Published in final edited form as:

Ann Rheum Dis. 2013 October ; 72(10): 1725–1731. doi:10.1136/annrheumdis-2012-202033.

High density lipoprotein is targeted for oxidation by myeloperoxidase in rheumatoid arthritis

Anuradha Vivekanandan-Giri¹, Jessica L. Slocum¹, Jaeman Byun¹, Chongren Tang⁶, Robin L. Sands^{4,5}, Brenda W. Gillespie^{4,5}, Jay W. Heinecke⁶, Rajiv Saran^{1,4}, Mariana J. Kaplan², and Subramaniam Pennathur^{1,3,*}

¹Division of Nephrology, University of Michigan, Ann Arbor, MI 48109

²Division of Rheumatology, University of Michigan, Ann Arbor, MI 48109

³Computational Medicine and Biology, University of Michigan, Ann Arbor, MI 48109

⁴Kidney Epidemiology and Cost Center, University of Michigan, Ann Arbor, MI 48109

⁵Biostatistics, University of Michigan, Ann Arbor, MI 48109

⁶Department of Medicine and Diabetes and Obesity Center of Excellence, University of Washington, Seattle, WA 98195

Abstract

Objective—Phagocyte-derived myeloperoxidase (MPO) and pro-inflammatory high density lipoprotein (HDL) associate with rheumatoid arthritis (RA), but the link between MPO and HDL has not been systematically examined. In this study we investigated whether MPO can oxidize HDL and determined MPO-specific oxidative signature by apoA1 by peptide mapping in RA subjects without and with known cardiovascular disease (CVD).

Methods—Two MPO oxidation products, 3-chlorotyrosine and 3-nitrotyrosine were quantified by tandem mass-spectrometry (MS/MS) in *in vitro* model system studies and in plasma and HDL derived from healthy controls and RA subjects. MPO levels and cholesterol efflux were determined. Site-specific nitration and chlorination of apo A-1 peptides were quantified by MS/MS.

* Address Correspondence to: Subramaniam Pennathur, 6120 Brehm Center, 1000 Wall Street, University of Michigan, Ann Arbor, MI, 48105, Fax: 734-763-0982, spennath@umich.edu.

AV-G and JLS contributed equally to this work.

Competing Interest: None declared.

Data Sharing Agreement: The mean data, mass spectral analytical parameters and statistical analysis from the study has been reported in the paper and the accompanying online supplement. After publication, the raw mass spectra and meta data will be deposited in the National Institutes of Health data repository.

Contributorship statement: A-VG developed sample preparation strategies and performed all the experiments and assisted in manuscript preparation. JL-S performed statistical analysis, data interpretation, and manuscript preparation. J-B developed MS/MS methodology. C-T and JW-H performed cholesterol efflux measurements and interpretation for all samples. R-S and MJ-K designed and performed study subject recruitment, sample collection, and data analysis from control and RA subjects respectively. RLS and BWG provided statistical analysis. S-P initiated the collaborative study process, monitored overall study design, data collection, data analysis, manuscript preparation and provided funding for the project. All authors participated in draft manuscript revision and final manuscript approval.

Results—RA subjects demonstrated higher levels of MPO, MPO-oxidized HDL, and diminished cholesterol efflux. There was marked increase in MPO-specific 3-chlorotyrosine and 3-nitrotyrosine content in HDL in RA subjects consistent with specific targeting of HDL, with increased nitration in RA subjects with CVD. Cholesterol efflux capacity was diminished in RA subjects and correlated inversely with HDL 3-chlorotyrosine suggesting a mechanistic role for MPO. Nitrated HDL was elevated in RACVD subjects compared with RA subjects without CVD. Oxidative peptide mapping revealed site-specific unique oxidation signatures on apoA1 for RA subjects without and with CVD.

Conclusion—We report an increase in MPO-mediated HDL oxidation that is regiospecific in RA and accentuated in those with CVD. Decreased cholesterol efflux capacity due MPO-mediated chlorination is a potential mechanism for atherosclerosis in RA and raises the possibility that oxidant-resistant forms of HDL may attenuate this increased risk.

Keywords

Rheumatoid Arthritis; Myeloperoxidase; High Density Lipoprotein; Mass Spectrometry; Oxidative stress

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disorder characterized by an excessive cardiovascular disease (CVD) burden.¹⁻³ Traditional risk factors do not account for the accelerated atherosclerosis observed in RA⁴ and growing evidence suggests a more critical role for chronic inflammation, immune dysregulation,⁵ and oxidative stress in disease pathogenesis.⁶ Myeloperoxidase (MPO), a phagocytic enzyme, is a major source of reactive oxidants within the human vasculature.⁷ MPO, classically thought of as macrophage-derived, has been localized to atherosclerotic plaques, and oxidants produced by this enzyme activate protease cascades and plaque rupture^{8, 9}. There is growing evidence suggests an importance of MPO-mediated oxidation of high density lipoprotein (HDL), a lipid protein complex well-recognized for its inverse association with CVD¹⁷⁻²¹

HDL's protective vascular effects are attributed to its ability to remove excess cholesterol from arterial wall macrophages, a process known as reverse cholesterol transport (RCT),¹⁰⁻¹² in addition to its anti-inflammatory and antioxidant effects^{13,14}. HDL can be oxidatively modified leading to enhanced^{15,16} or diminished efflux¹⁷.

RA is associated with altered pro-inflammatory HDL^{22,23} and increased MPO level and activity^{24,25}. Impaired RCT has been reported in RA patients with high disease activity, and levels of plasma MPO correlated with this impairment²⁶. Epidemiologic investigation has shown the association between plasma MPO and CVD in the general population,²⁷ and other studies have both supported and refuted this association suggesting that plasma MPO may be of importance in specific subsets of patients²⁸⁻³⁰. Recently, neutrophil-derived MPO was linked with early vascular dysfunction³¹. While the cellular source of circulating MPO is unclear, activated phagocytes in plasma and synovial fluid are an attractive source of MPO in RA. This raises the possibility that circulating plasma MPO, resident extravascular macrophage and neutrophil-derived MPO at sites of inflammation, may contribute to the

oxidation and dysfunction of HDL in RA. In this study, we utilized isotope-dilution tandem mass spectrometry (MS/MS) to quantify and map levels and sites of two oxidative reactions mediated by MPO: chlorination and nitration in RA patients with and without CVD.

Methods

Human subjects

Plasma samples were collected from 38 patients with rheumatoid arthritis (RA), fulfilling the 1987 American College of Rheumatology diagnostic criteria³² on stable (>3months on Disease modifying antirheumatic drugs (DMARDs) and/or biologics), and from 20 healthy control subjects. The study was approved by the University of Michigan's Institutional Review Board. Subjects were excluded for pregnancy/lactation, tobacco usage, diabetes, infection, liver or renal disease. Patients were defined as having CVD if they had documented history of any CV event (myocardial infarction, unstable angina, or cerebrovascular accident). Patients receiving lipid-lowering drugs were required to be on stable doses for at least 6-months. Laboratory tests such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), lipid panel were obtained on all RA subjects and clinical disease activity score (DAS-28) was computed.

General methods

Human neutrophil experiments, HDL isolation, cholesterol efflux assay and MPO quantification were performed as described previously^{44,45} and in supplementary methods.

Quantification of oxidized amino acids and tyrosine containing apoA-1 peptides

Oxidized amino acids and native and oxidized apoA-1 containing peptides were quantified by liquid chromatography-electrospray ionization (LC/ESI) MS/MS with multiple reaction monitoring (MRM) mode as described previously and in supplementary methods³³.

Data analysis

Pearson correlation coefficients were calculated between the outcomes studied and patient characteristics. Paired t-tests were used to compare plasma and HDL isolates within each subject. Multivariable linear models were used to explore significant predictors of the outcomes of interest. The method of best subsets with the R-squared selection criterion guided the model selection process.³⁴ These models were also used to estimate and test differences between the control and RA groups. Skewed variables were log transformed (ln) and a p-value < 0.05 was considered significant. All analyses were conducted using SAS, version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Study population

In order to examine the effects of MPO on circulating HDL *in vivo*, plasma was obtained from 20 healthy control and 38 RA subjects. Demographical and clinical data from all subjects are presented in table 1. Of the 38 subjects with RA, 18 had known CVD. There were no differences in age or proportion of males between healthy control and RA subjects.

Patients with RA had a higher prevalence of hypertension and lower cholesterol than healthy controls. Lipoprotein profiles including HDL were similar among the groups. However, RA subjects with CVD were older and had lower triglycerides compared to the RA subjects without CVD. There was greater use of DMARDs in RA subjects without CVD; however, RA disease activity, assessed by DAS-28, CRP levels and ESR values, were similar between the two RA groups (table 1).

The correlations of patient characteristics with each outcome studied are reported in supplementary table (ST1). DAS-28 score correlated with cholesterol efflux ($r=-0.28$, $p=0.036$) and MPO ($r=0.28$, $p=0.0349$) levels. There was a significant correlation between DAS-28 and HDL 3-chlorotyrosine (ST1) in RA patients. DMARDs, biologics and steroids were correlated with MPO, HDL oxidation products and efflux.

Plasma MPO is increased and RCT is impaired in RA

Plasma MPO was significantly higher in subjects with RA compared to healthy control subjects. This difference remained significant after adjustment for predictors of MPO (table 2). However, there was no difference between RA subjects with and without CVD (table 1 and ST2A). We measured cholesterol efflux capacity, a metric of HDL function, which has a strong inverse association with CVD, independent of the HDL cholesterol level³⁵³⁶. Patients with RA had significantly impaired cholesterol efflux when compared to healthy controls and this persisted in multivariable analysis (table 2). However, there was no difference in efflux capacity between RA subjects with and without CVD (table 1 and ST2A). These results suggest that dysfunctional HDL may contribute to increased atherogenic risk in RA patients.

MPO-specific oxidative modification is increased in RAHDL

As RA subjects displayed higher plasma MPO levels and impaired RCT, we measured HDL levels of 3-chlorotyrosine and 3-nitrotyrosine. Plasma MPO levels were significantly correlated with levels of HDL 3-chlorotyrosine and HDL 3-nitrotyrosine (figure 1A,B). Additionally, the levels of 3-chlorotyrosine and 3-nitrotyrosine were highly correlated ($r=0.51$, $p<0.0001$) suggesting that MPO is the source of both of these oxidants (figure 1C). Cholesterol efflux capacity had a strong negative correlation with MPO-specific HDL 3-chlorotyrosine ($r=-0.39$, $p=0.0025$) but not with HDL 3-nitrotyrosine ($r=-0.22$, $p=0.1044$) although there was a trend. These results support the notion that MPO-derived oxidants chlorinate and nitrate HDL, and that MPO-specific chlorination might impair HDL function *in vivo*. In order to ascertain whether MPO-derived from human neutrophils can generate nitrated and chlorinated HDL, we exposed activated neutrophils to HDL. There was a dramatic increase in 3-chlorotyrosine and 3-nitrotyrosine formation (supplementary figure S1A,B) which required MPO-H₂O₂ system. HDL from subjects with RA had a significantly higher 3-chlorotyrosine and 3-nitrotyrosine content compared with control subjects (figure 2A,B). In multivariable analysis, these differences remained significant (table 2). Furthermore, when patients on statins ($n=10$) were removed, these differences still persisted (ST2B).

To further evaluate the clinical spectrum of MPO-modified HDL, we compared the level of HDL oxidation in RA subjects with and without CVD. The HDL from RACVD subjects had significantly higher 3-nitrotyrosine content and a trend toward increased 3-chlorotyrosine when compared those without CVD (figure 2C,D). In multivariable analysis, HDL nitrotyrosine remained elevated in RACVD subjects compared with those without CVD (ST2A). This supports a strong association between levels of MPO oxidized HDL and the clinical phenotype of CVD.

MPO specifically targets HDL for oxidative modification in RA

In order to determine if MPO was specifically targeting HDL, the level of tyrosine-modification in isolated HDL was compared to that in total plasma proteins. In both healthy controls and RA subjects, there was higher MPO-derived oxidative damage to HDL compared to circulating plasma proteins (figure 3). Both 3-chlorotyrosine (figure 3A, B) and 3-nitrotyrosine (figure 3C,D) were enriched in HDL of RA subjects compared to controls. This suggests that HDL is a specific target for MPO oxidation with pronounced increases in RA.

MPO-Oxidative modification of HDL is regiospecific in RA

ApoA-1 is the major HDL protein responsible for RCT, and MPO can chlorinate and nitrate the seven tyrosine (Tyr) residues of this protein in a differential pattern. We sought to determine if regiospecific MPO oxidative modification occurs in RA. We utilized LC/ESI/MS/MS with MRM to quantify levels of chlorination and nitration of seven tyrosine residues at positions 18, 29, 100, 115, 166, 192 and 236. Supplementary figure 2 depicts the MS/MS spectra for the native, nitrated and chlorinated peptide LAEY¹⁹²HAK. The *m/z*, MRM transitions, retention time and the MS parameters used for the detection of the seven peptides with tyrosine residues are shown in ST3. Figure 4 and ST4 display fold changes in 3-chlorotyrosine and 3-nitrotyrosine at each apoA-1 tyrosine residue (log-scale; ln), compared with levels from the healthy control subjects. RAapoA-1 contains increased levels of chlorination in residues 18, 29, 100, 115, and 192 when compared with controls (figure 4A). The highest increases were seen at Tyr100, Tyr115 and Tyr192. Nitration levels were highest on Tyr18, Tyr29, Tyr 100, Tyr 115 and Tyr192 (figure 4B).

Differential oxidation was found when comparing RA subjects with and without CVD (figure 4C,D). RA-CVD subjects had significant increases in chlorination to Tyr18, 29, 100, and 166 compared to RA subjects without CVD. The highest increase was seen in Tyr18 chlorination. While Tyr115 showed increases in both RA subgroups, suggesting that this modification is specific for RA but not the CVD phenotype. Tyr166 was higher only in RACVD suggesting specificity for CVD but not RA. RA subjects with CVD also had significant increases in nitration of Tyr100, 115, 166, and 192 when compared to RA subjects without CVD. Nitration to Tyr18 and 29 appeared specific for the RA phenotype, while nitration to Tyr166 was only elevated in RACVD. Tyr18 and 192 showed a progressive increase in nitration from healthy controls, to RA subjects without CVD, to RA subjects with CVD, similar to regiospecificity of chlorination. Taken together, this data suggests that MPO oxidizes HDL in a regiospecific fashion, and that modification to Tyr 18, 29, 166 and 192 may be of particular interest in understanding the CVD pathogenesis of RA.

Discussion

The pathophysiologic mechanisms underlying RA-accelerated atherosclerosis remain incompletely understood. In this study, we examined whether phagocyte-derived MPO can oxidize HDL and whether MPO-specific oxidative modifications can explain the atherogenic risk in RA. Plasma levels of MPO were increased by over 2-fold in subjects with RA compared to healthy controls similar to recent studies²⁴⁻²⁶. Interestingly, MPO levels in RA, did not predict CVD-phenotype whereas MPO-oxidation of HDL (Nitration, a functional read-out of MPO activity) was associated with the CVD-phenotype. This might indicate that the plasma MPO levels may not fully reflect MPO burden. Extravascular MPO (derived from macrophages and inflammatory cells in sites of inflammation including joints and vasculature) may participate in oxidation reactions but may not contribute to plasma MPO. We previously reported that HDL 3-chlorotyrosine and 3-nitrotyrosine content from atheroma was substantially higher than those in plasma-derived HDL in subjects with CVD^{18,19}. In this study, circulating HDL 3-chlorotyrosine and 3-nitrotyrosine approximate levels seen within atheromatous lesions. This further supports the hypothesis that in RA, MPO may be modifying HDL within the plasma or in inflamed tissue, perhaps by neutrophils or macrophages. Interestingly, subjects with RA without CVD had greater degree of drug usage (DMARDs) suggesting that they might have more active disease contributing to an environment of increased oxidant stress compared with those with CVD. While, this might have blunted the differentiation between the two phenotypes, HDL nitration was significantly increased even after adjustment in those with CVD suggesting that this could be a CVD-specific signature. These observations support the hypothesis that levels and patterns of oxidation of circulating HDL may serve as unique mechanistic markers of CVD in this high risk population.

Two potential models have been proposed for the site-specific chlorination of apoA-I by MPO at Tyr192, which has been linked with impaired cholesterol efflux^{37,2138}. Studies with synthetic peptides³⁷ and mutations of apoA-I provided strong evidence that the YXXK/KXXY motif can direct the regiospecific chlorination of Tyr192. An alternative hypothesis suggests MPO specifically interacts with Tyr192²¹³⁸. Other studies have shown that modification of Tyr166 can inhibit lecithin:cholesterol acyltransferase (LCAT), an enzyme important for HDL maturation.^{39,40} Our recent analysis of HDL derived from human atherosclerotic lesions implicated Tyr18 in addition to Tyr192 as a site of nitration.²⁰ In this study, modification to Tyr 18, 29, 166 and 192 appeared to be more specific to CVD in RA. This data, while similar to previous studies correlating levels of Tyr192 and 166 with CVD in the general population and impaired cholesterol transport by the ABCA1 pathway,^{18,41} also points to a RA-specific distinct signature that requires exploration in future studies.

There are some limitations to the study. First, the study has a small sample size and therefore has to be confirmed in a larger cohort of subjects. Second, despite increased level of nitrated HDL in RA-CVD we cannot directly show a link between HDL nitration and diminished efflux capacity although there was a trend. While chlorinated HDL, a more specific MPO marker associates with efflux, this marker is not specifically associated with RACVD phenotype, perhaps reflecting the small sample size. Second, RA subjects without CVD may have a more active disease (as evidenced by increased use of medications such as

DMARDs and biologics) and/or subclinical atherosclerosis, which might promote an environment of increased oxidative stress by MPO, which could diminish differences between the groups. The current evidence-based recommendations for CV risk management in RA do not support screening patients with RA for evidence of subclinical atherosclerosis, unless as part of a clinical trial. As such, we did not obtain these tests in the RA group that had no history of overt CVD.⁴² Finally, the effects on efflux capacity reduction of 21% might appear modest. However, a recent clinical study on efflux capacity in subjects with traditional CVD³⁶ reported a reduction in mean normalized efflux capacity of 9% in CVD versus control subjects. A second study done by the same group in 122 patients with psoriasis reports 15% reduction in efflux capacity even in subjects not known to have CVD.⁴³ Our results therefore are in agreement with the above studies and highlight marked reduction in efflux greater than what is observed in traditional CVD, suggesting inflammation in RA state drives oxidative damage. The fact that DAS-28 correlates with HDL chlorination highlight this important mechanism.

The results of this study suggest that MPO-induced oxidation plays an important role in the genesis of dysfunctional-HDL in RA and may contribute to CVD risk. The levels and site-specific oxidative signature of chlorination and nitration of circulating HDL may serve as novel mechanism-based markers of clinically-significant atherosclerosis in this population. MPO specific chlorination strongly correlates with efflux potentially providing a mechanistic link for vascular dysfunction in RA. Finally, our results provide initial evidence that the systemic inflammation of RA could produce these changes outside of the vascular wall at sites of inflammation even in those without known CVD. Future prospective trials will be of great interest to advance these observations and to determine if therapeutics, such as oxidant resistant forms apoA-1, will alter the CVD pathogenesis in RA populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work is supported in part by grants from the National Institutes of Health (K12HD001438, DK089503, HL086553), the Doris Duke Foundation Clinical Scientist Development Award and the American College of Rheumatology Within Our Reach Award.

References

1. Holmqvist ME, Wedren S, Jacobsson LT, et al. Rapid increase in myocardial infarction risk following diagnosis of rheumatoid arthritis amongst patients diagnosed between 1995 and 2006. *J Intern Med*. 2010; 268:578–85. [PubMed: 20698926]
2. Meune C, Touze E, Trinquart L, et al. Trends in cardiovascular mortality in patients with rheumatoid arthritis over 50 years: a systematic review and meta-analysis of cohort studies. *Rheumatology (Oxford)*. 2009; 48:1309–13. [PubMed: 19696061]
3. Kaplan MJ. Cardiovascular complications of rheumatoid arthritis: assessment, prevention, and treatment. *Rheum Dis Clin North Am*. 2010; 36:405–26. [PubMed: 20510241]
4. del Rincon ID, Williams K, Stern MP, et al. High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. *Arthritis Rheum*. 2001; 44:2737–45. [PubMed: 11762933]

5. Lindstrom TM, Robinson WH. Rheumatoid arthritis: a role for immunosenescence? *J Am Geriatr Soc.* 2010; 58:1565–75. [PubMed: 20942872]
6. Hahn BH, Grossman J, Chen W, et al. The pathogenesis of atherosclerosis in autoimmune rheumatic diseases: roles of inflammation and dyslipidemia. *J Autoimmun.* 2007; 28:69–75. [PubMed: 17433865]
7. Berliner JA, Heinecke JW. The role of oxidized lipoproteins in atherogenesis. *Free Radic Biol Med.* 1996; 20:707–27. [PubMed: 8721615]
8. Daugherty A, Dunn JL, Rateri DL, et al. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J Clin Invest.* 1994; 94:437–44. [PubMed: 8040285]
9. Fu X, Kassim SY, Parks WC, et al. Hypochlorous acid oxygenates the cysteine switch domain of pro-matrilysin (MMP-7). A mechanism for matrix metalloproteinase activation and atherosclerotic plaque rupture by myeloperoxidase. *J Biol Chem.* 2001; 276:41279–87. [PubMed: 11533038]
10. Gordon DJ, Rifkind BM. High-density lipoprotein--the clinical implications of recent studies. *N Engl J Med.* 1989; 321:1311–6. [PubMed: 2677733]
11. Miller GJ, Miller NE. Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. *Lancet.* 1975; 1:16–9. [PubMed: 46338]
12. Tall AR, Yvan-Charvet L, Terasaka N, et al. HDL, ABC transporters, and cholesterol efflux: implications for the treatment of atherosclerosis. *Cell Metab.* 2008; 7:365–75. [PubMed: 18460328]
13. Besler C, Heinrich K, Rohrer L, et al. Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. *J Clin Invest.* 2011; 121:2693–708. [PubMed: 21701070]
14. Suzuki M, Pritchard DK, Becker L, et al. High-density lipoprotein suppresses the type I interferon response, a family of potent antiviral immunoregulators, in macrophages challenged with lipopolysaccharide. *Circulation.* 2010; 122:1919–27. [PubMed: 20974999]
15. Hossain MA, Ngeeth S, Chan T, Oda MN, Francis GA. Lipid-bound apolipoproteins in tyrosyl radical-oxidized HDL stabilize ABCA1 like lipid-free apolipoprotein A-I. *BMC Biochem.* 2012; 13:1. [PubMed: 22248050]
16. Wang WQ, Merriam DL, Moses AS, et al. Enhanced cholesterol efflux by tyrosyl radical-oxidized high density lipoprotein is mediated by apolipoprotein AI-AII heterodimers. *J Biol Chem.* 1998; 273:17391–8. [PubMed: 9651324]
17. Shao B, Heinecke JW. Impact of HDL oxidation by the myeloperoxidase system on sterol efflux by the ABCA1 pathway. *J Proteomics.* 2011; 74:2289–99. [PubMed: 21501700]
18. Bergt C, Pennathur S, Fu X, et al. The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. *Proc Natl Acad Sci U S A.* 2004; 101:13032–7. [PubMed: 15326314]
19. Pennathur S, Bergt C, Shao B, et al. Human atherosclerotic intima and blood of patients with established coronary artery disease contain high density lipoprotein damaged by reactive nitrogen species. *J Biol Chem.* 2004; 279:42977–83. [PubMed: 15292228]
20. Shao B, Pennathur S, Heinecke JW. Myeloperoxidase Targets Apolipoprotein A-I, the Major HDL Protein, for Site-Specific Oxidation in Human Atherosclerotic Lesions. *J Biol Chem.* 2012; 287:6375–86. [PubMed: 22219194]
21. Zheng L, Nukuna B, Brennan ML, et al. Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. *J Clin Invest.* 2004; 114:529–41. [PubMed: 15314690]
22. Charles-Schoeman C, Watanabe J, Lee YY, et al. Abnormal function of high-density lipoprotein is associated with poor disease control and an altered protein cargo in rheumatoid arthritis. *Arthritis Rheum.* 2009; 60:2870–9. [PubMed: 19790070]
23. McMahon M, Grossman J, FitzGerald J, et al. Proinflammatory high-density lipoprotein as a biomarker for atherosclerosis in patients with systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum.* 2006; 54:2541–9. [PubMed: 16868975]
24. Fernandes RM, Silva NP, Sato EI. Increased myeloperoxidase plasma levels in rheumatoid arthritis. *Rheumatol Int.* 2011; 32:1605–9. [PubMed: 21331575]

25. Torsteinsdottir I, Hakansson L, Hallgren R, et al. Serum lysozyme: a potential marker of monocyte/macrophage activity in rheumatoid arthritis. *Rheumatology (Oxford)*. 1999; 38:1249–54. [PubMed: 10587554]
26. Charles-Schoeman C, Lee YY, Grijalva V, et al. Cholesterol efflux by high density lipoproteins is impaired in patients with active rheumatoid arthritis. *Ann Rheum Dis*. 2012
27. Zhang R, Brennan ML, Fu X, et al. Association between myeloperoxidase levels and risk of coronary artery disease. *JAMA*. 2001; 286:2136–42. [PubMed: 11694155]
28. Baldus S, Heeschen C, Meinertz T, et al. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. *Circulation*. 2003; 108:1440–5. [PubMed: 12952835]
29. Baldus S, Rudolph V, Roiss M, et al. Heparins increase endothelial nitric oxide bioavailability by liberating vessel-immobilized myeloperoxidase. *Circulation*. 2006; 113:1871–8. [PubMed: 16606792]
30. Kubala L, Lu G, Baldus S, Berglund L, Eiserich JP. Plasma levels of myeloperoxidase are not elevated in patients with stable coronary artery disease. *Clin Chim Acta*. 2008; 394:59–62. [PubMed: 18440308]
31. von Leitner EC, Klinka A, Atzler D, et al. Pathogenic cycle between the endogenous nitric oxide synthase inhibitor asymmetrical dimethylarginine and the leukocyte-derived hemoprotein myeloperoxidase. *Circulation*. 2011; 124:2735–45. [PubMed: 22082678]
32. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum*. 1988; 31:315–24. [PubMed: 3358796]
33. Vivekanandan-Giri A, Byun J, Pennathur S. Quantitative analysis of amino Acid oxidation markers by tandem mass spectrometry. *Methods Enzymol*. 2011; 491:73–89. [PubMed: 21329795]
34. Myers, RH. Classical and model regression with applications. Boston: Duxbury Press; 1986.
35. de la Llera-Moya M, Drazul-Schrader D, Asztalos BF, Cuchel M, Rader DJ, Rothblat GH. The ability to promote efflux via ABCA1 determines the capacity of serum specimens with similar high-density lipoprotein cholesterol to remove cholesterol from macrophages. *Arterioscler Thromb Vasc Biol*. 2010; 30:796–801. [PubMed: 20075420]
36. Khera AV, Cuchel M, de la Llera-Moya M, et al. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. *N Engl J Med*. 2011; 364:127–35. [PubMed: 21226578]
37. Bergt C, Fu X, Huq NP, Kao J, et al. Lysine residues direct the chlorination of tyrosines in YXXK motifs of apolipoprotein A-I when hypochlorous acid oxidizes high density lipoprotein. *J Biol Chem*. 2004; 279:7856–66. [PubMed: 14660678]
38. Zheng L, Settle M, Brubaker G, et al. Localization of nitration and chlorination sites on apolipoprotein A-I catalyzed by myeloperoxidase in human atheroma and associated oxidative impairment in ABCA1-dependent cholesterol efflux from macrophages. *J Biol Chem*. 2005; 280:38–47. [PubMed: 15498770]
39. Shao B, Cavigliolo G, Brot N, et al. Methionine oxidation impairs reverse cholesterol transport by apolipoprotein A-I. *Proc Natl Acad Sci U S A*. 2008; 105:12224–9. [PubMed: 18719109]
40. Wu Z, Wagner MA, Zheng L, et al. The refined structure of nascent HDL reveals a key functional domain for particle maturation and dysfunction. *Nat Struct Mol Biol*. 2007; 14:861–8. [PubMed: 17676061]
41. Shao B, Bergt C, Fu X, et al. Tyrosine 192 in apolipoprotein A-I is the major site of nitration and chlorination by myeloperoxidase, but only chlorination markedly impairs ABCA1-dependent cholesterol transport. *J Biol Chem*. 2005; 280:5983–93. [PubMed: 15574409]
42. Peters MJ, Symmons DP, McCarey D, et al. EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis. *Ann Rheum Dis*. 2010; 69:325–31. [PubMed: 19773290]
43. Mehta NN, Li R, Krishnamoorthy P, et al. Abnormal lipoprotein particles and cholesterol efflux capacity in patients with psoriasis. *Atherosclerosis*. 2012; 224:218–21. [PubMed: 22858285]
44. Hazen SL, Heinecke JW. 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. *J Clin Invest*. 1997; 99:2075–81. [PubMed: 9151778]

45. Stahlman M, Davidsson P, Kanmert I, et al. Proteomics and lipids of lipoproteins isolated at low salt concentrations in D₂O/sucrose or in KBr. *J Lipid Res.* 2008; 49:481–90. [PubMed: 18025001]

Abbreviations

apoA-1	ApolipoproteinA-1
CVD	cardiovascular disease
DMEM	Dulbecco's Modified Eagle Medium
HDL	high density lipoprotein
LC-ESI-MS/MS	liquid chromatography-electrospray ionization tandem mass spectrometry
MS	mass spectrometry
MPO	myeloperoxidase
RA	rheumatoid arthritis
RCT	reverse cholesterol transport
Tyr	tyrosine

Figure 1. Correlation of myeloperoxidase and HDL Oxidation Products

Panel A-B: Correlations of levels of plasma myeloperoxidase (MPO) with 3-chlorotyrosine (A) and 3-nitrotyrosine (B) content of HDL and (C) levels of HDL 3-nitrotyrosine correlates with HDL 3-chlorotyrosine content in all subjects, determined by tandem mass spectrometry. Figure displays the scatter plot and least squares regression line.

Figure 2. Myeloperoxidase oxidizes HDL in patients with Rheumatoid arthritis

The figure displays levels of 3-chlorotyrosine and 3-nitrotyrosine, two myeloperoxidase (MPO) oxidative modifications, to high density lipoprotein (HDL) determined by tandem mass spectrometry, in control and rheumatoid arthritis (RA) subjects. The levels of 3-chlorotyrosine (A) and 3-nitrotyrosine (B) content of HDL are increased in RA subjects compared to healthy controls. Comparisons between RA subjects with and without known cardiovascular disease (CVD) are also shown (C, D). Box plots display the distributions of 3-chlorotyrosine and 3-nitrotyrosine (log scale; ln) for healthy and RA subjects (A,B) and for RA patients with and without CVD (C,D). The length of the box defines the interquartile

range (IQR). Outliers (values >1.5 times the IQR) are represented by diamonds. Medians (IQR) are reported for each group on the raw scale.

Figure 3. Myeloperoxidase targets HDL for oxidation

The levels of myeloperoxidase (MPO) oxidation products 3-chlorotyrosine and 3-nitrotyrosine in control (A,C) rheumatoid arthritis (RA) subjects (B,D) are significantly increased in HDL isolates compared to circulating plasma proteins. The highest increases are seen in RA subjects consistent with specific targeting of HDL by MPO. Box plots display the distributions of plasma and HDL levels (log scale; ln) of (A) 3-chlorotyrosine and (B) 3-nitrotyrosine for healthy and RA subjects. The length of the box defines the interquartile range (IQR). Outliers (values >1.5 times the IQR) are represented by diamonds. Medians (IQR) are reported for each group on the raw scale.

Figure 4. Quantification of the regiospecific chlorination and nitration of apoA-I peptides isolated from healthy controls and Rheumatoid arthritis patients

Isotopically labeled oxidized tyrosine containing (nitrated and chlorinated) as well as native apoA-1 peptides were spiked into HDL samples isolated from control and rheumatoid arthritis (RA) patients without and with cardiovascular disease (CVD) following trypsin digestion. The extracted ion chromatograms from specific fragment ions were used for quantitative analysis. Box plots display the distribution of fold changes of percent nitrated or chlorinated peptide for each peptide (log-scale; ln) compared to controls for all three groups: (A) 3-chlorotyrosine for healthy and RA subjects, (B) 3-nitrotyrosine for healthy and RA subjects, (C) 3-chlorotyrosine for RA subjects versus RA CVD subjects and (D) 3-nitrotyrosine for RA subjects versus RA CVD subjects. The length of the box defines the interquartile range (IQR). Asterisks indicate significant differences ($p < 0.01$).

Table 1
Patient characteristics for healthy controls and patients with rheumatoid arthritis (RA)
with and without CVD

Continuous variables are reported as mean $\pm$ standard deviation for normally distributed variables and as median (min, max) for skewed variables. Significant differences between the healthy control and RA patients and among the RA patients with and without cardiovascular disease (CVD) are shown in bold ($p < 0.05$). NA: not available

	Healthy Control (n=20)	Rheumatoid Arthritis (n=38)	Rheumatoid Arthritis	
			CVD (n=18)	No CVD (n=20)
Age (years)	53.5 $\pm$ 6.0	58.3 $\pm$ 12.2	62.7 $\pm$ 12.4	54.3 $\pm$ 10.8
Sex (males)	35.0% (7)	44.7% (17)	61.1% (11)	30.0% (6)
Body Mass Index (kg/m ²)	27.8 $\pm$ 7.4	27.9 $\pm$ 5.4	27.5 $\pm$ 4.6	28.3 $\pm$ 6.1
Cholesterol (mg/dL)	221.1 $\pm$ 43.2	188.2 $\pm$ 37.2	177.4 $\pm$ 31.4	196.4 $\pm$ 39.9
Triglycerides (mg/dL)	115 (4, 260)	110.0 (46, 296)	80 (46, 160)	117 (52, 296)
HDL (mg/dL)	60.2 $\pm$ 16.7	57.2 $\pm$ 16.0	53.5 $\pm$ 10.6	60.1 $\pm$ 18.9
LDL (mg/dL)	135 (74, 220)	103 (52, 293)	106 (62, 238)	102 (52, 293)
ESR	NA	11.5 (2.0, 44)	12.5 (2.0, 44.0)	9.0 (2.0, 44.0)
C-Reactive Protein	NA	0.7 (0.0, 9.3)	0.7 (0.1, 2.6)	0.6 (0.0, 9.3)
DAS-28	0	1.9 (0.5, 5.2)	1.9 (0.5, 5.2)	1.9 (0.5, 5.2)
Hypertension	0	44.7% (17)	44.4% (8)	45.0% (9)
Corticosteroid Use	0	73.7% (28)	66.7% (12)	80.0% (16)
DMARD/Biologic Use	0	81.6% (31)	66.7% (12)	95.0% (19)
Statin Use	0	26.3% (10)	44.4% (8)	10.0% (2)
NSAID Use	0	42.1% (16)	33.3% (6)	50.0% (10)
Beta Blockers	0	23.7% (9)	33.3% (6)	15.0% (3)
ACE inhibitor Use	0	18.4% (7)	22.2% (4)	15.0% (3)
MPO (fmol/ml)	246 (205, 499)	495.0 (161, 2,138)	473.0 (309, 2,138)	506 (161, 1,190)
Cholesterol Efflux (%)	9.5 $\pm$ 1.7	7.5 $\pm$ 1.1	7.6 $\pm$ 1.2	7.4 $\pm$ 1.1

Table 2

Differences in the estimated means between Controls and Rheumatoid Arthritis (RA) patients for each outcome studied (n=58).

	Unadjusted				Adjusted**			
	Controls	RA	p-value	Model R ²	Controls	RA	p-value	Model R ²
Cholesterol Efflux (%)	9.51 ± 0.31	7.49 ± 0.22	<0.0001	0.34	9.13 ± 0.45	7.82 ± 0.28	0.0481	0.51
MPO (fmol/ml) *	5.57 ± 0.10	6.31 ± 0.07	<0.0001	0.40	5.76 ± 0.12	6.21 ± 0.08	0.0069	0.54
HDL 3-chlorotyrosine *	2.39 ± 0.30	5.29 ± 0.22	<0.0001	0.51	2.04 ± 0.45	5.47 ± 0.28	<0.0001	0.57
HDL 3-nitrotyrosine *	4.35 ± 0.28	5.74 ± 0.21	0.0002	0.22	4.28 ± 0.40	5.78 ± 0.25	0.0107	0.36
Plasma 3-chlorotyrosine *	-0.54 ± 0.33	1.62 ± 0.24	<0.0001	0.33	0.32 ± 0.42	1.16 ± 0.27	0.1543	0.44
Plasma 3-nitrotyrosine *	3.51 ± 0.27	5.06 ± 0.20	<0.0001	0.28	3.78 ± 0.34	4.92 ± 0.22	0.0171	0.42

* log-scale (ln)

** Cholesterol Efflux (%): Adjusted for age, cholesterol, NSAID and DMARD/Biologic use (n=54).
MPO (fmol/ml): Adjusted for hypertension, ace inhibitor and corticosteroid use (n=58).
HDL-3-Chlorotyrosine: Adjusted for gender, DMARD/Biologic and corticosteroid use (n=58).
HDL-3-Nitrotyrosine: Adjusted for gender, DMARD/Biologic, NSAID and beta blocker use (n=58).
Plasma-3-Chlorotyrosine: Adjusted for corticosteroid, statin and beta blocker use (n=58).
Plasma-3-Nitrotyrosine: Adjusted for age, gender, corticosteroid and statin use (n=58).