosinophil effector responses. Our findings support our hypothesis and suggest a link between HDL functionality and AR.

2. Materials and methods

2.1. Ethical approvals

The Institutional Review Board of the Medical University of Graz approved the study protocol (27-528 ex 14/15) in accordance with the Declaration of Helsinki. Informed consent was obtained from all study subjects.

2.2. Study subjects

Blood was sampled from 43 patients with seasonal allergic rhinitis (AR-patients) and from 20 non-allergic healthy subjects in serum tubes (Greiner Bio-One, Kremsmünster, Austria). The mean ($\pm$ standard deviation) age of included subjects was 20.2 ± 6.5 and the age range was 8–36 years. Overweight subjects or subjects suffering from diabetes were excluded from the study. During childhood and adolescence the ratio between weight and height markedly varies with sex and age, so the cut-off values that determine the nutritional status of those aged 0–19 years are gender- and age-specific. Therefore, for participants ≤ 18 years of age, overweight was defined as a body mass index (BMI) > 90 th percentile. Study subjects were not under medication when blood was drawn. All included AR-patients had at least detectable serum sIgE levels in blood and positive skin prick tests to grass pollen. Non-allergic individuals were not sensitized to the most common allergens such as grass pollen, birch pollen or house dust mite and had low total IgE levels.

2.3. Blood sampling

After blood was drawn, serum tubes were incubated for 30 min at room temperature and subsequently centrifuged at $1800 \times g$ for 10 min at 4°C . Serum was collected and stored at -70°C . Specific and total IgE antibody levels in the AR-patients' sera were measured using the ImmunoCAP 250 (Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's instructions. SIgE values > 0.35 kU/L were considered positive [27]. AR-patients were sensitized to different pollens such as grass, birch, mugwort, ash and ragweed, to house dust mite and to mold.

2.4. Preparation of apoB-depleted serum

ApoB-depleted serum was prepared by addition of $40\ \mu\text{L}$ polyethylene glycol (Sigma-Aldrich, Darmstadt, Germany) (20% in 200 mmol/L Glycine buffer) to $100\ \mu\text{L}$ serum with gentle mixing. Serum was incubated at room temperature for 20 min and after centrifugation (10,000 rpm, 20 min, 4°C) the supernatant was recovered [28]. Samples were stored at -70°C until use.

2.5. Isolation of HDL

HDL was isolated from controls ($n = 20$) and AR-patients ($n = 43$). Density of serum was adjusted by addition of potassium bromide (Sigma-Aldrich, Darmstadt, Germany) to $1.24\ \text{g/mL}$ and a two-step density gradient was generated in centrifuge tubes ($16 \times 76\ \text{mm}$, Beckman Coulter) by layering the density-adjusted serum ($1.24\ \text{g/mL}$) underneath a KBr density solution ($1.063\ \text{g/mL}$) [29]. The tubes were sealed and centrifuged at 65,000 rpm for 6 h at 15°C in a 90Ti fixed angle rotor (Beckman Instruments, Krefeld, Germany). After centrifugation, the visible HDL-containing band (containing HDL2 and HDL3 subfractions) was collected, concentrated using Vivaspin Turbo 4 ultracentrifugation spin columns (Sartorius, Göttingen, Germany), desalted using the PD MiniTrap G-25 columns (GE Healthcare, Vienna, Austria) and stored at -70°C in the presence of 5% sucrose (Sigma-Aldrich, Darmstadt, Germany) as cryoprotectant, as described [30].

2.6. Biochemical quantification of HDL-associated proteins

ApoA-I, apoA-II, apoC-II and apoE were determined by immunoturbidimetry using reagents from DiaSys (Holzheim, Germany) and standards from Siemens (Marburg, Germany; apoA-I, apoE) and Kamiya Biomedical (Seattle, WA, USA; apoA-II, apoC-II). All lipoprotein analyses were performed on an Olympus AU640 analyzer (Beckman Coulter, Brea, CA), as previously described [31]. ApoC-III was quantified using the Human Apolipoprotein CIII ELISA Kit (Abcam, Cambridge Science Park, Cambridge, UK), a method that has a sensitivity of 0.3 ng/mL. SAA was evaluated using a human SAA ELISA Kit (Invitrogen, Carlsbad, California, USA), which has a sensitivity of 4 ng/mL, according to the manufacturer's instructions.

2.7. Determination of serum and HDL lipid composition

Non-esterified cholesterol, cholesteryl esters, total cholesterol and triglycerides were determined by commercially available kits (Diasys Diagnostic Systems GmbH, Holzheim, Germany). Low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald equation using HDL cholesterol values measured in apoB-depleted serum.

2.8. Mass spectrometry analysis of HDL lipid composition

Samples containing 100 µg protein (1 µg/µL aqueous solution) were extracted according to Matyash et al. [32] in 700 µL methyl-tert-butyl ether (MTBE)/methanol (3/1, v/v) containing 500 pmol butylated hydroxytoluene, 1% acetic acid, and internal standards [IS; 40 pmol 17:1 lyso-phosphatidylcholine (LPC), 50 pmol d18:1/17:0 sphingomyelin, Avanti Polar Lipids, Alabaster, AL, USA; 250 pmol 17:0/17:0 phosphatidylcholine (PC), Larodan, Solna, Sweden]. Total lipid extraction was performed under constant shaking for 30 min at room temperature. After addition of 40 µL dH₂O (140 µL total aqueous phase) and further incubation for 30 min at room temperature, samples were centrifuged at 1000 × g for 15 min. 500 µL of the upper, organic phase were collected and dried under a stream of nitrogen. Lipids were resolved in 250 µL MTBE/methanol (3/1, v/v) and diluted in 2-propanol/methanol/dH₂O (7/2.5/1, v/v/v) for UHPLC-qTOF analysis. Chromatographic separation was performed on an 1290 Infinity II LC system (Agilent, Santa Clara, CA, USA) equipped with a Luna omega C18 column (2.1 × 50 mm, 1.6 µm; Phenomenex, Torrance, CA, USA) running a 20 min linear gradient from 55% solvent A (H₂O; 10 mM ammonium acetate, 0.1% formic acid, 8 µM phosphoric acid) to 100% solvent B (2-propanol; 10 mM ammonium acetate, 0.1% formic acid, 8 µM phosphoric acid). The column compartment was kept on 50 °C. A 6560 Ion Mobility Q-TOF mass spectrometer (Agilent, Santa Clara, CA, USA) equipped with Dual AJS ESI source was used for detection of lipids in positive Q-TOF mode. Data acquisition was done by MassHunter Data Acquisition software (B.09, Agilent, Santa Clara, CA, USA). Lipids were manually identified and lipid feature extraction was performed using MassHunter Profinder (V.10, Agilent, Santa Clara, CA, USA). Data were normalized for recovery, extraction, and ionization efficacy by calculating analyte/IS ratios (AU) and expressed as AU/µg protein.

2.9. C-reactive protein (CRP) determination

CRP levels were evaluated using the CRP Human ELISA Kit (Thermo Scientific, Rockford, USA), which has a sensitivity of < 10 pg/mL.

2.10. Gradient gel electrophoresis

Isolated HDL (5 µg protein per lane) was separated by gradient gel electrophoresis (4–16% Native Page, Life Technologies, Vienna, Austria) under nonreducing and nondenaturing conditions, as described previously [30]. The HDL₂, medium sized HDL₃ and small HDL₃

particles were estimated using the Image Lab software, Bio-Rad, Austria, as described [33].

2.11. Arylesterase activity of PON

Ca²⁺-dependent arylesterase activity of PON was determined by a photometric assay using phenylacetate substrate, as described [16]. Activities were calculated from the slopes of the kinetic chart of four independent experiments, measured in duplicates.

2.12. Determination of the anti-oxidative capacity of HDL

The anti-oxidative activity of apoB-depleted serum and isolated HDL was determined as previously described with modifications [34]. Briefly, dihydrorhodamine was suspended in DMSO to a 50 mM stock, which was diluted in HEPES (20 mM HEPES, 150 mM NaCl, pH 7.4) containing 1 mM 2,2'-azobis-2-methyl-propanimidamide-dihydrochloride (Sigma-Aldrich, Darmstadt, Germany) to a 10 µM working reagent. Either 10 µL of apoB-depleted serum (1:10 diluted) or 10 µL of isolated HDL (1 mg/mL) were placed in 384-well plates (Greiner Bio-One, Kremsmünster, Austria) and the volume was adjusted to 100 µL with HEPES buffer containing 10 µM dihydrorhodamine. The increase in fluorescence due to oxidation of dihydrorhodamine was monitored using an xMark plate reader (Biorad, Vienna, Austria) for 90 min at 538 nm. After an initial lag phase of about 20 min, the rate of oxidation was linear. The increase in fluorescence was calculated from the linear range and used for comparison. The increase in fluorescence per minute of dihydrorhodamine in the absence of apoB-depleted serum or isolated HDL was set to 100%, and individual apoB-depleted serum (three independent experiments measured in duplicates) or isolated HDL (three independent experiments measured in duplicates) samples were calculated as percentage of inhibition of dihydrorhodamine oxidation.

2.13. Lp-PLA2 activity assay

Lp-PLA2 activity of apoB-depleted serum was measured using a commercially available photometric assay (Cayman, Europe) using 2-thio PAF as substrate. Activities were calculated from the slopes of the kinetic chart of three independent experiments, measured in duplicates.

2.14. LCAT activity

LCAT activity of serum was measured in duplicates, using a commercially available kit (Merck, Darmstadt, Germany) according to the manufacturer's instructions. Specifically, samples were incubated with LCAT substrate for 4 h at 37 °C. The fluorescent substrate emits fluorescence at 470 nm. When the substrate is hydrolyzed by LCAT, a monomer is released that emits fluorescence at 390 nm. The LCAT activity is assessed over time and expressed in change of 470/390-nm emission intensity [35].

2.15. CETP activity

CETP activity of serum was measured in duplicates, using a commercially available kit (Merck, Darmstadt, Germany) according to the manufacturer's instructions. Specifically, the assay uses a proprietary substrate that enables the detection of CETP-mediated transfer of neutral lipid from the substrate to a physiological acceptor. The transfer activity results in an increase in fluorescence intensity ($\lambda_{\text{ex}} = 465/\lambda_{\text{em}} = 535$ nm).

2.16. Determination of nuclear factor- κ B (NF- κ B) expression and cytokines release evaluation

U937 monocytic cells containing a 5 × NF- κ B green fluorescence protein (GFP) reporter cassette were cultivated in RPMI 1640 (Life

Technologies, Carlsbad, California, USA) containing 10% fetal bovine serum (FBS) (Life Technologies, Carlsbad, California, USA) and 1% penicillin/streptomycin (Pan-Biotech, Aidenbach, Germany). The cells were pretreated for 90 min with either 7% apoB-depleted serum or isolated HDL (50 µg/mL), in two and three independent experiments, respectively, measured in duplicates. Subsequently, the cells were stimulated for 24 h with lipopolysaccharide (LPS) (50 ng/mL) (Sigma, Darmstadt, Germany), collected by centrifugation at 400 × g for 7 min and fixed with 100 µL fixative solution, which was prepared as previously described [36]. The expression of NF-κB was assessed by flow cytometry and cytokines released by U937 monocytic cells were quantified using a multiplex bead-based immunoassay (Thermo Scientific, Rockford, USA). The assay range was 9.35–38,300 pg/mL for IL-4, 6.74–27,600 pg/mL for IL-6, 2.38–9750 pg/mL for IL-8, 1.56–6400 pg/mL for IL-12p70, 6.42–26,300 pg/mL for TNF-α and 2.39–9800 pg/mL for IL-1 beta.

2.17. HDL-cholesterol efflux capacity

HDL-cholesterol efflux capacity of apoB-depleted serum was assessed, as described [7,28]. Briefly, J774.2 cells (Sigma-Aldrich, Darmstadt, Germany) were maintained in Dulbecco's modified eagle medium (DMEM) (Life Technologies, Carlsbad, California, USA) in the presence of 10% FBS and 1% penicillin/streptomycin. Cells were plated on 48-well plates (Greiner Bio-One, Kremsmünster, Austria) (300,000 cells/well), cultured for 24 h and loaded with 0.5 µCi/mL radiolabeled [³H]-cholesterol (Hartmann Analytic, Braunschweig, Germany) in DMEM supplemented with 2% FBS and 1% penicillin/streptomycin in the presence of 0.3 mM 8-(4-chlorophenylthio)-cyclic AMP (Sigma-Aldrich, Darmstadt, Germany) overnight. After labeling, cells were rinsed with serum-free DMEM containing 1% penicillin/streptomycin and equilibrated with serum-free DMEM containing 1% penicillin/streptomycin and 2 mg/mL bovine serum albumin (Sigma-Aldrich, Darmstadt, Germany) for 2 h. Subsequently [³H]-cholesterol efflux was determined by incubating cells for 3 h with 2.8% apoB-depleted serum. Cholesterol efflux was expressed as radioactivity in the cell culture supernatant relative to total radioactivity (in the cell culture supernatant and cells) of two independent experiments, measured in duplicates. All steps were performed in the presence of 2 µg/mL of the acyl coenzyme A cholesterol acyltransferase inhibitor Sandoz 58-035 (Sigma-Aldrich, Darmstadt, Germany).

2.18. Preparation of human eosinophils

Human peripheral blood eosinophils were isolated, as described [37]. Briefly, blood was drawn from healthy, non-allergic subjects and platelet-rich plasma was separated by centrifugation. Dextran sedimentation was used to remove erythrocytes, while polymorphonuclear leukocytes were obtained by density gradient separation using Histopaque. Eosinophils were further purified using negative magnetic selection through a respective antibody cocktail coupled to magnetic beads (StemCell Technologies, Vancouver, Canada). Purity and viability were > 98% after isolation.

2.19. Shape change assay

Purified eosinophils were pretreated with either 2.5% apoB-depleted serum or isolated HDL (50 µg/mL) for 30 min at room temperature and stimulated afterwards with eotaxin-2/CCL24 (3 nM) (Immunotools, Friesoythe, Germany) for 5 min at 37 °C. Subsequently, the samples were fixed and the change in shape of eosinophils was estimated as increase of forward scatter [38,39]. Responses were expressed as percent of vehicle (eotaxin-2/CCL24 in the absence of apoB-depleted serum or isolated HDL) treated cells of five and three independent experiments, respectively, measured in duplicates with eosinophils from different donors.

2.20. Chemotaxis assay

Eosinophils were pretreated with either 2.5% apoB-depleted serum or isolated HDL (50 µg/mL) for 30 min at room temperature. Then, cells were placed in the upper compartment of Transwell inserts with 5 µm pore size filters (Corning, Acton, MA, USA) and were allowed to migrate towards eotaxin-2/CCL24 (30 nM) in the bottom compartment for 1 h at 37 °C. Afterwards, migrated cells were fixed and enumerated on a flow cytometer, while responses were expressed as percent of vehicle (eotaxin-2/CCL24 in the absence of apoB-depleted serum or isolated HDL) treated cells of three independent experiments, respectively, measured in duplicates with eosinophils from different donors.

2.21. Statistical analysis

Data were tested for normality using the Shapiro-Wilk normality test. Data are presented as means and standard deviations if normally distributed or medians and first and third quartile if not. Differences between the two groups were analyzed using the Student's *t*-test or the Mann-Whitney *U* test. Individual data are depicted on top of boxplots showing median and interquartile range as well as minimum and maximum values. Gender differences were evaluated using the Fisher's exact test. Correlations between apoB-depleted serum and isolated HDL were determined using Pearson's correlation coefficient *r*, whereas other correlations were assessed using Spearman's correlation coefficient *rho* due to the skewed nature of many of the parameters. The general significance level for the analyses was set to $\alpha = 0.05$. However, because of the large amount of correlation analyses performed for functional properties of HDL with HDL-composition and -distribution, the significance level was reduced to 0.01 for these analyses (presented in heat maps). Statistical analyses were performed using GraphPad Prism (Version 6.01, GraphPad Software), SPSS Statistics (Version 23) and R (Version 3.5.3).

3. Results

3.1. Clinical characteristics of study subjects

Patients with AR showed unchanged serum levels of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and CRP when compared to controls (Table 1). Total IgE levels of AR-patients were above the normal range for each subject, depending on their age, whereas the control group showed normal total IgE levels. AR-patients

Table 1
Clinical characteristics of study subjects.

	Controls	AR-patients	<i>p</i> -Value
n	20	43	
Age (y)	21.1 ± 1.38	19.7 ± 7.82	0.444
Gender Male (%)	7 (35.0)	24 (55.8)	0.177
Total cholesterol (mg/dL)	173.0 (155.2–214.4)	174.2 (146.0–192.3)	0.250
HDL-cholesterol (mg/dL)	57.4 (46.9–76.3)	50.4 (43.4–61.6)	0.097
LDL-cholesterol (mg/dL)	93.4 (80.6–113.0)	101.3 (83.2–114.5)	0.668
Triglyceride (mg/dL)	82.6 (68.5–121.0)	65.8 (51.3–105.3)	0.103
CRP (mg/L)	0.72 (0.46–1.82)	0.56 (0.15–2.26)	0.288
Total IgE (kU/L)	16.0 (5.6–73.2)	117.0 (48.0–206.0)	< 0.001
sIgE grass pollen (kU/L)	sIgE negative	21.5 (6.1–52.2)	

Data are presented as mean ± standard deviation, median with first and third quartiles, or as absolute and relative frequencies. Differences between the two groups were analyzed with Student's *t*-test, with Mann-Whitney *U* test, or with Fisher's exact test, respectively. n, number of subjects; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; IgE, immunoglobulin E; sIgE, specific immunoglobulin E; kU/L, kilounits per liter; AR, allergic rhinitis.

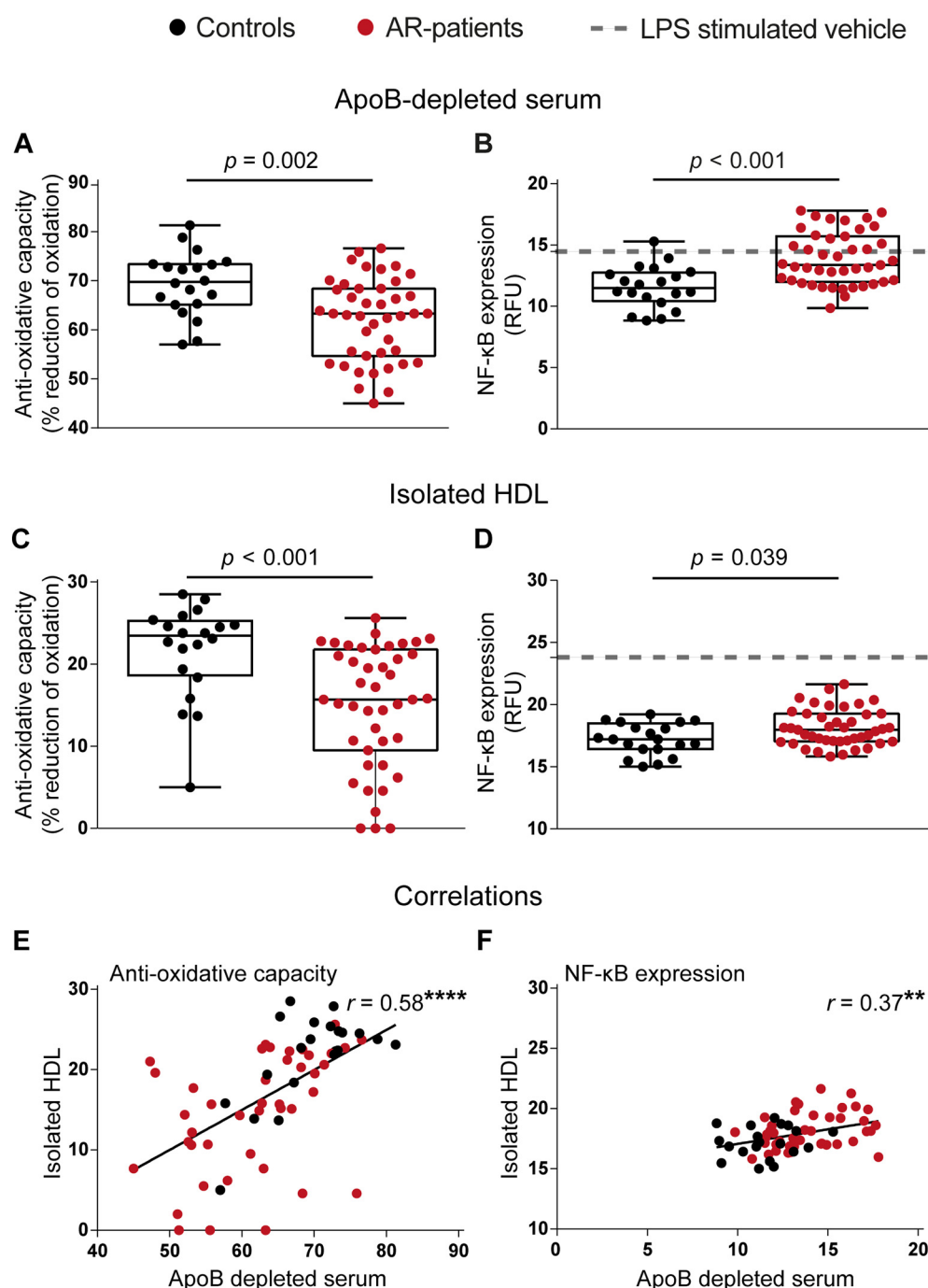


Fig. 1. Allergic rhinitis is associated with impaired anti-oxidative and anti-inflammatory capacity of apoB-depleted serum and isolated HDL.

ApoB-depleted serum or isolated HDL of controls ($n = 20$) and AR-patients ($n = 43$) was used in different assays. Inhibition of oxidation was measured by incubating apoB-depleted serum (A) or isolated HDL (C) with dihydrorhodamine in three independent experiments, respectively, measured in duplicates. The anti-inflammatory capacity of apoB-depleted serum (B) or isolated HDL (D) was evaluated using U937 monocytes containing a reporter cassette for NF- κ B. Cells were pretreated with 7% apoB-depleted serum or HDL (50 μ g/mL) for 90 min and subsequently stimulated with LPS (50 ng/mL) for 24 h. GFP-NF- κ B expression was assessed by flow cytometry in two and three independent experiments, respectively, measured in duplicates. The grey dashed line indicates LPS treated cells in the absence of apoB-depleted serum or isolated HDL. Scatter plots of the anti-oxidative capacity (E) and anti-inflammatory potential (F) of apoB-depleted serum and isolated HDL. (A–D) Differences between the two groups were analyzed with the Mann-Whitney U test. Individual data are depicted on top of boxplots showing median and interquartile range as well as minimum and maximum values. (E, F) The Pearson correlation coefficients (r) are noted. ** $p < 0.01$, **** $p < 0.0001$. AR, allergic rhinitis; RFU, relative fluorescence units; HDL, high-density lipoprotein; LPS, lipopolysaccharide; apoB, apolipoprotein B.

had elevated sIgE levels for grass pollen (Table 1, Supplementary Table 1), while all tested sIgE for controls were below the reference values. SIgE for grass pollen, birch pollen and house dust mite were the most frequent and abundant, while ash, mugwort and ragweed pollen as well as mold were less frequent (Supplementary Table 1).

3.2. Altered HDL functionality in AR-patients

In first experiments, we assessed whether AR affects the anti-oxidative and anti-inflammatory activity of apoB-depleted serum (containing all HDL subclasses, but no apoB-containing low-density lipoproteins) from AR-patients and control subjects. For that purpose, we evaluated the ability of apoB-depleted serum to inhibit free radical-induced oxidation of the fluorescent dye dihydrorhodamine [33]. Interestingly, we observed that apoB-depleted sera from AR-patients were

less potent in inhibiting dihydrorhodamine oxidation when compared to control sera (Fig. 1A). In subsequent experiments, we tested the anti-inflammatory activity of apoB-depleted serum by assessing its ability to inhibit LPS-induced activation of the pro-inflammatory transcription factor NF- κ B in monocytes [33,40]. We observed that almost all apoB-depleted sera of controls inhibited NF- κ B expression, whereas sera of AR-patients showed a significantly impaired inhibitory activity (Fig. 1B), and many AR samples even exhibited pro-inflammatory activity.

We isolated HDL from sera of AR-patients and controls to study whether these effects were directly caused by an altered HDL functionality. Similar to apoB-depleted sera, isolated HDL from AR-patients (AR-HDL) was less potent in inhibiting dihydrorhodamine oxidation (Fig. 1C) and showed an impaired ability to suppress NF- κ B in monocytes (Fig. 1D). Importantly, we observed a significant correlation

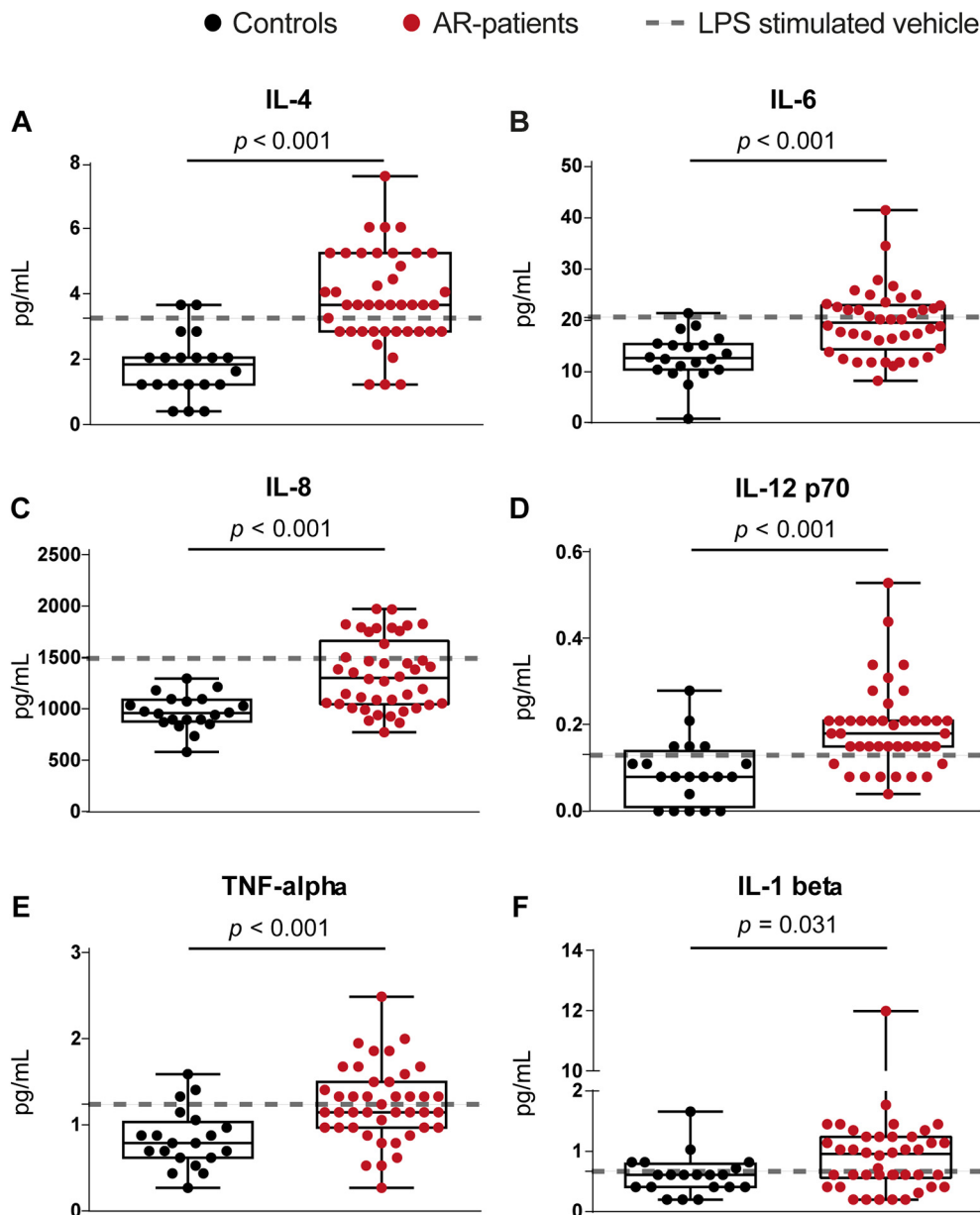


Fig. 2. Impaired ability of AR-HDL to suppress monocyte cytokine secretion.

Monocytes were pretreated with isolated HDL of controls (n = 20) and AR-patients (n = 42) and stimulated with LPS to induce cytokine release. Upon treatment the supernatants were collected and cytokines were assessed using a multiplex bead-based immunoassay. (A) Interleukin (IL-) 4, (B) IL-6, (C) IL-8, (D) IL-12 p70 (E), TNF- α and (F) IL-1 beta. (A–F) Differences between the two groups were analyzed with the Mann-Whitney U test. The grey dashed line indicates LPS treated cells in the absence of isolated HDL. Individual data are depicted on top of boxplots showing median and interquartile range as well as minimum and maximum values. AR, allergic rhinitis; IL, interleukin; TNF, tumor necrosis factor; LPS, lipopolysaccharide.

between apoB-depleted sera and isolated HDL in terms of their ability to inhibit dihydrorhodamine oxidation (Fig. 1E) and to suppress NF- κ B expression (Fig. 1F). Furthermore, AR-HDL was less potent in suppressing monocyte cytokine secretion (including IL-4, IL-6, IL-8, IL-12 p70, TNF- α and IL-1 beta) when compared to HDL of controls (Fig. 2A–F). The NF- κ B inhibitory activity of HDL was significantly correlated with the ability of HDL to modulate cytokine production (Supplementary Table 2).

3.3. Altered HDL-associated enzyme activities in AR-patients

HDL acts as reservoir for a number of biologically active enzymes that may be affected by AR. HDL-associated PON is an esterase with vascular protective activities [41]. Compared to apoB-depleted sera of controls, PON activity was significantly decreased in apoB-depleted serum of AR-patients (Fig. 3A). Another HDL-associated enzyme is Lp-PLA2, that hydrolyzes short- and medium-chain phospholipids [42]. Lp-PLA2 is thought to play a role in AR due to its ability to hydrolyze PAF, which is assumed to be involved in mucus secretion. Increased PAF levels have been found in nasal lavage of AR-patients after nasal

challenge with pollen [43]. Interestingly, we found that activity of Lp-PLA2 in hydrolyzing PAF was increased in apoB-depleted serum of AR-patients (Fig. 3B). These data suggest reduced anti-oxidative/anti-inflammatory activity of AR-HDL but an improved capability to decompose platelet-activating factor.

During HDL maturation, free cholesterol is esterified to cholesteryl ester by LCAT [44]. LCAT converts discoidal HDL into spherical HDL and increases the size of HDL through the conversion of HDL3 to HDL2 [45], which is considered to be an important step during reverse cholesterol transport. However, we observed no significant difference in the LCAT activity of sera derived from AR-patients in comparison to controls (Fig. 3C). CETP is responsible for the bidirectional transfer of cholesteryl esters and triglycerides between plasma lipoproteins [46]. We observed significantly decreased CETP activity in sera derived from AR-patients in comparison to control sera (Fig. 3D). Moreover, the ability of HDL to remove membrane cholesterol from cells (cholesterol efflux capacity, an important anti-atherogenic property of HDL) was not altered (Supplementary Fig. 1).

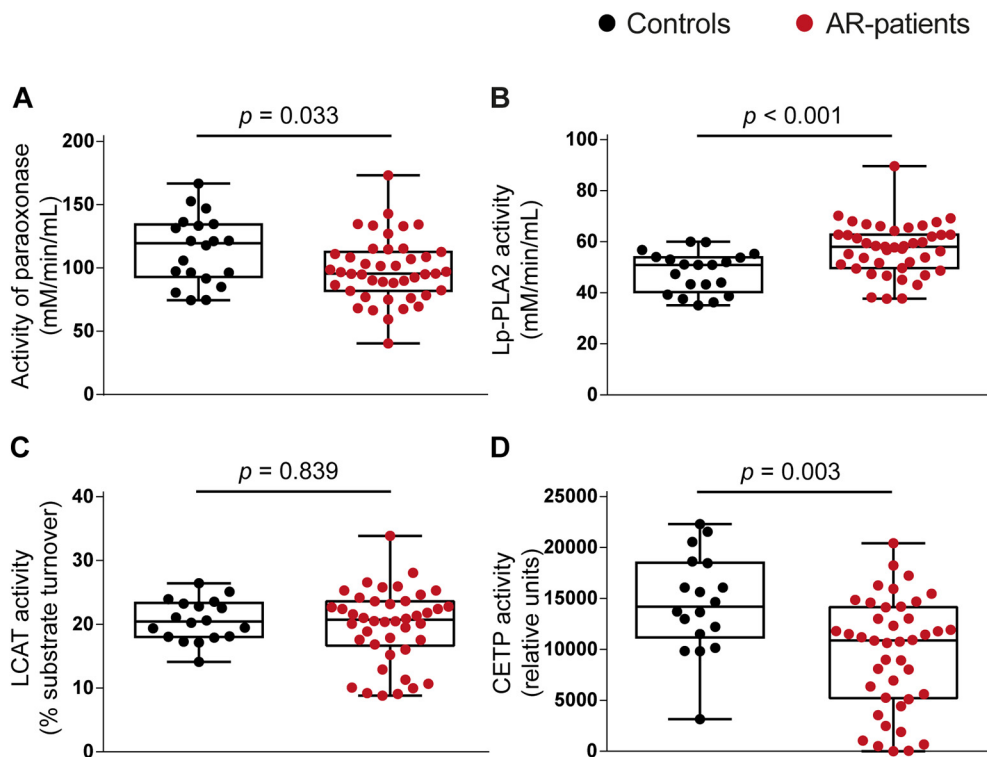


Fig. 3. AR shows altered PON, Lp-PLA2 and CETP activities.

(A, B) ApoB-depleted serum from controls ($n = 20$) and AR-patients ($n = 43$) was used in different assays. (A) Arylesterase activity of paraoxonase (PON) was measured using phenylacetate as substrate. Activities were calculated from the slopes of the kinetic chart of four independent experiments, measured in duplicates. (B) Lp-PLA2 activity was measured using 2-thio platelet-activating factor as substrate. Activities were calculated from the slopes of the kinetic chart of three independent experiments, measured in duplicates. (C) The LCAT activity in serum of controls ($n = 18$) and AR-patients ($n = 42$) was evaluated. (D) The CETP activity in serum of controls ($n = 18$) and AR-patients ($n = 42$) was evaluated. (A–D) Differences between the two groups were analyzed with the Mann-Whitney U test. Individual data are depicted on top of boxplots showing median and interquartile range as well as minimum and maximum values. AR, allergic rhinitis; Lp-PLA2, lipoprotein-associated phospholipase A2; LCAT, lecithin-cholesterol acyltransferase; CETP, cholesteryl ester transfer protein.

3.4. AR-HDL shows an improved ability to suppress eosinophil effector responses

Infiltrated inflammatory cells like eosinophils play a role in inducing and maintaining on-going allergic inflammation [47]. We investigated whether apoB-depleted sera or isolated HDL of AR-patients or controls can affect eosinophil activation (shape change) and chemotaxis. For that purpose, freshly isolated eosinophils from healthy subjects were pretreated with either apoB-depleted sera or isolated HDL, followed by stimulation with eotaxin-2/CCL24. The change in forward scatter was monitored by flow cytometry [48]. Surprisingly, apoB-depleted sera as well as isolated HDL of AR-patients showed an improved ability to suppress eotaxin-2/CCL24-induced eosinophil shape change when compared to controls (Fig. 4A, C).

To study the effects of AR on eosinophil chemotaxis, eosinophils were pretreated with either apoB-depleted sera or isolated HDL and allowed to migrate towards eotaxin-2/CCL24 in Transwell inserts. Similar to shape change experiments, apoB-depleted sera as well as isolated HDL of AR-patients showed an improved ability to suppress eotaxin-2/CCL24-induced eosinophil chemotaxis, when compared to controls (Fig. 4B, D). Moreover, the abilities of apoB-depleted sera and isolated HDL to inhibit agonist-induced eosinophil effector responses were significantly correlated (Fig. 4E, F).

3.5. Composition of AR-HDL is markedly altered

It is known that alterations of lipid metabolism, activation of the innate immune system, or inflammation, can modulate the content of apolipoproteins in HDL [19]. Owing to the marked changes observed in AR-HDL functionality, we were interested in assessing HDL composition. AR-patients showed decreased levels of apoA-I (Fig. 5A) and increased levels of apoA-II (Fig. 5B) compared to controls. No difference was observed in the apoC-II content (Fig. 5C), while a trend towards increased apoC-III ($p = 0.063$) content (Fig. 5D) was observed in AR-HDL. ApoE (Fig. 5E) and SAA (Fig. 5F) levels were not altered in HDL from AR-patients. When assessing the lipid composition of AR-HDL, we observed no change in free cholesterol (Fig. 6A) and cholesteryl ester

(Fig. 6B) content of AR-HDL, but markedly increased levels of triglycerides (Fig. 6C). AR-HDL-associated PC content was decreased (Fig. 6D), whereas LPC content was strikingly increased (Fig. 6E). Sphingomyelin content was not altered in AR-HDL (Fig. 6F). A more detailed analysis of the different PC, LPC and sphingomyelin species is given in Supplementary Fig. 2.

HDL particles are diverse in structure and composition representing the basis for their functional heterogeneity. HDL particles can be subdivided into large, cholesterol-rich spherical HDLs and more dense, protein-rich HDLs that appear to display the most potent atheroprotective properties [44]. When performing gradient gel electrophoresis to separate HDL subfractions, we observed a decrease in the medium sized HDL3 subclass in AR-patients (Fig. 7A), whereas other HDL subfractions, such as small HDL3 and large HDL2 particles were not altered (Fig. 7B, C). A representative gradient gel electrophoresis of HDL subfractions is shown in Fig. 7D.

3.6. Metrics of AR-HDL function are associated with AR-HDL composition and structure

Owing to the marked alterations in AR-HDL composition and function, we performed an in depth correlation analysis to determine which proteins and lipids in AR-HDL are associated with an altered function. We observed multiple and complex associations (Supplementary Fig. 3), suggesting that alterations in the composition and structure of AR-HDL affect distinct functional properties. The highest number of associations was seen with AR-HDL triglyceride and LPC content as well as with the PC/LPC and HDL2/HDL3 ratios. All these factors were associated with multiple functional properties of AR-HDL, such as anti-oxidative and anti-inflammatory activities, cholesterol efflux capacity, and inhibition of eosinophil shape change, as well as with enzyme activities, such as PON, CETP and LCAT.

We observed weaker associations between AR-HDL apoA-I and apoA-II content and AR-HDL PON activity along with the ability of AR-HDL to suppress eosinophil shape change. ApoC-II content of AR-HDL was associated with LCAT activity and anti-inflammatory potential of HDL, while apoE content was associated with the ability of apoB-

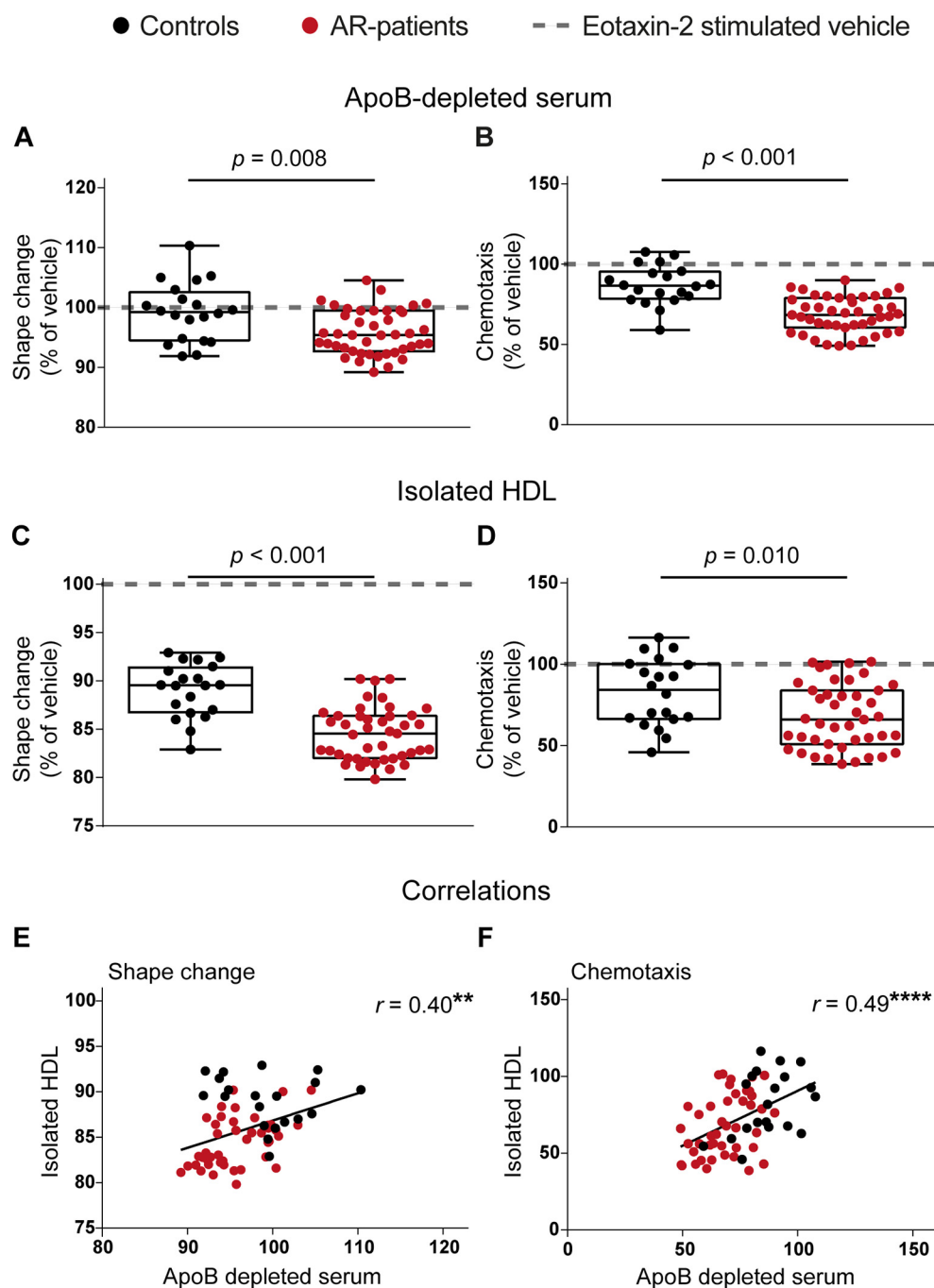


Fig. 4. AR-HDL shows improved ability to suppress eosinophil effector responses. ApoB-depleted serum or isolated HDL of controls ($n = 20$) and AR-patients ($n = 43$) was examined for their ability to modulate eosinophil effector responses. Isolated human eosinophils were pretreated with apoB-depleted serum (A) or isolated HDL (C) followed by stimulation with eotaxin-2/CCL24. Shape change was analyzed by flow cytometry. Responses were expressed as percent of vehicle (eotaxin-2/CCL24 in the absence of apoB-depleted serum or isolated HDL) treated cells (indicated by the grey dashed line) of five and three independent experiments, respectively (measured in duplicates with eosinophils from different donors). Eosinophils were pretreated with apoB-depleted serum (B) or isolated HDL (D), placed in Transwell inserts and allowed to migrate towards eotaxin-2/CCL24. Responses were expressed as percent of vehicle (eotaxin-2/CCL24 in the absence of apoB-depleted serum or isolated HDL) treated cells (indicated by the grey dashed line) of three independent experiments, respectively (measured in duplicates with eosinophils from different donors). Scatter plots of apoB-depleted serum and isolated HDL eosinophil shape change (E) and eosinophil chemotaxis (F). (A–D) Differences between the two groups were analyzed with the Mann-Whitney U test. Individual data are depicted on top of boxplots showing median and interquartile range as well as minimum and maximum values. (E, F) The Pearson correlation coefficients (r) are noted. $^{**}p < 0.01$, $^{****}p < 0.0001$. AR, allergic rhinitis; HDL, high-density lipoprotein; apoB, apolipoprotein B.

depleted serum of AR-patients to suppress eosinophil chemotaxis (Supplementary Fig. 3). Considerably weaker and sometimes qualitatively different associations, between HDL composition and functional properties, were observed in controls (Supplementary Fig. 4).

3.7. Specific IgE levels are associated with metrics of AR-HDL composition, distribution and function

Prompted by the marked alterations in AR-HDL composition, distribution and functionality, we next assessed potential associations with specific IgE levels of grass pollen, the most abundant allergen in the AR-patients group (Supplementary Table 3). We observed that specific IgE levels of grass pollen were significantly correlated with AR-HDL-associated apoE and weakly correlated with AR-HDL-associated SAA, AR-HDL-distribution, AR-HDL-associated PON and Lp-PLA2 activities as

well as the capacity of AR-HDL to modulate cholesterol efflux.

4. Discussion

In the present study, we provide first evidence that AR is associated with alterations in HDL metabolism, thereby affecting HDL composition and functionality (Fig. 8). Both isolated HDL, as well as apoB-depleted serum of AR-patients, showed decreased anti-oxidative and anti-inflammatory activities, but an improved ability to suppress agonist-induced eosinophil effector responses. Interestingly, the activities of apoB-depleted sera and isolated HDL were correlated, suggesting that AR-associated changes in HDL function are reflected in apoB-depleted serum.

An important observation of the present study was that AR-HDL is defective in the suppression of monocyte NF- κ B expression and

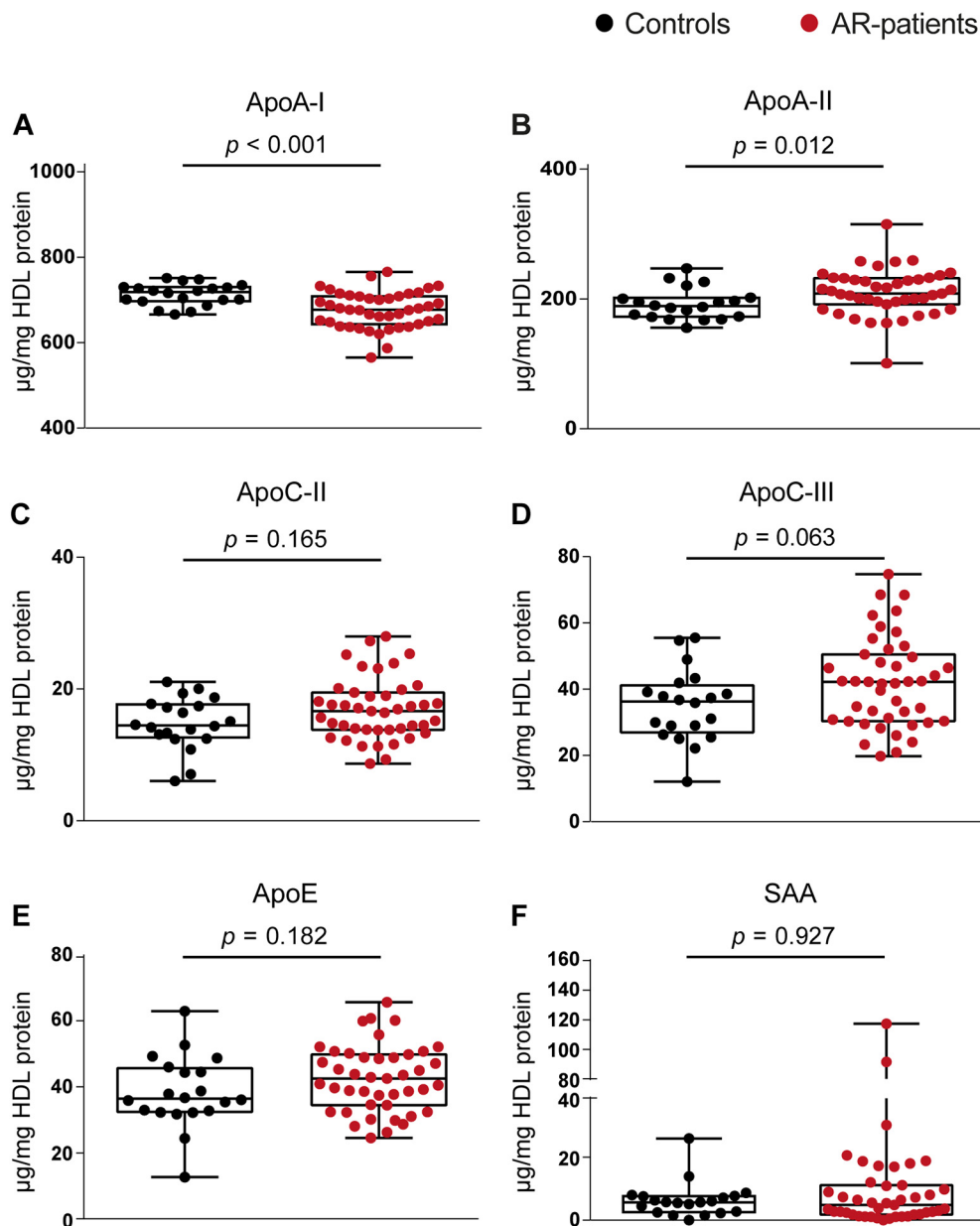


Fig. 5. Protein composition of HDL. HDL-associated proteins from controls ($n = 20$) and AR-patients ($n = 43$), including apoA-I (A), apoA-II (B), apoC-II (C) and apoE (E) were determined by immunoturbidimetry. ApoC-III (D) and serum amyloid A (SAA) (F) were measured by ELISA. (A–F) Differences between the two groups were analyzed with the Mann-Whitney U test. Individual data are depicted on top of boxplots showing median and interquartile range as well as minimum and maximum values. AR, allergic rhinitis; apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; apoC-II, apolipoprotein C-II; apoC-III, apolipoprotein C-III; apoE, apolipoprotein E; SAA, serum amyloid A; HDL, high-density lipoprotein.

subsequent cytokine secretion, including IL-4, IL-6, IL-8, IL-12 p70, TNF- α and IL-1 beta. The loss of anti-inflammatory activity of AR-HDL may contribute to the progression and severity of the disease during the late-phase response of AR, as most of these cytokines are involved in allergic inflammation. For example, TNF- α activates signaling pathways that regulate the release of eotaxin and the cell surface expression of the intercellular adhesion molecule 1 of eosinophils [49]. IL-4 is a cytokine released by Th2 cells and involved in the late-phase reaction of AR, leading to eosinophil recruitment, activation and mucosal influx [20]. In addition, IL-6 is a Th2-type cytokine involved in the induction of IgE synthesis, proliferation and maturation of mast cells, while IL-8 is a chemokine known to induce leukocyte migration, that has been reported to be involved in the late-phase allergic reaction [50]. IL-1 beta plays a role in different allergic disorders via inflammasome-dependent activation [51].

In the late-phase response of AR, cells that infiltrate the nasal mucosa produce a variety of mediators, including reactive oxygen species [52]. Previous studies have shown that the major HDL apolipoproteins, apoA-I and apoA-II, are significantly elevated in the mucus proteome of AR patients, suggesting that HDL-associated apolipoproteins directly

modulate the immune response [53–55]. In the present study, we observed that the anti-oxidative capacity and the PON activity of AR-HDL are reduced. PON is a calcium-dependent lactonase [56] with anti-oxidant [57], anti-inflammatory [58] and endothelial protective activities [59]. Importantly, previous studies reported that serum PON levels in children with AR were significantly lower [52], which is consistent with the reduced PON and anti-oxidative activity of AR-HDL observed in our study. These previous studies, together with our results, suggest a defective anti-oxidant capacity of HDL in AR, which may lead to an impaired ability to counteract the production of reactive oxygen species in the nasal mucosa of AR patients.

Surprisingly, we observed that AR-HDL showed an improved ability to suppress eotaxin-2/CCL24 induced eosinophil shape change and chemotaxis, when compared to HDL from controls. This supports the notion that AR is associated with complex alterations in HDL composition and function, linked to loss, as well as to gain of functional properties. Interestingly in this regard, the improved ability of AR-HDL to suppress eosinophil shape change was associated with apoA-II, apoC-III, LPC, PC and triglyceride content of AR-HDL.

A striking observation of the present study was the increased

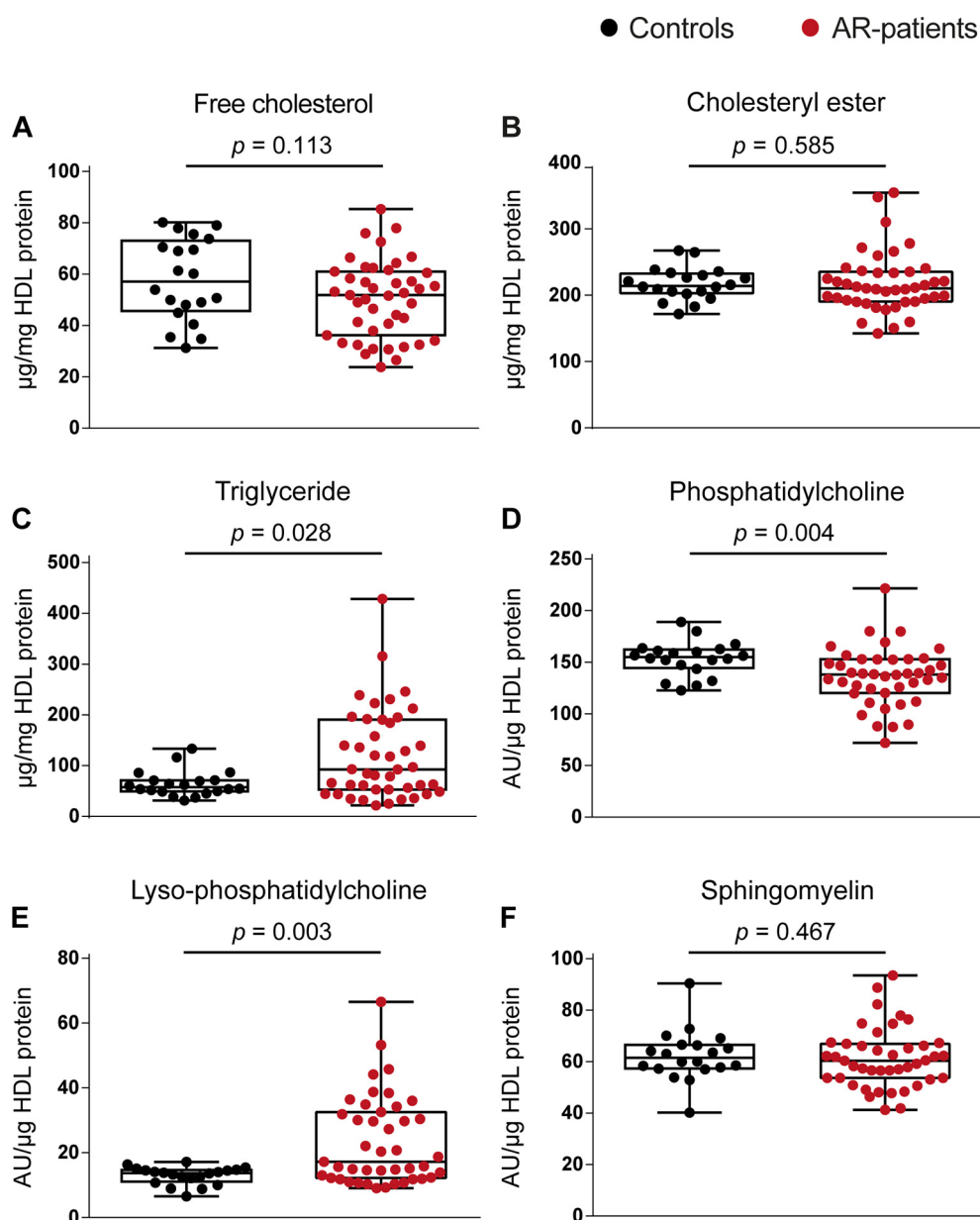


Fig. 6. Lipid composition of HDL.

HDL-associated lipids from controls ($n = 20$) and AR-patients ($n = 43$), including free cholesterol (A), cholesteryl ester (B) and triglyceride (C) were determined with commercially available kits. Phosphatidylcholine (D), lyso-phosphatidylcholine (E) and sphingomyelin (F) were determined with mass spectrometry. (A–F) Differences between the two groups were analyzed with the Mann-Whitney U test. Individual data are depicted on top of boxplots showing median and interquartile range as well as minimum and maximum values. AR, allergic rhinitis; AU, analyte/IS ratio; HDL, high-density lipoprotein.

triglyceride and LPC contents of AR-HDL, which were strongly associated with most metrics of AR-HDL function. The question now is why these complex lipid changes occur in AR-HDL. One reason might be that the triglyceride content is strongly associated with the apoC-III levels in AR-HDL ($p < 0.001$), consistent with the fact that apoC-III delays the catabolism of triglyceride-rich particles [60]. Triglyceride-rich HDL is a natural hepatic lipase substrate, that hydrolyses phospholipids and triglycerides from HDL [61]. In addition, we found that the Lp-PLA2 activity of AR-HDL was significantly increased. Lp-PLA2 hydrolyzes short- and medium-chain phospholipids and produces LPC [42]. Thus, hepatic lipase and Lp-PLA2 are likely candidates that induce, or at least contribute, to LPC formation in AR-HDL.

We have recently shown that HDL enriched with unsaturated LPC 16:0 effectively suppresses neutrophil [62] and eosinophil [14] effector responses. Since LPC 16:0 is significantly enriched in AR-HDL, our data

suggest that LPC 16:0 in AR-HDL contributes to AR-HDL's improved ability to suppress eosinophilic effector responses. In line with our results, LPC levels in plasma and bronchoalveolar fluid were found to be elevated in rhinitis patients [63]. Despite its established role in the formation of HDL-associated LPCs, we observed that LCAT activity in the AR-sera remained unchanged. Surprisingly, LCAT activity was negatively associated with LPC content in AR-HDL. One possible explanation for this contra-intuitive finding is that LPC can inhibit LCAT activity [64].

In addition to the changes in the lipid composition of AR-HDL, our data show that changes in the HDL3 subfraction are related to the impaired ability of AR-HDL to suppress the secretion of monocyte cytokines. This is consistent with previous reports that HDL3 particles are superior to larger and more lipid-rich HDL2 particles in suppressing inflammatory responses [44]. In addition, it has been reported that

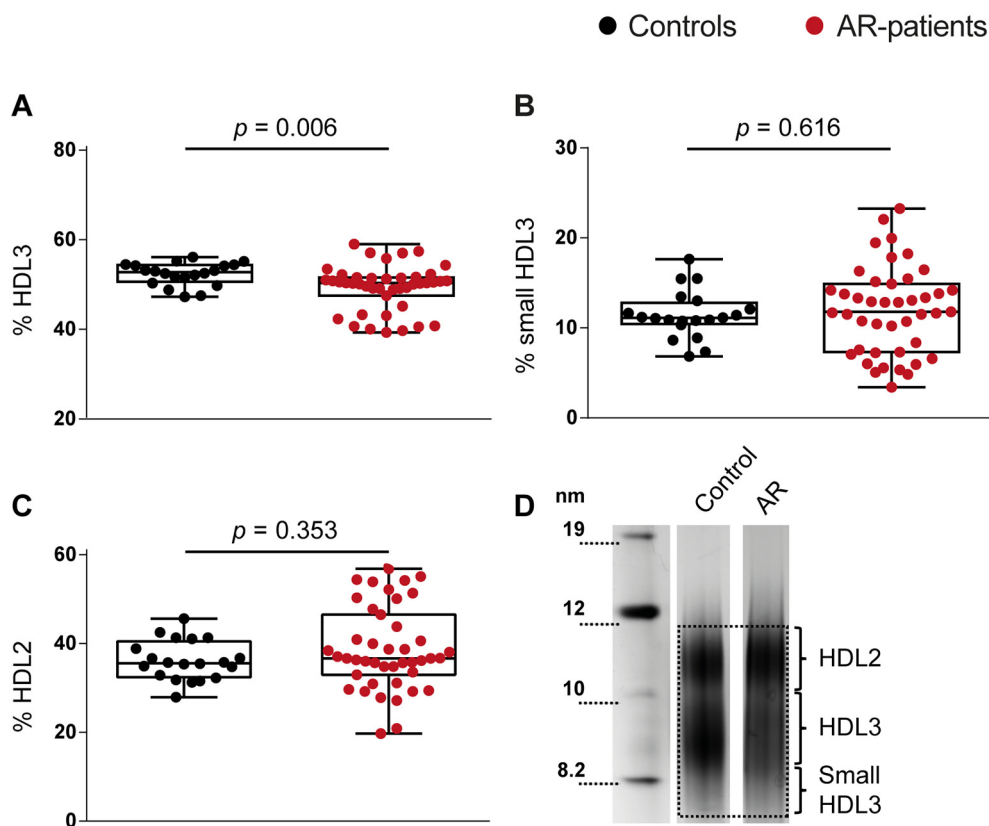


Fig. 7. Analysis of HDL subgroup distribution.

Isolated HDL of controls (n = 20) and AR-patients (n = 43) was separated by non-reducing and nondenaturing gradient gel electrophoresis. HDL-proteins were stained with Coomassie brilliant blue to determine distribution of HDL3 (A), small HDL3 (B) and HDL2 (C) subfractions. A representative gradient gel electrophoresis of HDL of one patient and one control sample is shown (D). (A–C) Differences between the two groups were analyzed with the Mann-Whitney U test. Individual data are depicted on top of boxplots showing median and interquartile range as well as minimum and maximum values. AR, allergic rhinitis; HDL, high-density lipoprotein.

small, dense HDL3 particles are superior to large, light HDL2 particles in removing oxidized lipids from other lipoproteins and cell membranes [44]. Therefore, our data suggest that the reduced content of the HDL3

subfraction contributes to the low anti-oxidant capacity of AR-HDL.

Another interesting observation of the present study was that CETP activity in sera of AR patients was significantly impaired. CETP

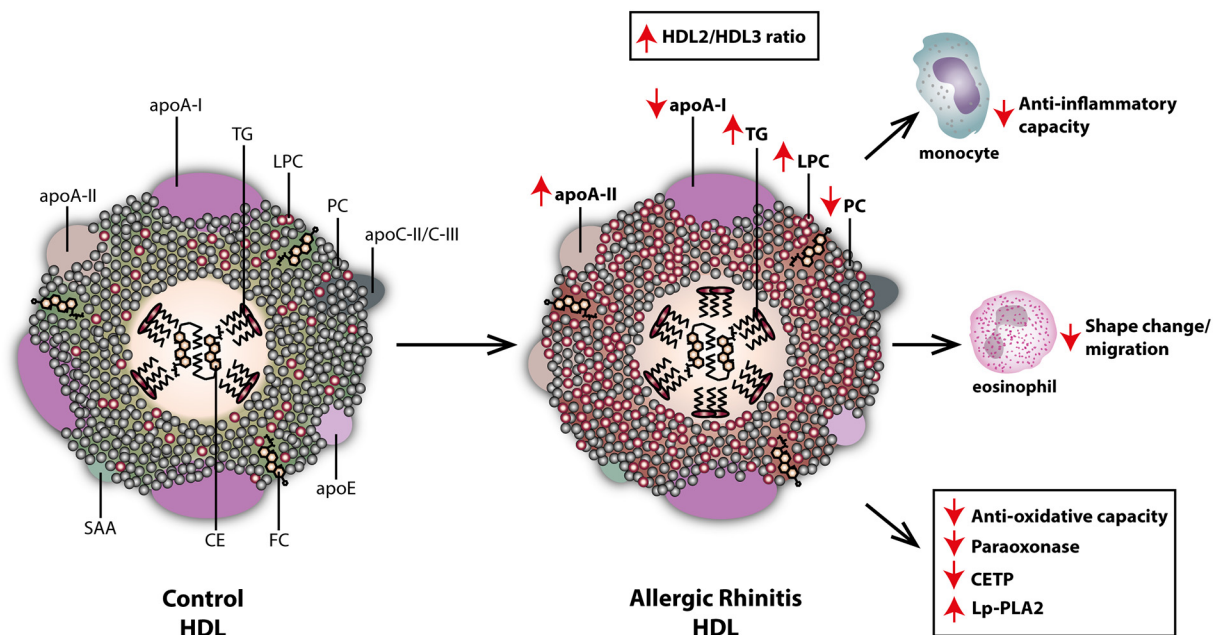


Fig. 8. Schematic illustration of HDL remodeling in AR-patients.

HDL from AR-patients exhibits various alterations in the protein and lipid composition linked to altered functional properties. Profound alterations in AR-HDL-lipid composition are observed, such as an increase in the AR-HDL-triglyceride and -LPC content and a decrease in the HDL-PC content. AR-HDL is significantly enriched in apoA-II, while apoA-I and the medium sized HDL3 are decreased. In AR-patients, the activity of Lp-PLA2 is markedly increased, while activities of paraoxonase and CETP are significantly decreased. AR-HDL shows impaired anti-oxidative and anti-inflammatory capacities, but an improved ability to inhibit eosinophil effector responses. HDL, high-density lipoprotein; apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; apoC-II, apolipoprotein C-II; apoC-III, apolipoprotein C-III; apoE, apolipoprotein E; SAA, serum amyloid A; TG, triglyceride; LPC, lyso-phosphatidylcholine; PC, phosphatidylcholine; CE, cholesteryl ester; FC, free cholesterol; CETP, cholesteryl ester transfer protein; Lp-PLA2, lipoprotein-associated phospholipase A2.

circulates in plasma and is mainly bound to HDL [65], while it distributes cholesterol esters, triglycerides and to a lesser extent phospholipids between plasma lipoproteins [46]. We observed that CETP activity clearly correlated with triglyceride and LPC levels of AR-HDL, but interestingly, not with HDL cholesterol levels. We currently have no explanation for this, and further studies are needed to understand underlying mechanisms. In summary, our results suggest that AR induces complex changes in serum enzyme activities that affect HDL composition and function.

After detecting significant changes in AR-HDL composition, distribution and functionality, we investigated possible associations with the specific IgE values of grass pollen, the most common allergen in the AR patient group. We observed that specific IgE values of grass pollen were significantly correlated with AR-HDL-associated apoE, while we observed weaker correlations with AR-HDL-associated SAA, AR-HDL distribution, AR-HDL-associated PON and Lp-PLA2 activities, and the ability of AR-HDL to modulate cholesterol efflux.

Interestingly, we observed no impact of gender on AR-HDL functional properties (Supplementary Table 4) and we observed no change in cholesterol efflux capacity of AR-HDL when compared to HDL of controls, indicating that this major anti-atherogenic property of HDL [66] is not affected.

We acknowledge limitations to this study. Due to the laborious experiments and analyses, we had to keep the patient number rather small. Moreover, our study is exploratory and due to the design limited to be correlative in nature, not permitting causal inference. Therefore, further studies in larger cohorts are warranted to confirm our results. Strengths of our study are that we measured multiple metrics of HDL functionality, as well as HDL composition, in relatively young individuals, excluding a major contribution of age-associated diseases to alterations in AR-HDL composition and function.

In summary, the results of our study provide new evidence that AR significantly influences HDL composition and functionality, suggesting a novel link between HDL metabolism and allergy.

Transparency document

The [Transparency document](#) associated with this article can be found, in online version.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbalip.2019.06.007>.

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Supplementary Material

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Supplementary Table 1. Sensitizations of AR-patients

Allergen	sIgE levels (kU/L)	n
Grass pollen	21.5 (6.1 - 52.2)	43
Birch pollen	9.6 (1.7 - 29.0)	19
House dust mite	3.2 (1.4 - 5.6)	7
Mugwort pollen	0.7 (0.5 - 1.2)	7
Mold	1.6 (0.4 - 5.4)	3
Ash pollen	1.1 (1.0 - 1.9)	4
Ragweed pollen	11.4 (11.4 - 11.4)	2

Supplementary Table 1. Data are presented as median with first and third quartile and number of sensitized subjects (n). sIgE, specific immunoglobulin E; kU/L, kilounits per liter; AR, allergic rhinitis.

Supplementary Table 2. Correlation of NF- κ B expression with cytokine release

Cytokine	NF- κ B expression	
	<i>rho</i>	<i>p</i> -value
IL-4	0.413	0.001
IL-6	0.289	0.023
IL-8	0.448	0.001
IL-12 p70	0.459	0.001
TNF-alpha	0.237	0.063
IL-1 beta	0.092	0.476

Supplementary Table 2 NF- κ B expression is shown as relative fluorescence units (RFU) and IL-4, IL-6, IL-8, IL-12 p 70, TNF-alpha and IL-1 beta are expressed in pg/mL. Spearman's rank correlation coefficients (*rho*) are presented with the corresponding *p*-values, considering controls and AR-patients as a single group. NF- κ B, nuclear factor- κ B; IL-4, interleukin 4; IL-6, interleukin 6; IL-8, interleukin 8; IL-12 p70, interleukin 12 p70; TNF-alpha, tumor necrosis factor alpha; IL-1 beta, interleukin 1 beta.

Supplementary Table 3. Correlation between specific IgE grass pollen and HDL compositional and functional parameters

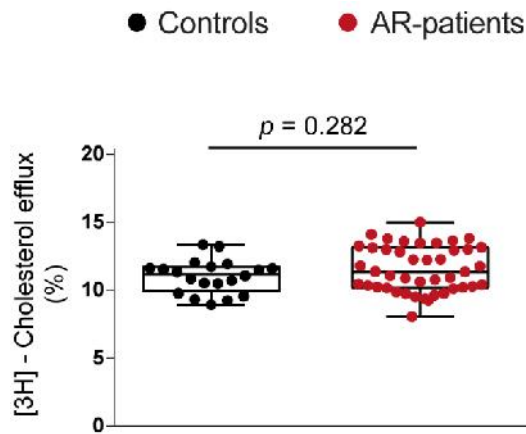
		Specific IgE grass pollen	
		<i>rho</i>	<i>p</i> -value
HDL-associated proteins	apoA-I	-0.062	0.697
	apoA-II	-0.202	0.201
	apoC-II	0.248	0.113
	apoC-III	-0.046	0.774
	apoE	0.305	0.049
	SAA	0.300	0.053
HDL-associated lipids	Cholesterol	-0.131	0.410
	Free cholesterol	-0.173	0.274
	Cholesteryl ester	-0.088	0.580
	Triglyceride	-0.199	0.207
	Phosphatidylcholine	0.071	0.658
	Lyso-phosphatidylcholine	-0.254	0.104
HDL-distribution	Sphingomyelin	-0.092	0.561
	HDL2/HDL3 ratio	-0.297	0.056
	Small HDL3 particles	0.295	0.058
HDL-associated enzymes	PON activity	0.289	0.063
	Lp-PLA2 activity	-0.302	0.052
	LCAT activity	0.097	0.546
	CETP activity	0.096	0.552
HDL-functional properties	Shape change	0.203	0.197
	Chemotaxis	0.112	0.480
	Anti-oxidative capacity	0.195	0.217
	NF-κB expression	-0.037	0.818
	Cholesterol efflux	-0.303	0.051

Supplementary Table 3. Specific IgE grass pollen is expressed in kU/L (kilounits per liter), HDL-associated proteins and lipids are expressed in µg/mg HDL protein or in AU/µg HDL protein, small HDL3 particles are expressed as %, PON activity is expressed in mM/min/mL, Lp-PLA2 activity is expressed in mM/min/mL, LCAT activity is expressed as % substrate turnover, CETP activity is expressed in relative units, shape change is expressed as % of vehicle, chemotaxis is expressed as % of vehicle, anti-oxidative capacity is expressed as % reduction of oxidation, NF-κB expression is shown as relative fluorescence units (RFU) and cholesterol efflux is expressed as %. Spearman's rank correlation coefficients (*rho*) are presented with the corresponding *p*-values, considering only AR-patients. HDL, high-density lipoprotein; apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; apoC-II, apolipoprotein C-II; apoC-III, apolipoprotein C-III; apoE, apolipoprotein E; SAA, serum amyloid A; PON, paraoxonase; Lp-PLA2, lipoprotein-associated phospholipase A2; LCAT, lecithin-cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; NF-κB, nuclear factor-κB; IgE, immunoglobulin E.

Supplementary Table 4. Gender comparison of HDL functional properties of controls and AR-patients

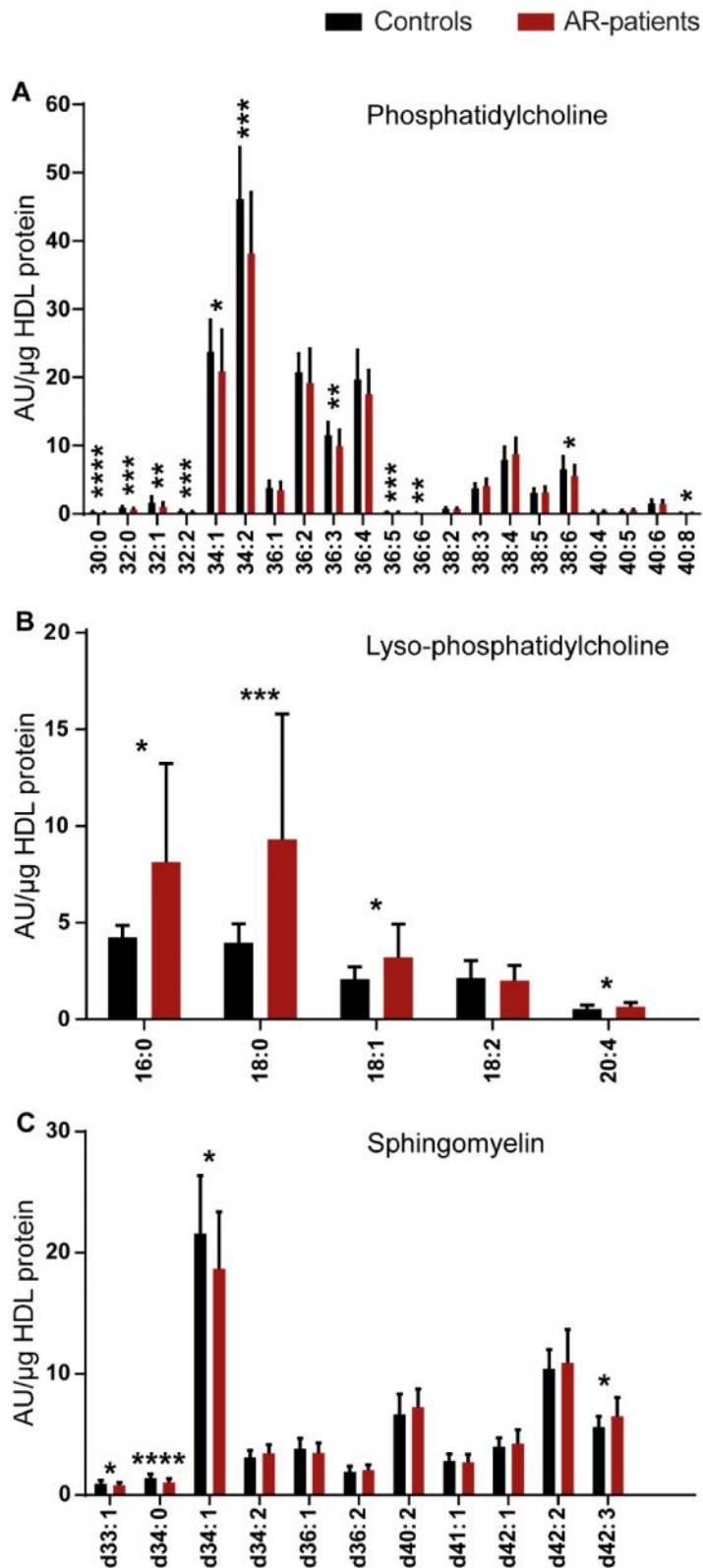
	Functional assays	Controls			AR-patients		
		Males	Females	<i>p</i> -value	Males	Females	<i>p</i> -value
Isolated HDL	Anti-oxidative capacity	21.9 (18.4 - 24.6)	23.8 (19.3 - 25.7)	0.341	15.5 (9.8 - 21.6)	15.7 (7.7 - 22.3)	0.842
	NF-κB expression	16.8 (16.4 - 17.2)	17.7 (16.2 - 18.7)	0.151	17.5 (17.0 - 18.5)	18.2 (17.1 - 19.8)	0.199
	Shape change	89.6 (88.4 - 92.2)	89.5 (86.5 - 90.9)	0.533	84.7 (82.6 - 86.3)	83.1 (81.9 - 87.2)	0.986
	Chemotaxis	70.3 (67.6 - 95.0)	92.3 (61.2 - 106.5)	0.784	75.7 (54.3 - 89.9)	56.2 (45.6 - 80.4)	0.093
Serum/apoB-depleted serum	PON activity	97.4 (80.5 - 121.2)	121.7 (96.4 - 141.0)	0.111	95.5 (82.0 - 115.2)	96.7 (76.3 - 108.4)	0.516
	Lp-PLA2 activity	53.0 (44.1 - 56.8)	47.4 (38.2 - 52.9)	0.189	58.1 (49.6 - 63.9)	57.8 (51.7 - 62.8)	0.966
	LCAT activity	22.0 (19.9 - 23.4)	19.4 (17.5 - 23.3)	0.335	21.6 (20.1 - 24.7)	18.9 (12.9 - 22.7)	0.094
	CETP activity	12935 (11172 - 17435)	15149 (10631 - 18599)	0.712	11206 (8093 - 14203)	8027 (3559 - 13036)	0.301

Supplementary Table 4. Anti-oxidative capacity is expressed as % reduction of oxidation, NF-κB expression is shown as relative fluorescence units (RFU), shape change is expressed as % of vehicle, chemotaxis is expressed as % of vehicle, PON activity is expressed in mM/min/mL, Lp-PLA2 activity is expressed in mM/min/mL, LCAT activity is expressed as % substrate turnover and CETP activity is expressed in relative units. Data are presented as median with first and third quartile. Differences between the genders were analyzed with the Mann-Whitney U test. HDL, high-density lipoprotein; apoB, apolipoprotein B; AR, allergic rhinitis; NF-κB, nuclear factor-κB; PON, paraoxonase; Lp-PLA2, lipoprotein-associated phospholipase A2; LCAT, lecithin-cholesterol acyltransferase; CETP, cholesteryl ester transfer protein.



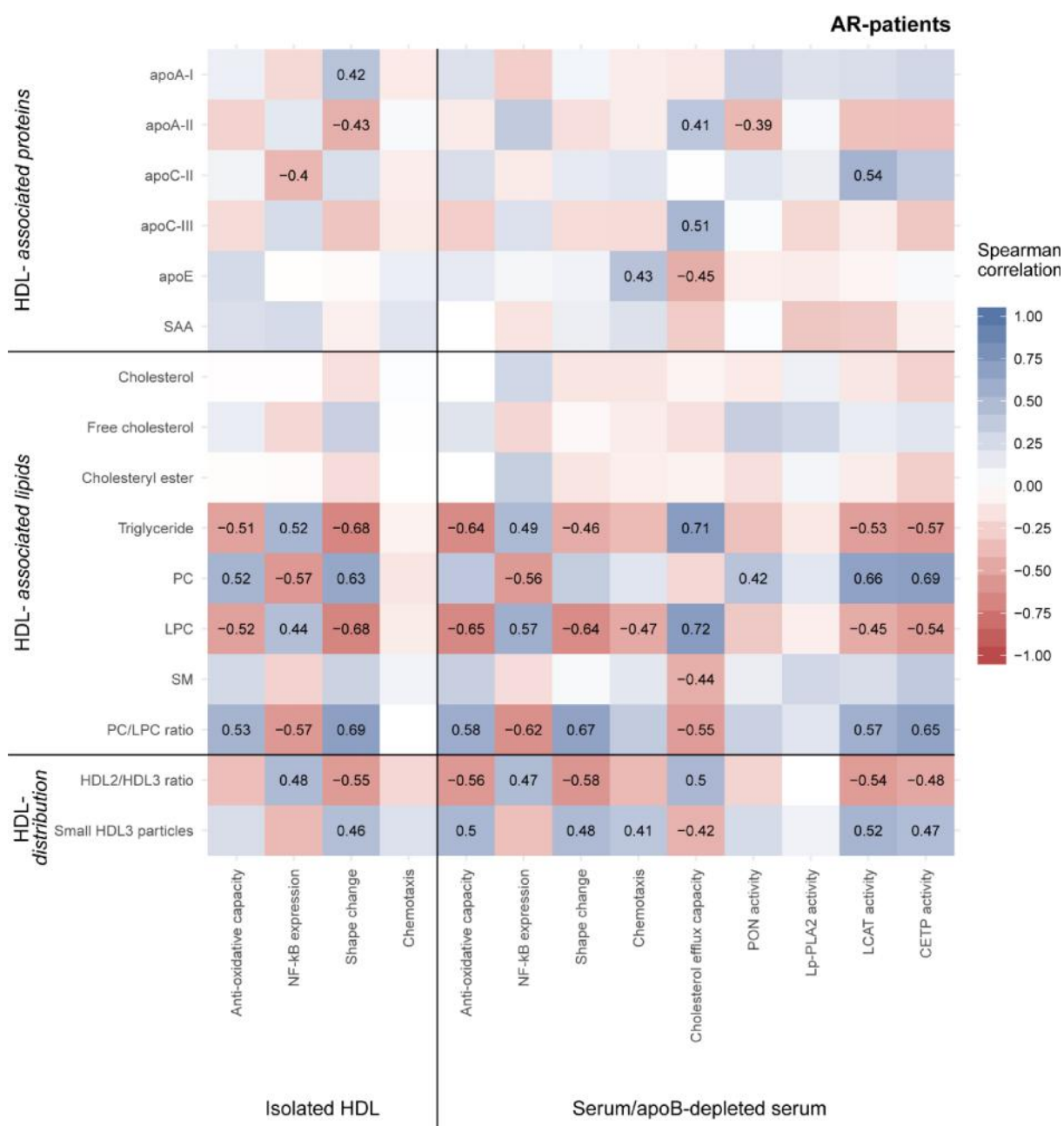
Supplementary Figure 1. HDL cholesterol efflux potential is not altered between AR-patients and controls

The ability of HDL to promote [3H]-cholesterol efflux from macrophages was evaluated. [3H]-cholesterol-labeled J774 macrophages were incubated with 2.8% apoB-depleted serum for 3 hours and the effluxed [3H]-cholesterol was quantified. Cholesterol efflux is expressed as radioactivity in the cell culture supernatant relative to total radioactivity (in the cell culture supernatant and cells) of two independent experiments measured in duplicates. Differences between the two groups were analyzed with the Mann-Whitney U test. Individual data are depicted on top of boxplots showing median with interquartile range as well a minimum and maximum values. AR, allergic rhinitis.



Supplementary Figure 2. Detailed analysis of phospholipid species of HDL of healthy controls and AR-patients

HDL-associated phospholipids from controls (n = 20) and AR-patients (n = 43) were evaluated with mass spectrometry. (A) Phosphatidylcholine, (B) Lyso-phosphatidylcholine and (C) Sphingomyelin. (A-C) Differences between the two groups were analyzed with the Mann-Whitney U test. Data are presented as mean (bars) and standard deviation (error bars). AR, allergic rhinitis; AU, analyte/IS ratio; HDL, high-density lipoprotein. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.



Supplementary Figure 3. Correlation between HDL-associated proteins, lipids and HDL-distribution and metrics of HDL function in AR-patients

Each cell of the heat map represents the pairwise Spearman correlation (rho) between the two parameters indicated in the respective row and column for AR-patients. The magnitude of the correlation is indicated by color. The significance level (α) is set to 0.01. Significant correlations ($p < 0.01$) are indicated with the corresponding Spearman rank correlation coefficient. Non-significant correlations can still be inferred from the color but are not explicitly indicated. Anti-oxidative capacity is expressed as % reduction of oxidation, NF-kB expression is shown as relative fluorescence units (RFU), shape change is expressed as % of vehicle, chemotaxis is expressed as % of vehicle, cholesterol efflux is expressed as %, PON activity is expressed in mM/min/mL, Lp-PLA2 activity is expressed in mM/min/mL, LCAT activity is expressed as % substrate turnover and CETP activity is expressed in relative units. NF-kB, nuclear factor-kB; PON, paraoxonase; Lp-PLA2, lipoprotein-associated phospholipase A2; LCAT, lecithin-cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; HDL, high-density lipoprotein; apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; apoC-II, apolipoprotein C-II; apoC-III, apolipoprotein C-III; apoE, apolipoprotein E; SAA, serum amyloid A; PC, phosphatidylcholine; LPC, lyso-phosphatidylcholine; SM, sphingomyelin; apoB, apolipoprotein B; AR, allergic rhinitis.

