# Lack of association between asymmetric dimethylarginine and *in vivo* microvascular and macrovascular endothelial function in patients with rheumatoid arthritis

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## Abstract

### Objectives

*The aim of the present study was to investigate if assymetric dimethylarginine (ADMA) is increased in patients with rheumatoid arthritis (RA) compared to healthy controls and to examine associations between ADMA, RA disease activity and in vivo assessments of microvascular and macrovascular endothelial function.*

### Methods

*Sixty-seven RA patients (age [mean ± standard deviation]: 56±12 years, disease duration median [25$^{th}$–75$^{th}$ percentile]: 8 [3–15] years, 48 women) and 29 healthy controls (age [mean ± standard deviation]: 42±12, 21 women) underwent assessments of microvascular endothelial function (Laser Doppler imaging with iontophoresis of acetylcholine and sodium-nitroprusside), and macrovascular endothelial function (flow-mediated dilatation and glyceryl-trinitrate-mediated dilatation) as well as arterial stiffness. ADMA levels were measured in contemporary specimens using an immunoassay ELISA kit.*

### Results

*ADMA levels were significantly higher (p=0.004) in RA patients compared with healthy controls after adjustment for age (difference=0.088, 95% confidence interval 0.029–0.147). ADMA levels did not correlate with demographic or disease characteristics. No correlation was found between ADMA and microvascular and macrovascular endothelial function or with arterial stiffness.*

### Conclusion

*ADMA levels are increased in patients with RA but there was no significant correlation with in vivo assessments of endothelial function. Further studies are needed to unfold the pathophysiological role of nitric oxide/ADMA pathway derangement in endothelial dysfunction and cardiovascular risk in RA.*

**Key words**

ADMA, endothelial function, rheumatoid arthritis, cardiovascular disease

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## Introduction

Accumulating evidence suggests that cardiovascular disease (CVD) is a significant contributor to excess morbidity and mortality in rheumatoid arthritis (RA) (1, 2). This is not fully explained by the presence of traditional cardiovascular risk factors (3, 4). Since RA and atherosclerosis share significant similarities, it has been suggested that inflammatory mechanisms responsible for synovial lesions might also occur in the vessel wall and facilitate the development of advanced atherosclerosis (5). Systemic inflammation plays a key role in atherogenesis by inducing endothelial dysfunction, secondary dyslipidemia and activating the coagulation process (6).

Disruption of normal endothelial function is considered a primary event in the early phases of the atherosclerotic process. Injury to the endothelium can lead to vasoconstriction, increased leukocyte adhesion, chemokine and cytokine release, all of which induce chronic inflammation, which can eventually result in plaque formation. Endothelial function and morphology have been reported to be significantly worse in RA patients compared to healthy controls and this may be associated with the chronic inflammatory state of RA (7). It has been shown that endothelial dysfunction occurs early in the course of RA, and may represent a predisposing substrate for accelerated atherosclerosis (8).

Several non-invasive assessments of endothelial function have been developed. Using ultrasonographically-measured brachial artery flow mediated dilatation (FMD) or vasodilatory responses to acetylocholine (ACh, endothelium dependent vasodilator) or glyceryl-trinitrate (GTN, endothelium-independent vasodilator), some (9, 10), but not all (11) studies have shown impaired endothelial function in RA. Augmentation index (AIx), a marker of arterial stiffness, and an independent risk factor for cardiovascular disease has also been reported to be increased in patients with RA when compared with healthy controls (12, 13).

An alternative method to assess endothelial function involves biomarkers. The free radical nitric oxide (NO) is an endothelium-derived vasodilatory mediator and is synthesised from $_{L}$-arginine by NO synthase. Assymetric dimethylarginine (ADMA), an endogenous non-selective inhibitor of the three isoforms of NO synthase (NOS), has emerged as a novel cardiovascular risk factor in the setting of diseases associated with endothelial dysfunction, including type II diabetes mellitus (14), coronary artery disease (15) and end-stage renal disease (16). In the context of rheumatic diseases, elevated ADMA levels have been reported in patients with RA (17) and ankylosing spondylitis (18). ADMA has also been associated with scleroderma-related heart disease and pulmonary hypertension (19, 20) as well as increased risk of CVD events and poor prognosis in systemic lupus erythematosus (21).

To date, very few studies have assessed the role of ADMA in RA (17, 22) and it remains unkown whether ADMA is associated with *in vivo* assessments of endothelial function. The present study aimed to: (i) compare ADMA levels in patients with RA and healthy controls, (ii) investigate the relationship between ADMA and indices of disease activity and severity, and (iii) explore the association between ADMA and *in vivo* assessments of endothelial function in the microvasculature and the macrovasculature.

## Materials and methods

### *Study population*

Sixty-seven RA patients were recruited from the rheumatology outpatient clinics of the Dudley Group of Hospitals NHS Trust, United Kingdom, between January 2008 and April 2009. All patients met the retrospective application of the 1987 revised RA criteria of the American College of Rheumatology (23). Patients were excluded if they had a previously confirmed acute coronary syndrome, established CVD, diabetes mellitus, renal dysfunction or serious psychiatric disorder as indicated in their medical notes and/or on questioning during the initial consultation. Twenty-nine healthy participants were recruited from hospital staff and their friends. The exclusion criteria were the same as for the RA patients. The study

received local Research Ethics Committee approval and all participants gave their written informed consent according to the Declaration of Helsinki.

*Protocol*

Patients reported to a temperature controlled laboratory (22ºC) after a 12-hour overnight fast. Patients were not asked to withhold RA disease-related or vasoactive medications for the assessment. Patients were asked to refrain from smoking for 12 hours prior to the test and physical activity 24 hours before the test. All patients underwent a detailed clinical examination including evaluation of their medical history and hospital records. Demographic information was collected by questionnaire. The disease activity score in 28 joints (DAS28) (24) and physical function using the anglicised version of the Health Assessment Questionnaire (HAQ) (25) were assessed. Blood pressure measurements were taken via an automated blood pressure monitor (Datascope Accutor, USA). Blood was collected from the patient's antecubital vein using a 23G butterfly needle (Greiner Bio One GmbH, Austria). All tests were carried out in the research laboratories of Russells Hall Hospital, Dudley Group of Hospital NHS Foundation Trust, UK, and were analysed for routine laboratory biochemistry, lipid and bone profile tests, haematology, Westergren erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). Rheumatoid factor (RF) and antibodies against cyclic citrullinated peptides (anti-CCP) were also measured.

*Measurement of ADMA*

ADMA levels were measured in serum samples using a commercial enzyme immunoassay ELISA kit (Immundiagnostik, Bensheim, Germany). The kit uses an immunoaffinity highly specific and sensitive rabbit anti-ADMA antibody. Blood was collected and centrifuged in the Vacutainer@ SSFM tubes, at room temperature and at 4°C. Serum was removed and stored at -80°C before being assayed for ADMA. The intra-assay co-efficient of variation was 7.6%.

*Arterial stiffness*

Non-invasive assessment of radial artery waveforms (pulse wave analysis) was recorded using an applanation tonometer (SphygmoCor Px Pulse Wave Analysis, ScanMed Medical Instruments, UK). After the recording of brachial blood pressure, the right radial artery was palpated to identify a suitable pulse. The applanation tonometer was positioned over the artery with enough pressure to flatten (but not occlude) the patient's radial artery. The applanation tonometer records the first and second systolic peaks and then displays the augmentation index (AIx). The AIx is calculated as the difference between the first and second systolic peak and is expressed as a percentage of the pulse pressure (26). The pressure waveforms in the radial artery were recorded for an 11-second period. The software integrated in the analyser displayed an operator index which reflects the quality of the recorded waveform. If the operator index was low (<65), another reading was taken. Three readings with an operator index >65 were used for analysis. The average AIx of these three readings was calculated.

*Microvascular endothelial function*

Endothelial function of the microvasculature was assessed non-invasively using LDI (Moor LDI 2 SIM, Moor Instruments Ltd, Devon, UK) with iontophoresis of 1% acetylcholine (ACh, endothelium-dependent) and 1% sodium-nitroprusside (SNP, endothelium-independent) (Sigma Chemical Co, Montvale, New Jersey, USA) in 0.5ml of saline by a single observer (AS). The technique was performed according to previously established guidelines (27) and was described in detail previously (28). Briefly, after a baseline scan, ten scans were recorded during iontophoresis of the vasoactive agents using a 30μA current, followed by two scans during recovery. This technique has an intra-observer co-efficient of variation (CV) for ACh and SNP of 6.5% and 5.9% respectively in our laboratory.

*Macrovascular endothelial function*

Assessment of macrovascular endothelial-dependent function was performed using FMD with high-resolution ultrasonography of the brachial artery (Acuson Antares ultrasound system, Siemens PLC, Camberley, UK) according to previously established guidelines (29). Following ten minutes of rest, endothelium-independent responses were examined by administration of 500 microgram sublingual glyceryl-trinitrate (GTN) tablet (Alpharma, Barnstaple, UK), while the brachial artery was imaged continuously for five minutes. The intra-observer CV was 10.7% for FMD and 11.8% for GTN assessments respectively. For all vascular tests, endothelial function was expressed as the percentage increase in perfusion or diameter from baseline, and all analysis was carried out offline by AS who was blinded to the identity of the patient.

*Statistical analysis*

Statistical analysis was performed using SPSS16 (SPSS Inc, Chicago, Illinois). Variables were tested for normality by the Kolmogorov-Smirnov test. Means and standard deviations (SD) were calculated for normally distributed continuous variables and proportions for categorical variables. Log transformation was performed for skewed variables as appropriate. Differences between patients and healthy controls for ADMA and vascular parameters were tested using univariate analysis of co-variance (ANCOVA). The ANCOVA was preferred over the *t*-test, as it allows factors that differ between groups to be co-varied in the analysis. An age-matched comparison of ADMA for patients and controls was also performed.The dates of birth of participants were used to age-match two patients with each control wherever possible. This process was carried out systematically, with closest matches being performed first. It continued until there were no further matches to within the 3 years tolerance that had been specified in advance. Individuals who were not part of a triplet of two patients and a control were excluded from the age-matched analysis.

The Chi-squared test was used to compare categorical variables. Pearson correlations were used to assess the relationships between each parameter of endothelial function and ADMA.

## Results

*Participant characteristics and general demographics*

The general characteristics and demographic data are presented in Table I. Univariate ANOVA showed that age ($p$=0.000), and resting SBP ($p$=0.001) were greater in RA patients when compared to healthy controls, however, when correcting for age, resting SBP was no longer significantly different (Table I). None of the healthy control participants were on any medications.

*Routine biochemical profile and vascular function in RA patients and controls*

Univariate ANCOVA with age entered as a covariate revealed that inflammatory markers (ESR and CRP) were significantly higher in RA patients than controls (Table I). With the exception of triglycerides, RA patients did not differ on other cholesterol parameters or endothelial function. However, arterial stiffness was greater and microvascular endothelial-dependent function was lower in patients with RA, but neither difference was statistically significant ($p$=0.05 and $p$=0.07 respectively) (Table II). ADMA levels were significantly higher ($p$=0.004) in RA patients compared with healthy controls after adjustment for age (difference=0.088, 95% confidence interval 0.029–0.147). From the age-matched analysis the difference in ADMA levels was found to be 0.083 (0.018–0.148) based on 17 triplets of two patients and a control ($p$=0.016). Pearson correlations revealed that ADMA values, microvascular and macrovascular endothelial function were not associated with any demographic variables or with parameters of disease activity (LogESR, logCRP, DAS28), or severity (HAQ). In addition, ADMA was not associated with RF or anti-CCP antibodies.

*Association between ADMA and assessments of endothelial function*

To identify associations between ADMA and assessments of endothelial function in RA, Pearson correlation was performed (Fig. 1). ADMA was not associated with microvascular endothelial-dependent function ($r$ [66]

**Table I.** The general characteristics of both groups.

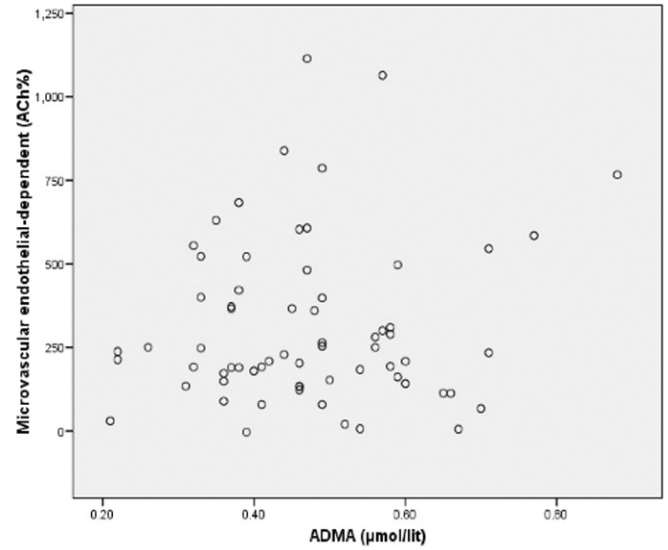
| | RA patients | n. | Healthy controls | n. | $p$-value |
|---|---|---|---|---|---|
| *Participant characteristics* | | | | | |
| Age (years) | 56± 12 | 67 | 42± 12 | 29 | 0.000 |
| Sex female (%) | 48(72) | 67 | 21(72) | 29 | 0.94 |
| Height (cm) | 164± 9 | 63 | 167± 10 | 27 | 0.32* |
| BMI (kg/m$^2$) | 30± 7 | 62 | 28± 7 | 27 | 0.38* |
| Resting SBP (mmHg) | 134± 15 | 66 | 121± 15 | 28 | 0.02* |
| Resting DBP (mmHg) | 82± 10 | 66 | 77± 10 | 28 | 0.26* |
| *Disease characteristics* | | | | | |
| RF positive (%) | 48(72) | 67 | – | | |
| Anti-CCP positivity (%)** | 13(57) | 23 | – | | |
| Disease duration (years) | 8(3 –15) | 50 | – | | |
| LogESR | 1.17± 0.45 | 64 | 0.74± 0.37 | 29 | 0.001* |
| LogCRP | 0.78± 0.51 | 67 | 0.52± 0.15 | 29 | 0.005* |
| DAS28 | 3.6± 1.4 | 67 | – | | |
| HAQ | 1.7± 0.82 | 66 | – | | |
| *Medications* | | | | | |
| Methotrexate (%) | 41(61) | 67 | – | | |
| Sulfasalazine (%) | 17(25) | 67 | – | | |
| Hydroxychloroquine (%) | 18(27) | 67 | – | | |
| Leflunomide (%) | 4(6) | 67 | – | | |
| Oral steroids (%) | 13(19) | 67 | – | | |
| NSAIDs (%) | 14(21) | 67 | – | | |
| COX-II inhibitors (%) | 20(30) | 67 | – | | |
| Anti-hypercholesterolemics (%) | 9(13) | 67 | – | | |
| Anti-hypertensives (%) | 17(25) | 67 | – | | |
| Beta-blockers (%) | 4(6) | 67 | – | | |
| Calcium-channel blockers (%) | 5(8) | 67 | – | | |

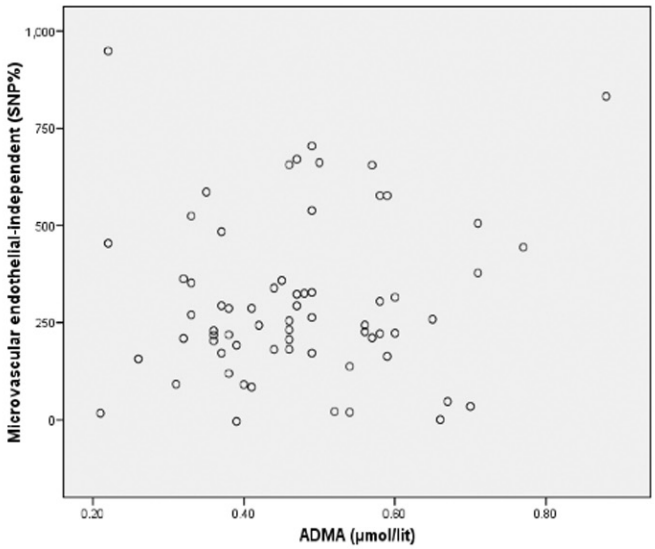
Results are expressed as number (percentage), median (25$^{th}$–75$^{th}$ percentile) or mean ± SD as appropriate. *Analysis adjusted for age. **Anti-CCP antibodies were available only in 23/67 patients.
BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; RF: rheumatoid factor; DAS28: disease activity score in 28 joints; HAQ: health assessment questionnaire; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; COX: cyclooxygenase, NSAIDs: non-steroidal anti-inflammatory drugs.

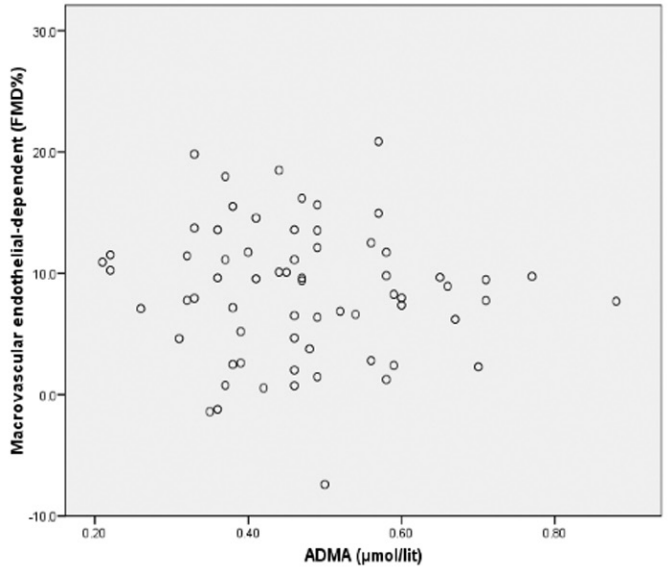
**Table II.** Laboratory parameters and endothelial function in both groups.

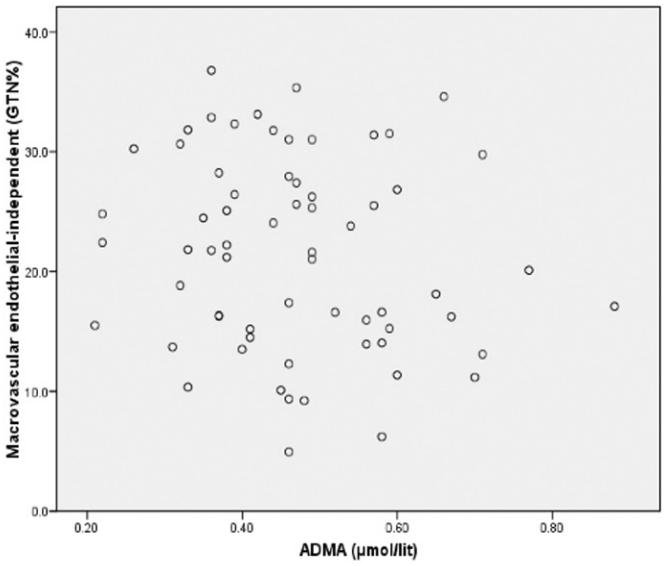
| | RA patients | n. | Healthy controls | n. | $p$-value* |
|---|---|---|---|---|---|
| *Blood tests* | | | | | |
| Total cholesterol (mmol/l) | 5.1 ± 1.0 | 67 | 4.9 ± 0.94 | 29 | 0.99 |
| HDL (mmol/l) | 1.5 ± 0.36 | 67 | 1.4 ± 0.36 | 29 | 0.93 |
| Triglycerides (mmol/l) | 1.4 ± 0.66 | 67 | 0.99 ± 0.49 | 29 | 0.02 |
| TC:HDL ratio | 3.6 ± 0.84 | 67 | 3.5 ± 0.84 | 29 | 0.85 |
| ADMA | 0.47 ± 0.13 | 67 | 0.37 ± 0.07 | 29 | 0.004 |
| *Endothelial function* | | | | | |
| Arterial stifness (AIx) | 32 ± 9 | 55 | 25 ± 10 | 11 | 0.05 |
| Microvascular endothelial-dependent (ACh %) | 313 ± 245 | 66 | 541 ± 316 | 27 | 0.07 |
| Microvascular endothelial-independent (SNP %) | 306 ± 208 | 66 | 391 ± 260 | 27 | 0 .72 |
| Macrovascular endothelial-dependent (FMD %) | 8.5 ± 5.5 | 66 | 11.8 ± 5.5 | 29 | 0.16 |
| Macrovascular endothelial-independent (GTN %) | 21.6 ± 8.0 | 65 | 25.0 ± 6.0 | 28 | 0.84 |

Results are expressed mean ± SD. *Analysis adjusted for age.
HDL: high density lipoprotein; TC: total cholesterol; AIx: augmentation index; ACh: acetylcholine; SNP: sodium nitroprusside; FMD: flow-mediated dilatation; GTN: glyceryl-trinitrate mediated dilatation.



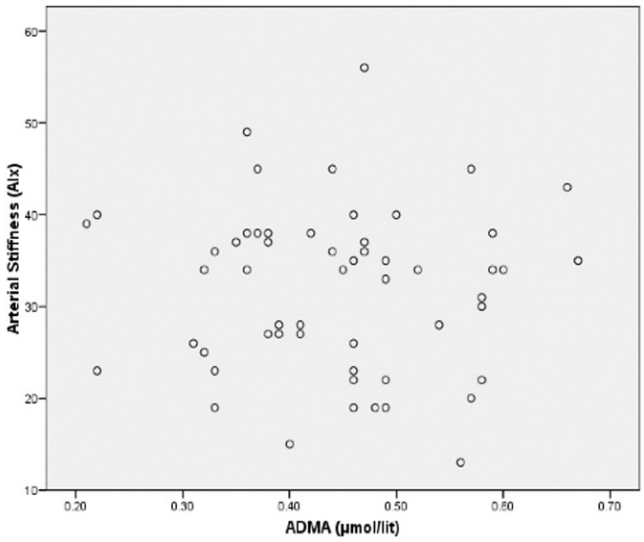




**Fig. 1.** Associations between parameters of endothelial function and ADMA.

=0.1, $p$=0.42), microvascular endothelial-independent function ($r$ [66] =0.09, $p$=0.49), macrovascular endothelial-dependent function ($r$ [66] =-0.08, $p$=0.53), macrovascular endothelial-independent function ($r$ [65] =-0.16, $p$=0.20) or arterial stiffness ($r$ [65] =0.19, $p$=0.89).

## Discussion

The present study investigated ADMA as a biomarker of endothelial dysfunction in patients with RA. Although ADMA was found to be significantly higher in RA patients than in healthy controls, no associations were found between ADMA and either disease characteristics or *in vivo* assessments of microvascular and macrovascular endothelial function within the RA population studied.

The current findings concur with those of previous studies which have demonstrated increased ADMA in patients with RA (17, 22). Elevation of ADMA levels in RA reflects increased production and/or reduced degradation. The activity of the enzymes involved in the formation – protein arginine N-methyltransferases (PRMTs) – and degradation – dimethylarginine dimethylaminohydrolase (DDAH) – of ADMA have been shown to be regulated in a redox-sensitive fashion (30). Since the production of reactive oxygen species is increased in the synovium of patients with RA, oxidative stress may result in accumulation of ADMA by the activation of the PRMTs (30), augmentation of protein arginine type I N-methyltransferase (31) and the reduction of DDAH activity (30). Additionally increased TNF-alpha expression (32) and hypoxia within the inflamed synovium (33) have been reported to downregulate DDAH, thus contributing to limited catabolism of ADMA. The increased endothelial cell turnover in the inflamed synovial tissue and the consequent release of ADMA during protein degradation may represent another possible mechanism of increased ADMA in RA (34).

Sixty-one percent of the patients in the present study were receiving methotrexate (MTX), a disease modifying drug which inhibits dihydrofolate deructase. MTX may affect the synthesis of ADMA via depressed remethylation of homocysteine to methionine, which can reduce circulating levels of ADMA. However, in a recent study, MTX did not decrease the concentration of ADMA in patients with early rheumatoid arthritis, though it considerably controlled disease activity (35). In the current study, despite a high proportion of patients receiving MTX, ADMA levels were still higher than in controls.

The effects of medications used to lower CVD risk factors might also affect ADMA levels and endothelial function. It has been shown that a significant number of patients with RA without CVD are at sufficiently high risk of developing CVD and require statin treatment (36). In the current study, 25% of patients were receiving lipid lowering treatment. Statins can improve endothelial function by depressing the activity of NAD(P)H oxidase and stimulating the production of NO (37). Simvastatin has been reported to inhibit *in vitro* ADMA-induced inflammatory reactions by the p38 Mitogen Activated Protein Kinase pathway in cultured endothelial cells (38). Clinical and experimental studies, however, have revealed that statins have no effect on plasma concentrations of ADMA (39, 40).

Hypertension is a well established risk factor for atherosclerosis which has been linked to increased ADMA levels (41). The prevalence of hypertension in RA is high, estimated between 52%–73% (3). In the present cohort, 24% of patients were hypertensive, and the majority of them were receiving angiotensin converting enzyme or angiotensin II subtype-1 receptor inhibitors and beta-blockers, all of which may improve endothelial function and lower the concentration of ADMA (42). In our study, hypertensive patients did not differ from non-hypertensive patients for any of the parameters of vascular function assessed or for ADMA levels (data not shown). Therefore, elevated levels of ADMA were probably not due to hypertension in our population.

We did not demonstrate any relationship between ADMA and inflammatory indices of the disease (ESR, CRP, DAS28), which is in line with previous studies (17, 22). Similarly, a recent systematic review of the literature has revealed that the majority of studies do not support an association between RA-disease-related inflammation and *in vivo* assessments of endothelial function (43). ADMA has only been found to associate with the number of swollen and tender joints in RA of short duration (<12 months) (22). Studies have also described correlations between ADMA and hs-CRP and ESR in patients with ankylosing spondylitis (18, 44). The reasons for the differing findings are unclear, but may relate to differences in the populations assessed (for example, established *vs*. early disease, with *vs*. without prevalent CVD), the magnitude of the inflammatory response or different treatment regimens.

Surdacki *et al*. (17) have shown that ADMA is associated with carotid artery intima-media thickness in patients with early RA who were free of CVD or significant CVD risk factors. In the same study, decreased endothelial progenitor cells correlated with ADMA, suggesting a novel potential mechanism of proatherogenic ADMA activity. Recently, Antoniades *et al*. have reported elevated ADMA levels and abnormal macrovascular endothelial function in patients with coronary artery disease and RA (45). In terms of endothelial function, Turiel *et al*. (22) noticed a statistically significant negative effect of ADMA on coronary flow reserve in 25 patients with early RA. In our study, endothelium dependent and independent microvascular function and macrovascular function as well as arterial stiffness did not correlate with ADMA levels.

Differences between our study and others may be due to several reasons. Firstly, our patients had relatively moderate disease activity (DAS 3.6±1.4). In previous studies investigating ADMA in RA, patients with DAS score <3.2 were excluded, and mean DAS was significantly higher (17, 22). Since endothelial function has been hypothesised to associate with inflammation, it is possible that the endothelial function of our patients may have been sufficiently preserved due to their modest levels of systemic inflammation. This is supported by the finding of compara-

ble levels of microvascular and macrovascular endothelial function between RA patients and controls in the present study. ADMA has been negatively correlated with FMD in renal disease (46) and hypercholesterolemia (47), but in these studies significant impairments in FMD had been observed relative to the control groups. Interestingly, no relationship between ADMA and FMD has been shown in patients with diabetes mellitus (48), a condition that bares significant similarities with RA in terms of cardiovascular risk (49, 50).

Recent studies in patients with systemic lupus erythematosus have demonstrated significant correlations between ADMA and arterial stiffness (51), as well as coronary calcium (52). In our patients, although there was a trend for significant increase of arterial stiffness in the group of RA patients, no association with ADMA was established. This may suggest that the impaired endothelial function in these individuals is not a result of NOS inhibition by ADMA. Mäki-Petäjä *et al*. (53) found that inducible NOS activity is increased in patients with RA and contributes to endothelial dysfunction, suggesting that inflammation might play a role in potentiating endothelial damage which subsequently develops into CVD. It is tempting to speculate that increased ADMA levels and arterial stiffness might reflect different stages of atherosclerosis, as arterial stiffness is believed to be a late functional-early morphological measure of atherosclerosis (54), while increases in ADMA may reflect early atherosclerotic stages characterised by the loss of NO-dependent vasodilatory function. However, the influence of endothelial NOS gene polymorphisms on the risk of CVD events in RA remains controversial (55, 56). Clinical and genetic studies which assessed the association between adipokines and carotid artery intima-media thickness, as well as CVD risk in patients with RA, demonstrated negative results (57, 58).

The present study has several strengths. It is the largest study to date to investigate a broad spectrum of vascular *in vivo* and biochemical *ex vivo* parameters for assessing endothelial dysfunction in a population of well established RA. To the best of our knowledge, this is also the first study which explored the association between ADMA and non-invasive assessments of endothelial function in both the microvasculature and the macrovasculature, as well as with arterial stiffness, in RA. On the other hand, this study also has limitations. Firstly, it was a cross sectional study which included patients with long-term RA suffering from comorbidities that can influence ADMA levels. In particular, triglycerides levels were higher in RA patients than healthy controls. Lundman *et al*. (59) have reported that hypertriglyceridemia is associated with elevated ADMA in a small sample (n=15) of young adults. However, in that study, patients with hypertriglyceridemia had reduced macrovascular endothelial function when compared with healthy controls, which is in contrast to our study where there was no difference in endothelial function between patients and controls. In addition, participants were considerably younger than the present cohort making it difficult to compare the findings between the studies and to determine the contribution of hypertriglyceridemia in our results. Secondly, *a priori* power calculations were not performed for the 3 main objectives, however, post-hoc power analysis revealed that the study was able to detect a 0.08 difference in ADMA with 80% power at the 5% significance level. For the correlation between endothelial function variables and ADMA, the study had 80% power at the 5% significance level to detect a correlation of 0.34 (for those variables with n=65 or 66) or 0.37 (for AIx with n=55). Finally, some patients received treatment with steroids or other disease modifying anti-rheumatic drugs resulting in a low disease activity score which may have impacted upon the findings. However, the aim of the current study was to investigate, as best as possible, endothelial function in a 'real life' population of RA.

## Conclusion

In conclusion, the present findings confirm that ADMA levels are significantly higher in patients with RA compared to healthy controls but do not appear to correlate either with disease activity or with *in vivo* assessments of endothelial function in RA. The role of ADMA in endothelial dysfunction warrants further investigation to delineate whether it is a credible serum biomarker of vascular disease in patients with RA.

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