Published in final edited form as:

Atherosclerosis. 2017 November ; 266: 74–80. doi:10.1016/j.atherosclerosis.2017.09.030.

Effect of statin on arginine metabolites in treated HIV-infection

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Abstract

Background and aims—Asymmetric dimethylarginine (ADMA) is an inhibitor of nitric oxide and an independent risk factor for cardiovascular disease. We examined the effect of statin on ADMA in HIV+ patients on stable ART, and whether such an effect contributes to the favorable changes on carotid intima media thickness.

Methods—This is a secondary analysis of SATURN-HIV, in which HIV+ adults on stable ART with HIV-1 RNA < 1,000 copies/mL and LDL-cholesterol < 130 mg/dL were randomized to 10 mg daily rosuvastatin or placebo. Arginine metabolites, ADMA, and markers of inflammation were assessed at baseline and 48 weeks. Carotid intima media thickness (c-IMT) was measured at baseline, 48 and 96 weeks. Spearman correlations, and linear mixed-effect models were used to study relationships among variables.

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⁶This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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Conflicts of interest

GAM served as a consultant for Gilead, BMS, GSK/Viiv, and ICON, and has received research funding from Gilead, Merck, GSK/Viiv, and BMS. CTL has consulted for Gilead Sciences and has received research funding from Bristol-Myers Squibb and Medtronic Philanthropy. NF has consulted for Gilead.

Author contributions:

GM designed the study, obtained funding and supervised all aspects of the trial. KA and AS provided statistical support. DL and LP assisted with subject enrollment. NF assisted with the inflammation and immune activation marker assays. WHWT provided support with the arginine metabolomics profile. CTL performed vascular ultrasound measurements. SDF and VEK wrote the first draft of the manuscript. All authors contributed to data analysis and reviewed the manuscript for intellectual content.

Results—Overall, 79% were male, 68% African Americans, with a median age of 46 years. In the statin arm, no change in ADMA levels was observed at 48 weeks (0.70%), whereas a trend towards an increase in ADMA levels (23.78%) was observed in the placebo group ($p=0.06$). Elevated baseline ADMA (highest tertile) was associated with a 0.04 mm increase in c-IMT ($p=0.03$) after adjusting for statin and study duration. No interaction was seen between baseline ADMA and statin randomization on change in c-IMT ($p=0.21$).

Conclusions—In HIV+ subjects on ART, rosuvastatin suppressed the increase over time in ADMA levels. Elevated baseline levels of ADMA were associated with increases in c-IMT, regardless of statin assignment. The favorable effect of rosuvastatin on c-IMT appears to be independent of the arginine pathway.

Keywords

Arginine; HIV; ADMA; Cardiovascular Diseases; Inflammation

Introduction

Despite antiretroviral therapy (ART), patients with HIV are still at an increased risk of developing cardiovascular disease (CVD) compared to the general population^{1–3}. Increasing evidence suggests that chronic inflammation and endothelial dysfunction may play an important role in the increased CVD risk in this population^{4,5}.

Endothelial dysfunction is related to a decrease in nitric oxide (NO) availability⁶. NO has a crucial role in maintaining normal vascular function and has anti-atherogenic effects⁷. The major source of NO is through the oxidation of L-arginine by the endothelial nitric oxide synthase (eNOS) enzyme, which is located predominantly in endothelial cells (ECs)⁸. eNOS is inhibited endogenously by asymmetric dimethylarginine (ADMA)⁹. Elevated levels of ADMA in the general population have been associated with high rate of cardiovascular events, independent of traditional risk factors¹⁰. Reducing serum ADMA levels has been suggested as a potential therapeutic target in patients with endothelial dysfunction¹¹.

HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductases inhibitors or statins have been shown to exert protective effects beyond cholesterol lowering, which are mediated through the vasculature. Several studies suggest that hydrophilic statins (such as Rosuvastatin) can also significantly decrease the plasma levels of ADMA^{12–15}.

In HIV-infected patients, while little is known about ADMA, studies have found increased levels in HIV-infected patients compared to uninfected controls^{16,17}. Levels of ADMA also decrease with ART initiation¹⁸. Our group has shown that statin therapy in HIV-infected patients can decrease inflammation, monocyte and lymphocyte immune activation, and carotid vascular disease^{19–22}, however, to our knowledge, the effect of statin therapy on ADMA concentration in HIV-infected patients has never been studied.

Here we present the results of an exploratory secondary analysis aimed at assessing the effect of rosuvastatin on plasma ADMA concentration over 48 weeks in a randomized, placebo-controlled trial of HIV-infected adults on stable ART.

Materials and methods

Study design

The above aims were assessed using data from SATURN-HIV trial which is a randomized, double-blind, placebo-controlled study to assess the effect of Rosuvastatin on markers of cardiovascular risk, skeletal health, and immune activation in HIV-infected adults²¹. The results presented herein represent a secondary analysis that assessed changes in arginine metabolites from entry to week 48. The study is registered on clinicaltrials.gov (NCT01218802). The Institutional Review Board of University Hospitals Case Medical Center (Cleveland, Ohio) approved the study. Written consents were obtained from all subjects. The study conforms to the ethical guidelines of the Declaration of Helsinki (1975). Randomization was conducted on a 1:1 basis with rosuvastatin 10 mg vs matching placebo, after stratification by protease inhibitor use. The active drug and placebo were provided by AstraZeneca.

Study population

Out of a total 147 participants in SATURN-HIV, 114 were eligible for this analysis; 58 subjects were included in the rosuvastatin arm and 56 in the placebo arm.

Briefly, all study subjects met the following criteria: infection with HIV-1, on ART (stable for at least 3 months; cumulative duration of at least 6 months), age ≥ 18 years, HIV-1 RNA <1000 copies/mL, triglyceride ≤ 500 mg/dL, LDL-cholesterol (LDL-C) ≤ 130 mg/dL, heightened T-cell activation (CD38⁺HLA-DR⁺ CD8⁺ T cells ≥ 19%) or high-sensitivity C-reactive protein (hs-CRP) ≥ 2 mg/L. Exclusion criteria included: diabetes or history of coronary artery disease, current pregnancy or lactation, active inflammatory or infectious process. The inclusion and exclusion criteria were previously published in full detail²¹. Only subjects with available plasma samples at week 0 and week 48 were included in this analysis.

Study evaluation

Evaluation for the study included obtaining medical history and demographic information, a physical examination that included weight, height, and waist and hip measurements. At entry and week 48, fasting (> 12 hours) blood draws were obtained for the following: lipid profile, renal profile, glucose level, insulin level, immune markers and arginine metabolite levels. HIV-1 RNA levels and CD4⁺ cell counts were drawn as part of routine clinical care and measures closest to study visits were adopted for the analysis. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as described²³.

Arginine metabolites—Measurement of asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), monomethylarginine (MMA), arginine, ornithine and citrulline, were performed by stable-isotope-dilution high-performance liquid chromatography with online tandem mass spectrometry. Four volumes of methanol containing 13C6-Arg, d6-Orn, d4-Cit, d7-ADMA each 10 µM were added to plasma to precipitate proteins, and the supernatant was collected for liquid chromatography/tandem mass spectrometry assay after centrifugation at 13,000 rpm for 10 minutes. 10 µL

supernatant was injected onto a HPLC column. The levels of the arginine and related metabolites were quantified by liquid chromatography/electrospray ionization/tandem mass spectrometry analysis using API 365 triple quadrupole mass spectrometer (Applied Biosystems, Foster, CA) with Ionics EP 10+ upgrade (Concord, Ontario, CA) and Cohesive Technologies Aria LX Series HPLC multiplexing system (Franklin, MA). Amino acids were separated with a 250 × 4.6 mm Rexchrom S5-100-P phenyl column (Product number 728207 Regis Chemical, Morton Grove, IL, USA) with a 1 mm × 15 mm Opti-Guard Phenyl guard column (Optimize Technologies, Oregon City, OR, USA). Formic acid (0.1%) and ammonium formate (10 mM) in water (solvent A) and formic acid (0.1%) and ammonium formate (10 mM) in methanol (solvent) were used as solvents. The gradient consisted of: 1) 100% solvent A at 800 µL/min held for 0.5 min after injection, 2) linear gradient run to 50% solvent B (50% solvent A) over 3 min, 3) held at 50% B for 3.5 min at a flow rate of 800 µL/min, 4) flow rate increased to 1000 µL/min with solvent composition changed to 100% B in linear fashion over 1 min, 5) linear gradient run to 100% A at 1000 µL/min over 0.5 min, 6) held at this composition and flow rate for 6 min. Overall, the 8.5 min data window was started at 3.5 min after injection, and mass spectrometric analyses were performed using electrospray ionization tandem mass spectrometry in the positive ion mode with multiple reaction monitoring. We optimized cone potentials and collision energy for each analyte. Each demonstrated quantitative recovery, linearity across the concentration range over multiple orders of magnitude, and intra- and inter- assay coefficients of variance <10%.

Inflammation and soluble immune activation markers—Several biomarkers of systemic inflammation and immune activation were measured in plasma, including: interleukin-6 (IL-6), soluble tumor necrosis factor-α receptors I and II (sTNFR-I and II), and interferon γ inducible protein-10 (IP-10), soluble vascular and intracellular cell adhesion molecule-1 (sVCAM-1 and sICAM-1, respectively), and sCD14 and sCD163 – soluble markers of monocyte activation. All were determined using ELISA (R&D Systems; Minneapolis, MN, USA). High sensitivity C-reactive protein (hsCRP), was measured by particle enhanced immunonephelometric assay on a BNII nephelometer (Siemens; Munich, Germany). Serum oxidized-LDL levels were measured using an ELISA assay (Mercodia, Uppsala, Sweden).

Cellular markers of T-cell activation—T-cell phenotypes were assessed using flow cytometry as previously reported²⁴. The percentage of CD4+ or CD8+ cells expressing both CD38 and HLA-DR was used to quantify T-cell activation. Entry CD8+ activation was measured from frozen PBMCs.

Cardiovascular measures—The Framingham risk score (10-year) was calculated²⁵. Mean-mean common carotid artery intima media thickness (c-IMT) was measured at baseline and at weeks 48 and 96 as described previously²¹ offline by a single reader (CTL; blinded to treatment assignment and participant characteristics) using semi-automated edge-detection software (Medical Imaging Applications; Coralville, IA, USA).

Statistical analysis

This is an exploratory analysis to assist in developing hypotheses for future confirmatory studies. The main objective of this study was to compare changes in ADMA and arginine metabolites from baseline to 48 weeks between rosuvastatin and placebo groups. Secondary objectives were to evaluate changes in these biomarkers within the groups at baseline and 48 weeks, and to examine their associations to markers of systemic inflammation, immune activation, coagulation as well as measures of cardiovascular disease risk, and to explore predictors of change in ADMA levels.

Demographics, clinical characteristics, fasting metabolic parameters and inflammatory and coagulation markers were described by study groups. Continuous biomarkers or variables were reported using the mean and standard deviation, while categorical variables were reported with frequency and percent. Descriptive statistics, t-tests, chi-square tests, were used for comparing baseline HIV parameters, cardiovascular risk factors, inflammation and immune activation, and arginine metabolites in two groups. Effect sizes (absolute change/SD) were computed for ADMA and arginine metabolites. Within groups changes were tested using Wilcoxon signed-rank, and between groups changes (effect size) were tested using z-tests. According to the American Statistical Association statement on *p*-value, we emphasize on the absolute and relative changes in the biomarkers in two groups over the study period, and effect sizes in terms of nonzero beta regression coefficients and its 95% confidence interval, rather than adjusted significance levels²⁶.

Spearman correlation analyses were used to assess the relationships among arginine metabolites, markers of inflammation, immune activation, coagulation and cardiovascular risk. Linear regressions were used in studying associations between ADMA levels (measured at 48 weeks) and baseline ADMA levels, inflammatory markers, monocyte activation, HIV related factors and CVD risk factors. Linear mixed effects model with random intercept and slope was used in studying association between changes in c-IMT (over 96 weeks, response variable) and ADMA after controlling for Rosuvastatin therapy and duration on study.

Results

Baseline characteristics

Out of a total 147 participants in SATURN-HIV, 114 were eligible for this analysis: 19 participants dropped out by week 48 and 14 participants did not have available plasma for testing arginine metabolites. Therefore, this analysis included 58 subjects in the rosuvastatin arm and 56 in the placebo arm. Subjects included in this analysis were compared to the original SATURN-HIV cohort. The two groups were similar, except the arginine metabolites cohort was younger (mean 45 vs. 48 years, $p=0.03$) and had lower viral load (57 vs. 81 copies/ml, $p=0.01$).

Demographic information and characteristics are displayed in Table 1. Overall, the mean age of the participants was 44.60 years and their mean BMI was 28.24, 79% were male and 68% were African American. HIV disease was well controlled (patients were on ART by design; 81% of participants had a viral load of less than 50 copies/ml (total range was from 20–530

copies/ml). Lipid profile and 10 year Framingham scores were low risk. The characteristics between the arms were similar except for plasma LDL cholesterol which was higher in placebo arm ($p=0.04$) and SDMA which was higher in statin arm ($p=0.02$).

Baseline association of arginine metabolites with immune activation

As seen in Table 2, ADMA at baseline tended to positively correlate with markers of systemic inflammation and immune activation, although correlations were modest and only statistically significant for sTNFR-I ($p<0.01$), CD4+CD38+HLADR+ T-cells ($p=0.04$) and CD8+CD38+HLADR+ T-cells ($p=0.05$) (Table 2). Baseline ADMA was not associated with any demographics, HIV factors, or markers of cardiovascular risk (all $p > 0.05$).

Changes in arginine metabolites on statin

No significant change in ADMA levels was observed at 48 weeks in the statin arm (0.70%, $p=0.74$); whereas a trend towards an increase in ADMA levels (23.78%; $p=0.06$) was observed in the placebo group (Fig. 1). The difference between the two groups was statistically significant ($p<0.01$). Amongst the arginine catabolic byproducts, ornithine increased significantly in both arms ($p = 0.02$, $p=0.26$ between the arms), however citrulline increased significantly in the statin arm ($p<0.01$), but not in the placebo arm ($p=0.09$) ($p<0.01$ between the arms). On the other hand, no change in L-arginine was observed at 48 weeks in both arms ($p>0.60$, $p=0.57$ between arms). Changes in arginine metabolites are shown in Fig. 1.

Predictors of ADMA change over 48 weeks

In an unadjusted regression analysis, as seen in Table 2, 48-week ADMA concentration was associated with baseline ADMA ($\beta=0.33$, $p<0.01$), inflammatory markers: sTNFR-I ($\beta=0.03$, $p=0.03$) and sTNFR-II ($\beta=0.02$, $p=0.04$), oxidized LDL (oxLDL) ($\beta=0.03$, $p=0.03$), and a marker of monocyte activation, sCD163 ($\beta=0.03$, $p=0.04$). None of the HIV-related factors (nadir or current CD4+ cell count, viral load, ART duration) or traditional CVD risk factors (family history of Myocardial Infarction, BMI, smoking, alcohol) examined at baseline predicted change in ADMA levels at 48 weeks in the two groups ($p = 0.07$).

In a linear regression model, after adjusting for these HIV-related factors, as well as CVD risk factors, inflammatory, immune markers, age, gender and BMI, only baseline ADMA levels was significantly associated with ADMA levels at 48 weeks ($p=0.012$).

Association between changes in ADMA and inflammatory factors

Changes in ADMA positively correlated with changes in soluble intracellular cell adhesion molecule sICAM-1 ($p=0.02$) and T cell activation markers (CD8+CD38+ T-cells, $p=0.05$ and CD4+CD38+HLA-DR+ T-cells, $p<0.01$).

Relationship between changes of IMT with statin and ADMA

Elevated baseline ADMA levels (highest tertile) was associated with 0.03mm increase over 96 weeks in c-IMT ($p=0.05$) in the two arms. In a linear mixed-effect regression model, this association remained significant after adjusting for statin therapy and study duration

($\beta=0.04$, $p=0.03$). However, there was no interaction seen between baseline levels of ADMA and statin therapy on change in c-IMT ($p=0.21$).

Discussion

In this exploratory subgroup analysis we investigated for the first time in a population of people living with HIV, the effects of 48 weeks of rosuvastatin therapy on arginine metabolites levels in treated HIV infection. We found that in HIV-infected participants on ART with LDL < 130 mg/dL, statin therapy halted the increase in ADMA levels seen in the placebo arm. In addition, baseline levels of ADMA were positively associated with changes in carotid intima-media thickness over time, but did not seem to alter the beneficial statin effect on subclinical carotid disease progression.

We have previously shown in SATURN-HIV that 10 mg of rosuvastatin daily slowed progression of c-IMT over 2 years in patients with treated HIV infection. Patients within the placebo group had significant progression of c-IMT over the course of 96 weeks but c-IMT was unchanged in the statin group²¹. The exact mechanism by which rosuvastatin exerts its beneficial effect on c-IMT in the SATURN-HIV population remains unknown. Plasma ADMA levels have been shown to be significantly correlated with c-IMT in the HIV-uninfected population²⁷, and progression of c-IMT have also been related to serum ADMA levels. In the PREVENCIÓN study, a population based study of 922 uninfected adults, ADMA significantly predicted c-IMT even after adjustment for cardiovascular risk factors²⁸. The aim of our exploratory analysis is to investigate potential pathways that might modulate the effect of statin on c-IMT, by specifically examining the role of ADMA in the pathogenesis of c-IMT progression in the HIV-infected population.

Our results show that statin had a favorable effect on ADMA levels among HIV-infected subjects on ART. Daily rosuvastatin prevented the increase over time in plasma ADMA levels that was seen in the placebo arm. A possible proposed mechanism by which statins influence ADMA levels is via the increase of dimethylarginine dimethylaminohydrolase (DDAH), an enzyme involved in the metabolism of ADMA¹². The specific pathway involved in the metabolism of ADMA occurs via hydrolytic degradation to citrulline and dimethylamine catalyzed by the enzyme DDAH²⁷. DDAH overexpression has been shown to reduce ADMA levels and increase NO levels²⁹. In animal studies DDAH overexpression has been shown to promote endothelial repair after vascular injury, to suppress myocardial reperfusion injury and to inhibit ADMA induced endothelial dysfunction²⁷. In cell culture studies pro-oxidant and pro-inflammatory stimulants inhibit DDAH activity³⁰.

In our study, ADMA levels remained unchanged over 48 weeks and a statistically increase in citrulline levels was observed in the rosuvastatin arm. We therefore hypothesized through its anti-inflammatory effects, rosuvastatin might have halted the progression of ADMA levels indirectly by up regulating DDAH levels and leading to an increase in metabolism of ADMA levels and a subsequent increase in citrulline.

Previous studies have demonstrated improvement in ADMA plasma levels with ART initiation¹⁸, however the natural progression of plasma ADMA levels has not been

investigated in HIV-infected subjects on stable ART. In our study, the increase in ADMA levels seen in the placebo arm seems to behave in a similar manner as the increase of oxidative stress markers and inflammatory markers reported previously in HIV-infected patients on stable ART over 48 weeks^{24,31}, as well as the progression of c-IMT seen in the placebo arm over 96 weeks in SATURN-HIV.

ADMA is known to exert numerous vascular effects³², and elevated ADMA levels have been associated with high rates of cardiovascular events independently of traditional risk factors. The association of ADMA with cardiovascular disease in HIV-infected patients is not well understood³³; however, it has been associated with coronary calcium³⁴ and with pulmonary hypertension³⁵. In our present study, in accordance to what has been seen in HIV-uninfected patients^{36–39} elevated baseline levels of ADMA (highest tertile) were independently associated with increases in c-IMT overtime; however, the favorable effect of rosuvastatin on c-IMT did not differ according to baseline ADMA level. In accordance to what has been shown in the HIV-uninfected population our results suggest that elevated baseline levels of ADMA may play a causal role in the pathogenesis of c-IMT progression. These results should be interpreted with caution as the relationship between inflammation and cardiovascular disease in HIV is complex.

In the general population, systemic inflammation has been associated with decreased arginine and increased ADMA levels⁴⁰. Similar findings have been noted in disease states known to be associated with elevated systemic inflammation including diabetes, hypercholesterolemia, hypertension⁴¹, rheumatoid arthritis⁴² and systemic lupus erythematosus⁴³, but this was not previously investigated in HIV. In this study, we found that higher levels of ADMA were associated with sTNFR-I and with T cell activation markers.

Our study has several strengths, including the double-blind placebo controlled randomized trial design and the comprehensive evaluations of arginine metabolites, inflammation, immune activation and cardiovascular disease risk. Although it is the largest placebo-controlled statin trial conducted in HIV-infected persons to date, we limited our sample sizes for this secondary analysis and our focus was on the effect size estimates. We also investigated a specific HIV-infected population: those receiving stable ART with a low or undetectable HIV-1 RNA level, normal LDL cholesterol level, and heightened baseline immune activation. These patients are also mostly African American men; these factors may potentially limit the generalizability of these results to the HIV-infected population as a whole. Rosuvastatin halted the increase in c-IMT and ADMA overtime; however our study might be underpowered to detect any clinically significant causal inference between the favorable effect of rosuvastatin on c-IMT and ADMA levels.

Conclusions

In summary, rosuvastatin 10 mg daily in HIV+ subjects prevented the increase overtime in ADMA levels on ART. Elevated baseline levels of ADMA were associated with increases in c-IMT, regardless of statin use; however, the favorable effect of rosuvastatin on c-IMT was independent of the arginine pathway. This study contributes to the understanding of ADMA as a predictor of cardiovascular risk in patients with treated HIV infection. Further studies

should investigate the exact role of ADMA on the pathogenesis of c-IMT with statin therapy in the HIV-infected population and should also consider testing whether adjunctive L-arginine could improve the effectiveness of statin therapy to slow vascular disease progression.

Acknowledgments

The authors would like to thank the patients who participated in this research.

Financial support:

The work was supported by National Institutes of Health grant # R01 NR012642, R56HL126539 to GM and K23 HL123341 to CTL. This publication was made possible by the Clinical and Translational Science Collaborative of Cleveland, 4UL1TR000439 from the National Center for Advancing Translational Sciences (NCATS) component of the National Institutes of Health and NIH roadmap for Medical Research. Technical support was provided by the Center for AIDS Research, Case Western Reserve University (P30 AI36219). This publication was made possible by the Clinical and Translational Science Collaborative of Cleveland, UL1TR000439 from the National Center for Advancing Translational Sciences (NCATS) component of the National Institutes of Health and NIH roadmap for Medical Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

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Highlights

- Markers of systemic inflammation and immune activation correlate with ADMA levels.
- Rosuvastatin halted the increase in ADMA levels over time in HIV+ subjects on ART.
- Elevated baseline levels of ADMA were associated with an increase in cIMT.
- The favorable effect of rosuvastatin on cIMT was independent of the arginine pathway.

Figure 1.
Box-plot of arginine metabolites changes for Placebo and Statin group from 0–48 weeks.

Figure 2. Box-plot of changes in carotid intima media thickness (c-IMT) over 96 weeks relative to baseline ADMA levels.

Table 1

Baseline characteristics of study participants by assigned treatment group.

	Placebo (N=56) Mean (SD) or N (%)	Rosuvastatin (N=58) Mean (SD) or N (%)	<i>p</i> -value
Demographics			
Age (years)	44.53 (10.70)	44.67 (8.29)	0.94
Female sex (%)	13 (23.2)	11 (19.0)	0.58
African American race (%)	36 (64.3)	42 (72.4)	0.35
HIV parameters			
Current CD4+ count (cells/ μ L)	585.88 (277.73)	641.05 (293.16)	0.29
Viral load (copies/mL)	57.71 (63.83)	56.71 (70.28)	0.94
Cardiovascular risk factors			
Body Mass Index (kg/m ²)	28.30 (6.84)	28.18 (6.79)	0.95
Systolic blood pressure (mmHg)	121.20 (15.08)	124.21 (18.16)	0.36
Diastolic blood pressure (mmHg)	77.61 (10.06)	79.72 (9.40)	0.25
HDL cholesterol (mg/dL)	47.57 (15.16)	48.38 (16.55)	0.94
LDL cholesterol (mg/dL)	98.18 (25.96)	88.55 (23.73)	0.04
HOMA-IR ^a	4.36 (5.34)	2.87 (4.09)	0.09
Current smoking (%)	38 (67.9)	33 (56.9)	0.48
Current alcohol (%)	21 (37.5)	34 (58.6)	0.07
Family history of myocardial infarction (%)	18 (32.1)	18 (31.0)	0.9
Framingham Risk Score (% 10-year risk)	4.88 (4.31)	4.40 (4.25)	0.5
Mean-mean common carotid artery IMT (mm)	0.68 (0.11)	0.71 (0.16)	0.28
Inflammation and immune activation			
IL6 ^a (pg/mL)	3.54 (2.33)	4.69 (10.27)	0.79
CD8+CD38+HLA-DR+ T-cells (%)	13.43 (8.58)	15.16 (8.56)	0.2
CD4+CD38+HLA-DR+ T-cells (%)	5.15(2.34)	6.05 (3.49)	0.17
High sensitivity C-reactive protein (mmol/L)	5.18 (8.56)	5.74 (9.54)	0.95
Oxidized LDL (mU/L)	50561.92 (21341.51)	45855.52 (20440.37)	0.13
TNF α - receptor I ^a (pg/mL)	1774.11 (813.18)	1847.63 (724.69)	0.21
TNF α - receptor II ^a (pg/mL)	2200.15 (783.85)	2399.41 (854.75)	0.22
VCAM ^a (ng/mL)	663.48 (192.80)	719.46 (266.61)	0.33
sCD14 ^a (ng/mL)	2123.23 (636.07)	2479.60 (2023.94)	0.12
sCD163 ^a (ng/mL)	731.00 (311.06)	713.52 (315.28)	0.94
IP10 ^a (pg/mL)	309.83 (260.74)	341.09 (392.37)	0.52
Arginine metabolites			
ADMA ^a (μ mol/L)	0.65 (0.14)	0.67 (0.12)	0.38
Citrulline (μ mol/L)	33.68 (10.88)	33.00 (9.61)	0.81

	Placebo (N=56) Mean (SD) or N (%)	Rosuvastatin (N=58) Mean (SD) or N (%)	p-value
SDMA ^a (μmol/L)	0.50 (0.15)	0.55 (0.12)	0.02
L-arginine (μmol/L)	52.27(18.79)	52.97(19.17)	0.78

^aHOMA-IR, homeostatic model assessment of insulin resistance; IL6, Interleukin 6; TNFα-receptor I, tumor necrosis factor α-receptor-I; TNFα-receptor II, tumor necrosis factor α-receptor-II; VCAM, vascular cell adhesion molecule; sCD14, soluble CD14; sCD163, soluble CD163; IP10, interferon γ inducible protein-10; ADMA, asymmetric dimethylarginine; SDMA, symmetrical dimethyl arginine.

Table 2

Baseline factors correlated with baseline ADMA and ADMA levels at 48 weeks.

	Baseline ADMA ^a	48-week ADMA levels
ADMA at baseline	–	0.33 ($p<0.01$)
Statin	–	–0.019 ($p=0.61$)
sCD163 ^b (ng/mL)	–	0.03 ($p=0.04$)
IP10 ^b (pg/mL)	0.17 ($p=0.07$)	–
Oxidized LDL (mU/L)	–	0.03 ($p=0.03$)
IL6 ^b (pg/mL)	0.16 ($p=0.08$)	–
TNF α -receptor II ^b (pg/mL)	0.17 ($p=0.07$)	0.0245 ($p=0.04$)
TNF α -receptor I ^b (pg/mL)	0.37 ($p<0.01$)	0.0267 ($p=0.03$)
CD4+CD38+HLADR+ (%)	0.20 ($p=0.04$)	–
CD8+CD38+HLADR+ (%)	0.19 ($p=0.05$)	–

^aValues shown are parameters estimate and (p -values) from unadjusted regression analysis on ADMA measure at baseline and week 48. Only randomization group and variables with $p<0.1$ are shown.

^bsCD163, soluble CD163; IP-10; IP10, interferon γ inducible protein-10; IL6, Interleukin 6; TNF α -receptor II, tumor necrosis factor α -receptor-II; TNF α - receptor I, tumor necrosis factor α -receptor-I.