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Remodeling of the HDL Proteome with Treatment Response to Abatacept or Adalimumab in the AMPLE Trial of Patients with Rheumatoid Arthritis

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Abstract

Background and Aims—To evaluate changes in the high-density lipoprotein (HDL) proteome and HDL function in active RA patients initiating therapy with abatacept or adalimumab in the Abatacept Versus Adalimumab Comparison in Biologic-Naïve RA Subjects with Background Methotrexate (AMPLE) study.

Methods—Ultra high-pressure liquid chromatography (UHPLC) coupled with ion mobility mass spectrometry (LC-IM-MS) was used to analyse proteins associated with immunoaffinity-captured HDL from plasma of 30 patients with RA randomized to either abatacept (n=15) or adalimumab (n=15) therapy. Paraoxonase 1 (PON1) activity, HDL anti-oxidant capacity, cholesterol profiles, and homocysteine levels were also measured at baseline and following treatment. Repeated-measures analyses were performed using mixed-effect linear models to model the within-subject covariance over time.

Disclosures

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Conflicts of Interest and Funding:

Bristol-Myers Squibb (BMS) provided funding for the AMPLE trial. The current work was funded through an investigator-initiated research grant from BMS to Dr. Charles-Schoeman. Dr. Charles-Schoeman also received support from the NHLBI (5K23HL094834, R01HL123064). Dr. Wang received support from NCRR (1UL1RR033176). Dr. Furst received grant and research support from AbbVie, Actelion, Amgen, Bristol-Myers Squibb, Gilead, GSK, NIH, Novartis, Pfizer, Roche/Genentech, UCB, is a consultant for AbbVie, Actelion, Amgen, Bristol-Myers Squibb, Cytori, Janssen, Gilead, GSK, NIH, Novartis, Pfizer, Roche/Genentech, and is on the speaker's bureau for AbbVie, Actelion, UCB (all CME only). Dr. Ranaganth received grant and research support from Bristol-Myers Squibb and Genentech. Dr. Maldonado is a shareholder and employee from Bristol-Myers Squibb. Dr. Reddy received support from NHLBI 5R01HL082823, 71776.

Results—In models controlling for age, sex and treatment group, improvement in inflammation measured by decreases in CRP was associated with improvement in HDL function and changes in several HDL-associated proteins including significant decreases in lipopolysaccharide-binding protein, serum amyloid A-I (SAA-I) and inter-alpha-trypsin inhibitor heavy chain H4 (p values <0.05). Improvement in disease activity was also associated with changes in multiple HDL-associated proteins. Adalimumab was associated with higher PON1 activity, HDL-associated serotransferrin, and HDL-associated immunoglobulin J chain, and lower HDL-associated SAA-I over time compared with abatacept.

Conclusions—Improvement in inflammation associated with treatment of RA, using either abatacept or adalimumab in the AMPLE study, was associated with improvement in HDL function and significant alterations in the HDL proteome, including proteins involved in the immune response, proteinase inhibition, and lipid metabolism.

Introduction

Patients with rheumatoid arthritis (RA) have a markedly increased cardiovascular (CV) risk compared to the general population, which is associated with high RA disease activity (1–3). In contrast to the general population, dyslipidemia in active RA patients has been characterized by low total and LDL cholesterol levels, which are primarily associated with an elevated inflammatory state (4). Further investigation of alternative mechanisms and CV biomarkers in RA patients with active disease is greatly needed.

High density lipoprotein (HDL) is an anti-atherogenic molecule that regulates systemic inflammation by promoting cholesterol efflux and preventing oxidation of low density lipoproteins (LDL) (5–8). The HDL particle contains multiple HDL-associated proteins, which are integral to its anti-inflammatory or pro-inflammatory functions (9). Significant work has suggested strong associations between HDL-associated proteins, HDL function, and CV risk in the general population, independent of HDL cholesterol levels (8;10;11). In the current work, we evaluated the proteome and particle function of HDL in patients with active RA entering a substudy of the Abatacept versus Adalimumab Comparison in Biologic-Naïve RA Subjects with Background Methotrexate (AMPLE) trial (12).

Patients and Methods

Study Design

The AMPLE trial (NCT00929864) was a two-year phase IIIb, randomized clinical trial with a 1-year primary endpoint of biologic naive patients with active RA and an inadequate response to methotrexate (MTX) who were randomized to 125 mg subcutaneous (SC) abatacept weekly or 40 mg adalimumab bi-weekly, both on background MTX. The AMPLE trial design and patient eligibility criteria have been previously described (12). Patients met the 1987 American Rheumatism Association (ARA) criteria for RA, had active disease for ≤5 years despite MTX therapy and were naive to biologic therapy. Of the 646 patients who were randomized and treated in the study, 86.2% of patients receiving SC abatacept and 82% receiving SC adalimumab completed 12 months of treatment. Full results of the clinical trial have been previously published (12).

The current work was a pre-defined cardiovascular substudy of the AMPLE trial which included 30 patients from sites located in Southern California. An amendment to the main AMPLE trial was made to add this pilot substudy. Twelve patients with RA were randomized in the main study and eighteen additional patients were randomized in an extension study, utilizing the same eligibility criteria as the main study protocol and following the same procedures in the first 365 days or until early termination with minor exceptions. Patients provided consent for participation in the AMPLE trial and separately, for the cardiovascular substudy.

Laboratory Testing

Fasting plasma and serum samples from a total of 30 patients participating in the AMPLE CV substudy study were available for analysis. Samples were collected over the study period at day 0, 85, 365, and 729 (main study patients only) and stored at −80°Celsius until analysis. Samples from all time points for individual patients were run together for the HDL proteomics and each of the HDL function assays. Traditional cholesterol, C-reactive protein (CRP) and homocysteine levels were assessed by standard central laboratory protocols. 28 tender and swollen joint counts, and patient/physician global assessments were assessed locally at each site and the (DAS28[CRP]) calculated.

Proteomics analysis—Proteomics studies were performed as described previously with modifications (13). In brief, HDL was isolated by anti-HDL IgY spin columns from plasma according to manufacturer's protocol (GenWay Biotech, San Diego, CA). The protein concentration of HDL captured by the IgY column was determined by Nanodrop spectrophotometer (Thermo, Wilmington, DE). Ion mobility-supported liquid chromatography-mass spectrometry (LC-IM-MS) was used to analyze proteins associated with immunoaffinity-captured HDL. The same amount of protein from each sample was reduced, alkylated and digested with trypsin, and then each sample digest was spiked with a Yeast dehydrogenenase digest (YADH-1) at 250 fmol/inj, separated by a Waters NanoAquity UHPLC system and subsequently analyzed, by a Waters Synapt G2 high definition mass spectrometer fitted with an Ionkey nanoelectrospray source.

The peptides were loaded onto a 180 μm × 20 mm, 5 μm Symmetry C_{18} silica Waters trapping column at a flow rate of 6 μL/min 99% buffer A (0.1% formic acid in water) and 1% buffer B (0.1% formic acid in acetonitrile), then separated on a Waters C18 HSS T3 ion Key containing a 85 μm × 100 mm, 1.8 μm particle size analytical column maintained at 45 °C at a flow rate of 450 nL/min, using the following gradient: from 3% buffer B to 35% buffer B in 30 min, then at 34 min 50% B, from 35 to 38 min 90% B. Peptide ions were separated by ion mobility and subsequently measured in low/high collision energy alternating scans (MS^e) in the range 50 – 2000 m/z and a collision energy of 4 eV and 15 – 40 eV respectively, with a scan time of 0.75s in resolution mode. The [Glu_1]-Fibrinopeptide B, +2 ion was used as a lock mass and was measured every 60s.

Tandem mass spectra were extracted by Waters Protein Links Global Server (PLGS) v 2.5.2. All MS/MS samples were analyzed using IdentityE (Waters Corporation, Milford, MA; version iaDBs:2.135.2.0). IdentityE was set up to search the SwissProt human database

assuming the digestion enzyme trypsin. IdentityE was searched with a fragment ion mass tolerance of 0.025 Da and a parent ion tolerance of 0.0100 Da. Carbamidomethyl of cysteine was specified in IdentityE as a fixed modification. Amidation+C-TERM of, oxidation of methionine and phosphoryl STY of serine, threonine and tyrosine were specified in IdentityE as variable modifications. Scaffold (version Scaffold_4.2.5, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 99.0% probability to achieve an FDR less than 1.0% by the Scaffold Local FDR algorithm. Protein identifications were accepted if they could be established at greater than 99.0% probability to achieve an FDR less than 1.0% and contained at least 4 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (14, 15). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

Determination of Paraoxonase 1 (PON1) Activity—PON1 activity was quantified as previously (14) using paraoxon as the substrate and measuring the increase in the absorbance at 405 nm due to the formation of 4-nitrophenol over a period of 12 minutes (at 20 second intervals). Paraoxon was purchased from Sigma (St. Louis, MO) and further purified using chloroform extraction. A unit of PON1 activity was defined as the formation of 1 nmol of 4-ntirophenol per minute per milliliter of sample used.

Evaluation of HDL's Anti-Oxidant Function—The cell free assay (CFA) was a modification of a previously published method (15) using LDL as the fluorescence-inducing agent. Values for intra- and interassay variability were 0.5 ± 0.37% and 3.0 ± 1.7%, respectively (16). [Further assay description deleted]

HDL-Associated Haptoglobin (Hp) and Apolipoprotein A-I (ApoA-I)—HDL-associated Hp (HDL-Hp) and apoA-I (HDL-ApoA-I) assays were performed as described previously (17). [Further assay description deleted]

Myeloperoxidase(MPO) activity—The activity of MPO was measured using the InnoZyme™ MPO activity assay kit (EMD Chemicals, Darmstadt, Germany). In brief, patient plasma was added to a 96 well plate with an immobilized polyclonal antibody specific for human MPO. Activity of captured MPO was measured using a detection reagent that includes TMB and hydrogen peroxide. Following color development, the reaction was stopped with sulfuric acid and the absorbance of the oxidized TMB detected at 450 nm.

Statistical analysis

Clinical characteristics and biomarkers were compared between treatment groups and time points using paired t-tests for continuous variables and Chi-Square test for categorical variables. To determine the relative contribution of RA treatments, RA-associated inflammation/disease activity, and other patient characteristics to changes in HDL function and HDL-associated proteins over time, repeated measures analysis with linear mixed effect models (18) was used to model the within-subject covariance over time. Measures of inflammation/disease activity and HDL function/HDL-associated proteins at up to four

points were included in the models as fixed effects. Separate models were constructed for each HDL outcome and inflammation/disease activity measures. Other fixed-effect patient covariates included treatment assignment, age, and sex. Log transformation was performed on all outcome measurements. All statistical testing was two-sided with 0.05 alpha level threshold for declaring significance. Statistical analyses were carried out using SAS version 9.3 (SAS Institute Inc. 2012).

RESULTS

Demographic, Laboratory, and Clinical Characteristics

The baseline clinical characteristics of the AMPLE patients participating in the bio-repository study with samples available for analyses are shown in Table 1. The population studied was similar in demographics to the main AMPLE trial population (12). No significant differences between treatment arms were observed in important demographic and clinical variables including age, sex, race, and body mass index (BMI). Disease duration was less than two years in both groups and patients had active disease at baseline with the mean DAS28 scores greater than or equal to 5 in each group. The baseline CV risk factors including smoking, hypertension, diabetes, and prednisone/NSAID use were similar between the groups. No differences in baseline HDL function, traditional cholesterol levels, or homocysteine levels were observed. One patient in the abatacept group had a history of CV disease which was a previous stroke.

Proteomics Analysis

Using immunoaffinity-purified HDL isolated from patient plasma, 77 different proteins were identified in association with HDL particles. These proteins included proteins involved in complement regulation and the immune response, lipid metabolism, proteinase inhibition/clotting, and the acute phase response (Table 2).

Changes in the HDL proteome with improvement in inflammation and disease activity—Response to treatment measured by decreases in CRP or disease activity was significantly associated with changes in several HDL-associated proteins. Specifically, decreases in CRP by 10 units were associated with a 22.6% decrease in lipopolysaccharide (LPS)-binding protein, 41.6% decrease in serum amyloid A-I (SAA-I) and a 16.8% decrease in inter-alpha-trypsin inhibitor heavy chain H4 (p values <0.05) (Table 3). Trends for decreases in several other HDL-associated proteins were also noted in association with improvement in CRP including complement C4, α1-anti-chymotrypsin, fibrinogen, galectin 3 binding protein, carboxypeptidase N Subunit 2, and fibulin. In contrast, a trend for increases in apolipoprotein C-IV was noted with decrease in CRP with treatment (p values=0.09–0.14; Table 3).

Decreases in disease activity measured by DAS28, swollen and tender joint counts, and physical function (HAQ-DI) were also associated with changes in the HDL proteome (Table 4A). Similar to the CRP analysis, decreases in DAS28 and HAQ-DI were both associated with decreases in HDL-associated SAA-I. Decreases in disease activity and disability measured by DAS28, tender joint counts, and/or HAQ-DI were also associated with modest

decreases in HDL-associated alpha1-antichymotrypsin and fibrinogen, similar to the CRP analysis (p values 0.07–0.14) (Table 4A). A trend was noted for increases in HDL-associated apoAI with decrease in DAS28, but was not statistically significant (Table 4A).

Changes in several additional HDL-associated proteins occurred with response to treatment measured by improvement in disease activity measures. Decreases in the 68 tender joint count were associated with significant decreases in HDL-associated apolipoprotein M (apoM) (p=0.03) and similar trends were noted with apoM and several other disease activity assessments including DAS28 and the 28 tender and swollen joint counts (Table 4A). Levels of HDL-associated fibronectin, kininogen, and prenylcysteine oxidase were also significantly decreased with improvement in disease activity measured by tender and swollen joint counts and/or DAS28 (p values= 0.007–0.048). Improvement in disease activity was also associated with modest changes in other HDL-associated proteins including decreases in cathelicidin antimicrobial peptide and clusterin, as well as increases in transthyretin and vitronectin (p= 0.06–0.11) (Table 4A).

Changes in HDL-associated proteins with abatacept versus adalimumab therapy—Adalimumab was associated with greater decreases in HDL-associated SAA-I over time compared with abatacept in multiple models including different measures of disease activity (p values 0.02–0.03) as well as CRP (p=0.06) (Table 4B). In contrast, adalimumab was associated with higher levels of HDL-associated immunoglobulin J (IgJ) and serotransferrin over time compared to abatacept (p values = 0.008–0.03) (Table 4B). A modest association between adalimumab and greater HDL-associated CD5 antigen-like protein compared to abatacept was also noted in multiple models (p values 0.049–0.06) (Table 4B).

Changes in Cholesterol and HDL Function with Treatment

Improvement in inflammation measured by decreases in CRP over time was associated with significant increases in cholesterol and improvement in HDL function. Specifically, for a 10 unit decrease in CRP over 1 year follow-up, total cholesterol increased by 3.72% (p=0.01) irrespective of treatment (Table 5A). Use of adalimumab versus abatacept was not associated with differences in TC, LDL-C, HDL-C, Tg, or homocysteine levels over one year of follow-up. Prednisone dose remained stable during the study period for all except for three patients. Female sex was associated with lower homocysteine levels (p=0.01), and older age with associated with higher homocysteine levels (p=0.001) (Table 5A).

Improvement in inflammation during the treatment period was associated with improvement in the HDL-function profile. Decreases in CRP were associated with significant improvement in the overall anti-oxidant capacity of HDL as shown by a decrease in HII, regardless of therapy (p=0.01). Specifically, a 10 unit decrease in CRP over one year was associated with a 20% decrease in HII. Treatment assignment was not associated with changes in the HII over follow-up (Table 5B). Improvement in inflammation was also associated with significant decreases in HDL-Hp and increases in PON1 activity over follow-up (p values <0.05). For every 10 unit decrease in CRP there was a 4.43% decrease in HDL-Hp and a 6.56% increase in PON1 activity. Adalimumab was associated with greater

decreases in HDL-Hp and greater increases in PON1 activity over time compared to abatacept (p values <0.05) (Table 5B).

Safety Outcomes

SC abatacept and adalimumab were well tolerated with no new safety concerns observed during the substudy. All patients had adverse events (AEs); the most common AEs were headache and nasopharyngitis in the abatacept group (5 patients [33%] each) and upper respiratory tract infections in the adalimumab group (4 patients [27%]). Two SAEs (appendicitis and benign meningioma, one patient each) and 1 discontinuation of study drug due to a treatment-related AE (convulsions) were reported, all in the adalimumab group.

Discussion

The current work is the first study to characterize changes in the HDL proteome in active RA patients following treatment with two different immunomodulatory therapies in a randomized, controlled clinical trial. Proteins cover over 75% of the HDL particle surface and play integral roles not only in lipid metabolism, but may also participate directly in the immune response and other physiologic functions.

Over 70 HDL-associated proteins were identified in the current work and changes in multiple proteins were identified in response to treatment with either abatacept or adalimumab. Specifically, decreases in inflammation measured by CRP over the treatment period were associated with significant decreases in LPS-binding protein, SAA-I, and inter-alpha-trypsin inhibitor heavy chain H4. LPS-binding protein normally binds to bacterial LPS in order to elicit immune responses by presenting LPS to different cell surface pattern recognition receptors such as CD14 and TLR4 (19). Decreases in HDL-associated LPS-binding protein with treatment response may suggest a decrease in the pro-inflammatory properties of the HDL particle. HDL-associated SAA-I has previously been described as a marker of dysfunctional, pro-inflammatory HDL in both human and animal studies (20;21), and was significantly decreased with response to treatment in the current work. Inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4) is a 120 kDa plasma glycoprotein that acts as an acute phase protein and a liver-restricted serine protease inhibitor (22). We previously identified significantly higher levels of ITIH4 in dysfunctional, pro-inflammatory HDL from active RA patients (23). In the current work, effective control of inflammation with either abatacept or adalimumab was associated with significant decreases ITIH4 levels in HDL.

Trends for decreases in several other HDL-associated proteins unrelated to lipid metabolism including complement C4A, alpha1-antichymotrypsin, fibrinogen, galectin 3 binding protein, carboxypeptidase N subunit 2, and fibulin I were also noted in association with response to treatment with either abatacept or adalimumab. Similar to ITIH4, alpha1-antichymotrypsin is a protease inhibitor, and carboxypeptidase N also has a role in protecting proteins from degradation. Fibrinogen is as an independent CV risk factor in the general population (24) and fibulin I is a glycoprotein, which can bind to fibrinogen and thereby incorporate into thromboses. Galectin 3 binding protein has been linked to thromboses as well as mortality in patients with coronary artery disease (25;26). No work to date has previously studied these proteins in HDL of RA patients including the changes

following treatment. This information may be important not only to the development of a more clinically feasible, less cumbersome, protein-based assay of HDL function, but also to our understanding of the precise role HDL plays in both in the immune response of atherosclerosis as well joint inflammation in RA patients.

Significant differences in three HDL-associated proteins were noted between adalimumab and abatacept therapy. While abatacept works in treatment of RA through modulation of T-cell co-stimulation, adalimumab is a monoclonal antibody, which directly binds the inflammatory cytokine, TNF-alpha. Adalimumab was associated with lower HDL-associated SAA-I compared with abatacept and higher HDL-associated IgJ chain and serotransferrin over the study period after controlling for disease activity in multivariate models. SAA has been previously associated with dysfunctional HDL through displacement of the central HDL-associated protein, apolipoprotein AI (27), however, no difference in the overall anti-oxidant function of HDL was observed between the treatment groups in this work. Interestingly, inflammatory cytokines such as TNF-alpha have been directly associated with regulation of the SAA genes (28), perhaps suggesting a mechanism whereby abatacept and adalimumab may differ in their effects on the HDL proteome. IgJ chain is a polypeptide expressed by mucosal and glandular plasma cells, which regulates formation of IgA and IgM (29). Serotransferrin is an iron-binding transport protein implicated in the innate immune response by binding free iron in the mucosa to impede bacterial survival (30). Further investigation is warranted to confirm these protein changes in larger patient cohorts and determine the functional significance to the HDL particle in the immune response.

Cardiovascular disease (CVD) is the leading killer of patients with RA who have a 2–3 fold increased risk of myocardial infarction compared to members of the general population (1–3). Growing evidence suggests that effective RA therapy decreases CV risk in RA patients despite increases in traditional cholesterol levels, which may not accurately reflect CV risk in this population (31;32). No work to date has previously evaluated the effects of abatacept on standard lipoprotein cholesterol levels or lipoprotein function/proteomics in active RA patients in a randomized controlled clinical trial. Mathieu et al. previously studied 21 RA patients treated for 6 months with abatacept in an open label study, showing that abatacept was associated with a significant increase in HDL-C levels and a non-significant increase in TC and LDL-C levels. However, associations of cholesterol changes with measures of disease activity or inflammation were not reported (33). In the current study, improvement in inflammation with abatacept or adalimumab therapy over one year of follow-up was associated with significant increases in total cholesterol levels, which were not different between treatment groups. Improvement in inflammation was also associated with significant improvement in the anti-oxidant capacity of HDL, including increases in PON1 activity and a “remodelling” of the HDL proteome.

There are some limitations to the current work. A correction for multiple outcome analyses such as bonferroni was not employed given the exploratory nature of the study. The overall substudy cohort size was small and the findings warrant confirmation in larger cohorts. A robust repeated measures analysis evaluated multiple time points for each study patient over one year of follow-up and first validated expected relationships with traditional CV risk factors. In particular, decreases in inflammation over time were associated with increases in

cholesterol, which is an association previously reported in larger studies with different RA therapeutics (34). In addition, higher homocysteine levels were associated with older age and male sex, which are known associations in the general population (35). Finally, HDL is very heterogeneous in particle composition, both in size and density, and future work is needed to evaluate specific changes in protein cargo that are associated with specific HDL particles in RA patients. Additional studies may also include further purification techniques following IgY column isolation of HDL-associated proteins to assure that only HDL-associated proteins are measured. HDL isolation methodologies are of frequent debate by experts in the field, and some contamination including abundant serum proteins such as albumin and apoB-100 has been recognized as in the current work.

In conclusion, the current work is the first to describe changes in the HDL proteome after immunomodulatory therapy with two different treatments in a randomized, controlled clinical trial. The findings demonstrate that improvement in inflammation and disease activity in RA patients is associated with multiple, potentially favourable protein changes in the HDL particle. Differences in three HDL-associated proteins over time were noted between abatacept and adalimumab therapies after controlling for inflammation/disease activity, and the significance of these differences warrants further investigation. Clinical response to either therapy was associated with improvement in HDL function, despite increases in cholesterol levels. In the general population, work has strongly supported the assessment HDL particle function and its associated proteins as alternative CV risk assessments as well as targets for intervention (8–10). Further investigation of the findings reported in the current work may be warranted to define a potential HDL protein “profile” as a surrogate CV risk assessment in patients with RA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Reference List

1. Agca R, Heslinga SC, van Halm VP, Nurmohamed MT. Atherosclerotic cardiovascular disease in patients with chronic inflammatory joint disorders. Heart. 2016; 102(10):790–5. [PubMed: 26888573]
2. Maradit-Kremers H, Nicola PJ, Crowson CS, Ballman KV, Gabriel SE. Cardiovascular death in rheumatoid arthritis: a population-based study. Arthritis Rheum. 2005; 52(3):722–32. [PubMed: 15751097]
3. Nurmohamed MT, Heslinga M, Kitas GD. Cardiovascular comorbidity in rheumatic diseases. Nat Rev Rheumatol. 2015; 11(12):693–704. [PubMed: 26282082]
4. Robertson J, Peters MJ, McInnes IB, Sattar N. Changes in lipid levels with inflammation and therapy in RA: a maturing paradigm. Nat Rev Rheumatol. 2013; 9(9):513–23. [PubMed: 23774906]
5. Navab M, Hama SY, Anantharamaiah GM, Hassan K, Hough GP, Watson AD, et al. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: steps 2 and 3. J Lipid Res. 2000; 41(9):1495–508. [PubMed: 10974057]
6. Navab M, Hama SY, Cooke CJ, Anantharamaiah GM, Chaddha M, Jin L, et al. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: step 1. J Lipid Res. 2000; 41(9):1481–94. [PubMed: 10974056]
7. Mackness MI, Arrol S, Durrington PN. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. FEBS Lett. 1991; 286(1–2):152–4. [PubMed: 1650712]

8. Khera AV, Cuchel M, de lL-M, Rodrigues A, Burke MF, Jafri K, et al. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med. 2011; 364(2):127–35. [PubMed: 21226578]
9. Heinecke JW. HDL's protein cargo: friend or foe in cardioprotection? Circulation. 2013; 127(8): 868–9. [PubMed: 23439444]
10. Bhattacharyya T, Nicholls SJ, Topol EJ, Zhang R, Yang X, Schmitt D, et al. Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. JAMA. 2008; 299(11):1265–76. [PubMed: 18349088]
11. Annema W, Willemsen HM, de Boer JF, Dikkers A, van der Giet M, Nieuwland W, et al. HDL function is impaired in acute myocardial infarction independent of plasma HDL cholesterol levels. J Clin Lipidol. 2016; 10(6):1318–28. [PubMed: 27919348]
12. Schiff M, Weinblatt ME, Valente R, van der Heijde D, Citera G, Elegbe A, et al. Head-to-head comparison of subcutaneous abatacept versus adalimumab for rheumatoid arthritis: two-year efficacy and safety findings from AMPLE trial. Ann Rheum Dis. 2014; 73(1):86–94. [PubMed: 23962455]
13. Watanabe J, Charles-Schoeman C, Miao Y, Elashoff D, Lee YY, Katselis G, et al. Proteomic profiling following immunoaffinity capture of high-density lipoprotein: association of acute-phase proteins and complement factors with proinflammatory high-density lipoprotein in rheumatoid arthritis. Arthritis Rheum. 2012; 64(6):1828–37. [PubMed: 22231638]
14. Charles-Schoeman C, Lee YY, Shahbazian A, Gorn AH, Fitzgerald J, Ranganath VK, et al. Association of paraoxonase 1 gene polymorphism and enzyme activity with carotid plaque in rheumatoid arthritis. Arthritis Rheum. 2013; 65(11):2765–72. [PubMed: 23917967]
15. Navab M, Hama SY, Hough GP, Subbanagounder G, Reddy ST, Fogelman AM. A cell-free assay for detecting HDL that is dysfunctional in preventing the formation of or inactivating oxidized phospholipids. J Lipid Res. 2001; 42(8):1308–17. [PubMed: 11483633]
16. Charles-Schoeman C, Khanna D, Furst DE, McMahon M, Reddy ST, Fogelman AM, et al. Effects of High-dose Atorvastatin on Antiinflammatory Properties of High Density Lipoprotein in Patients with Rheumatoid Arthritis: A Pilot Study. J Rheumatol. 2007; 34(7):1459–64. [PubMed: 17552046]
17. Charles-Schoeman C, Yin LY, Shahbazian A, Wang X, Elashoff D, Curtis JR, et al. Improvement of High-Density Lipoprotein Function in Patients With Early Rheumatoid Arthritis Treated With Methotrexate Monotherapy or Combination Therapies in a Randomized Controlled Trial. Arthritis Rheumatol. 2017; 69(1):46–57. [PubMed: 27483410]
18. Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics. 1982; 38(4):963–74. [PubMed: 7168798]
19. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science. 1990; 249(4975):1431–3. [PubMed: 1698311]
20. Van Lenten BJ, Wagner AC, Nayak DP, Hama S, Navab M, Fogelman AM. High-density lipoprotein loses its anti-inflammatory properties during acute influenza a infection. Circulation. 2001; 103(18):2283–8. [PubMed: 11342478]
21. Han CY, Tang C, Guevara ME, Wei H, Wietecha T, Shao B, et al. Serum amyloid A impairs the antiinflammatory properties of HDL. J Clin Invest. 2016; 126(2):796. [PubMed: 26829628]
22. Noh CK, Kim SS, Kim DK, Lee HY, Cho HJ, Yoon SY, et al. Inter-alpha-trypsin inhibitor heavy chain H4 as a diagnostic and prognostic indicator in patients with hepatitis B virus-associated hepatocellular carcinoma. Clin Biochem. 2014; 47(13–14):1257–61. [PubMed: 24836184]
23. Watanabe J, Charles-Schoeman C, Miao Y, Elashoff D, Lee YY, Katselis G, et al. Proteomic profiling following immunoaffinity capture of HDL: Association of acute phase proteins and complement factors with pro-inflammatory HDL in rheumatoid arthritis. Arthritis Rheum. 2012
24. Stec JJ, Silbershatz H, Tofler GH, Matheney TH, Sutherland P, Lipinska I, et al. Association of fibrinogen with cardiovascular risk factors and cardiovascular disease in the Framingham Offspring Population. Circulation. 2000; 102(14):1634–8. [PubMed: 11015340]

25. Gleissner CA, Erbel C, Linden F, Domschke G, Akhavanpoor M, Doesch AO, et al. Galectin-3 binding protein plasma levels are associated with long-term mortality in coronary artery disease independent of plaque morphology. Atherosclerosis. 2016; 251:94–100. [PubMed: 27295060]
26. DeRoo EP, Wrobleski SK, Shea EM, Al-Khalil RK, Hawley AE, Henke PK, et al. The role of galectin-3 and galectin-3-binding protein in venous thrombosis. Blood. 2015; 125(11):1813–21. [PubMed: 25428218]
27. Van Lenten BJ, Hama SY, de Beer FC, Stafforini DM, McIntyre TM, Prescott SM, et al. Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocultures. J Clin Invest. 1995; 96(6): 2758–67. [PubMed: 8675645]
28. Thorn CF, Lu ZY, Whitehead AS. Regulation of the human acute phase serum amyloid A genes by tumour necrosis factor-alpha, interleukin-6 and glucocorticoids in hepatic and epithelial cell lines. Scand J Immunol. 2004; 59(2):152–8. [PubMed: 14871291]
29. Johansen FE, Braathen R, Brandtzaeg P. Role of J chain in secretory immunoglobulin formation. Scand J Immunol. 2000; 52(3):240–8. [PubMed: 10972899]
30. Ritchie RF, Palomaki GE, Neveux LM, Navolotskaia O. Reference distributions for the negative acute-phase proteins, albumin, transferrin, and transthyretin: a comparison of a large cohort to the world's literature. J Clin Lab Anal. 1999; 13(6):280–6. [PubMed: 10633295]
31. Choi HK, Hernan MA, Seeger JD, Robins JM, Wolfe F. Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study. Lancet. 2002; 359(9313):1173–7. [PubMed: 11955534]
32. Roubille C, Richer V, Starnino T, McCourt C, McFarlane A, Fleming P, et al. The effects of tumour necrosis factor inhibitors, methotrexate, non-steroidal anti-inflammatory drugs and corticosteroids on cardiovascular events in rheumatoid arthritis, psoriasis and psoriatic arthritis: a systematic review and meta-analysis. Ann Rheum Dis. 2015; 74(3):480–9. [PubMed: 25561362]
33. Mathieu S, Couderc M, Glace B, Pereira B, Tournadre A, Dubost JJ, et al. Effects of 6 months of abatacept treatment on aortic stiffness in patients with rheumatoid arthritis. Biologics. 2013; 7:259–64. [PubMed: 24324327]
34. Charles-Schoeman C, Wang X, Lee YY, Shahbazian A, Navarro-Millan I, Yang S, et al. Association of Triple Therapy with Improvement in Cholesterol Profiles over Two Year Follow-up in the TEAR Trial. Arthritis Rheumatol. 2015
35. Okura T, Miyoshi K, Irita J, Enomoto D, Nagao T, Kukida M, et al. Hyperhomocysteinemia is one of the risk factors associated with cerebrovascular stiffness in hypertensive patients, especially elderly males. Sci Rep. 2014; 4:5663. [PubMed: 25012721]

Highlights

- Patients with rheumatoid arthritis (RA) have high cardiovascular (CV) risk.
- Increased CV risk in RA patients is associated with high RA disease activity.
- The HDL proteome is abnormal in active RA patients.
- Treatment of RA was associated with improvement in HDL function.
- Treatment of RA was associated with favorable changes in the HDL proteome.

Table 1

Baseline patient demographics

	SC abatacept (n=15)	SC adalimumab (n=15)
Age, years	42.5 (15.4)	47.7 (10.5)
Female, %	12 (80.0)	11 (73.3)
White, %	13 (86.7)	12 (80.0)
BMI, kg/m^2	27.3 (4.5)	29.5 (4.5)
Disease duration, years	1.4 (1.1)	1.8 (1.4)
TJC	20.3 (12.5)	29.3 (16.9)
SJC	13.7 (11.2)	14.7 (14.4)
PGA	52.1 (29.9)	57.8 (16.3)
CRP, mg/dl	1.7 (2.4)	0.7 (0.8)
DAS28 (CRP)	5.0 (1.0)	5.2 (0.8)
History of CV disease, %	1 (6.7)	0
History of hypertension, %	4 (26.7)	6 (40.0)
Smoking history, %	0	4 (26.7)
Diabetes, %	2 (13.3)	1 (6.7)
Statin/other cholesterol-lowering therapy, %	2 (13.3)	1 (6.7)
Oral steroid use, %	7 (46.7)	7 (46.7)
Smoking status, %	0	2 (13.3)
NSAIDs use, %	9 (60.0)	10 (66.7)
Aspirin use, %	2 (13.3)	3 (20.0)

	SC abatacept (n=15)	SC adalimumab (n=15)
Folic acid use, %	13 (86.7)	13 (86.7)
HDL anti-oxidant function		
Proinflammatory HDL, %	5 (33.3)	5 (33.3)
Anti-inflammatory HDL, %	10 (66.7)	10 (66.7)
HDL inflammatory index (HII)	1.1 (1.5)	0.8 (0.6)
Total cholesterol, mg/dL	172.8 (45.7)	195.3 (37.4)
HDL cholesterol (HDL-C), mg/dL	49.7 (15.0)	50.0 (13.6)
LDL cholesterol (calculated), mg/dL	93.8 (30.0)	117.5 (34.7)
Triglycerides, mg/dL	146.9 (84.5)	139.5 (67.0)
Homocysteine, μmol/L	8.72 (2.97)	10.17 (3.91)

Values are mean (SD) unless otherwise stated.

BMI= body mass index; TJC= tender joint count; SJC= swollen joint count; PGA=physician global assessment; CRP= C-reactive protein; DAS28= disease activity scale using a 28 joint count; CV=cardiovascular; HDL=high density lipoprotein; LDL=low density lipoprotein.

Table 2

Proteins Associated with High Density Lipoproteins Isolated by Immuno-affinity Capture from Plasma of Patients with Rheumatoid Arthritis in the AMPLE trial

Alpha-1-acid glycoprotein 2
Alpha-1-antichymotrypsin
Alpha-1-antitrypsin
Alpha-2-antiplasmin OS
Alpha-2-HS-glycoprotein
Alpha-2-macroglobulin
Angiotensinogen
Antithrombin-III
Apolipoprotein A-I
Apolipoprotein A-II
Apolipoprotein A-IV
Apolipoprotein B-100
Apolipoprotein C-I
Apolipoprotein C-II
Apolipoprotein C-III
Apolipoprotein C-IV
Apolipoprotein D
Apolipoprotein E
Apolipoprotein F
Apolipoprotein L1
Apolipoprotein M
Beta-2-glycoprotein 1
Beta-Ala-His dipeptidase
C4b-binding protein alpha chain
Carboxypeptidase N subunit 2
Cathelicidin antimicrobial peptide
CD5 antigen-like
Clusterin
Complement C3
Complement C4-A
Complement C5
Cytoplasmic dynein 1 heavy chain 1
Fibrinogen alpha chain
Fibrinogen beta chain
Fibrinogen gamma chain
Fibronectin
Fibulin-1
Galectin-3-binding protein
Haptoglobin

Haptoglobin-related protein
Hemopexin
Heparin cofactor 2
Ig alpha-1 chain C region
Ig alpha-2 chain C region
Ig gamma-1 chain C region
Ig gamma-2 chain C region
Ig gamma-3 chain C region
Ig gamma-4 chain C region
Ig kappa chain C region
Ig kappa chain V-III region SIE
Ig kappa chain V-IV region Len
Ig lambda-2 chain C regions
Ig mu chain C region
Ig mu heavy chain disease protein
Immunoglobulin J chain
Immunoglobulin lambda-like polypeptide 5
Insulin-like growth factor-binding protein complex acid labile subunit
Inter-alpha-trypsin inhibitor heavy chain H1
Inter-alpha-trypsin inhibitor heavy chain H2
Inter-alpha-trypsin inhibitor heavy chain H4
Kininogen-1
Lipopolysaccharide-binding protein
Phospholipid transfer protein
Plasminogen
Pregnancy zone protein
Prenylcysteine oxidase 1
Protein AMBP
Prothrombin
Serotransferrin
Serum albumin
Serum amyloid A-1
Serum amyloid A-2 protein
Serum amyloid A-4 protein
Serum paraoxonase/arylesterase 1
Transthyretin
Vitamin D-binding protein
Vitronectin

Table 3

Changes in HDL-associated proteins over time by covariate in multivariate repeated measures analyses-CRP model

	Lipopolysaccharide-binding protein		Serum amyloid A-I		Inter-alpha-trypsin inhibitor heavy chain H4		Complement C4A		α1-antichymotrypsin		Fibrinogen gamma chain		Galectin 3 binding protein		Carboxypeptidase N subunit 2		Apolipoprotein C-IV		Fibulin I		ApoAI	
	Est	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***	**Est**	***p***
CRP (per 10 unit decrease)	−22.59%	**0.004**	46.92%	**0.004**	16.99%	**0.04**	16.08%	0.09	−21.52%	0.09	−8.22%	0.10	20.43%	0.14	−12.79%	0.12	7.66%	0.14	6.96%	0.14	2.82%	0.30
Female *vs.* male (0)	38.69%	0.33	37.60%	0.45	1.48%	0.96	13.85%	0.69	−16.47%	0.65	21.35%	0.45	37.04%	0.41	60.63%	0.11	6.94%	0.78	0.01%	1.00	18.72%	0.33
Age	−0.71%	0.54	−1.71%	0.40	0.20%	0.84	−0.64%	0.54	0.26%	0.84	0.00%	1.00	−0.85%	0.66	−24.02%	0.48	0.59%	0.49	0.64%	0.33	−1.32%	0.07
Adalimimumab *vs.* abatacept (0)	−8.94%	0.73	59.38%	0.06	12.91%	0.54	15.78%	0.48	−2.18%	0.94	19.87%	0.34	5.51%	0.90	−1.33%	0.30	−17.40%	0.32	1.05%	0.94	13.51%	0.42

CRP= C-reactive protein, Est= Estimate.

Table 4

Changes in HDL-associated proteins over time by (A) disease activity and (B) treatment in multivariate repeated measures analyses.

A			
Protein	**Disease activity measure model**	**Disease activity estimate**	***p*-value**
Apolipoprotein AI	DAS28	−0.047	0.19
Apolipoprotein M	DAS28	0.049	0.058
	HAQ-DI	0.07	0.11
	68 Tender	0.0038	0.058
	28 Tender	0.0093	0.03
	28 Swollen	0.0076	0.178
Apolipoprotein E	HAQ-DI	0.088	0.09
Alpha1-antichymotrypsin	DAS28	0.34	0.14
	28 Tender	0.037	0.13
	HAQ-DI	0.21	0.12
Beta 2 glycoprotein 1	66 swollen	0.02	0.08
Cathelicidin antimicrobial peptide	DAS28	0.19	0.065
Clusterin	28 Tender	0.01	0.10
	28 Swollen	0.01	0.08
Fibronectin	28 Tender	0.07	0.018
	68 Tender	0.034	0.009
Fibrinogen alpha chain	28 Tender	0.02	0.068
	68 Tender	0.009	0.11
Fibulin	HAQ-DI	0.14	0.12
Ig gamma-1 chain C region	DAS28	−0.0679	0.02
Ig gamma-2 chain C region	DAS28	0.136	0.08
	68 Tender	0.0095	0.11
Ig gamma-4 chain C region	DAS28	0.22	0.11
	28 Swollen	0.06	0.045
	28 Tender	0.029	0.19
	68 Tender	0.016	0.13

A

Protein	Disease activity measure model	Disease activity estimate	*p*-value
Immunoglobulin J Chain	DAS28	0.085	0.16
Kininogen	68 Tender	0.0157	0.043
	66 Swollen	0.02	0.0481
Prenylcysteine oxidase 1	DAS28	0.24	0.03
	28 Tender	0.05	0.007
	68 Tender	0.02	0.01
Serum Amyloid A-1	DAS28	0.36	0.07
	HAQ-DI	0.55	0.07
Serum Amyloid A-4	DAS28	0.07	0.09
Transthyretin	28 Swollen	−0.031	0.078
	66 Swollen	−0.016	0.06
Vitronectin	28 Swollen	−0.021	0.11
	66 Swollen	−0.011	0.08

B

Protein	Disease activity measure model	Treatment estimate (Adalimumab *versus* Abatacept	*p*-value
Apolipoprotein AI	DAS28	0.14	0.37
	28 Tender	0.14	0.39
	28 Swollen	0.14	0.36
	68 Tender	0.14	0.39
	66 Swollen	0.15	0.36
	HAQ-DI	0.15	0.34
	CRP	0.14	0.37
CD5 Antigen like	DAS28	0.33	0.054
	28 Tender	0.33	0.059
	28 Swollen	0.34	0.053
	68 Tender	0.33	0.055
	66 Swollen	0.33	0.054
	HAQ-DI	0.17	0.064

B			
Protein	**Disease activity measure model**	**Treatment estimate (Adalimumab *versus* Abatacept**	***p*-value**
	CRP	0.34	0.049
Immunoglobulin J chain	DAS28	0.57	0.009
	28 Tender	0.57	0.01
	28 Swollen	0.58	0.009
	68 Tender	0.58	0.009
	66 Swollen	0.57	0.008
	HAQ-DI	0.56	0.01
	CRP	0.59	0.008
Serotransferrin	DAS28	0.43	0.02
	28 Tender	0.43	0.02
	28 Swollen	0.42	0.02
	68 Tender	0.43	0.02
	66 Swollen	0.43	0.02
	HAQ-DI	0.47	0.01
	CRP	0.40	0.03
Serum amyloid A-1	DAS28	−1.16	0.02
	28 Tender	−1.21	0.02
	28 Swollen	−1.15	0.02
	68 Tender	−1.13	0.03
	66 Swollen	−1.17	0.02
	HAQ-DI	−1.24	0.02
	CRP	−0.86	0.06

DAS28= disease activity scale using a 28 joint count; HAQ-DI- Health assessment questionnaire disability index. Data not shown for sex, age, and treatment variables.

DAS28= disease activity scale using a 28 joint count; HAQ-DI-Health assessment questionnaire disability index. CRP= C-reactive protein. Data not shown for sex, age, and disease activity variables.

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Table 5A

Model estimates for changes in (A) traditional CV biomarkers and (B) HDL function biomarkers by covariate in multivariate repeated measures analyses.

A										
	Total cholesterol		**LDL-C**		**HDL-C**		**Triglycerides**		**Homocysteine**	
	Estimate	***p***	**Estimate**	***p***	**Estimate**	***p***	**Estimate**	***p***	**Estimate**	***p***
CRP (per 10 unit decrease)	3.72%	**0.01**	2.03%	0.39	0.38%	0.83	−1.10%	0.88	4.25%	0.11
Female *vs* male (0)	−4.08%	0.71	−6.78%	0.68	23.17%	0.14	9.04%	0.75	−23.39%	**0.01**
Age (per year increase)	0.43%	0.25	0.77%	0.18	0.68%	0.15	0.24%	0.79	1.23%	**0.001**
Adalimimumab *vs* abatacept (0)	2.81%	0.74	12.00%	0.37	−4.84%	0.63	8.05%	0.70	−2.40%	0.75

B										
	HII		**PON1 activity**		**HDL-ApoAI**		**HDL-Hp**		**MPO activity**	
	Estimate	***p***	**Estimate**	***p***	**Estimate**	***p***	**Estimate**	***p***	**Estimate**	***p***
CRP (per 10 unit decrease)	−20.55%	**0.01**	6.56%	**0.003**	2.61%	0.47	−4.43%	**0.04**	−4.97%	0.48
Adalimimumab *vs* abatacept (0)	17.64%	0.50	68.68%	**0.03**	2.32%	0.83	−17.61%	**0.04**	−5.82%	0.81
Age (per year increase)	0.14%	0.90	−1.72%	0.10	0.01%	0.99	0.47%	0.26	0.16%	0.89
Female *vs* male (0)	−30.60%	0.27	27.33%	0.44	16.58%	0.29	12.91%	0.33	−39.97%	0.15
HDL (per 10% increase)	2.83%	0.44	4.00%	0.02	10.49%	<**0.001**	−1.84%	0.13	−2.24%	0.54

CV=cardiovascular; HDL-C=high density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; CRP= C-reactive protein

CV=cardiovascular; HDL=high density lipoprotein; HDL-ApoAI=HDL-associated apolipoprotein A-I; HDL-Hp=HDL-associated haptoglobin; HII= HDL Inflammatory Index; MPO=myeloperoxidase; PON1=paraoxonase 1; CRP= C-reactive protein