

Oxidation Products of Arachidonic Acid and Linoleic Acid Are Increased in High Density Lipoprotein and Low Density Lipoprotein From Patients with Active Rheumatoid Arthritis

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Rheumatoid Arthritis and CVD

- RA Associated with Significantly Increased CV Morbidity and Mortality
- Adjustment for CHD Risk Factors Including Dyslipidemia Does Not Fully Explain the Increased Atherosclerosis.

Sattar et al, *Circulation* 2003, Gonzalez-Gay et al, *Seminars Arthritis Rheum* 2005

RA Disease Activity and CV Risk

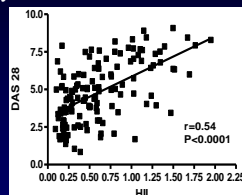
- High Levels of Systemic Inflammation Associated with CV Death in Patients with RA.
- Inflammatory Markers (ESR/CRP) Associated with Subclinical Atherosclerosis in RA.

Del Rincon I et al, *Arthritis Rheum* 2003, Maradit-Kremers et al, *Arthritis Rheum* 2004, 2005

Abnormal HDL Function in RA

- Impaired Anti-oxidant Capacity of HDL Significantly Associated with Disease Activity:

→ Higher Disease Activity
= Worsened Anti-Oxidant
HDL Function



Charles-Schoeman et al, *Arthritis Rheum*, 2009 Oct;60(10):2870-9.

Oxidative Stress Increased in RA

- Oxidative enzyme activity increased in RA sera and synovial fluid (SF).
- ROS-Direct damage to RA cartilage.
- Increased lipid peroxidation products in RA SF: oxidized low-density-lipid proteins (LDL) and others.

Hitchon et al., *Arth Care and Research* 2004.

Oxidative Stress and Atherosclerosis

- Oxidation products of arachidonic acid and linoleic acid:
 - Free Oxidized Fatty Acids:
 - Hydroxyeicosatetraenoic acids (HETES)
 - Hydroxyoctadecadienoic acids (HODES)
 - Accumulation in high density lipoprotein (HDL) has been proposed to inhibit HDL function, increasing atherosclerotic risk.
 - Contribute to the oxidation of LDL.

Imaizumi et al. Drug Metab Lett. 2010; 4(3):139-48), Morgantini et al. Diabetes. 2011; 60(10): 2617-23

Aim:

- To Evaluate the Levels of HETES and HODES in HDL and LDL Isolated from Patients with Active Rheumatoid Arthritis (RA) Compared to Healthy Controls.

Hypothesis:

- HETES and HODES are Increased in HDL and LDL from Active RA Patients and are Associated with Abnormal HDL Anti-oxidant Function.

Methods

- Fast Protein Liquid Chromatography:
 - Isolation of HDL and LDL from Plasma
 - 10 Active RA patients
 - 8 Age and Sex Matched healthy controls
- Mass Spectrometry of HDL and LDL Fractions:
 - 5-HETE 9-HODE
 - 12-HETE 13-HODE
 - 15-HETE

Imaizumi et al. Drug Metab Lett. 2010; 4(3):139-48),

Methods

- HDL's Anti-oxidant Function
 - Measured by a cell free assay which assesses the ability of patient HDL to inhibit oxidation of a stock LDL.
- Lipoprotein Cholesterol Levels
 - Determined by standard methods.

Charles-Schoeman et al. Arthritis Rheum. 2009 Oct;60(10):2870-9.

Patient Clinical Characteristics

	Rheumatoid Arthritis (n=10)	Healthy Control (n=8)
Age (years)	49.6 ± 11.8	48.4 ± 15.9
Sex (% female)	80%	75%
Ethnicity (% caucasian)	80%	63%

Total Cholesterol (mg/dL)	178 ± 52	165 ± 22
LDL Cholesterol (mg/dL)	104 ± 35	89 ± 22
HDL Cholesterol (mg/dL)	52 ± 15	56 ± 14
Triglycerides (mg/dL)	116 ± 59	101 ± 84

Patient Clinical Characteristics

	Rheumatoid Arthritis (n=10)	Healthy Control (n=8)
ESR (mm/hr)	71 ± 24*	6 ± 5
HS-CRP (mg/L)	42.1 ± 40.1*	0.91 ± 1.5
HDL Inflammatory Index (HII)	2.21 ± 0.52*	0.56 ± 0.09

* p<0.05

Free Oxidized Fatty Acids Are Increased in HDL from Active RA Patients

	Rheumatoid Arthritis (n=10)	Healthy Control (n=8)
HDL 5-HETE (ng/ml plasma)	14.4 ± 5.8*	5.8 ± 2.6
HDL 12-HETE (ng/ml plasma)	155.9 ± 338.6	33.3 ± 8.0
HDL 15-HETE (ng/ml plasma)	3.8 ± 2.2*	1.4 ± 0.5
HDL 9-HODE (ng/ml plasma)	27.7 ± 14.8*	9.5 ± 5.7
HDL 13-HODE (ng/ml plasma)	38.2 ± 19.9*	13.7 ± 6.1

All values mean ± standard deviation. *p value ≤ 0.005 compared to controls.

Free Oxidized Fatty Acids Are Increased in LDL from Active RA Patients

	Rheumatoid Arthritis (n=10)	Healthy Control (n=8)
LDL 5-HETE (ng/ml plasma)	0.58 ± 0.28*	0.22 ± 0.14
LDL 12-HETE (ng/ml plasma)	7.6 ± 9.5	3.4 ± 3.1
LDL 15-HETE (ng/ml plasma)	0.47 ± 0.17*	0.21 ± 0.14
LDL 9-HODE (ng/ml plasma)	5.3 ± 3.6*	1.8 ± 1.4
LDL 13-HODE (ng/ml plasma)	6.8 ± 4.5*	2.3 ± 1.6

All values mean ± standard deviation. *p value ≤ 0.005 compared to controls.

Correlations of HDL HETES/HODES with Systemic Inflammation and HDL's Anti-Oxidant Properties

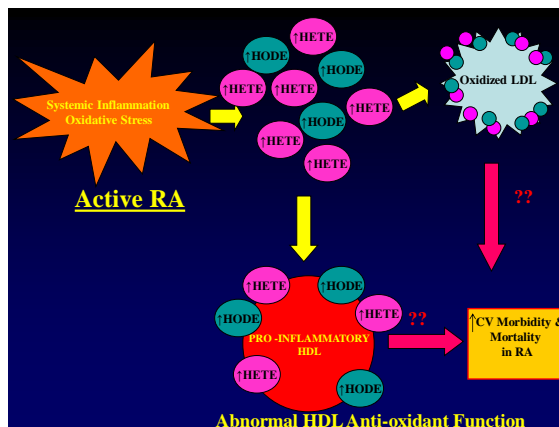
- Higher ESR → Higher HDL HETES/HODES:
r values= 0.70-0.80, p values <0.004
- Higher hsCRP → Higher HDL HETES/HODES:
r values= 0.65-0.74, p values <0.004
- Higher HDL HETES/HODES → Higher HII:
r values= 0.54-0.58; p values <0.03

Correlations of LDL HETES/HODES with Systemic Inflammation

- Higher ESR → Higher LDL HETES/HODES:
r values= 0.30-0.48, p values 0.006-0.05
(5HETE/ESR, r= 0.30, p=0.25)
- Higher hs-CRP → Higher LDL HETES/HODES:
r values= 0.34-0.62, p values 0.006-0.02
(15HETE/hsCRP, r= 0.34, p=0.16)

Conclusions

- Oxidized Fatty Acids (HETES/HODES) are Increased in HDL and LDL from Patients with Active Rheumatoid Arthritis compared to Healthy Controls.
- HETES/HODES are Strongly Correlated with Levels of Systemic Inflammation, Particularly in HDL.
- Elevations of 5-HETE, 15-HETE, 9-HODE and 13-HODE in HDL are also Significantly Associated with Impaired HDL Anti-oxidant Function.



Study Limitations:

- Pilot Study-Small Number of Patients.
→Confirmation of Initial Findings in Larger RA Cohorts.
- Need Direct Link of Potential Atherogenic Pathways to Cardiovascular Outcomes in RA.

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