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High Density Lipoprotein Function is

Abnormal in Idiopathic Inflammatory Myopathies

A dissertation submitted in partial satisfaction of the requirements for the degree Master

of Science in Clinical Research

by

Sangmee Bae

2019

ABSTRACT OF THE DISSERTATION

High Density Lipoprotein Function is

Abnormal in Idiopathic Inflammatory Myopathies

by

Sangmee Bae

Master of Science in Clinical Research

University of California, Los Angeles, 2019

Professor Eleazar Eskin, Chair

Purpose: Damage to the vascular endothelium is strongly implicated in the pathogenesis of idiopathic inflammatory myopathies (IIM). Normal high density lipoprotein (HDL) protects the vascular endothelium from damage from oxidized phospholipids, which accumulate under conditions of oxidative stress. The current work evaluated the function of HDL in IIM patients.

Methods: HDL's anti-oxidant function was measured in IIM patients using a cell free assay, which assesses the ability of isolated patient HDL to inhibit oxidation of low density lipoproteins (LDL). Cholesterol profiles were measured for all patients and subgroup analysis included assessment of oxidized fatty acids in HDL by mass spectrometry and plasma myeloperoxidase (MPO) activity. A subgroup of IIM patients were compared to a group of healthy controls (HC).

Results: HDL was dysfunctional in patients with IIM compared to HC and associated with higher plasma MPO activity and higher oxidized fatty acids in HDL. Higher 5-HETE in HDL

correlated with impaired lung diffusion capacity in patients with interstitial lung disease, and HDL function was most impaired in patients with MDA5 or anti-synthetase antibodies. In multivariate analysis including 182 IIM patients, dysfunctional HDL was associated with higher disease activity and DM diagnosis.

Conclusions: The anti-oxidant function of HDL is abnormal in IIM patients and may warrant further investigation for its role in propagating microvascular inflammation and damage in this patient population.

The dissertation of Sangmee Bae is approved.

David Elashoff

Christina Charles-Schoeman

Eleazar Eskin, Committee Chair

University of California, Los Angeles

2019

Table of Contents

List of Figures and Tables

Tables

Figures

Chapter 1. Introduction

Dermatomyositis (DM) is an idiopathic inflammatory myopathy (IIM), which is associated with marked activation and damage to small blood vessels in connective tissue of the muscle, skin, and gastrointestinal tract (1, 2). Capillary loss is believed to precede other pathological muscle changes, and the inflammatory infiltrate in DM is predominantly perivascular and perymysial (3). Vascular damage is considered integral to the disease pathogenesis in DM, and to a lesser degree in polymyositis (PM) and inclusion body myositis (IBM).

High density lipoprotein (HDL) protects the vascular endothelium from damage due to oxidized phospholipids, which accumulate under conditions of oxidative stress. Impaired function of HDL has previously been identified in inflammatory diseases including systemic lupus erythematosus, rheumatoid arthritis, and atherosclerosis (4). Oxidized fatty acids including hydroxyeicosatetraenoic acids (HETEs) and hydroxyoctadecadienoic acids (HODEs) not only contribute to the oxidation of low density lipoprotein (LDL) but their accumulation in HDL also impairs HDL function, increasing risk of vascular damage (5, 6).

Although it is well established that oxidized lipids and HDL strongly influence the vascular endothelium (4, 5), the level and function of these lipoproteins in adult patients with DM and other IIM has not been previously evaluated. In the current work, we investigate HDL function in IIM patients compared to healthy controls, and in a large cohort of IIM patients.

Chapter 2. Methods

2.1. Study Design

Myositis patients and healthy controls were recruited from the rheumatology offices at the University of California, Los Angeles (UCLA). All myositis patients met European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) Classification Criteria for at least "probable" IIM and subclasses including DM, PM, and IBM which was verified by chart review (7). All subjects gave written informed consent for the study under a protocol approved by the Human Research Subject Protection Committee at UCLA.

Initial analysis was designed to compare IIM patients to healthy controls (*IIM/HC comparison cohort*). The first 95 patients enrolled in the UCLA IIM cohort were compared to 41 healthy controls who were recruited for the study during the same time period. Subgroup analyses for oxidized lipids were performed from the initial patient cohorts and included 12 DM, 12PM, and 12 HC matched for age and sex. Subsequently, we further expanded the IIM cohort to all patients enrolled in the study through the time of analysis (*Expanded disease specific cohort*) to assess the association of HDL function with disease specific characteristics and disease activity level.

Patients provided a blood sample and completed questionnaires as described below. Assessment of creatine phosphokinase (CPK) levels, inflammatory markers including high-sensitivity C-reactive protein (hSCRP), Westergren erythrocyte sedimentation rate (ESR), and fasting lipid profiles were performed by the UCLA clinical laboratory using standard methods. Myositis specific antibodies (MSA) were assessed using standardized lab protocols (95 by the Oklahoma Medical Research Foundation and 16 from other clinical labs). Additional blood was collected in heparinized tubes (Becton Dickinson) and stored at -80°C for additional assays as described below. Disease activity and damage were assessed using physician global myositis disease activity and damage scales by 100mm visual analog scale (VAS) and 5 point Likert scales(8). Cardiovascular (CV) risk and health information was obtained by questionnaire and chart review. Interstitial lung disease (ILD) was defined by radiographic findings consistent with

2

ILD on high-resolution CT (HRCT) showing at least one of the following features: 1) reticulation and fibrosis, 2) traction bronchiectasis, 3) honeycombing, or 4) ground glass opacification (9).

2.2. Evaluation of HDL Antioxidant Function

The cell free assay was a modification of a previously published method using LDL as the fluorescence-inducing agent (10). To determine the anti-inflammatory properties of HDL, the change in fluorescence intensity as a result of the oxidation of 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) (ThermoFisher Scientific) to 2',7'-dichlorofluorescein (DCF) in incubations with a standard LDL in the absence or presence of the test HDL was assessed and the HDL inflammatory index (HII) calculated. Values for intra- and interassay variability were $0.5 \pm 0.37\%$ and $3.0 \pm 1.7\%$ respectively (11).

2.3. Determination of HETES and HODES in HDL

HDL was isolated by ultracentrifugation from plasma and 5-HETE, 12-HETE, 15-HETE, 9-HODE, and 13-HODE levels in HDL were measured by liquid chromatography–electrospray ionization, tandem mass spectroscopy as described previously (6, 12).

2.4. MPO Activity

The activity of MPO was measured using the InnoZyme MPO activity assay kit (EMD Chemicals, Darmstadt, Germany) as described previously (13).

2.5. Statistical analysis

Data were analyzed using JMP IN 13.0 (SAS Institute Inc., Cary, NC, USA). Patient groups were compared using Student's t-test for continuous variables and the chi-square test of association for categorical variables. When needed, nonparametric Wilcoxon rank-sum tests were used to analyze continuous variables. For IIM/HC comparison cohort, we compared IIM and HC,

3

then further pairwise testing within different IIM subtypes were performed for covariates that were significant in initial comparison. Correlations between variables were evaluated using the Pearson's correlation coefficient for normally distributed data and Spearman's correlation coefficient for nonparametric data. The significance level was pre-specified at two sided $p<0.05$. For multivariate regression analyses, model covariates included demographics, patient variables which were significantly associated with HDL function in univariate analysis and variables known to be associated with HDL function including age and statin use. HDL function was examined both as a continuous variable in linear regression analysis (HII), and as a categorical variable (tertile HII) in logistic regression analyses of the IIM cohort. In linear models, HII, hscrp, CPK were log transformed due to skewness. Because physician global disease activity and CPK levels were significantly correlated, two separate multivariate models were performed to evaluate each disease activity measure individually.

Chapter 3. Results

3.1. Abnormal HDL Function in IIM compared to healthy controls *(IIM/HC comparison cohort)*

The anti-oxidant function of HDL as measured by its ability to inhibit oxidation of LDL (HII) was significantly worse in patients with IIM (n=95) when compared to age and sex matched healthy controls (HC, n=41) (Table 1, Figure 1-a). Plasma MPO activity was higher in DM/PM patients compared to HC (Figure 1-b) and correlated with impaired HDL function measured by a higher HII (r = 0.35 p=0.0001, Figure 1-c).

Traditional cholesterol levels and CV risk factors were similar between IIM patients and HC with the exception of higher triglycerides in IIM patients, higher total cholesterol in PM (Table 1). DM and PM patients also had higher levels of systemic inflammation measured by hsCRP and ESR compared to HC (Table 1), which did not correlate with HII (r=0.11, p=0.20 for hsCRP, r=0.13, p=0.14 for ESR).

Multivariate regression analysis of HDL function (HII) was performed to evaluate whether IIM diagnosis and higher MPO activity remained associated with abnormal HDL function after adjustment for other differences between the HC and IIM groups. IIM diagnosis (vs HC) and higher plasma MPO activity remained significantly associated with HDL dysfunction measured by a higher HII in multivariate analysis after controlling for demographics (age, sex), other variables that were significant in univariate analysis (ever smoker, triglycerides), and statin use (Table 2).

3.2. Increased oxidation products of arachidonic acid and linoleic acid in HDL in IIM

Oxidized fatty acids were assessed in age-matched DM, PM and HCs (n=12 in each group) (Table 3). Levels were significantly higher in HDL from IIM patients compared to HC and associated with abnormal HDL function. In particular, 5-HETE, 15-HETE, 9-HODE and 13-HODE levels were significantly increased in HDL from patients with PM compared to HC (Figure 2) and higher 5-HETE significantly associated with worse HDL function measured by a higher HII (r =

5

0.41, p=0.01, Figure 3-a). HDL-associated 13-HODE also showed a modest trend for association with worse HDL function (r = 0.30, p=0.08). Higher MPO activity correlated with higher HDL-associated 5-HETE (r=0.46, p=0.007 Figure 3-b), 12-HETE (r=0.23, p=0.19), 15-HETE (r=0.38, p=0.03), 9-HODE (r=0.40, p=0.02) and 13-HODE (r=0.41, p=0.02).

Patients with ILD showed modest trends for higher 5-HETE, 12-HETE, 15-HETE, and 13-HODE levels in HDL compared to myositis patients without ILD (n=10/24) (Table 3). More patients with PM had ILD (9/12) compared to patients with DM (5/12) (p=0.09), which may have resulted in higher oxidized fatty acid levels in PM compared to DM patients. Patients with moderate to severe ILD (DLCO<50%) also showed trends for higher HDL-associated 5-HETE, 12-HETE, and 13-HODE levels compared to ILD patients with less severe ILD (DLCO >50%) (Table 3). HDL-associated 5-HETE (Figure 3-c) and 12-HETE levels were inversely correlated with DLCO (r = -0.58, p=0.02 and r = -0.49, p=0.05 respectively); higher levels of these oxidized fatty acids correlated with lower DLCO in ILD patients.

3.3. Clinical and laboratory characteristics of IIM patients associated with abnormal HDL antioxidant function *(Expanded disease specific cohort)*

HDL's antioxidant capacity was evaluated in 182 patients with IIM. IIM patients were divided into three groups by the HII. Tertile 1 contained patients with the highest HII, consistent with severe HDL dysfunction, tertile 2 with lower HII consistent with less HDL dysfunction, and tertile 3 contained patients with the lowest HII consistent with the most protective, anti-oxidant HDL. No significant differences in demographics (age, sex, race, or ethnicity), traditional cholesterol levels or other co-morbidities including CV risk factors were noted between patients in the different tertiles (Table 4).

The proportion of patients with DM was lowest in patients with the most anti-inflammatory HDL (tertile 3 HII). Patients with the most dysfunctional HDL (tertile 1 HII) had higher myositis disease activity levels compared to patients with the most anti-inflammatory HDL (tertile 3 HII),

as measured by physician global disease activity scales as well as serum CPK levels. Tertile 1 patients also had the longest disease duration of the groups, and there was a modest trend for higher global disease damage scores in these patients with poor HDL function.

3.4. Association of myositis disease activity and DM diagnosis with dysfunctional HDL in IIM patients

Multivariate logistic regression analysis was performed to evaluate the association of myositis disease activity with HDL function in IIM patients after controlling for significant correlates of HDL function noted in univariate analyses as well as variables previously associated with HDL function (age and statin use). Higher physician global disease activity measured by VAS was significantly associated with dysfunctional HDL (tertile 1 HII) when compared to least dysfunctional HDL (tertile 3) after controlling for age, statin use, DM diagnosis, disease duration, and MTX use (Table 5). A second multivariate model was performed to assess the relationship of abnormal HDL function with myositis disease activity measured by CPK levels. Higher CPK levels were also significantly associated with dysfunctional HDL (tertile 1 HII) after controlling for the same covariates. The association between dysfunctional HDL (higher HII) and higher disease activity remained significant in multivariate linear models including all patients in the expanded disease cohort with HII as a continuous variable (supplementary table).

In all models described above, the diagnosis of DM was significantly associated with more dysfunctional HDL (tertile 1 HII for logistic model, higher HII for linear model) after controlling for myositis disease activity measured by physician global disease assessments or CPK levels, as well as other variables including age, statin use, disease duration, and MTX use. A diagnosis of DM conferred a 4-5 fold risk of dysfunctional HDL (tertile 1 HII) compared to a diagnosis of PM in the multivariate logistic model (Table 5).

3.5. Association of myositis autoantibodies with HDL function

111/182 IIM patients had myositis antibody profiles available for review at the time of this analysis. HDL function was compared between patients grouped by autoantibody status (Table 6). Patients with no autoantibodies had the lowest HII values consistent with the most antioxidant HDL. In contrast, patients with anti-MDA5 and anti-synthetase antibodies had the highest mean HII values consistent with the worst HDL anti-oxidant function. Anti-synthetase ab had significantly higher mean HII compared to patients with no autoantibodies (p=0.02) while MDA5 group had a smaller sample size and did not reach statistical significance (p=0.09). Other MSA/MAA group also had significantly higher mean HIIs compared to patients with no autoantibodies (p=0.02).

Chapter 4. Discussion

IIM are inflammatory diseases of the muscle, which may be associated with severe, multi-organ damage and high morbidity and mortality (14). Marked activation and damage to small blood vessels in connective tissue of the muscle, skin, lung, and gastrointestinal tract (1) occurs in DM, which is considered by most to be a systemic vasculopathy. While the involvement of the microvasculature is most severe in DM patients, activated capillaries with increased adhesion molecule expression are also present in patients with PM and IBM (15). The mechanisms for ongoing capillary activation, damage and drop-out in DM, and to a lesser extent other IIM, are currently not well understood.

Normal HDL protects the vascular endothelium from damage due to oxidized phospholipids, which may accumulate under conditions of oxidative stress such as atherosclerosis, as well as viral infections, and chronic inflammatory conditions such as rheumatoid arthritis (RA) (16, 17). However, during acute and chronic inflammatory states, HDL may become dysfunctional (13, 18). Such non-protective "pro-inflammatory" HDL has been shown to promote, rather than prevent vascular inflammation and damage, and associates with increased atherosclerotic risk (19-21).

In the current work, HDL from IIM patients was dysfunctional with impaired capacity to prevent lipid oxidation compared to healthy controls. This difference remained significant after multivariate adjustment for other patient factors, which were different between IIM patients and controls. MPO activity was also higher in DM and PM patients compared to healthy controls, with the highest levels noted in DM patients. Higher plasma activity of MPO associated with worse HDL function.

MPO is a peroxidase enzyme, which is abundantly expressed in leukocytes such as neutrophils, and generates hypochlorous acid, which is cytotoxic to bacteria, but also causes oxidative damage to host tissues. Previous work has shown that MPO oxidizes HDL's major protein, apolipoprotein AI (apoAI), generating a dysfunctional HDL particle (22). MPO levels have

9

been strongly linked to acute coronary events in the general population (23, 24), however, no work to date has previously investigated MPO and HDL's anti-oxidant function in patients with IIM. In the current work, higher plasma MPO activity remained significantly associated with dysfunctional HDL in IIM patients after multivariate adjustment. Further investigation of the relationship between MPO, HDL dysfunction and microvascular disease in larger IIM cohorts may be warranted.

Oxidation products of arachidonic acid (HETEs) and its precursor linoleic acid (HODEs) are bioactive lipid mediators produced by enzymes such as MPO under conditions of oxidative stress (25). In the vascular endothelium, HETEs and HODEs contribute to monocyte and macrophage differentiation (26) as well as to the increased expression of adhesion molecules and release of inflammatory, chemotactic and pro-thrombotic mediators (27). Previous work has linked these lipid mediators to the development of atherosclerosis (6), and work suggests that their accumulation in HDL contributes to HDL dysfunction (5, 28).

In the current study, 5-HETE, 15-HETE, 9- HODE, and 13-HODE levels were higher in HDL from patients with IIM compared to healthy controls. Higher plasma MPO activity in IIM patients correlated with higher HDL-associated HETEs and HODEs, and 5-HETE in particular was significantly correlated with impaired anti-oxidant capacity of HDL. IIM patients with ILD had trends for higher 5-HETE levels in HDL compared to IIM patients without ILD, and higher 5-HETE correlated with more severe lung disease measured by a lower DLCO. Work by Bittleman and colleagues has previously demonstrated that 5-HETE may play an important role in neutrophil rich lung inflammatory responses such as asthma and alveolitis associated with pulmonary fibrosis (29). 12-HETE was also numerically higher in IIM patients, particularly in IIM patients with ILD. 12-HETE is another known pro-inflammatory chemoattractant for neutrophils that was shown to be a key mediator of vascular permeability in acute lung injury mouse models (30-32).

DM diagnosis was associated with worse HDL function compared to PM or IBM diagnosis after multivariate analysis of a cross sectional cohort of 182 IIM patients. Higher disease activity

measured by physician global assessment and CPK levels was also associated with abnormal HDL function after multivariate adjustment. Work by Zheng et al. previously reported that the MPO-induced oxidation of HDL may occur primarily in the artery wall rather than in circulation due to the multiple "anti-oxidant" pathways in circulation (33). We hypothesize that the relatively predominant vascular inflammation in DM patients compared to PM patients may predispose them to site–specific oxidation of apoAI in the skin and muscle microvasculature, contributing to HDL dysfunction.

Finally, when HDL function was analyzed by autoantibody group, patients with either MDA5 or anti-synthetase antibodies had the highest mean HIIs, consistent with worst HDL function. IIM patients without antibodies had the most anti-inflammatory, protective HDL, which was significantly better in function compared to patients with anti-synthetase antibodies and other MSA. While this data must be interpreted as pilot in nature, we hypothesize that these myositis autoantibodies may be surrogate markers of more severe disease, which associate with HDL dysfunction. Interestingly, MDA5 and anti-synthetase antibodies are also markers of myositis ILD, and MDA5 has been specifically linked to vascular dysfunction (34). Further work is ongoing to confirm these findings.

There are some limitations to the current work. Our data is from an observational cohort of a single center and further prospective studies in other IIM cohorts with additional patients are needed. It is important to recognize that with limited prior knowledge of HDL function in IIM, univariate and multivariable analyses were meant to be hypothesis generating, and covariate searching was exploratory in nature. Many comparisons were made and significance level was not adjusted for multiple hypothesis testing. Further longitudinal and mechanistic studies are in progress to validate our findings. Future studies will examine specific pathways by which HDL is altered in active IIM patients, and determine the consequences of dysfunctional HDL to disease progression including ILD. Immune mediated necrotizing myopathy (IMNM) has been recently identified as a unique disease entity (35). In the current work, patients with IMNM were not

11

analyzed separately given small numbers. Studies with larger numbers of patients in IMNM and specific MSA subgroups are needed.

In conclusion, the current work is the first study to characterize HDL's anti-oxidant function in a large cohort of patients with IIM. The protective function of HDL in IIM patients was abnormal compared to non-myositis controls, and associated with higher disease activity and DM diagnosis. Higher circulating MPO activity associated with accumulation of oxidized fatty acids in HDL, and worse HDL function. The current data may suggest a mechanism for propagation of microvascular damage seen in IIM through failure of HDL to metabolize pro-inflammatory, oxidized lipids. Further prospective studies are necessary to determine the specific role of dysfunctional HDL particles in IIM and IIM-associated ILD. Better understanding of disease pathogenesis may lead to development of alternative, targeted therapeutics. Synthetic apoA-1 mimetic peptides, which bind oxidized fatty acids, attenuate atherosclerosis and lung injury in animal models and have shown to improve HDL function in humans (28, 36). Our results suggest that the use of such therapeutic agents that can reduce vascular damage may warrant further investigation in patients with IIM.

Chapter 5. Tables and Figures

Table 1. HDL function in IIM patients and healthy controls (*IIM/HC comparison cohort, n=136*)

	DM (N=55)	PM (N=30)	IBM (N=10)	Control (N=41)
HII*	$1.09 \pm 0.49^{\dagger}$	$1.20 \pm 0.84^{\dagger}$	$1.05 \pm 0.31^{\dagger}$	0.82 ± 0.13
Age, years	48 ± 15	54 ± 13	65 ± 14	49 ± 14
Gender, Female n(%)	43(78)	20(67)	6(60)	28(68)
Race, n(%)*				
White	49(89)	16(54)	7(70)	32(78)
Black	2(4)	10(33)	2(20)	0(0)
Asian	4(7)	4(13)	1 (10)	9(22)
Ethnicity, n(%)				
Hispanic	16(29)	3(10)	1(10)	11(27)
Hs-CRP (mg/L)*	$7.8 \pm 11.3^{\dagger}$	$6.7 \pm 9.0^{\dagger}$	$1.8 \pm 2.4^{\#}$	2.7 ± 3.8
ESR (mm/hr)*	$29 \pm 29^{\dagger}$	$30 \pm 20^{*\dagger}$	25 ± 21	12 ± 13
CPK(U/L)	325 ± 795	573 ± 759	474 ± 508	-
Lipid panel (mg/dL)				
Total cholesterol*	199 ± 41	$215 \pm 61^{\dagger}$	186 ± 46	191 ± 33
LDL cholesterol	114 ± 36	117 ± 46	95 ± 40	109 ± 28
HDL cholesterol	55 ± 18	62 ± 29	55 ± 13	58 ± 19
Triglycerides*	$157 \pm 95^{\dagger}$	$186 \pm 146^{\dagger}$	$175 \pm 89^{\dagger}$	123 ± 82
CVD risk factors, n(%)				
History of MI	1(2)	1(3)	0	0
Hypertension*	13(24)	10(33)	5(50)	8(20)
Diabetes*	5(9)	8(27)	3(30)	1(2)
Ever smoker	1(2)	2(7)	1(10)	1(2)
Family history of MI	5(9)	3(10)	1(10)	3(7)
Body mass index	31 ± 8	30 ± 8	25 ± 4	27 ± 7
Statin use	5(9)	2(7)	2(20)	2(5)
MPO (ng/ml)*	$14 \pm 9^{\dagger}$	$13 \pm 10^{\dagger}$	11 ± 7	9 ± 5
Medications n(%) use				
Methotrexate	16(29)	5(20)	0	-
TNF inhibitor	3(5)	2(6)	0	-
Leflunomide	2(4)	1(3)	0	-
Mycophenolate	9(16)	9(30)	0	-
Azathioprine	9(16)	7(23)	0	-
Hydroxychloroquine	12(22)	6(20)	1(10)	-
Immunoglobulins	13(24)	5(17)	0	-
Rituximab	1(2)	4(13)	0	-
Cyclophosphamide	4(7)	2(6)	0	-
Prednisone	37(67)	27(90)	5(50)	-
Prednisone dose	14 ± 16	21 ± 27	6 ± 7	-
ILD, n(%)	18(33)	14(47)	0(0)	-

Values are mean ± standard deviation unless specified. T test (or Wilcoxon test for nonparametric variables), or chi square test for categorical variables were performed between IIM and HC. For

covariates that were significant, further pairwise testing within different IIM subtypes were performed.

* p<0.05 IIM compared to HC and further testing within different IIM subgroups performed

† p<0.05 compared to HC

#p<0.05 compared to PM

Abbreviations: HII: HDL inflammatory index, hs-CRP: high sensitivity C-reactive protein, ESR: sedimentation rate, CPK: creatine phosphokinase, MPO: myeloperoxidase, CVD: cardiovascular disease, MI: myocardial infarction. Patients with anti-HMCGR or anti-SRP mediated immune mediated necrotizing myopathy (IMNM) were included as DM (N=2) or PM (N=11).

Table 2. Linear regression analysis of variables associated with HDL function (HII) (IIM/HC comparison cohort, n=136)

Predictor variable	Univariate analysis		Multivariate analysis	
	Regression coefficient (95%CI)	P value	Regression coefficient (95%CI)	P value
IIM diagnosis(vs HC)	0.102(0.046,0.159)	**<0.001**	0.119(0.004, 0.234)	**0.04**
Age, 10 years	0.01(-0.1,0.02)	0.31	0.011(-0.024,0.048)	0.52
Sex, Female (vs male)	-0.007(-0.07,0.06)	0.83	-0.053(-0.171,0.064)	0.37
Race, White	-0.03(-0.09,0.03)	0.31	-	
Ethnicity, Hispanic	0.004(-0.08,0.09)	0.92	-	
Body Mass Index	0.002(-0.002,0.005)	0.33	-	
Hypertension, yes	0.028(-0.333,0.090)	0.36	-	
Diabetes, yes	0.037(-0.044,0.118)	0.37	-	
Ever Smoker, yes	0.141(0.006,0.276)	**0.04**	0.313(0.072,0.554)	**0.01**
H/o MI, yes	0.131(-0.076,0.338)	0.21	-	
Statin, yes	0.101(-0.112,0.314)	0.35	0.153(-0.049,0.355)	0.14
Total cholesterol, 10mg/dl	0.000(-0.006,0.007)	0.90	-	
LDL-C, 10mg/dl	-0.003(-0.010,0.004)	0.45	-	
HDL-C, 10mg/dl	-0.008(-0.021,0.005)	0.25	-	
Triglycerides, 10mg/dl	0.004(0.001,0.007)	**<0.001**	-0.001(-0.008,0.005)	0.65
ESR,10mm/hr	0.011(-0.001,0.022)	0.07	-	
hsCRP, log mg/L	0.03(-0.005, 0.07)	0.09	-	
MPO, ng/ml	0.007(0.004,0.010)	**<0.001**	0.013(0.006, 0.020)	**<0.001**

HII and hsCRP were log transformed
Abbreviations: HC: healthy controls, h/o MI: history of myocardial infarction, LDL-C: LDL cholesterol, HDL-C: HDL cholesterol, MPO: myeloperoxidase

Table 3. Oxidized fatty acid levels in HDL from IIM patients and HC

	IIM (N=24)		HC (N=12)
Age, years	50(8)		48(13)
Sex, female n(%)	17(71)		8(67)
Race, White n(%)	14(58)		10(53)
Ethnicity, Hispanic n(%)	7(29)		5(42)
Hs-CRP (mg/L)	10.8 (11.7)*		0.6(0.5)
ESR (mm/hr)	46(24)*		6(7)
CV risk factors			
History of MI n(%)	1(5)		0(0)
Hypertension n(%)	8(47)*		1(8)
Diabetes n(%)	5(28)		0(0)
Ever smoker n(%)	2(14)		0(0)
FHx of MI n(%)	4(27)*		0(0)
BMI	33±9*		24±4
Statin use n(%)	1(4)		0(0)
Lipid panel (mg/dL)			
Total cholesterol	197±45		184±28
LDL cholesterol	118±44		112±25
HDL cholesterol	59±29		55±13
Triglycerides	137±57*		90±39
HII	1.34±0.56*		0.80±0.15
MPO (ng/ml)	19±9*		8±3
HETE/HODE (pg/75µg HDL-C)			
5-HETE	48792±28922*		12721±4493
12-HETE	698±650*		321±234
15-HETE	27±21*		12±9
9-HODE	161±148*		57±97
13-HODE	390±279*		158±111
	ILD (N=14)	**No ILD (N=10)**	
5-HETE	54968 ± 25630	40147 ± 32351	
12-HETE	938 ± 766**	362 ± 136	
15-HETE	30 ± 24	22 ± 14	
9-HODE	133 ± 87	200± 205	
13-HODE	481 ± 279**	263 ± 236	
	DLCO <50% predicted (N=7)	**DLCO ≥ 50% predicted (N=7)**	
5-HETE	65414±22488	44521±25747	
12-HETE	1102 ± 603	772 ± 918	
15-HETE	26 ± 16	35 ± 31	
9-HODE	115 ± 56	183 ± 126	
13-HODE	488 ± 304	475 ± 276	

Values reported in mean ± SD unless specified
Comparing means tested in HETEs/HODEs
*p<0.05 **p<0.1

Table 4 Clinical data of IIM patients by tertiles of HDL anti-inflammatory function (*Expanded disease specific cohort*, n=182)

	Tertile 1 (n=61)	Tertile 2 (n=61)	Tertile 3 (n=60)
HDL Inflammatory Index (HII)	1.21 ± 0.62	0.53 ± 0.09	0.23 ± 0.05
Age, years	50 ± 14	51 ± 15	51 ± 15
Gender, n(%) Female	44(72)	42(69)	44(73)
Race, n(%) White	43(72)	49(83)	44(73)
Ethnicity, n(%) Hispanic	13(22)	11(18)	10(17)
ESR (mm/hr)	34 ± 29	28 ± 24	27 ± 25
Hs-CRP (mg/L)	7.7 ± 12.0	4.7 ± 8.3	6.5 ± 9.7
Lipid panel (mg/dL)			
Total Cholesterol	209 ± 50	210 ± 52	205 ± 50
LDL Cholesterol	121 ± 44	128 ± 46	113 ± 3
HDL Cholesterol	59 ± 24	57 ± 20	58 ± 19
Triglycerides	182 ± 137	156 ±93	169 ± 124
CVD risk factors, n(%)			
History of MI, yes	1(2)	1(2)	2(3)
Hypertension	16(26)	18(30)	17(28)
Diabetes	7(11)	7(11)	10(17)
Ever Smoker	11(18)	19(32)	12(20)
Body Mass Index, kg/m^2	27.7 ± 6.3	27.8 ± 6.5	27.6 ± 5.8
Statin use	7(11)	5(8)	8(14)
IIM characteristics			
Disease Duration, years	4.5 ± 7.8*	3.6 ± 7.4	4.3 ± 5.0†
DM Disease Diagnosis, n(%) DM	46(75)	46(75)	30(50)#
CPK, (U/L)	867 ± 1989†	405 ± 1123	562 ± 1199
Physician Global Activity (VAS 0-100mm)	43 ± 21*	40 ± 18	34 ± 17
Physician Global Activity (Likert 1-5)	1.89 ± 0.95	1.69 ± 0.73	1.56 ± 0.65
Physician Global Damage (VAS 0-100mm)	37 ± 23	31 ± 25	34 ± 21
Physician Global Damage (Likert 1-5)	1.63 ± 0.96	1.40 ± 1.11	1.51 ± 0.85
ILD, n(%) present	23(47)	14(28)	20(47)
Medications, n(%) use			
Rituximab	6(10)	3(5)	3(5)
Cyclophosphamide	4(7)	4(7)	2(3)
Hydroxychloroquine	11(18)	11(18)	17(28)
Immunoglobulins	13(21)	14(23)	11(19)
Mycophenalate	17(28)	11(18)	15(25)
Prednisone	43(70)	39(64)	45(76)
Prednisone dose (daily)	19 ± 24	15 ± 19	15 ± 18
Methotrexate	11(18)	22(36)	11(19)#
Leflunomide	1(1)	0(0)	3(5)
Azathioprine	7(12)	3(5)	11(19)

Values reported are Mean ± SD unless specified. P value by t test or Wilcoxon test for continuous variables, chi-square for categorical variables.

*p < 0.05 compared to tertile 3

†p <0.05 compared to tertile 2

#p <0.05 by Chi-square test

Table 5. Multivariate logistic regression models of dysfunctional HDL (tertile 1 vs tertile 3 HII)

Predictor Variable	Model 1	Model 2
Age, years	1.009 (0.98-1.04)	1.004 (0.98-1.03)
Disease duration, years	1.03 (0.96-1.10)	1.02(0.95-1.09)
DM diagnosis (vs PM)	3.73 (1.51-9.78)*	4.9 (1.7-15.8)*
MTX use	0.84 (0.30-2.34)	0.84 (0.29-2.39)
Statin use	0.43 (0.11-1.64)	0.94 (0.24-3.78)
Physician Global Disease Activity VAS (0-100mm), 10mm	10.67 (1.60-71.22)*	-
CPK level, 10 U/L	-	1.002 (0.999-1.006)*

Values presented are unit odds ratios and 95% confidence intervals
*p<0.05
Due to collinearity between VAS and CPK we constructed separate models for each outcome measure. Model 1: Physician global disease activity in visual analog scale (VAS), Model 2: CPK

Table 6. HDL function in IIM by autoantibody subgroups

Antibody group	N	HII, Mean ± SD
Anti-synthetase ab	20	0.93±0.81*
MDA5 ab	9	0.95±1.02
HMGCR ab/SRP ab	15	0.72±0.66
Unidentified ab	10	0.71±0.33
Other MSA/MAA	47	0.70±0.28*
No autoantibody	10	0.46±0.23

*p<0.05 compared to No autoantibody group by Wilcoxon test. Other MSA (myositis specific antibodies)/MAA(myositis associated antibodies) included Mi2, MJ, SSA, PM-SCL, and TIF1 γ

Supplementary Table. Multivariate linear regression models of dysfunctional HDL (log[HII])

| Predictor Variable	Model 1	Model 2
Age, 10 years	0.026 (-0.050, 0.101)	0.014 (-0.061,0.089)
Disease duration, years	0.009 (-0.007,0.026)	0.006 (-0.009,0.022)
DM diagnosis, yes	0.261 (0.025,0.498)*	0.388 (0.118,0.658) *
MTX use	-0.065 (-0.316,0.185)	-0.071 (-0.3190.178)
Statin use	-0.281 (-0.641,0.079)	-0.064 (-0.432,0.305)
Physician Global Disease Activity VAS (0-100mm), 10mm	0.079(0.022,0.137)*	-
CPK, Log U/L	-	0.091 (0.007,0.177)*

*p<0.05, outcome variable (HII) and CPK was transformed to log scale

Due to collinearity between VAS and CPK we constructed separate models for each outcome measure. Model 1: Physician global disease activity in visual analog scale (VAS), Model 2: CPK level
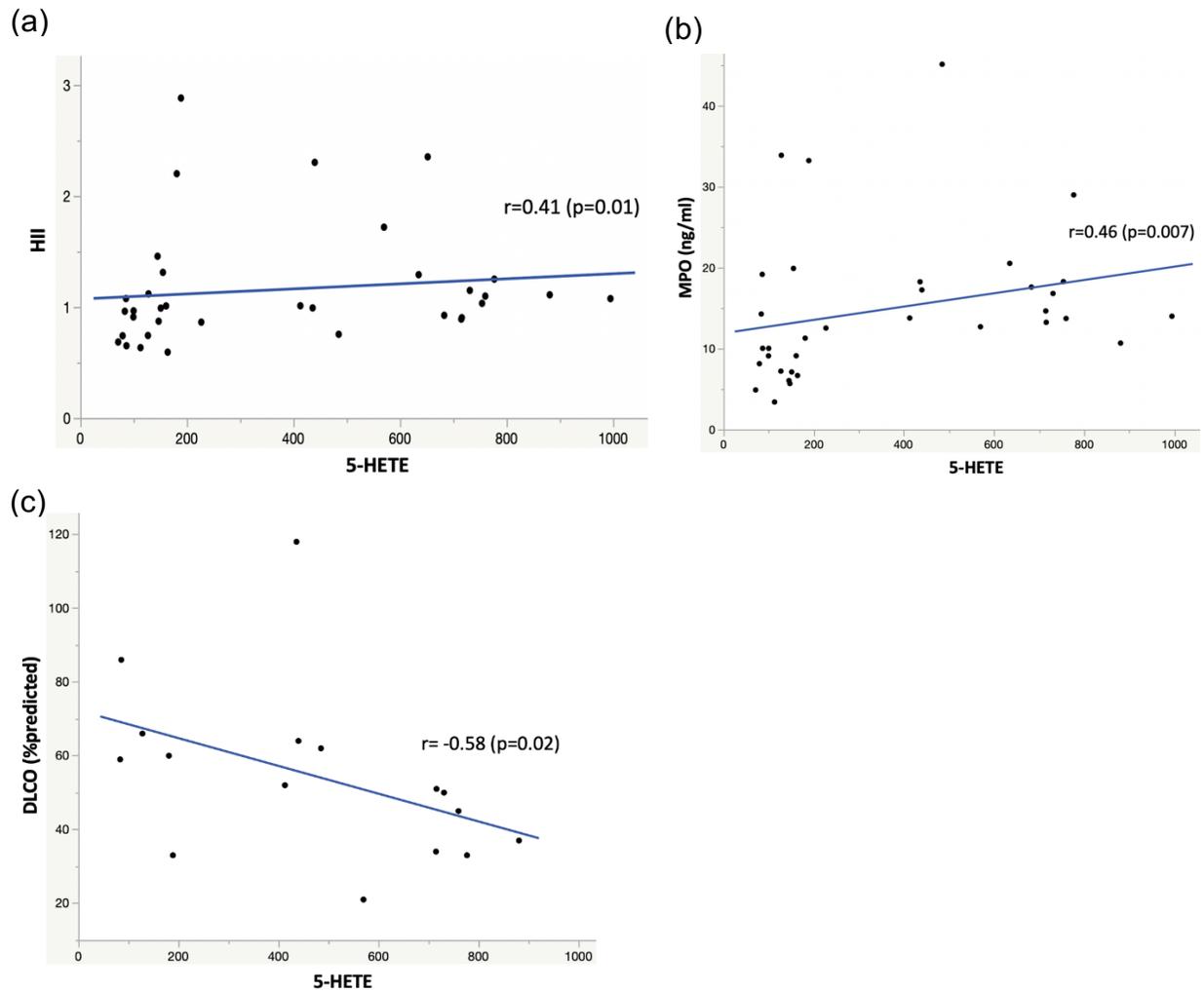
Figures
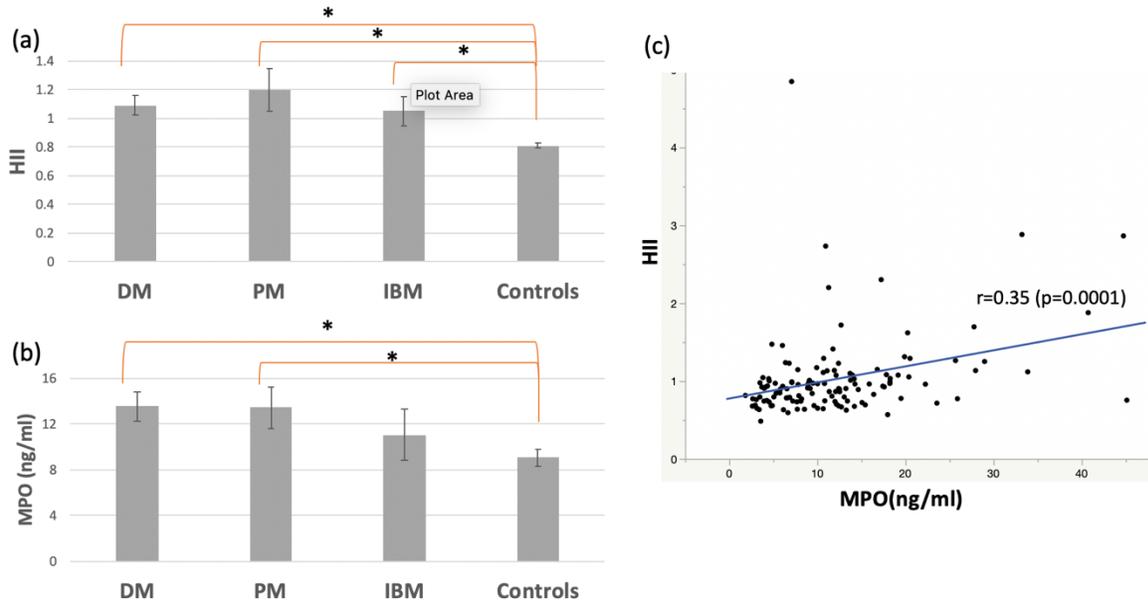
Figure 1. (a) HDL function (HDL inflammatory index; HII) in IIM (55 DM, 30 PM, 10 IBM) and controls (n=41), Mean±SE (b) Myeloperoxidase (MPO) in IIM and controls, Mean±SE, (c) correlation between HII and MPO, *P<0.05
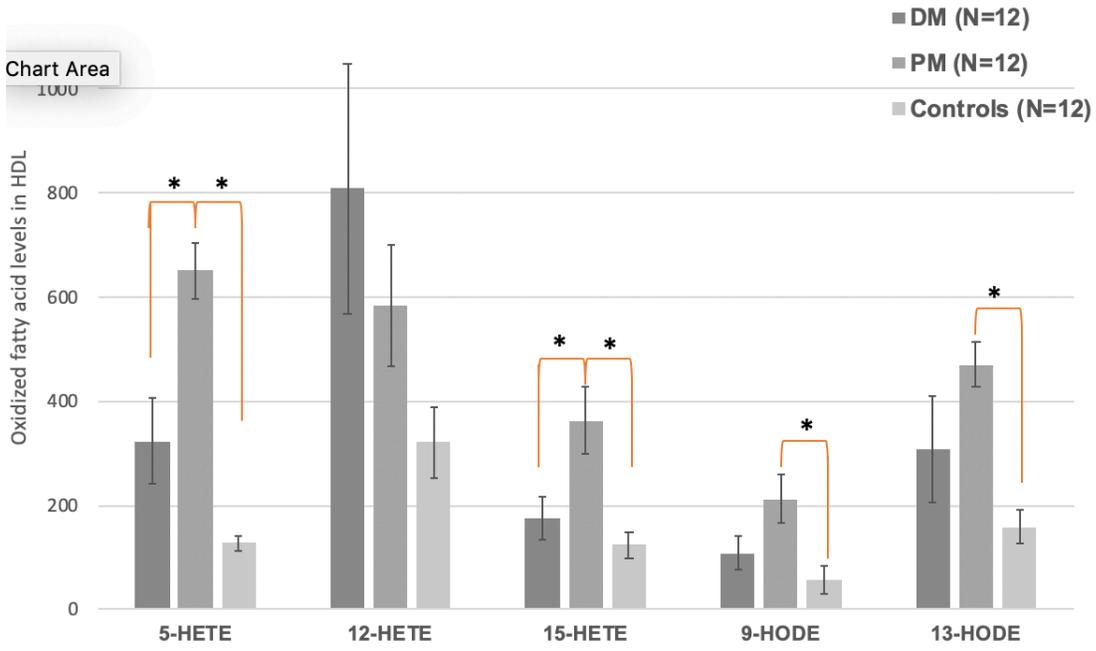
Figure 2. Oxidized fatty acid levels in HDL from IIM patients and healthy controls.

*P<0.05

Figure 3. Correlations with HDL associated 5-HETE and (a) HII; HDL inflammatory index, (b) MPO (Myeloperoxidase) and (c) diffusion capacity (DLCO)

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