C-TERMINAL TRUNCATION OF APOLIPOPROTEIN A1 GLUTAMATE RESIDUE 243 IS A BIOMARKER FOR OXIDATIVE STRESS IN CORONARY ARTERY DISEASE AND CHRONIC KIDNEY DISEASE

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Dedications

To my parents, Jeff Wilson, Tina Myers, Barry Myers and Annie Wilson, my sister Rachel Myers and my wife Kelly Wilson, thank you for supporting me in all that I do. To all my teachers and mentors thank you for showing me the value of lifelong learning.

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Conflicts of Interest

The authors declare that there are no conflicts of interest with this study.

Abstract

Background:

High density lipoprotein (HDL) oxidation is a potential biomarker for coronary artery disease (CAD) severity. Methionine sulfoxidation, tyrosine chlorination and C-terminal truncation are Apo A- I modifications that inactivate HDL and lead to pro-oxidant action. We hypothesize that C-terminal truncation of apolipoprotein A1 glutamate residue 243 (Apo A-I Des-Q243) is a byproduct of a protease, such as a matrix metalloprotease (MMP), and it is associated with the presence and severity of coronary artery disease and chronic kidney disease (CKD).

Methods:

We enrolled 103 patients presenting for evaluation of chest pain in this cross-sectional study at Maricopa Medical Center. Plasma and serum samples were collected, processed, and transferred to Arizona State University (ASU) Biodesign Institute for high pressure liquid chromatography-mass spectrometry (HPLC-MS). A statistical analysis was conducted with a spearman's coefficient, two-tailed linear regression and multivariate analysis of the relative fractional abundance (RFA) of Apo A-I Des-Q243 and clinical variables.

Results:

Multivariate analysis revealed significantly reduced levels of Apo A-I Des-Q243 in the presence of male gender (-1.5%, P=0.035), atrial fibrillation (-2.8%, P=0.04), and ACEi/ARB use (-2.4%, P=0.001). Additionally, a diagnosis of CKD (2.3%, P=0.037) and the presence of four (9.6%, P=0.005) or five (4.7%, P=0.045) coronary stents, regardless of vessel location, were associated with significantly increased levels of Apo A-I Des-Q243. American Indian/Alaskan race as compared to Caucasian race (Plasma -5.8%, 95% CI -9.9- -1.8%, P=0.005; Serum -4.6%, 95% CI -8.5- -0.8%, P=0.02), and the eGFR (Plasma ρ=-0.024, P=0.014, Serum ρ=-0.291, P=0.003) only reached significance in the linear regression and spearman's correlation analysis respectively.

Conclusion:

Apo A-I Des-Q243 is elevated in patients with multiple coronary stents, and thus may be contributing to vascular inflammation and plaque formation. Furthermore, Apo A-I Des-Q243 is elevated in CKD and is directly correlated with its severity as determined by eGFR. These findings highlight the renin-aldosterone system's (RAS) role in HDL oxidation and the anti-oxidant action of ACEi/ARBs. Apo A-I Des-Q243 appears to be an important link between CAD and CKD and is a promising biomarker that warrants further study.

Highlights:

- Apo A-I Des-Q243 is hypothesized to be an oxidation product of a protease such as matrix metalloprotease (MMP).
- Apo A-I DesQ243 is increased in patients with multiple coronary stents.
- Apo A-I Des-Q243 is increased in patients with CKD and is negatively correlated with eGFR.
- Apo A-I Des-Q243 is decreased in patients on ACE/ARB therapy.

Table of Contents

List of Tables and Figures

Introduction

Atherosclerosis is one of the greatest health burdens in the modern world. It is the number one cause of all type mortality in the United States since 1921[1]. Oxidative stress plays a key role in the pathogenesis of atherosclerotic cardiovascular disease (ASCVD). Many of the "traditional" risk factors, e.g. diabetes mellitus (DM), obesity, hypertension (HTN), hyperlipidemia (HLD), smoking and alcohol consumption either directly or indirectly lead to higher levels of oxidative stress. Still, despite the evidence for the role of oxidation in ASCVD, the dominate treatment strategy involves modification of lipoprotein levels. Hyperlipidemia is, without a doubt, a major contributor to ASCVD because it provides the substrate required for plaque deposition. Although treatment of HLD with statin therapy led to significantly reduced cardiovascular events, there exists a subset of patients with "normal" lipid levels that present with worsening ASCVD and major adverse cardiac events[2]. Lipoprotein function and/or oxidation status seems to be a new risk factor and drug target in these patients.

High density lipoprotein (HDL), when functioning normally, prevents atherosclerosis. Normal HDL function is dependent on Apolipoprotein A-I (Apo A-I), the primary protein constituent of HDL (~95% of the protein in HDL is Apo A-I and to a lesser extent Apo A-II)[3]. The functions of HDL include: reverse cholesterol exchange, reduction of endothelial dysfunction, pro-antioxidant, anti-inflammatory and anti-apoptotic effects. In particular, reverse cholesterol exchange is an essential function of HDL. This involves removal of cholesterol from macrophage foam cells in the arterial cell walls and subsequent transport to the liver for disposal in the bile (Figure 1). This process requires proper interaction between ATP-binding cassette transporter A1 (ABCA1), ATP-binding cassette transporter G1 (ABCG1), lecithin: cholesterol acyltransferase (LCAT) and ApoA-1[4].

A review by Robert Rosenson and colleagues addresses the role of oxidation-induced high density lipoprotein (HDL) dysfunction in ASCVD[5]. They believe oxidized ApoA-1 no longer protects against atherosclerosis but paradoxically exhibits pro-atherosclerotic activity. This phenomenon was first described by the Van Lenten group in 1995[6]. In the study, HDL no longer prevented LDL from oxidation during an acute phase reaction in both human and rabbit models. Instead, HDL

increased release of pro-inflammatory molecules, such as serum amyloid A (SAA), from arterial cell walls.

There are several types of oxidative modifications of Apo A-I that can disrupt the normal function of HDL. These include methionine oxidation (Met(O)), tyrosine chlorination and/or C-terminal truncation. Myeloperoxidase (MPO), a heme protein expressed by macrophages in atherosclerotic lesions, produces hydrogen peroxide. This leads to reactive intermediates that modify methionine residues 86, 112, and 148 of Apo A-I[7]. Similarly, tyrosine residue 192 is an additional target of MPO. Both methionine oxidation and tyrosine chlorination of Apo A-I have been suggested as biomarkers given evidence of an association with increased ASCVD risk[8-9].

Of particular interest to our present study, is C-terminal truncation of Apo A-I glutamine residue 243. Apo A-I C-terminal truncation was first identified by three different groups using liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS)[10-12]. The truncation was identified in multiple patient samples, suggesting that it was a post-translational modification of Apo A-I that occurs with natural aging. It is likely caused by enzymatic degradation. To our knowledge no such enzyme has been identified based on the literature. Potential agents could include a chymase[13] or matrix metalloproteinase (MMP)[14] both of which have been found to cause C-terminal truncation and subsequent reduction in reverse cholesterol efflux (Figure 1). We believe Apo A-I Des-Q243 is an oxidation product of a protease and that it also causes HDL dysfunction[15-16].

HDL dysfunction and oxidation is elevated in individuals with ASCVD[21-23]. Therefore determining the extent of dysfunctional HDL could be a valuable marker of CVD risk and/or severity in addition to quantitative measurements of HDL cholesterol. The purpose of this study was to determine if Apo A-I Des-Q243 is a biomarker for oxidative stress and disease severity of CAD and/or CKD. Whether this extends to other inflammatory conditions, e.g. hypertension (HTN), congestive heart failure (CHF), diabetes mellitus (DM) and/or various types of cancer is yet to be determined.

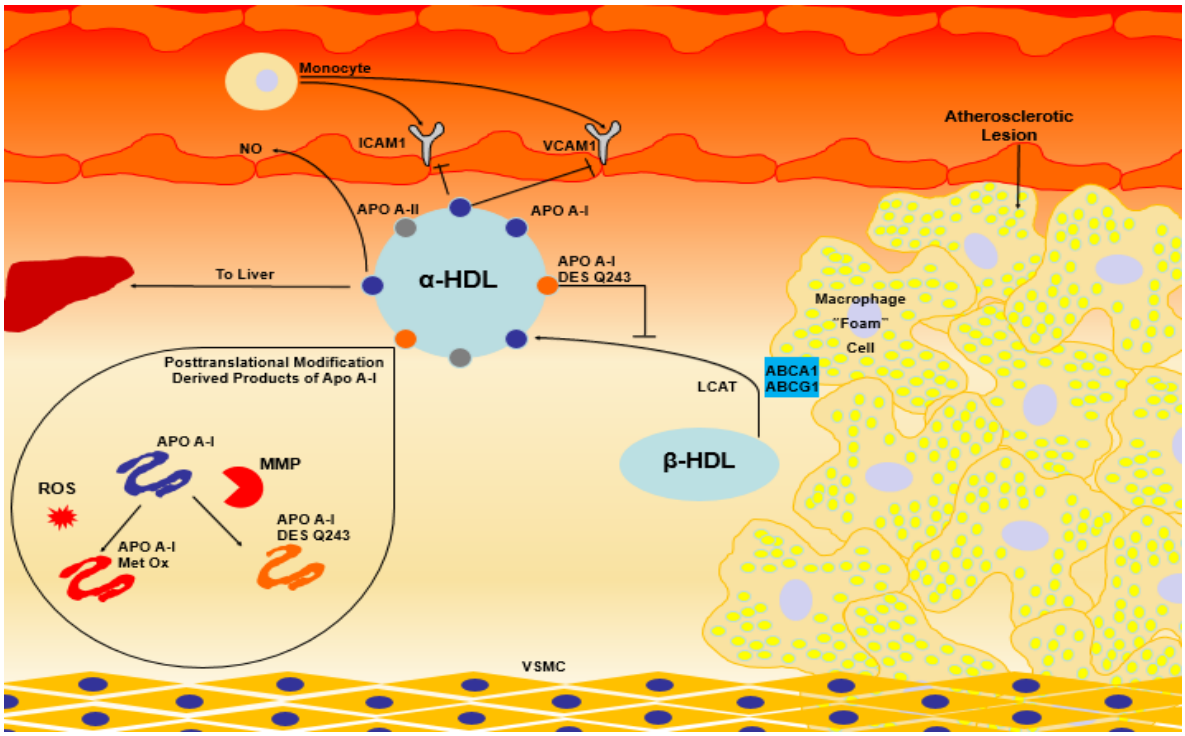

Figure 1: HDL and Apo A-I Normal Function and PTMDP in atherosclerotic lesions. HDL = high density lipoprotein, Apo A-I = Apolipoprotein A-I, ROS = Reactive Oxygen Species, MMP = Matrix Metalloproteinase, LCAT = Lectin-Cholesterol Acyltransferase, ABCA-1 = ATP-binding cassette A1, ABCG1 – ATP-binding cassette G1, NO = nitric oxide

Materials and Methods

Study Design, Patient Enrollment and Clinical Data Collection:

This is a single center cross-sectional study of 103 patients who presented for evaluation of CAD. The patients received a single diagnostic cardiac test or some combination of either an exercise stress test, nuclear perfusion scan, computed tomography coronary angiogram (CCTA), or coronary angiogram performed at Maricopa Medical Center (MMC). Patients were included in the study if their plasma and serum samples met our laboratory protocol specifications. Patients were excluded if they did not want to participate and/or sign the consent form, were pregnant, minors, prisoners and/or did not meet laboratory specifications for inclusion (e.g., hemolysis >50mg/dl, insufficient quantity, unacceptable deviations from lab protocol). Consent was gathered by either a resident physician, or clinical research coordinator for all patients as defined by the MMC institutional review board. Enrollment began in August of 2016 and was ongoing at the time of this analysis, reaching 103 patients during October of 2018. All clinical data were gathered up to the point of their current cardiovascular evaluation via the electronic medical record (Epic Systems, Verona, WI). Chart review was performed by two individuals and compared for fidelity.

Material and Reagents:

All non-LC-ESI-MS solvents were of HPLC grade. Refer to Borges et al for complete solvent list[24].

Sample Collection and Processing:

Patients received a single blood specimen collection of one 10mL serum tube and one pre-chilled 10mL K_2EDTA plasma using standard venipuncture procedure. Upon collection, tubes underwent 10 immediate inversions. Samples were then centrifuged at 2,000g for 20 minutes at 4°C either after clotting for 30 minutes for serum tubes, but no longer than 45 minutes after draw time, or immediately for plasma tube, but no later than 30 minutes after draw time. Each tube was then immediately aliquoted and stored at -80^{O}C (within -65°C to -90°C) within ~40 minutes of collection. Samples were stored for approximately 1-2 months and then sent for analysis at

Arizona State University (ASU) Biodesign Institute. Times of draw, centrifugation and placement under deep freeze were recorded for each sample. Sample hemolysis was determined by comparison to a color chart, minimal, mild, and moderate or greater hemolysis, ranging from < 20 mg hemoglobin/dL, 20-50 mg/dL and > 50 mg/dL, respectively.

Liquid chromatography-electrospray ionization-mass spectrometry:

LC-ESI-MS was gathered using the same technique as previously mentioned in Borges et al[24]. In brief, P/S samples are prepared for injection onto the LC-ESI-MS by 1000x dilution in 0.1% (v/v) TFA after incubation at 37 °C for 18 hrs. Apo A-I Des Q243 was gathered via LC-ESI-MS on a Dionex Ultimate 3000 HPLC equipped with a 1:100 flow splitter connected to a Bruker maXis 4G quadrupole-time-of-flight (Q-TOF) mass spectrometer. A trap-and-elute form of LC-MS was carried out in which 5 µL of sample was loaded and solubilized via a loading pump at 10 µl/min in 80% water containing 0.1% formic acid (Solvent A) / 20% acetonitrile (Solvent B) as per standard protocol. The mass spectrometer was set to operate in positive ion, Q-TOF-only mode, acquiring spectra in the m/z range of 300 to 3000. Relative fractional abundance (RFA) of apolipoprotein A-I Des-Q243 was determined by dividing the mass of Apo A-I Des-Q243 by unmodified Apo A-I. Levels of Apo A-I Met(O) were gathered simultaneously and reported as weighted relative fractional abundance (WRFA) ((1xMet(O)*1/3)+(2xMet(O)*2/3)+(3xMet(O)*1) /Total Fractional Abundance (TFA) of Apo A-I + all Met(O)) and weighted relative percent abundance (WRPA) (WRFA*100%).

Statistical Analysis:

Two-tailed linear regression was calculated for between Apo A-I Des-Q243 and the categorical clinical variables. Spearman's correlation was used to assess for any relationship between Apo A-I Des-Q243 and continuous clinical variables. All statistically significant results and those within ~5% were subsequently analyzed with multivariate regression.

Results

Patient demographics:

Of the 103 patients included in the analysis, a minority of patients were male (41.8%) with a mean age of 59.2 ± 9.1. The most common medical conditions were HTN (88.4%), HLD (77.7%), DM (49%) and CAD (44.7%) (Table 1a). CKD was present in 10.7% of the patients with a mean eGFR of 87.8 ± 30.2 (Table 1b); none of those patients were in end-stage renal disease or receiving hemodialysis. The most commonly prescribed medications were aspirin (72.8%), statins (79.6%), angiotensin converting enzyme/angiotensin receptor blockers (47.6%) and beta blockers (47.6%). The patients presented with a variety of cardiovascular complaints: no chest pain (24.5%), atypical chest pain (49%), typical chest pain (20.6%), unstable angina (1%) or NSTEMI (4.9%). None of the patients presented with a STEMI. The average ASCVD risk score was high (15.3±15.6). Fifty patients underwent coronary angiography showing a pattern of less severe disease with major stenosis in either one vessel (34%), two vessels (34%), three vessels (6%) or the left main coronary artery (6%).

Spearman's Correlation:

The spearman's correlation analysis (Table 2) revealed a statistically significant negative association between weight (Plasma ρ -0.306, P=0.002; Serum ρ=-0.323, P=0.001) and body mass index (BMI) (Plasma ρ=-0.214, P=0.03; Serum ρ= -0.258, P=0.009). EGFR was negatively associated with Apo A-I Des-Q243 (Plasma ρ=-0.024, P=0.014, Serum ρ=-0.291, P=0.003). No other variables reached statistical significance.

Linear Regression:

Table 3 demonstrates the linear regression of the clinical variables and Apo A-I Des-Q243. Race/ethnicity demonstrated little difference except for American Indian/Alaskan patients which had significantly lower values as compared to Caucasian patients (Plasma -5.8%, 95% CI -9.9- -

Table 1a: Patient Demographics and Clinical Data	
Variable	**Summary Statistics**
Gender	
Male	43 (41.8%)
Female	60 (58.3%)
Race/Ethnicity	
Caucasian, Non-Hispanic/Latino	32 (31.1%)
Caucasian, Hispanic/Latino	47 (45.6%)
African American	20 (19.4%)
American Indian/Alaskan	4 (3.9%)
CAD Test Indication	
No Chest Pain/Risk Stratification	25 (24.5%)
Atypical Chest Pain	50 (49%)
Typical Angina	21 (20.6%)
Unstable Angina	1 (1%)
NSTEMI	5 (4.9%)
Coronary Artery Disease	46 (44.7%)
Chronic Kidney Disease*	11 (10.7%)
Hypertension	91 (88.4%)
Hyperlipidemia	80 (77.7%)
Diabetes Mellitus	50 (49%)
Atrial Fibrillation	7 (6.8%)
History of MACE	35 (34%)
Statin	82 (79.6%)
ACE/ARB	54 (47.6%)
Number of Severely Obstructive Vessels (>70%)	
None	17 (34%)
One	17 (34%)
Two	10 (20%)
Three	3 (6%)
Left Main	3 (6%)
Number of Stents	
None	80 (78.4%)
One	6 (5.9%)
Two	9 (8.8%)
Three	4 (3.9%)
Four	1 (1%)
Five	2 (2%)

*No patients with end-stage renal disease or on hemodialysis.

Table 1b: Patient Demographics and Clinical Data	
Variable	**Summary Statistics**
Age (Years)	59.2 ± 9.1
Wt (kg)	95.8 ± 27.4
BMI	33.9 ± 8.6
eGFR (mL/min/1.73m^2)	87.8 ± 30.2
Creatinine (mg/dL)	0.9 ± 0.3
Total cholesterol (mg/dL) (mg/dL)	169.6 ± 40.7
Triglycerides (mg/dL) (mg/dL)	157.9 ± 97.9
High Density Lipoprotein (mg/dL)	45.9 ± 13.6
Low Density Lipoprotein (mg/dL)	98.2 ± 32.2
HgbA1C (%)	7.0 ± 1.8
Acute Peak Troponin (ng/mL)	0.7 ± 2.9
ASCVD Risk Estimator Plus (%)	15.3 ± 15.6

Table 2 Spearman's Coefficient of Apo A-I Des-Q243 and Clinical Variables					
Patient Characteristics	**Sample Size (n =)**	**Relative Fractional Abundance of Apo A-I Des-Q243 (Plasma)**		**Relative Fractional Abundance of Apo A-I des Q243 (Serum)**	
		Spearman's ρ	**P value**	**Spearman's ρ**	**P value**
Demographics					
Age (Years)	103			0.064	0.523
Weight (kg)	103	-0.306	0.002*	-0.323	0.001*
Body Mass Index	103	-0.214	0.023*	-0.258	0.009*
Tobacco Pack Years	94	-0.110	0.293	-0.066	0.528
Years Since Quitting Smoking	31	-0.066	0.724		
Standard Drinks per Week	91	0.045	0.672	0.046	0.666
Framingham 10-year cardiovascular risk score (%)	56	0.016	0.909	-0.082	0.550
ASCVD Risk Estimator Plus (%)	55	0.037	0.786	-0.039	0.778
Labs and Vitals					
Systolic Blood Pressure (mmHg)	103	-0.007	0.944	-0.045	0.652
Diastolic Blood Pressure (mmHg)	103	0.122	0.219	0.059	0.551
Heart Rate (BPM)	103	0.033	0.738	0.068	0.496
Estimated Glomerular Filtration Rate (mL/min/1.73m^2)	103	-0.242	0.014*	-0.291	0.003*
Creatinine (mg/dL)	103	0.080	0.422	0.144	0.148
N-terminal Pro B-type Natriuretic Peptide (pg/mL)	40	0.038	0.817	-0.037	0.822
C-Reactive Protein (mg/l)	13	0.088	0.775	0.11	0.721
Erythrocyte Sedimentation Rate (mm/hr)	17	-0.237	0.360	-0.2	0.442
Total Cholesterol (mg/dL)	101	0.049	0.628	0.08	0.428
Triglycerides (mg/dL)	101	-0.002	0.981	-0.001	0.993
High Density Lipoprotein (mg\dL)	101	-0.077	0.939	-0.039	0.7
Low Density Lipoprotein (mg/dL)	101	-0.024	0.811	0.05	0.618
Hemoglobin A1c (%)	91	0.081	0.446	0.061	0.564
Acute Peak Troponin (ng/mL)	27	0.082	0.684	0.170	0.396

Echo					
Systolic Function (%)	92	-0.035	0.742	-0.016	0.882
Exercise Stress Test					
Duke Treadmill Score	16	-0.247	0.356	-0.328	0.214
Metabolic Equivalents	16	-0.366	0.164	-0.425	0.101
Rate Pressure	16	-0.277	0.3	-0.285	0.284
Myocardial Perfusion Imaging					
Number of Ischemic Regions	86	0.158	0.147	0.039	0.724
Summed Stress Score	82	0.110	0.324	0.012	0.918
Summed Difference Score	76	0.123	0.292	0.047	0.686
Ischemic Total Perfusion Defect (%)	76	0.117	0.314	0.031	0.789

Table 3 Linear Regression of Apo A-I Des-Q243 and Clinical Variables										
Patient Characteristics		**Sample Size (n=)**	**Percent Difference of Apo A-I Des-Q243 (Plasma)**				**Percent Difference of Apo A-I Des-Q243 (Serum)**			
			Difference	**95% CI**		**P value**	**Difference**	**95% CI**		**P value**
Demographics										
Gender	Male vs Female	103	-1.5%	-3.0%	0.1%	0.06	-1.3%	-2.8%	0.1%	0.07
Race/Ethnicity	Caucasian vs Hispanic Latino	103	-0.4%	-2.2%	1.3%	0.62	-0.5%	-2.2%	1.1%	0.52
	Caucasian vs African American		-1.0%	-3.2%	1.1%	0.34	-0.6%	-2.7%	1.4%	0.54
	Caucasian vs American Indian/Alaskan		-5.8%	-9.9%	-1.8%	0.005*	-4.6%	-8.5%	-0.8%	0.02*
FH of Premature CAD	Presence vs Absence	100	-1.2%	-4.0%	1.5%	0.38	-1.9%	-4.5%	0.8%	0.16
	Present vs Unknown Age of FH		-0.7%	-3.7%	2.3%	0.64	-1.1%	-3.9%	1.8%	0.46
CAD Test Indication	No Chest Pain vs Atypical Chest Pain	102	0.7%	-1.2%	2.6%	0.46	-0.3%	-2.1%	1.5%	0.74
	No Chest Pain vs Typical Chest Pain		1.3%	-1.0%	3.6%	0.27	0.7%	-1.5%	2.9%	0.55
	No Chest Pain vs Unstable Angina		-3.5%	-11.5%	4.5%	0.38	-3.2%	-10.8%	4.4%	0.40

	No Chest Pain vs NSTEMI		0.9%	-2.9%	4.7%	0.64	0.0%	-3.6%	3.7%	0.99
CCS Angina Grade	Asymptomatic vs Strenuous Exertion	49	-1.2%	-4.9%	2.4%	0.50	-1.4%	-4.7%	1.9%	0.39
	Asymptomatic vs Moderate Exertion		0.1%	-3.0%	3.2%	0.93	-0.1%	-2.9%	2.7%	0.96
	Asymptomatic vs Light Exertion		4.4%	-0.5%	9.3%	0.08	2.6%	-1.8%	7.0%	0.24
	Asymptomatic vs Rest		-1.9%	-6.8%	3.0%	0.45	-1.6%	-6.0%	2.8%	0.47
Substance Use										
Tobacco	Current vs Former Use	103	-0.3%	-2.4%	1.8%	0.78	-0.1%	-2.1%	2.0%	0.96
	Current vs No Use		0.9%	-1.1%	3.0%	0.36	0.8%	-1.2%	2.7%	0.43
Alcohol Use	Current vs Former Use	102	-0.3%	-4.1%	3.4%	0.86	-0.5%	-4.1%	3.1%	0.78
	Current vs No Use		-0.7%	-2.4%	0.9%	0.39	-0.8%	-2.4%	0.8%	0.30
Cocaine	Current vs No Use	102	1.5%	-2.4%	5.5%	0.45	3.2%	-0.6%	6.9%	0.10
Methamphetamine	Current vs No Use	102	0.5%	-3.5%	4.4%	0.82	1.2%	-2.6%	5.0%	0.54
Opioid	Current vs No Use	102	-2.3%	-7.8%	3.3%	0.42	-3.8%	-9.1%	1.4%	0.15
Marijuana	Current vs No Use	102	1.1%	-1.3%	3.6%	0.36	1.8%	-0.5%	4.2%	0.13
Medical History										
Coronary Artery Disease	Presence vs Absence	103	0.5%	-1.1%	2.0%	0.58	0.4%	-1.0%	1.9%	0.55

Hypertension	Presence vs Absence	103	1.1%	-1.3%	3.5%	0.36	0.8%	-1.5%	3.1%	0.50
Diabetes Mellitus	Presence vs Absence	102	-0.1%	-1.6%	1.4%	0.87	-0.2%	-1.6%	1.2%	0.79
Hyperlipidemia	Presence vs Absence	103	-0.5%	-2.3%	1.4%	0.61	-0.6%	-2.3%	1.2%	0.51
Cerebrovascular Accident	Presence vs Absence	103	1.4%	-1.5%	4.3%	0.34	0.6%	-2.1%	3.4%	0.64
Atrial Fibrillation	Presence vs Absence	103	-2.8%	-5.8%	0.2%	0.07	-2.8%	-5.6%	0.1%	0.06
Chronic Kidney Disease	Presence vs Absence	103	2.5%	0.0%	4.9%	0.05*	2.9%	0.6%	5.6%	0.014*
Obstructive Sleep Apnea	Presence vs Absence	103	-0.7%	-2.5%	1.2%	0.47	-0.8%	-2.6%	1.0%	0.37
Chronic Obstructive Pulmonary Disease	Presence vs Absence	103	2.2%	-1.1%	5.5%	0.18	2.3%	-0.8%	5.4%	0.14
Peripheral Artery Disease	Presence vs Absence	103	-0.3%	-4.0%	3.3%	0.86	-1.6%	-5.0%	1.8%	0.36
Carotid Artery Disease	Presence vs Absence	103	1.2%	-3.4%	5.8%	0.61	1.5%	-2.8%	5.9%	0.48
Major Depressive Disorder	Presence vs Absence	103	0.7%	-1.0%	2.5%	0.41	0.9%	-0.7%	2.6%	0.27
Generalized Anxiety Disorder	Presence vs Absence	103	1.1%	-1.0%	3.1%	0.30	0.8%	-1.1%	2.8%	0.38
Bipolar Disorder	Presence vs Absence	103	-2.8%	-8.4%	2.8%	0.33	-2.9%	-8.2%	2.3%	0.27
Hypothyroidism	Presence vs Absence	103	-0.9%	-4.0%	2.2%	0.56	-1.4%	-4.4%	1.5%	0.33
HTN Treatment	Treated vs Untreated	103	1.1%	-2.5%	4.8%	0.54	0.8%	-2.6%	4.2%	0.64
	Treated vs Undiagnosed		-1.4%	-3.8%	1.0%	0.26	-0.9%	-3.2%	1.4%	0.43

History of MACE	Presence vs Absence	103	0.5%	-1.1%	2.1%	0.54	0.1%	-1.5%	1.6%	0.94
Inflammatory Condition	Presence vs Absence	103	-0.3%	-2.0%	1.5%	0.76	-0.7%	-2.3%	0.9%	0.37
Autoimmune Condition	Presence vs Absence	103	0.1%	-2.8%	3.1%	0.92	-1.4%	-4.1%	1.3%	0.31
Cancer	Presence vs Absence	103	0.2%	-2.6%	2.9%	0.91	0.2%	-2.4%	2.8%	0.89
Infection	Acute vs Chronic Infection	103	3.6%	-2.4%	9.6%	0.24	3.5%	-2.1%	9.2%	0.22
	Acute vs No Infection		3.6%	-2.0%	9.2%	0.21	3.8%	-1.5%	9.1%	0.16
Hx of Gout	Presence vs Absence	103	0.5%	-2.8%	3.9%	0.75	1.0%	-2.2%	4.1%	0.54
Medication Use										
Aspirin	Current vs No Use	103	0.1%	-1.6%	1.9%	0.91	-0.1%	-1.7%	1.6%	0.91
Statin	Current vs No Use	103	0.4%	-1.5%	2.3%	0.68	0.4%	-1.4%	2.2%	0.67
ACEi/ARB	Current vs No Use	103	-2.1%	-3.6%	-0.6%	0.006*	-1.7%	-3.1%	-0.2%	0.022*
Beta Blocker	Current vs No Use	103	0.4%	-1.2%	1.9%	0.64	0.1%	-1.3%	1.6%	0.85
P2Y2 Inhibitor	Current vs No Use	103	0.4%	-1.6%	2.5%	0.68	-0.7%	-2.6%	1.3%	0.49
Short Acting Nitrate	Current vs No Use	103	0.6%	-1.5%	2.6%	0.58	0.2%	-1.8%	2.1%	0.87
Long Acting Nitrate	Current Use	103	1.2%	-1.1%	3.4%	0.31	0.8%	-1.3%	3.0%	0.44
Angiogram										
Diffuse Disease	Presence vs Absence	48	-1.2%	-4.0%	1.6%	0.39	-1.0%	-3.4%	1.3%	0.38

Number of Minimally Obstructive Vessels (<50%)	None vs One	49	-0.9%	-3.6%	1.7%	0.49	-0.7%	-2.9%	1.5%	0.53
	None vs Two		1.9%	-2.2%	6.0%	0.36	1.3%	-2.2%	4.7%	0.47
	None vs Three		7.3%	1.2%	13.5%	0.021*	5.6%	0.4%	10.8%	0.035*
	None vs Four		-2.7%	-11.2%	5.9%	0.53	-3.2%	-10.4%	4.0%	0.37
Number of Moderately Obstructive Vessels (50-70%)	None vs One	50	-0.7%	-3.9%	2.6%	0.68	-1.0%	-3.7%	1.7%	0.46
	None vs Two		0.0%	-8.9%	8.9%	1.00	0.9%	-6.5%	8.3%	0.81
Number of Severely Obstructive Vessels (>70%)	None vs One	50	0.3%	-2.8%	3.4%	0.86	-0.3%	-2.9%	2.3%	0.81
	None vs Two		1.2%	-2.4%	4.8%	0.51	-0.3%	-3.3%	2.7%	0.85
	None vs Three		0.6%	-5.0%	6.3%	0.82	0.3%	-4.4%	5.0%	0.89
	None vs Left Main		0.7%	-4.9%	6.4%	0.79	0.2%	-4.5%	4.9%	0.93
Interventions	PCI vs Stenting	53	1.5%	-5.0%	8.0%	0.65	2.0%	-3.5%	7.4%	0.47
	PCI vs Medical Therapy		-0.7%	-7.1%	5.6%	0.82	-0.1%	-5.4%	5.2%	0.97
History of Stenting	Presence vs Absence	103	1.1%	-0.8%	2.9%	0.25	0.7%	-1.0%	2.5%	0.40
Number of Stents	None vs One	102	2.8%	-0.3%	5.9%	0.075	2.4%	-0.6%	5.4%	0.12
	None vs Two		-1.2%	-3.8%	1.4%	0.35	-1.0%	-3.5%	1.6%	0.45
	None vs Three		-1.8%	-5.6%	1.9%	0.34	-1.8%	-5.4%	1.9%	0.35
	None vs Four		12.0%	4.6%	19.4%	0.002*	9.3%	2.0%	16.5%	0.013*
	None vs Five		4.8%	-0.5%	10.1%	0.073	2.8%	-2.3%	8.0%	0.28

Stent Restenosis	Total vs None	16	3.2%	-5.0%	11.5%	0.41	2.9%	-4.3%	10.2%	0.40
	Total vs Partial		-0.5%	-13.8%	12.9%	0.94	0.7%	-11.0%	12.4%	0.90
Echo										
Diastolic Function	Normal vs Grade 1	86	1.1%	-1.8%	4.0%	0.45	1.4%	-1.3%	4.1%	0.30
	Normal vs Grade 2		0.1%	-2.2%	2.5%	0.91	-0.8%	-3.0%	1.4%	0.47
	Normal vs Grade 3		-3.0%	-8.7%	2.7%	0.30	-0.2%	-5.5%	5.1%	0.94
	Normal vs Grade 4		-0.7%	-3.4%	2.1%	0.63	-1.0%	-3.6%	1.6%	0.44
	Normal vs Indeterminate		3.5%	-0.6%	7.6%	0.09	4.3%	0.5%	8.2%	0.028*
Stress Imaging										
Exercise Stress Test	Positive vs Negative	16	-4.3%	-11.4%	2.8%	0.22	-4.1%	-11.8%	3.6%	0.27
	Positive vs Indeterminate		-0.8%	-8.4%	6.9%	0.83	-0.7%	-8.9%	7.6%	0.87
Myocardial Perfusion Imaging	Positive vs Negative	87	0.4%	-1.6%	2.5%	0.70	0.2%	-1.8%	2.2%	0.84
Type of Lesion	Fixed vs Reversible	28	-1.4%	-4.6%	1.8%	0.37	-0.3%	-3.6%	3.0%	0.85
	Fixed vs Both		2.3%	-3.8%	8.4%	0.44	3.5%	-2.6%	9.7%	0.25
Size of Lesion	Small vs Moderate	28	0.4%	-2.7%	3.5%	0.79	0.2%	-3.0%	3.4%	0.90
	Small vs Large		1.4%	-7.0%	9.7%	0.74	-0.8%	-9.2%	7.6%	0.85

Multivariate Analysis:

Table 4 demonstrates the multivariate analysis of the significant clinical variables and those that were within ~5% of statistical significance. Levels of Apo A-I Des-Q243 were significantly lower in male patients (-1.5%, P=0.035). Patients with CKD (2.3%, P=0.037) exhibited a higher level of Apo A-I Des-Q243, while those with atrial fibrillation (-2.8%, P=0.04) had lower levels of Apo A-I Des-Q243. ACEi/ARB therapy (-2.4%, P=0.001) was associated with lower levels of Apo A-I Des-Q243. Finally, patients with four (9.6%, P=0.005) or five (4.7%, P=0.045) stents were found to have significantly elevated levels of Apo A-I Des-Q243.

Apo A-I Methionine Oxidation:

Levels of Apo A-I Met(O) for the first 30 patients were within the signal-to-noise ratio for the LC-ESI-MS (< 5% relative abundance) (Table 5). Samples 31-103 were ran on a different instrument due to mechanical failure of the previous LC-ESI-MS. The low abundance of Apo A-I Met(O) (+16 Da) was obscured by sodium adducts (+22 Da) of approximately the same size. This resulted in unmeasurable levels of Apo A-I Met(O).

Table 4: Multivariate Analysis of Clinical Parameters					
Patient Characteristics	Difference in Percentage of Apo A-I Des Q243 (%)	95% CI			P value
BMI	-0.1	-0.2	-	-0.1	0.001*
Male Gender	-1.5	-2.8	-	-1	0.035*
Chronic Kidney Disease	2.3	1.4	-	4.3	0.037*
Atrial Fibrillation	-2.8	-5.4	-	-0.1	0.04*
ACE/ARB Use	-2.4	-3.8	-	-1	0.001*
Number of Stents					
One	2.8	0	-	5.7	0.057
Two	-0.6	-3	-	1.8	0.637
Three	-0.4	-3.8	-	3	0.801
Four	9.6	3	-	16.2	0.005*
Five	4.7	0.1	-	9.4	0.045*
Not included in multivariate: eGFR, Spearman's ρ = -0.29; p=0.003					

Table 5: Weighted Relative Percent Abundance (WRPA) of Oxidized Apo A-I		
Patient Number	Plasma	Serum
1	2.9%	3.6%
2	3.7%	3.1%
3	3.0%	3.0%
4	3.4%	2.6%
5	3.2%	4.1%
6	5.1%	4.0%
7	2.6%	3.6%
8	4.5%	3.9%
9	3.6%	3.3%
10	3.0%	3.5%
11	2.9%	3.5%
12	2.9%	3.5%
13	3.5%	3.4%
14	3.2%	3.0%
15	2.8%	3.1%
16	2.9%	3.5%
17	3.9%	4.0%
18	6.0%	3.1%
19	3.2%	3.2%
20	2.6%	2.9%
21	3.5%	6.9%
22	3.6%	3.6%
23	3.7%	3.0%
24	3.6%	4.0%
25	4.1%	4.2%
26	3.2%	2.9%
27	3.9%	3.4%
28	4.6%	2.7%
29	4.1%	3.2%
30	8.1%	3.7%

Discussion

The major findings of this study were: 1) increased levels of Apo A-I Des-Q243 in patients with multiple stents, 2) increased levels of Apo A-I Des-Q243 in patients with CKD, which is linearly associated with worsening renal failure (eGFR), 3) decreased levels of Apo A-I Des-Q243 in patients on ACEi/ARB therapy. And 4) Apo A-I Des-Q243 appears to be a stable measureable biomarker.

Apo A-1 Des Q243 and CAD:

Previous studies by Nikolova et al noted a positive association between Apo A-I Des-Q243 and the presence of angiographically confirmed CAD, particularly in the right coronary artery (RCA) and left circumflex artery (LCX) regions[16]. Additionally, the HDL/Apo A-I Des-Q243 ratio was found to be elevated in patients with CAD and DM[15]. The findings of these studies laid the ground work for the current study. This paper expands upon the previous work to include additional clinical variables that could affect oxidation status.

This study demonstrates a significant positive association between Apo A-I Des-Q243 and the number of coronary stents, regardless of the type of stent (bare-metal or drug eluting). One potential explanation is that the stents themselves could be acting as a nidus for oxidative damage by increasing enzymatic activation of MMP or similar proteases. There is known activation of leukocytes and inflammatory mediators in the literature and it is believed to be a component of in-stent restenosis[29-31]. Our results didn't reach significance for stent restenosis but the sample size was small. Another explanation could be that levels of Apo A-I Des-Q243 were higher with presence of multiple stents as a function of more severe underlying CAD and/or other inflammatory conditions of the patient.

Apo A-1 Des-Q243 and CKD:

A new finding of the study was the relationship between Apo A-I Des-Q243 and chronic kidney disease. Our results suggest that CKD-mediated oxidative stress significantly increases the levels of Apo A-I Des-Q243 and is inversely correlated with the eGFR. These are novel findings, which haven't been previously described in the literature to our knowledge.

For patients taking ACEi/ARBs the levels of Apo A-I Des-Q243 were found to be significantly lower. The renin-angiotensin system (RAS) is likely involved and there is literature demonstrating that activation of RAS in hypertension leads to the formation of reactive oxygen species (ROS)[25-27]. Thus ACEi/ARB therapy is protective against oxidation. This data supports other literature of an anti-oxidant mechanism of ACEi/ARB[33-35]. This is an additional benefit for patients with CAD and CKD.

These results suggests a common pathway between chronic kidney disease (CKD), coronary artery disease (CAD) and Apo A-I Des-Q243. CKD is well known to cause accelerated atherosclerosis. This is due to oxidative stress, uremic toxins and metabolic derangements[17-19]. A review by Nans Florens in Toxins discusses the effects on HDL activity in CKD[20]. These include decreased levels of Apo A-I, decreased function of Apo A-I-mediated reverse cholesterol efflux and either decreased levels and/or function of essential enzymes, e.g. LCAT, cholesterol-ester transfer protein (CTEP), nitric oxide (NO), paraoxonase (PON) and glutathione peroxidase (GPX). The Florens group used the term "posttranslational modification derived products" (PTMDPs) to encapsulate the various byproducts that occur in CKD (Figure 1). It is likely that Apo A-I Des-Q243 is another PTMDP byproduct of CKD in addition to CAD.

Apo A-I Oxidation Products:

Much of the focus in the literature on HDL dysfunction has been on Met(O) and to a lesser extent, tyrosine chlorination[8-9,22,28]. As previously mentioned, Met(O) has been shown to be a potential biomarker of CAD. Unfortunately, a major limitation is that oxidation of methionine residues does not stop until the samples are frozen at approximately -80^{O}C, so called ex vivo oxidation[12,24]. Without proper handling, ex vivo oxidation inflates the levels of Met(O). In fact, levels of methionine oxidation became nearly undetectable via HPLC-MS when utilizing fresh plasma and serum samples combined with careful processing (table 5). This includes strict time and sample integrity metrics as adapted by Borges et al[24]. There is convincing evidence that Met(O) is a result of in vivo oxidative stress; however, its sensitivity to ex vivo oxidation makes it less stable and therefore an unreliable biomarker. This could explain why attempts to quantify any consistent clinical correlation have yielded mixed results at best[8-9,22]. Thus other markers of oxidative stress that are more stable would be desirable if the goal is to develop a clinically useful biomarker.

One such candidate may be Apo A-I C-terminal truncation of glutamate residue 243. Our studies have yet to show ex vivo changes in the total fractional abundance of Apo A-I Des-Q243. With proper handling of fresh samples, we have no reason to believe that Apo A-I Des-Q243 undergoes any substantial modifications. It is important to note, that this hasn't been systematically studied and it is a potential area of future study. Another consideration when measuring Apo A-I Des-Q243, is that methionine oxidation can be superimposed upon it. The effect of this can be avoided by calculating the total fractional abundance, which takes into account both Apo A-I Des-Q243 with and without superimposed Met(O).

Limitations:

Based on our original power calculations a target sample size of 166 patients was determined necessary to reach a β >80%. Due to time constraints our final small sample size only reached 103 patients. Some of our clinical variables may have been underpowered, specifically our angiographic data (n=50). This may explain why several variables didn't reach statistical significance, despite the fact that Apo A-I Des-Q243 was elevated and these variables had reached significance in Nikolova et al[15-16]. Another limitation of this single center study was that there was a disproportionate number of Hispanic patients (45.6%) as compared to the general United States population (~18.1% based 2017 US census data) and this could influence the generalizability of the study.

Finally a major limitation was the lack of a healthy control population. It is very likely that most, if not all of the patients, had elevations in the levels of Apo A-I Des-Q243, given the number of co-morbid inflammatory conditions present. This could have limited the magnitude of change in Apo A-I Des-Q243 and made any attempt to distinguish between levels undistinguishable.

Future Directions

Future areas of study could include: 1) determining the reference ranges of Apo A-I Des Q243 in healthy individuals and those with oxidative disease states, e.g. CAD, CKD, DM, and CHF. 2) studying the mechanism of Apo A-I Des-Q243 formation which could lead to potential pharmaceutical targeting, 3) further characterization of ex vivo stability of Apo A-I Des-Q243 and optimal sample handling which would improve its utility as a biomarker and 4) confirming causality with a prospective cohort study.

Conclusion

This cross-sectional study of fresh plasma and serum samples further demonstrates the positive association between Apo A-I Des-Q243 and CAD severity, as determined by the quantity of coronary stents. Additionally, this article gives preliminary evidence of a positive association between Apo A-I Des-Q243 and the presence of CKD, which is found to be inversely correlated to its severity as determined by eGFR. The negative association between ACEi/ARB therapy and Apo A-I DesQ243 implicates RAS-mediated oxidation as a common link between CAD and CKD pathogenesis. Thus Apo A- I Des-Q243 may be of particular utility as a stable biomarker in this population.

Abbreviations

ABCA1	ATP-binding cassette transporter A1
ABCG1	ATP-binding cassette transporter G1
ACE/ARB	Angiotensin converting enzyme inhibitor/Angiotensin receptor blocker
Apo A-I	Apolipoprotein A-I
Apo A-I Des-Q243	Apo lipoprotein A-I C-terminal truncation of glutamine residue 243
Apo A-II	Apolipoprotein A-II
ASCVD	Atherosclerotic cardiovascular disease
BMI	Body mass index
CAD	Coronary artery disease
CKD	Chronic kidney disease
CHF	Congestive Heart Failure
CCTA	Computed tomography coronary angiogram
CTEP	Cholesterol-ester transfer protein
DM	Diabetes Mellitus
eGFR	Estimated glomerular filtration rate
ESRD	End stage renal disease
GPX	Glutathione peroxidase
HDL	High density lipoprotein
HLD	Hyperlipidemia
HTN	Hypertension
LC-ESI-MS	Liquid chromatography-electrospray ionization-mass spectrometry
LCAT	Lecithin: cholesterol acyltransferase
LCX	Left circumflex artery
Met(O)	Methionine oxidation
MMC	Maricopa Medical Center
MMP	Matrix Metalloprotease
MPO	Myeloperoxidase
NO	Nitric Oxide

NSTEMI	Non-ST elevation myocardial infarction
PON	Paraoxonase
PTMDP	Posttranslational modification derived products
Q-TOF	Quadrupole-time-of-flight
RCA	Right coronary artery
ROS	Reactive oxygen species
RFA	Relative fractional abundance
SAA	Serum amyloid A
STEMI	ST elevation myocardial infarction
WRFA	Weighted relative fractional abundance
WRPA	Weighted relative percent abundance

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