Hindawi
Mediators of Inflammation
Volume 2018, Article ID 3897295, 13 pages
https://doi.org/10.1155/2018/3897295

Review Article

Asymmetric Dimethyl Arginine as a Biomarker of Atherosclerosis in Rheumatoid Arthritis

Manuela Di Franco, Bruno Lucchino, Fabrizio Conti, Guido Valesini, and Francesca Romana Spinelli

Dipartimento di Medicina Interna e Specialità Mediche-Reumatologia, Sapienza Università di Roma, Rome, Italy

Correspondence should be addressed to Bruno Lucchino; lucchino.b@gmail.com

Received 13 September 2017; Accepted 27 November 2017; Published 18 January 2018

Academic Editor: Mirella Giovarelli

Cardiovascular disease is the main cause of morbidity and mortality in rheumatoid arthritis (RA). Despite the advent on new drugs targeting the articular manifestations, the burden of cardiovascular disease is still an unmet need in the management of RA. The pathophysiology of accelerated atherosclerosis associated to RA is not yet fully understood, and reliable and specific markers of early cardiovascular involvement are still lacking. Asymmetric dimethylarginine is gaining attention for its implication in the pathogenesis of endothelial dysfunction and as biomarkers of subclinical atherosclerosis. Moreover, the metabolic pathway of methylarginines offers possible targets for therapeutic interventions to decrease the cardiovascular risk. The purpose of this review is to describe the main causes of increased methylarginine levels in RA, their implication in accelerated atherosclerosis, the possible role as biomarkers of cardiovascular risk, and finally the available data on current pharmacological treatment.

1. Introduction

Patients with rheumatoid arthritis (RA) have a significantly higher risk of cardiovascular diseases (CVD) compared to general population, comparable to patients with diabetes mellitus or non-RA subjects 10 years older [1]. In RA patients, cardiovascular events account for over 50% of the excess premature mortality [2]. Accelerated atherosclerosis plays a pivotal role in the pathogenesis of RA-related CVD: indeed, in RA patients, the atherosclerotic process starts in the early phases of the disease and it is determined by both an increased prevalence of traditional risk factors and the inflammatory nature of RA itself [3, 4]. The systemic inflammation has a major role in the pathogenesis of accelerated atherosclerosis. Proinflammatory cytokines involved in the pathogenesis of RA, such as TNF, IL-1, and IL-6, are also involved in the development and in the progression of atherosclerotic plaque. The first step in plaque development is the activation of endothelial cells and the induction of endothelial dysfunction (ED) by proinflammatory cytokines. The proatherogenic and prothrombotic endothelium is characterized by upregulation of adhesion molecules, raised vascular permeability, cytokine and chemokine expression, and reduced production of vasodilatory molecules, such as nitric oxide [5]. ED is the earliest, reversible, preclinical phase of plaque development, leading to the accumulation of lipoproteins and inflammatory cells in the subendothelial layer and to subsequent plaque formation [5]. Other than activating endothelial cells, TNF and IL-6 activate monocytes and immune cells contributing to the progression of the atherosclerotic disease, until rupture and thrombotic complication of the plaque [6]. There is a growing interest around the prevention of CVD in RA patients, although there is no clear evidence that any intervention can actually reduce that risk [7]. Early identification of ED may allow clinicians to characterize patients with subclinical atherosclerosis, establishing early risk factor modification or pharmacological intervention [5]. The imbalanced production of endothelial vasoactive mediators is a key step in the development of ED. Nitric oxide (NO) is the main endothelial-derived vasodilatory and antiproliferative molecule, inhibiting activation and vessel wall adhesion of

leukocytes and platelets [8]. The impaired ability of endothelial cells to produce NO is a main driver of ED. Dysregulation of other vasoactive mediators of NO metabolism predispose to subsequent pathological abnormalities such as platelet activation, abnormal fibrinolytic activity, lipoprotein deposition, and oxidative stress: all these modifications contribute to impaired vascular integrity [5, 9]. The role of endogenous inhibitors of NO synthase (NOS) activity in the induction of ED has gained the attention of rheumatologists. Asymmetric dimethylarginine (ADMA) is an analogue of L-arginine—the precursor of NO—naturally released in biological fluids following proteolysis; it inhibits NO synthesis by competing with L-arginine at the active site of NOS [10]. ADMA emerged as novel markers of ED and cardiovascular risk in RA [11]. The aim of this review is to summarize the available data on the role of ADMA in the pathogenesis of ED in RA patients, its role as potential biomarkers of CVD risk, and the possible therapeutic interventions.

2. Methylarginine Metabolism

Dimethylarginines are naturally occurring endogenous products of the degradation of methylated proteins. Methylation of arginine residues is a posttranslational modification catalyzed by a family of enzymes called protein arginine methyltransferases (PRMTs) which use S-adenosylmethionine as source of methyl groups; methylation of arginine is a two-step process of monomethylation [12, 13]. The first methylation leads to the formation of monomethylarginine (MMA), while the second one can produce either symmetric dimethylarginine (SDMA) or ADMA, according to the PRMT isoform involved in the methylation reaction [14]. After their proteolysis, MMA, SDMA, and ADMA are released in the cytosol, where the asymmetric methylarginines (MMA and ADMA) inhibit NOS activity by competing with L-arginine for the active site of the enzyme [15]. Cationic amino acid transporters (CATs) are the transmembrane enzymes which carry out methylarginines and arginine from the cellular cytosol to extracellular fluids and then in the bloodstream [16]. In physiological conditions, intracellular levels of arginine are much higher than those required for NOS activity; however, intravenous supplementation of arginine can increase endothelial-dependent vasodilatation [17]. This apparently incongruous phenomenon is called "arginine paradox": several hypotheses have been proposed to explain this effect. The activity of the enzyme arginase, which converts arginine in ornithine and urea, may reduce the availability of arginine, decreasing NOS activity. However, arginine is converted by NOS in an intermediate state, the hydroxy-L-arginine, which inhibits arginase, increasing substrate bioavailability for NOS. Another possible explanation is the competitive occupation of CATs by arginine excess for intracellular space transportation instead of other cationic amino acids [17]. CATs and NOS are located in the plasmatic membrane caveolae, ensuring a stable supply of the substrate (i.e., arginine) from the plasmatic compartment [18]. A relative abundance of plasmatic arginine may overtake NOS inhibition by raising intracellular arginine/ADMA ratio in the strict proximity of NOS [19]. However, using the same transporter, plasmatic ADMA may also gain a selective access to NOS, thus reducing NO bioavailability and explaining the association with the ED and, subsequently, with the increase in cardiovascular risk [16]. Once in the circulation, methylarginine can be eliminated through renal excretion or tissue catabolic pathways [13]. About 20% of ADMA is removed from plasma by the kidney while SDMA is mostly excreted unmodified through the urine [20]. The main pathway for asymmetric methylarginine catabolism is the hydrolytic reaction mediated by dimethylarginine dimethylaminohydrolase (DDAH) enzymes which catalyze the degradation of MMA and ADMA to citrulline and monomethylamine or dimethylamine, respectively [21]. Different tissues and cells express DDAH including heart, endothelium, kidney, lung, pancreas, liver, brain, and placenta as well as macrophages and neutrophils; however, ADMA is mostly catalyzed by the kidney and liver [22, 23]. A further catabolic pathway for both symmetric and asymmetric methylarginines is the transamination mediated by alanine-glyoxylate aminotransferase; however, the contribution of transamination to ADMA metabolism has not been fully investigated [24]. The methylarginine metabolism is depicted in Figure 1.

3. Physiopathology of ADMA and Endothelial Dysfunction in Rheumatoid Arthritis

3.1. Factors Affecting ADMA Levels in RA Patients. Different mechanisms can account for the increase in ADMA levels detected in RA patients. The inducible NOS (iNOS) is an isoform that can be induced in various cellular types under inflammatory stimuli; iNOS has a crucial role in the intracellular clearance of pathogens and in the vasodilatation of inflamed tissues [25]. However, the increased production of NO by iNOS, primed by inflammatory cytokines, leads to an S-nitrosylation of reactive cysteine in DDAH, inhibiting ADMA catabolism, thus increasing its levels and lastly inhibiting all three isoforms of NOS [26]. *In vitro* studies on endothelial cells demonstrated that TNF, a cytokine playing a key role in RA pathogenesis, exerts an inhibitory effect on DDAH leading to the impairment in ADMA degradation [27]. In RA patients, free radicals and nitrotyrosine produced by rheumatoid synovia as well as by the reduced expression of DDAH enzyme in the hypoxic environment of inflamed synovia may further contribute to DDAH inhibition and rise in plasmatic ADMA levels [28–30]. Another explanation for the high ADMA levels is an increase in its production by PRMT activity: Böger et al. described an enhanced production of ADMA in endothelial cells exposed to native and oxidized LDL (oxLDL), partially due to enhanced PRMT gene expression [31]. oxLDL levels are higher in RA patients than in healthy subjects because of the oxidative stress coexisting with the inflammatory state [32, 33]. Moreover, other posttranslational modifications of LDL may also account for NO uncoupling [34]. In the rheumatoid synovia, endothelial cells undergo a phenotypic change characterized by an increase in activation, angiogenesis, and apoptosis [35]. The increased turnover of endothelial cells as well as the increased number of proliferating cells associated with angiogenetic microenvironment of the

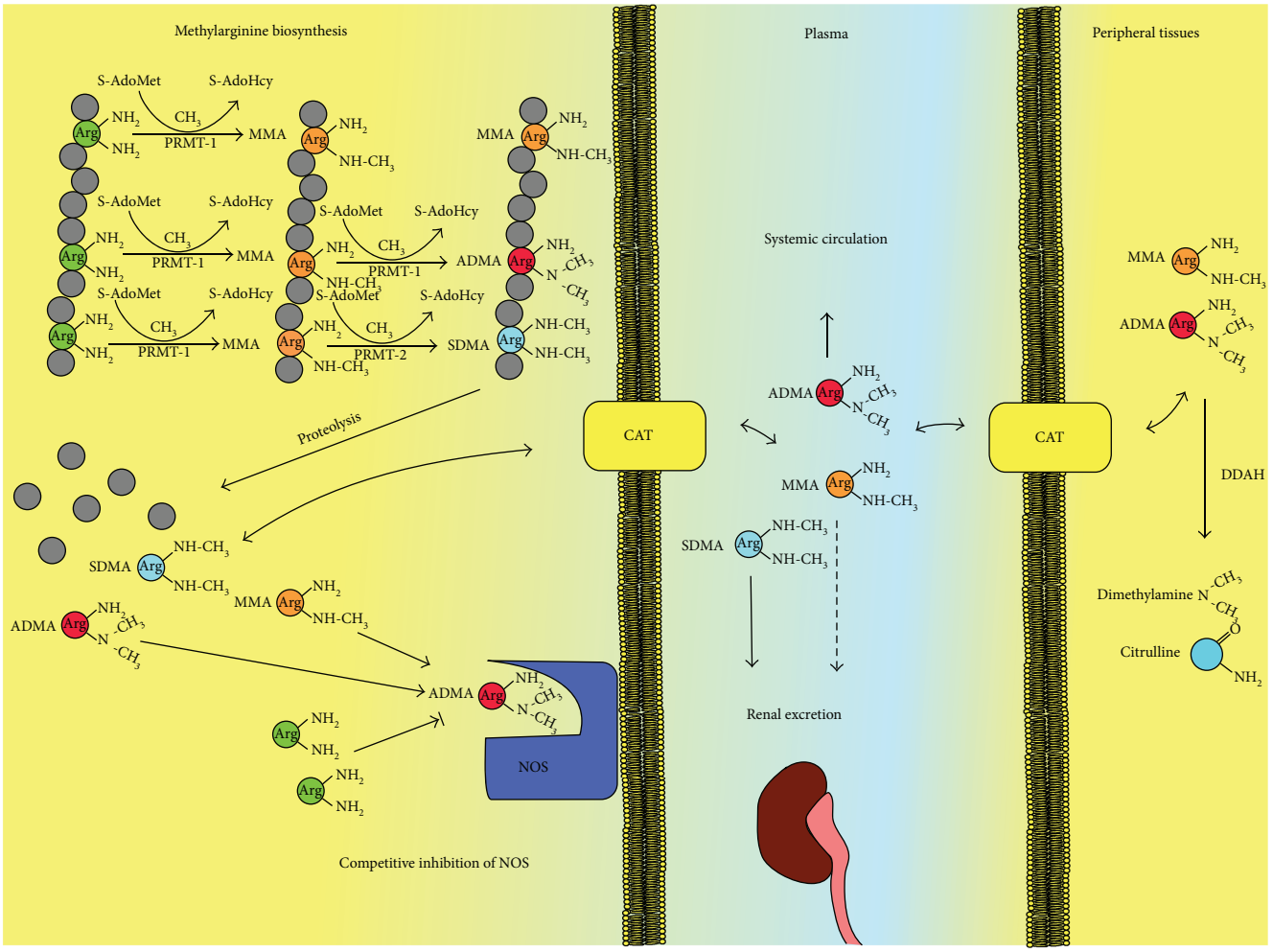

Figure 1: Metabolic pathways of methylarginines. S-AdoMet: S-adenosyl-l-methionine; S-AdoHcy: S-adenosylhomocysteine; PRMT: protein arginine methyltransferases; MMA: monomethyl arginine; ADMA: asymmetric dimethyl arginine; SMDA: symmetric dimethyl arginine; CAT: cationic amino acid transporter; DDAH: dimethylarginine dimethylaminohydrolase; NOS: nitric oxide synthase.

inflamed joint may be a source of methylarginines. ADMA production is enhanced in apoptotic and senescent endothelial cells, as a result of methylated protein turnover [36].

Patients with RA have a high basal level of insulin and a tendency toward insulin resistance which is associated with the inflammatory status and seems to be reverted by TNF inhibitors [37, 38]. Proinflammatory cytokines such as TNF and IL-6 prevent muscular glucose uptake and induce lipolysis in adipocytes, leading to an impaired plasmatic glucose regulation; moreover, free fatty acids released by stimulated adipocytes determine a positive feedback loop both by inducing an insulin-resistant phenotype of skeletal muscle and liver and by stimulating TNF and IL-6 production by macrophages [39, 40]. In diabetic patients, both increased and decreased levels of ADMA were reported [41, 42]. Chronic hyperglycemia increases ADMA levels by inhibiting DDAH activity [43]. On the contrary, insulin upregulates CAT expression in various cell types [44]. In healthy subjects and in type 1 diabetic patients, acute hyperinsulinemia reduces ADMA levels, probably increasing the cellular uptake related to CAT regulation [45, 46]. Raising the production of ADMA (via DDAH inhibition) and increasing cellular uptake (via CAT upregulation), insulin resistance may contribute to ADMA-mediated NOS inhibition [16].

Homocysteine (Hcy) is a sulfhydryl-containing amino acid mainly produced from the essential amino acid methionine. Several factors affect Hcy levels, including age, sex, lifestyle factors (coffee consumption, smoking habit, physical activity, and alcohol), genotype of the enzymes involved in Hcy catabolism, drugs and diseases interfering with its metabolism, and most importantly group B vitamins (folic acid, pyridoxine, and cobalamin) [47]. Hyperhomocysteinemia (HHcy) is a well-known risk factor for CVD in general population and in patients with RA [48, 49]. Some authors suggested a link between HHcy and increased ADMA levels; indeed, Hcy inhibits DDAH activity and the endoplasmic reticulum stress response in the dysfunctional endothelium seems to increase proteolysis, and thus ADMA levels [50, 51]. In RA patients, several factors contribute to the increase in Hcy serum levels. Chronic inflammation enhances immune cell turnover increasing the folate requirement, and the use of methotrexate contributes to folate deficiency

by inhibiting the enzyme dihydrofolate reductase [52, 53]. The reduced bioavailability of the methylenetetrahydrofolate, the key substrate of methylenetetrahydrofolate reductase, limits the conversion of Hcy to methionine, causing HHcy [53]. The link between NO metabolism and HHcy is not completely clear since Hcy-lowering agents seem not to significantly affect ADMA levels [54].

3.2. Linking ADMA to Endothelial Dysfunction in RA. Normal endothelium is responsible for many physiological functions needed to maintain vascular integrity, such as regulation of vascular tone and anticoagulating and anti-inflammatory functions [55]. NO is a key mediator of many functions of a healthy and functional endothelium, and consequently, the impaired ability to produce NO is a main feature of ED [56]. A dysfunctional endothelium is characterized by cytokine and chemokine production, adhesion molecule expression, platelet activation, abnormal fibrinolytic activity, lipoprotein deposition, and immune cell migration in the subendothelial layer leading to the early and subclinical phases of the atherosclerosis and driving all the steps of CVD until acute complications [5, 8, 9, 55].

Methylarginines affect endothelial function in different ways. Asymmetric methylarginines inhibit the three isoforms of NOS, reducing the NO production [15]. Furthermore, ADMA and MMA can compete with arginine for transmembrane transport through CAT, reducing the availability of the substrate for NO synthesis [57, 58]. Besides the interference with arginine-dependent NO production, ADMA determine "NOS uncoupling," a shift in NOS enzymatic activity from reductase to oxidase [59]. In the absence of its substrate, NOS transfers electrons to molecular oxygen, instead of arginine, leading to the formation of superoxide, instead of NO [59]. Superoxide is a free radical which rapidly combines with NO producing peroxynitrite, a highly reacting intermediate and powerful source of oxidative stress that entails DNA and protein oxidation and at high concentration, cytotoxicity [60]. Therefore, superoxide and peroxynitrite produced by ADMA-related NOS uncoupling contribute to oxidative stress and endothelial cell dysfunction [61].

Endothelial progenitor cells (EPCs) are bone marrow derived, circulating endothelial precursors able to differentiate in situ in functional endothelium, contributing to endothelial injury recovery and limiting atherosclerotic plaque formation; in the light of their repairing effect, EPCs are biomarkers of endothelial health [62]. A reduced number of circulating EPCs has been described in a number of conditions associated with an increased cardiovascular risk, including RA [63, 64]. In patients with RA, different authors observed an inverse correlation between ADMA levels and the number of circulating EPCs which can be reversed by TNF inhibitors [64–67]. Since NO is a key regulator of EPC migration and differentiation, lowering endogenous production of NO by the endothelium, ADMA can markedly reduce the mobilization and function of EPCs, impairing the protective effect [67, 68]. Figure 2 summarizes the physiopathology of ADMA in ED development in patients with RA.

4. ADMA as Biomarker of Cardiovascular Risk in Rheumatoid Arthritis

In the last years, the potential role of ADMA as a biomarker of cardiovascular risk has been investigated in several conditions. Recently, a meta-analysis of about 20,000 nonoverlapping participants enrolled in 22 cohort studies and long-term follow-up demonstrated an association between circulating levels of ADMA and cardiovascular outcomes, including coronary heart disease and stroke [69]. ADMA was also correlated with noninvasive markers of subclinical atherosclerosis such as flow-mediated dilation (FMD) and intima-media thickness (IMT). Brachial artery FMD is a noninvasive method to evaluate NO-mediated flow response to subischemic stimuli. FMD is a useful marker of CVD risk since it correlates with more invasive measurement of ED, with cardiovascular risk, and with coronary artery vasodilatory function [70]. In healthy subjects, elevated ADMA levels are associated with a reduced FMD, suggesting that ADMA may represent a biomarker of ED [71, 72]. In RA patients, the decrease of the endothelium-dependent macrovascular function starts to be evident within the first year of the disease; some authors detected an association with disease activity, not confirmed by others, and with serology [73, 74]. Some reports suggested an inverse correlation between ADMA levels and FMD, not confirmed by other studies [66, 75–77] (Table 1).

Ultrasonographic evaluation of carotid IMT is a reliable marker of cardiovascular outcome correlating with traditional risk factors and with the incidence of clinical cardiovascular events [78, 79]. A meta-analysis of the literature published in 2015 reported an increased carotid IMT with a higher prevalence of carotid plaque in RA patients compared to control subjects [80]. A meta-analysis of over 6,000 patients showed a positive relation between carotid IMT and ADMA, suggesting a role for the latter as a serological biomarker of cardiovascular risk [81]. As for RA, literature data seems not to confirm the association between carotid IMT and ADMA levels [77, 82–84] (Table 1). A single recent study, investigating biomarkers of micro- and macrovascular function in 197 RA patients, demonstrated a significant correlation between ADMA levels and noninvasive markers of endothelial dysfunction, in those patients showing a high disease activity: the authors showed a positive correlation between ADMA levels and cIMT and between arterial stiffness and ADMA/SDMA ratio, especially in patients with high inflammatory markers [85].

The studies investigating a possible association between markers of disease activity and ADMA led to conflicting results. A few studies on RA patients demonstrated a positive correlation between ADMA levels and C-reactive protein and disease activity score (DAS28) values, suggesting a link between a high inflammatory state, ADMA levels, and CVD in active RA; however, other studies failed to replicate these results [77, 82, 86–89]. Similarly, some reports described an association between anticitrullinated peptide antibodies (ACPA) titer and ADMA levels, especially in patients with early disease [87, 90, 91]. In RA patients, ADMA showed a positive correlation with Hcy

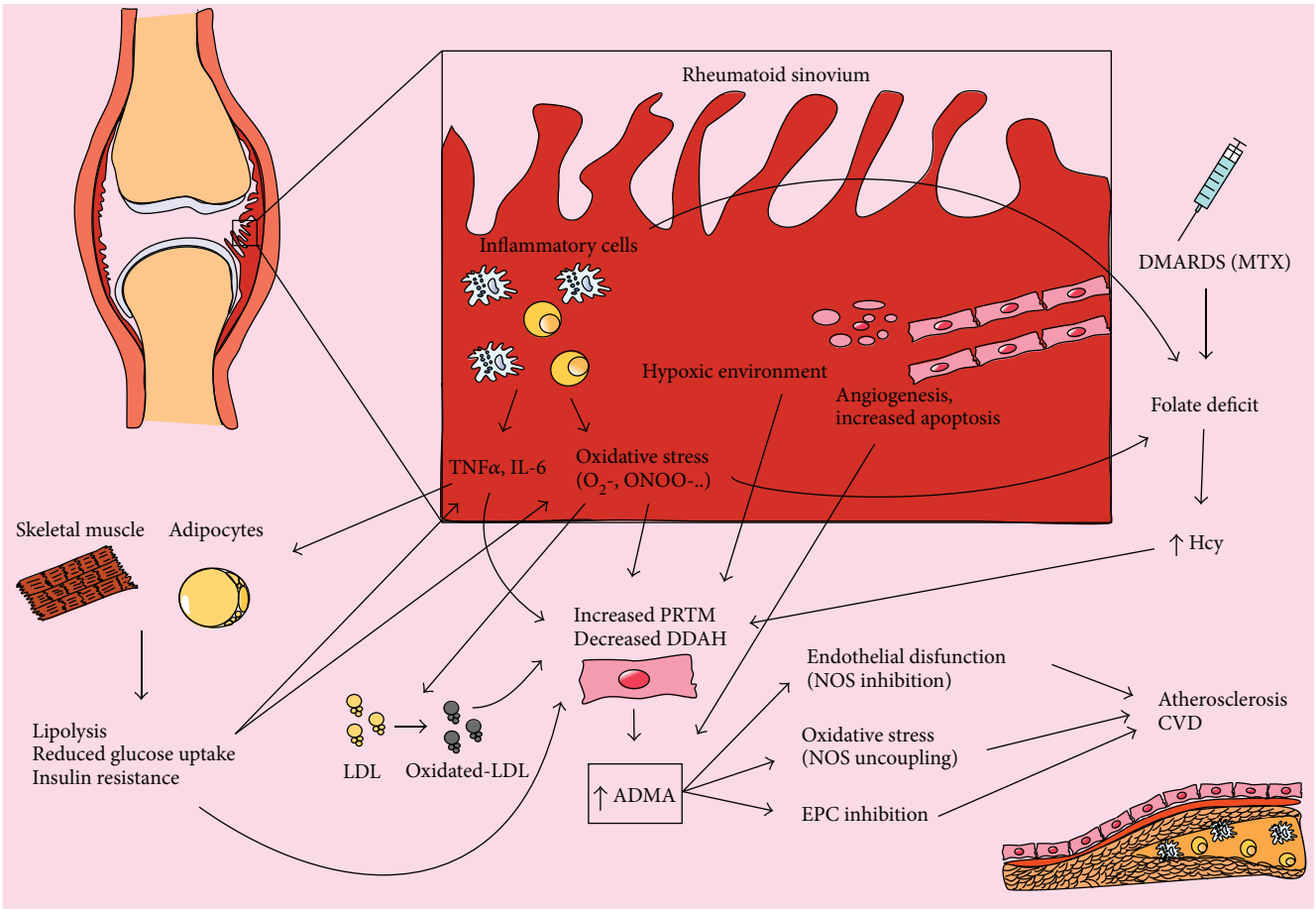

Figure 2: Mechanisms of ADMA-induced endothelial dysfunction in rheumatoid arthritis. The inflammatory microenvironment of the inflamed synovia produces cytokines and reactive oxygen species which directly stimulate PRTM and inhibits DDAH in endothelial cells, increasing ADMA production. Cytokines influence metabolically active tissues like skeletal muscle and adipocytes, inducing insulin resistance. This can generate a positive feedback loop, with an increased release of cytokines from macrophages and increased ADMA production in endothelial cells as well. The increased levels of oxidated lipoproteins, as a consequence of the oxidative stress linked to synovitis, furthermore contribute to ADMA synthesis, as well as the increased apoptosis of endothelial cells in inflamed synovium. At last, folate deficit, related to the increased cellular turnover, to the oxidation of folate from reactive oxygen species and to the methotrexate treatment, induces an increased generation of homocysteine, which contributes to ADMA increase. ADMA increase can induce endothelial dysfunction, oxidative stress, and EPCs inhibition, conducing atherosclerosis development and cardiovascular complications. PRMT: protein arginine methyltransferases; DDAH: dimethylarginine dimethylaminohydrolase; NOS: nitric oxide synthase; ADMA: asymmetric dimethyl arginine; EPCs: endothelial progenitor cells; MTX: methotrexate; Hcy: homocysteine; CVD: cardiovascular disease.

levels and it is associated with insulin resistance (the homeostasis model assessment (HOMA) being a strong predictor of ADMA serum levels) [92, 93]. Finally, ADMA serum levels correlate with other markers of endothelial health status, such as EPCs: the reduction in circulating EPCs, correlating with high plasmatic level of ADMA and high DAS28 values, was restored by a short course of TNF inhibitors [65]. In summary, these observations strongly suggest a possible role of ADMA as a reliable biomarker of early atherosclerosis in RA patients, especially in the context of an active disease.

ADMA levels start increasing in the early phase of disease, and the introduction of disease-modifying antirheumatic drug (DMARD) treatment seems to decrease the levels compared to those observed in the control group [77]. In a study on 20 early, untreated RA patients, our group demonstrated that therapeutic intervention with conventional synthetic DMARDs or TNF inhibitors significantly reduced ADMA serum levels [77]. Even in long-standing RA patients, the treatment with TNF inhibitors seems to reduce ADMA levels: this effect was shown in a study on 33 RA patients starting etanercept or adalimumab but was not confirmed by other authors [66, 83, 84, 91]. Table 1 summarizes the main findings of the studies investigating ADMA serum levels in the context of RA [65, 66, 75–77, 82–98].

The heterogeneity of methods used to assess subclinical atherosclerosis and the different contributions of traditional and disease-related risk factors in a complex disease such as RA may account for the lack of concordance of the results and limit the usefulness of ADMA as a marker for atherosclerotic risk stratification. In this regard, a cutoff level of ADMA defining a dysfunctional endothelium could be helpful.

Table 1: Main findings of the studies investigating ADMA in rheumatoid arthritis.

Number of RA patients (controls)	Main findings	Reference
91 (31)	No correlation between ADMA and subendocardial viability ratio	Anyfanti et al. [94]
201	No association between ADMA and genetic variants of the AGXT2 gene	Dimitroulas et al. [95]
197	Association between microvascular function, arterial stiffness, and cIMT and ADMA/SDMA levels in RA patients with high inflammatory marker	Dimitroulas et al. [85]
40 (29)	Inverse correlation between ADMA and FMD; positive correlation between ADMA and disease duration; no correlation with CRP	Sentürk et al. [88]
30 (30)	No relationship between ADMA concentration and aortic augmentation; no difference in ADMA levels between patients and controls	Erre et al. [96]
201	Difference in ADMA levels according to MTHFR; positive correlation between ADMA and Hcy and ESR	Dimitroulas et al. [92]
100	No correlation between ADMA and thCys at baseline and after omega-3 fatty acids, vitamin E, vitamin A, copper, and selenium, or placebo; correlation between ADMA and arginine	Kayacelebi et al. [98]
201	Positive correlation between ADMA and ESR and ADMA and CRP	Sandoo et al. [89]
33	Correlation between ADMA and DAS28; reduction of ADMA levels after 3 months of anti-TNF	Spinelli et al. [66]
201	No significant relationship between DDAH genetic variables and ADMA levels	Dimitroulas et al. [97]
17 (12)	Inverse correlation between ADMA levels and circulating EPC number	Spinelli et al. [65]
35 (35)	ADMA and RF have similar sensitivity and specificity in the detection of endothelial dysfunction	Spasovski and Sotirova [91]
67	HOMA, an indicator of insulin resistance, predicts elevated ADMA levels	Dimitroulas et al. [93]
48 (32)	Association between baseline PWV and ADMA but no correlation with cIMT; anti-TNF therapy increased L-arginine/ADMA ratio but not ADMA after 3 months	Angel et al. [84]
20 (20)	Significantly higher ADMA levels in RA than controls; significant reduction after 12 months of treatment	Di Franco et al. [77]
35	No change in ADMA levels after 2 weeks and 3 months of anti-TNF treatment	Sandoo et al. [75]
46 (50)	Higher ADMA levels in RA than in controls; correlation with CRP, DAS28, and 8-isoprostanes	Kwaśny-Krochin et al. [86]
60 (29)	Significantly higher ADMA levels in RA compared with controls; no correlation with demographic or disease characteristics	Sandoo et al. [83]
25	No change in ADMA levels and cIMT after treatment	Turiel et al. [82]
25 (25)	Higher ADMA levels in early RA than in controls. Significant negative correlation between ADMA levels and CFR; no correlation with IMT	Turiel et al. [90]
20	Positive correlation between ACPA and ADMA levels; no correlation with disease activity indices	Surdacki et al. [87]
36 (20)	Chronic low-dose prednisolone lower ADMA levels	Radhakutty et al. [109]

ADMA = asymmetric dimethyl arginine; AGXT2 = alanine-glyoxylate aminotransferase 2; SDMA = symmetric dimethyl arginine; cIMT = carotid intima media thickness; FMD = flow-mediated dilation; CRP = C-reactive protein; MTHFR = methylenetetrahydrofolate reductase; Hcy = homocysteine; ESR = erythrocyte sedimentation rate; thCys = total L-homocysteine; DAS28 = disease activity score 28; TNF = tumor necrosis factor; DDAH = dimethylaminohydrolase; EPCs = endothelial progenitor cells; RF = rheumatoid factor; HOMA = homeostasis model assessment; PWV = pulse wave velocity; CFR = coronary flow reserve; ACPA = anticitrullinated peptide antibodies.

5. Possible Therapeutic Intervention

Since methylarginines play a key role in the physiopathology of ED and ADMA levels have been strictly associated to cardiovascular risk, several pharmacological interventions have been investigated on the possible effect on ADMA levels and cardiovascular outcomes. However, taking into account the wide spectrum of indications of the drugs investigated and of the inter-study result variability, the actual relation between ADMA level reduction and cardiovascular benefits is still inconclusive [13]. Effect of statins on methylarginine metabolism has been investigated in different conditions such as diabetes, stroke, and hypercholesterolemia, demonstrated to effectively reduce plasmatic ADMA levels in recent controlled trials [99–101]. *In vitro*, statins increase the expression of DDAH genes and the bioavailability of tetrahydrobiopterin (BH4), which is a critical eNOS cofactor inhibiting NOS uncoupling phenomenon [102]. A recent

Table 2: ADMA lowering effect and possible pharmacodynamic mechanism of different drugs.

Drug	Investigated conditions	Hypothesized mechanism	Results	References
Statins	Diabetes mellitus, stroke, hypercholesterolemia	Increase DDAH expression, increased bioavailability of tetrahydrobiopterin	Decreased ADMA serum levels (18–50%)	[100, 109]
Fibrate	Hypertriglyceridemia	Increase DDAH activity through NF-kB suppression via PPAR-α receptors	Uncertain effect on ADMA serum levels, increase L-arginine/ADMA ratio	[111]
Niacine	Dyslipidemia	Depletion of methyl groups for niacine metabolism and consequent reduction in ADMA synthesis	Decreased ADMA serum levels (10%)	[112]
ACE inhibitors/ARB	Chronic glomerulonephritis, hypertension	Decreased NADPH oxidase upregulation by RAA system, with consequent reduced ROS-mediated DDAH inhibition	Decreased ADMA serum levels (10–16%)	[113, 114]
Thiazolidinediones	Diabetes mellitus	Through PPAR-γ receptor activation: reduced insulin resistance, increased expression of DDAH in renal tubules, suppressed activity of NF-kB	Controversial; from no reduction to reduction of ADMA serum levels (10%), possible protection against ADMA effect	[115]
Metformin	Diabetes mellitus Polycystic ovarian syndrome	Partially unknown, apparently not mediated by PRTM or DDAH Competitive antagonist of ADMA	Decreased ADMA serum levels (27%)	[116]
Nebivolol	Hypertension	Upregulation of DDAH, downregulation of PRTM	Decreased ADMA serum levels (37–44%)	[117, 118]
Acetylsalicylic acid	Coronary artery disease	Upregulation of DDAH and eNOS	Decreased ADMA serum levels (30%)	[119]
Estrogens	Postmenopausal women	Upregulation of DDAH via ERα	Decreased ADMA serum levels (18–20%)	[120, 121]
Folate and B group vitamins	Hypertension, hyperhomocysteinemia, chronic heart failure	Increased bioavailability of methylenetetrahydrofolate	Decreased ADMA serum levels (14%), acute decrease during e.v. infusion	[122, 123]
α-Lipoic acid	End-stage renal disease, diabetes mellitus	Activation and upregulation of DDAH via STAT3	Decreased ADMA serum levels (9%)	[124]
N-Acetylcysteine	End-stage renal disease	Partially unknown, direct activation DDAH, or ROS scavenging	Decreased ADMA serum levels (30%)	[125]

double-blind randomized study demonstrated that supplementation of oral tetrahydrobiopterin significantly improved the endothelial function measured by FMD in a small cohort of RA patients [103]. The authors did not investigate the effect on ADMA levels but, considering the implication of folate in methylarginine metabolism, an ADMA-lowering effect could be expected. This is also supported by the consolidated evidence of the role of folate supplementation on plasmatic Hcy lowering, in consideration of the interplay between HHcy and raised ADMA levels [47]. This suggests that larger and targeted studies, addressing the potential effect of tetrahydrobiopterin supplementation on ADMA levels in relation to ED and risk of CVD, are desirable.

In a small study on RA patients, atorvastatin effectively reduced arterial stiffness measured by pulse wave analysis, without affecting acute-phase reactants [104]. The lipid-lowering agent ezetimibe showed the ability to lower ADMA levels and to ameliorate renal function in patients with chronic kidney disease, probably by protecting DDAH enzymatic site from oxidative inactivation [105]. Besides the lipid-lowering effect, ezetimibe, as well as simvastatin, demonstrated to reduce disease activity and C-reactive protein levels and to improve the endothelial function and the arterial stiffness in patients with RA [106].

The evidence that lipid-lowering drugs couple an anti-inflammatory effect with an improvement of endothelial function, by modulation of ADMA metabolism, may suggest a role for these drugs in the management of cardiovascular risk associated to RA. The ADMA-lowering effect of several other agents have been investigated in conditions different from RA. Only few studies addressed the effects of therapeutic intervention for RA on ADMA levels. Treatment with DMARDs, especially anti-TNF agents, demonstrated a lowering effect on ADMA levels, more pronounced in high inflammatory conditions (patients with high levels of acute-phase reactants) [85]. A recent meta-analysis showed that treatment with TNF inhibitors improves endothelial function in patients with RA [107]. It is very likely that effect of TNF inhibitors on cardiovascular risk is multifactorial, acting on different steps of the atherosclerotic process. Longitudinal studies demonstrated a short-term effect of TNF inhibitors on ADMA levels, not confirmed in studies with different

follow-up [77, 83, 84]. Nevertheless, in a 12 month follow-up study, TNF inhibitors improved the arginine/ADMA ratio despite not impacting on ADMA absolute levels [84]. These results imply that the modulation of ADMA metabolism could partially account to the atheroprotective effect of TNF inhibitors.

The effect of folate supplementation on plasmatic Hcy is well known and some authors hypothesized an interplay between HHcy and raised ADMA [47]. A single study on a large population of RA patients ($n = 201$) demonstrated that Hcy levels are significantly related to serum ADMA, contrasting with previous data obtained in a smaller group of patients [92, 98]. The relationship between ADMA and Hcy levels is intriguing since the latter is affected by the use of methotrexate, a milestone in the RA treatment. In a recent study, Dimitroulas et al. demonstrated a trend of the MTHFR polymorphism to influence ADMA levels, with the C667T polymorphism associated to higher ADMA levels, only at the univariate analysis [92]. Interestingly, C677T polymorphism was associated with subclinical atherosclerosis and CVD risk in a study on 612 RA patients followed up for 5 and 10 years [108]. These evidences may support the protective, antiatherogenic effect of methotrexate.

A very recent study investigated the effect of low-dose glucocorticoids on arginine metabolisms by comparing patients who were chronically treated or not with prednisolone and demonstrated higher levels of ADMA and MMA in those patients who were not taking glucocorticoids; the authors conclude that long-term glucocorticoid treatment could help in protecting endothelial health in RA patients [109].

Table 2 summarizes potential therapeutic intervention with ADMA-lowering effect.

6. Conclusion

CVD risk reduction is still an unmet need in the long-term management of RA patients and, despite the great improvement of RA treatment, CVD is still the main cause of death. In 2016, the European League Against Rheumatism (EULAR) updated the recommendations for the management of CVD in rheumatic disease firstly published in 2009, suggesting the need for an aggressive and targeted risk management [110]. The research agenda still includes issues about the precise effect of antirheumatic drugs with different modes of action and the additional value of novel biomarkers for CVD risk prediction on CVD risk [110]. The physiopathology of ED in chronic inflammatory diseases such as RA is still largely unknown, and biomarkers to efficiently stratify patients according to their CV risk are scant. ADMA seems to have the potential to solve part of these issues. The apparent physiopathological role of ADMA in endothelial NO deficit as well as the correlation between the circulating ADMA levels and cardiovascular outcomes suggest that ADMA could be a good candidate for further basic research. Moreover, better understanding the role of ADMA in ED could also provide potential target of pharmacological intervention to lower the cardiovascular risk in RA.

Conflicts of Interest

All the authors declare that there is no conflict of interest regarding the publication of this paper.

References

[1] J. Lindhardsen, O. Ahlehoff, G. H. Gislason et al., "The risk of myocardial infarction in rheumatoid arthritis and diabetes mellitus: a Danish nationwide cohort study," *Annals of the Rheumatic Diseases*, vol. 70, no. 6, pp. 929–934, 2011.

[2] J. A. Aviña-Zubieta, H. K. Choi, M. Sadatsafavi, M. Etminan, J. M. Esdaile, and D. Lacaille, "Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies," *Arthritis Care & Research*, vol. 59, no. 12, pp. 1690–1697, 2008.

[3] S. Hannawi, B. Haluska, T. H. Marwick, and R. Thomas, "Atherosclerotic disease is increased in recent-onset rheumatoid arthritis: a critical role for inflammation," *Arthritis Research & Therapy*, vol. 9, no. 6, article R116, 2007.

[4] I. Del Rincón, K. Williams, M. P. Stern, G. L. Freeman, and A. Escalante, "High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors," *Arthritis & Rheumatology*, vol. 44, no. 12, pp. 2737–2745, 2001.

[5] X. Z. Yang, Y. Chang, and W. Wei, "Endothelial dysfunction and inflammation: immunity in rheumatoid arthritis," *Mediators of Inflammation*, vol. 2016, Article ID 6813016, 9 pages, 2016.

[6] E. Choy, "Understanding the dynamics: pathways involved in the pathogenesis of rheumatoid arthritis," *Rheumatology*, vol. 51, Supplement 5, pp. v3–v11, 2012.

[7] H. R. Kramer and J. T. Giles, "Cardiovascular disease risk in rheumatoid arthritis: progress, debate, and opportunity," *Arthritis Care & Research*, vol. 63, no. 4, pp. 484–499, 2011.

[8] A. Sandoo, J. J. C. S. Veldhuijzen van Zanten, G. S. Metsios, D. Carroll, and G. D. Kitas, "The endothelium and its role in regulating vascular tone," *The Open Cardiovascular Medicine Journal*, vol. 4, no. 1, pp. 302–312, 2010.

[9] M. S. Chimenti, P. Triggianese, P. Conigliaro, E. Candi, G. Melino, and R. Perricone, "The interplay between inflammation and metabolism in rheumatoid arthritis," *Cell Death & Disease*, vol. 6, no. 9, article e1887, 2015.

[10] V. D. Colonna, M. Bianchi, V. Pascale et al., "Asymmetric dimethylarginine (ADMA): an endogenous inhibitor of nitric oxide synthase and a novel cardiovascular risk molecule," *Medical Science Monitor*, vol. 15, pp. RA91–RA101, 2009.

[11] R. H. Böger, "The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor," *Cardiovascular Research*, vol. 59, no. 4, pp. 824–833, 2003.

[12] Y. Morales, T. Cáceres, K. May, and J. M. Hevel, "Biochemistry and regulation of the protein arginine methyltransferases (PRMTs)," *Archives of Biochemistry and Biophysics*, vol. 590, pp. 138–152, 2016.

[13] D. Tousoulis, M. Georgakis, E. Oikonomou et al., "Asymmetric dimethylarginine: clinical significance and novel therapeutic approaches," *Current Medicinal Chemistry*, vol. 22, no. 24, pp. 2871–2901, 2015.

[14] A. E. McBride and P. A. Silver, "State of the Arg: protein methylation at arginine comes of age," *Cell*, vol. 106, no. 1, pp. 5–8, 2001.

[15] R. H. Böger, P. Vallance, and J. P. Cooke, "Asymmetric dimethylarginine (ADMA): a key regulator of nitric oxide synthase," *Atherosclerosis Supplements*, vol. 4, no. 4, pp. 1–3, 2003.

[16] T. Teerlink, Z. Luo, F. Palm, and C. S. Wilcox, "Cellular ADMA: regulation and action," *Pharmacological Research*, vol. 60, no. 6, pp. 448–460, 2009.

[17] H. L. Gornik and M. A. Creager, "Arginine and endothelial and vascular health," *The Journal of Nutrition*, vol. 134, pp. 2880S–2887S, 2004.

[18] K. K. McDonald, S. Zharikov, E. R. Block, and M. S. Kilberg, "A caveolar complex between the cationic amino acid transporter 1 and endothelial nitric-oxide synthase may explain the "arginine paradox"," *Journal of Biological Chemistry*, vol. 272, no. 50, pp. 31213–31216, 1997.

[19] T. A. Hardy and J. M. May, "Coordinate regulation of L-arginine uptake and nitric oxide synthase activity in cultured endothelial cells," *Free Radical Biology & Medicine*, vol. 32, no. 2, pp. 122–131, 2002.

[20] R. J. Nijveldt, P. A. van Leeuwen, C. van Guldener, C. D. Stehouwer, J. A. Rauwerda, and T. Teerlink, "Net renal extraction of asymmetrical (ADMA) and symmetrical (SDMA) dimethylarginine in fasting humans," *Nephrology Dialysis Transplantation*, vol. 17, no. 11, pp. 1999–2002, 2002.

[21] F. Palm, M. L. Onozato, Z. Luo, and C. S. Wilcox, "Dimethylarginine dimethylaminohydrolase (DDAH): expression, regulation, and function in the cardiovascular and renal systems," *American Journal of Physiology - Heart and Circulatory Physiology*, vol. 293, no. 6, pp. H3227–H3245, 2007.

[22] R. J. Nijveldt, T. Teerlink, C. van Guldener et al., "Handling of asymmetrical dimethylarginine and symmetrical dimethylarginine by the rat kidney under basal conditions and during endotoxaemia," *Nephrology Dialysis Transplantation*, vol. 18, no. 12, pp. 2542–2550, 2003.

[23] R. J. Nijveldt, T. Teerlink, M. P. Siroen, A. A. Van Lambalgen, J. A. Rauwerda, and P. A. Van Leeuwen, "The liver is an important organ in the metabolism of asymmetrical dimethylarginine (ADMA)," *Clinical Nutrition*, vol. 22, no. 1, pp. 17–22, 2003.

[24] A. Kittel, F. Müller, J. König et al., "*Alanine-glyoxylate aminotransferase 2* (*AGXT2*) polymorphisms have considerable impact on methylarginine and β-aminoisobutyrate metabolism in healthy volunteers," *PLoS One*, vol. 9, no. 2, article e88544, 2014.

[25] J. N. Sharma, A. Al-Omran, and S. S. Parvathy, "Role of nitric oxide in inflammatory diseases," *Inflammopharmacology*, vol. 15, no. 6, pp. 252–259, 2007.

[26] J. Leiper, J. Murray-Rust, N. McDonald, and P. Vallance, "S-nitrosylation of dimethylarginine dimethylaminohydrolase regulates enzyme activity: further interactions between nitric oxide synthase and dimethylarginine dimethylaminohydrolase," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 21, pp. 13527–13532, 2002.

[27] A. Ito, P. S. Tsao, S. Adimoolam, M. Kimoto, T. Ogawa, and J. P. Cooke, "Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase," *Circulation*, vol. 99, no. 24, pp. 3092–3095, 1999.

[28] H. Kaur and B. Halliwell, "Evidence for nitric oxide-mediated oxidative damage in chronic inflammation nitrotyrosine in serum and synovial fluid from rheumatoid patients," *FEBS Letters*, vol. 350, no. 1, pp. 9–12, 1994.

[29] D. Spasovski, A. Latifi, B. Osmani et al., "Determination of the diagnostic values of asymmetric dimethylarginine as an indicator for evaluation of the endothelial dysfunction in patients with rheumatoid arthritis," *Arthritis*, vol. 2013, Article ID 818037, 10 pages, 2013.

[30] L. J. Millatt, G. S. Whitley, D. Li et al., "Evidence for dysregulation of dimethylarginine dimethylaminohydrolase I in chronic hypoxia–induced pulmonary hypertension," *Circulation*, vol. 108, no. 12, pp. 1493–1498, 2003.

[31] R. H. Böger, K. Sydow, J. Borlak et al., "LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of S-adenosylmethionine–dependent methyltransferases," *Circulation Research*, vol. 87, no. 2, pp. 99–105, 2000.

[32] S. H. Kim, C. K. Lee, E. Y. Lee et al., "Serum oxidized low-density lipoproteins in rheumatoid arthritis," *Rheumatology International*, vol. 24, no. 4, pp. 230–233, 2004.

[33] E. Profumo, M. Di Franco, B. Buttari et al., "Biomarkers of subclinical atherosclerosis in patients with autoimmune disorders," *Mediators of Inflammation*, vol. 2012, Article ID 503942, 8 pages, 2012.

[34] F. R. Spinelli, A. Pecani, F. Conti, R. Mancini, C. Alessandri, and G. Valesini, "Post-translational modifications in rheumatoid arthritis and atherosclerosis: focus on citrullination and carbamylation," *Journal of International Medical Research*, vol. 44, Supplement 1, pp. 81–84, 2016.

[35] J. Middleton, L. Americh, R. Gayon et al., "Endothelial cell phenotypes in the rheumatoid synovium: activated, angiogenic, apoptotic and leaky," *Arthritis Research & Therapy*, vol. 6, no. 2, pp. 60–72, 2004.

[36] A. Surdacki, "L-Arginine analogs – inactive markers or active agents in atherogenesis?," *Cardiovascular & Hematological Agents in Medicinal Chemistry*, vol. 6, no. 4, pp. 302–311, 2008.

[37] G. Paolisso, G. Valentini, D. Giugliano et al., "Evidence for peripheral impaired glucose handling in patients with connective tissue diseases," *Metabolism*, vol. 40, no. 9, pp. 902–907, 1991.

[38] M. Gonzalez-Gay, J. M. De Matias, C. Gonzalez-Juanatey et al., "Anti-tumor necrosis factor-alpha blockade improves insulin resistance in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 24, no. 1, pp. 83–86, 2006.

[39] G. Boden and G. I. Shulman, "Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and β-cell dysfunction," *European Journal of Clinical Investigation*, vol. 32, no. s3, pp. 14–23, 2002.

[40] G. S. Hotamisligil, P. Arner, J. F. Caro, R. L. Atkinson, and B. M. Spiegelman, "Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance," *The Journal of Clinical Investigation*, vol. 95, no. 5, pp. 2409–2415, 1995.

[41] H. Päivä, T. Lehtimäki, J. Laakso et al., "Plasma concentrations of asymmetric-dimethyl-arginine in type 2 diabetes associate with glycemic control and glomerular filtration rate but not with risk factors of vasculopathy," *Metabolism*, vol. 52, no. 3, pp. 303–307, 2003.

[42] F. Abbasi, T. Asagmi, J. P. Cooke et al., "Plasma concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes mellitus," *The American Journal of Cardiology*, vol. 88, no. 10, pp. 1201–1203, 2001.

[43] B. Ellger, M. C. Richir, P. A. M. van Leeuwen et al., "Glycemic control modulates arginine and asymmetrical-dimethylarginine levels during critical illness by preserving dimethylarginine-dimethylaminohydrolase activity," *Endocrinology*, vol. 149, no. 6, pp. 3148–3157, 2008.

[44] M. González, C. Flores, J. D. Pearson, P. Casanello, and L. Sobrevia, "Cell signalling-mediating insulin increase of mRNA expression for cationic amino acid transporters-1 and -2 and membrane hyperpolarization in human umbilical vein endothelial cells," *Pflügers Archiv*, vol. 448, no. 4, pp. 383–394, 2004.

[45] H. M. A. Eid, H. Reims, H. Arnesen, S. E. Kjeldsen, T. Lyberg, and I. Seljeflot, "Decreased levels of asymmetric dimethylarginine during acute hyperinsulinemia," *Metabolism*, vol. 56, no. 4, pp. 464–469, 2007.

[46] M. L. Marcovecchio, B. Widmer, D. B. Dunger, and R. N. Dalton, "Effect of acute variations of insulin and glucose on plasma concentrations of asymmetric dimethylarginine in young people with type 1 diabetes," *Clinical Science*, vol. 115, no. 12, pp. 361–369, 2008.

[47] M. Essouma and J. J. N. Noubiap, "Therapeutic potential of folic acid supplementation for cardiovascular disease prevention through homocysteine lowering and blockade in rheumatoid arthritis patients," *Biomarker Research*, vol. 3, no. 1, 2015.

[48] A. De Bree, W. M. Verschuren, D. Kromhout, L. A. Kluijtmans, and H. J. Blom, "Homocysteine determinants and the evidence to what extent homocysteine determines the risk of coronary heart disease," *Pharmacological Reviews*, vol. 54, no. 4, pp. 599–618, 2002.

[49] P. E. Lazzerini, P. L. Capecchi, E. Selvi et al., "Hyperhomocysteinemia: a cardiovascular risk factor in autoimmune diseases?," *Lupus*, vol. 16, no. 11, pp. 852–862, 2007.

[50] C. Korandji, M. Zeller, J. C. Guilland et al., "Asymmetric dimethylarginine (ADMA) and hyperhomocysteinemia in patients with acute myocardial infarction," *Clinical Biochemistry*, vol. 40, no. 1-2, pp. 66–72, 2007.

[51] C. van Guldener, P. W. B. Nanayakkara, and C. D. A. Stehouwer, "Homocysteine and asymmetric dimethylarginine (ADMA): biochemically linked but differently related to vascular disease in chronic kidney disease," *Clinical Chemical Laboratory Medicine*, vol. 45, pp. 683–1687, 2007.

[52] P. E. Lazzerini, P. L. Capecchi, E. Selvi et al., "Hyperhomocysteinemia, inflammation and autoimmunity," *Autoimmunity Reviews*, vol. 6, no. 7, pp. 503–509, 2007.

[53] Z. Ortiz, B. Shea, M. Suarez Almazor, D. Moher, G. Wells, and P. Tugwell, "Folic acid and folinic acid for reducing side effects in patients receiving methotrexate for rheumatoid arthritis," *Cochrane Database of Systematic Reviews*, vol. 2, article CD000951, 1999.

[54] S. Dayal and S. R. Lentz, "ADMA and hyperhomocysteinemia," *Vascular Medicine*, vol. 10, Supplement 2, pp. S27–S33, 2005.

[55] S. Skeoch and I. N. Bruce, "Atherosclerosis in rheumatoid arthritis: is it all about inflammation?," *Nature Reviews Rheumatology*, vol. 11, no. 7, pp. 390–400, 2015.

[56] D. Tousoulis, A. M. Kampoli, C. Tentolouris, N. Papageorgiou, and C. Stefanadis, "The role of nitric oxide on endothelial function," *Current Vascular Pharmacology*, vol. 10, no. 1, pp. 4–18, 2012.

[57] R. H. Böger, R. Maas, F. Schulze, and E. Schwedhelm, "Asymmetric dimethylarginine (ADMA) as a prospective marker of cardiovascular disease and mortality—an update on patient populations with a wide range of cardiovascular risk," *Pharmacological Research*, vol. 60, no. 6, pp. 481–487, 2009.

[58] T. M. C. Brunini, M. B. Moss, M. A. S. Siqueira et al., "Inhibition of l-arginine transport in platelets by asymmetric dimethylarginine and NG-monomethyl-l-arginine: effects of arterial hypertension," *Clinical and Experimental Pharmacology and Physiology*, vol. 31, no. 10, pp. 738–740, 2004.

[59] J. Vasquez-Vivar, B. Kalyanaraman, P. Martasek et al., "Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 16, pp. 9220–9225, 1998.

[60] J. S. Beckman and W. H. Koppenol, "Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly," *American Journal of Physiology - Cell Physiology*, vol. 271, pp. C1424–C1437, 1996.

[61] K. Sydow and T. Münzel, "ADMA and oxidative stress," *Atherosclerosis Supplements*, vol. 4, no. 4, pp. 41–51, 2003.

[62] T. Asahara, T. Murohara, A. Sullivan et al., "Isolation of putative progenitor endothelial cells for angiogenesis," *Science*, vol. 275, no. 5302, pp. 964–966, 1997.

[63] F. Du, J. Zhou, R. Gong et al., "Endothelial progenitor cells in atherosclerosis," *Frontiers in Bioscience*, vol. 17, no. 7, pp. 2327–2349, 2012.

[64] J. Grisar, D. Aletaha, C. W. Steiner et al., "Endothelial progenitor cells in active rheumatoid arthritis: effects of tumour necrosis factor and glucocorticoid therapy," *Annals of the Rheumatic Diseases*, vol. 66, no. 10, pp. 1284–1288, 2007.

[65] F. R. Spinelli, A. Metere, C. Barbati et al., "Effect of therapeutic inhibition of TNF on circulating endothelial progenitor cells in patients with rheumatoid arthritis," *Mediators of Inflammation*, vol. 2013, Article ID 537539, 8 pages, 2013.

[66] F. R. Spinelli, M. Di Franco, A. Metere et al., "Decrease of asymmetric dimethyl arginine after anti-TNF therapy in patients with rheumatoid arthritis," *Drug Development Research*, vol. 75, no. S1, pp. S67–S69, 2014.

[67] T. Thum, D. Tsikas, S. Stein et al., "Suppression of endothelial progenitor cells in human coronary artery disease by the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine," *Journal of the American College of Cardiology*, vol. 46, no. 9, pp. 1693–1701, 2005.

[68] A. Aicher, C. Heeschen, C. Mildner-Rihm et al., "Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells," *Nature Medicine*, vol. 9, no. 11, pp. 1370–1376, 2003.

[69] P. Willeit, D. F. Freitag, J. A. Laukkanen et al., "Asymmetric dimethylarginine and cardiovascular risk: systematic review and meta-analysis of 22 prospective studies," *Journal of the American Heart Association*, vol. 4, no. 6, article e001833, 2015.

[70] J. T. Kuvin, A. R. Patel, K. A. Sliney et al., "Peripheral vascular endothelial function testing as a noninvasive indicator of coronary artery disease," *Journal of the American College of Cardiology*, vol. 38, no. 7, pp. 1843–1849, 2001.

[71] M. Juonala, J. S. A. Viikari, G. Alfthan et al., "Brachial artery flow-mediated dilation and asymmetrical dimethylarginine in the cardiovascular risk in young Finns study," *Circulation*, vol. 116, no. 12, pp. 1367–1373, 2007.

[72] R. H. Böger, S. M. Bode-Böger, W. Thiele, W. Junker, K. Alexander, and J. C. Frölich, "Biochemical evidence for impaired nitric oxide synthesis in patients with peripheral arterial occlusive disease," *Circulation*, vol. 95, no. 8, pp. 2068–2074, 1997.

[73] L. Moroni, C. Selmi, C. Angelini, and P. L. Meroni, "Evaluation of endothelial function by flow-mediated dilation: a comprehensive review in rheumatic disease," *Archivum Immunologiae et Therapiae Experimentalis*, vol. 65, no. 6, pp. 463–475, 2017.

[74] F. R. Spinelli, A. Pecani, F. Ciciarello et al., "Association between antibodies to carbamylated proteins and subclinical atherosclerosis in rheumatoid arthritis patients," *BMC Musculoskeletal Disorders*, vol. 18, no. 1, p. 214, 2017.

[75] A. Sandoo, T. Dimitroulas, J. V. van Zanten et al., "Lack of association between asymmetric dimethylarginine and in vivo microvascular and macrovascular endothelial function in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 30, no. 3, pp. 388–396, 2012.

[76] T. Dimitroulas, A. Sandoo, J. Hodson, J. P. Smith, and G. D. Kitas, "*In vivo* microvascular and macrovascular endothelial function is not associated with circulating dimethylarginines in patients with rheumatoid arthritis: a prospective analysis of the DRACCO cohort," *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 76, no. 4, pp. 331–337, 2016.

[77] M. Di Franco, F. R. Spinelli, A. Metere et al., "Serum levels of asymmetric dimethylarginine and apelin as potential markers of vascular endothelial dysfunction in early rheumatoid arthritis," *Mediators of Inflammation*, vol. 2012, Article ID 347268, 7 pages, 2012.

[78] R. Campuzano, J. L. Moya, A. García-Lledó et al., "Endothelial dysfunction, intima–media thickness and coronary reserve in relation to risk factors and Framingham score in patients without clinical atherosclerosis," *Journal of Hypertension*, vol. 24, no. 8, pp. 1581–1588, 2006.

[79] A. Simon, J. Gariepy, G. Chironi, J. L. Megnien, and J. Levenson, "Intima–media thickness: a new tool for diagnosis and treatment of cardiovascular risk," *Journal of Hypertension*, vol. 20, no. 2, pp. 159–169, 2002.

[80] P. P. Ambrosino, R. Lupoli, A. Di Minno, M. Tasso, R. Peluso, and M. N. D. Di Minno, "Subclinical atherosclerosis in patients with rheumatoid arthritis," *Thrombosis and Haemostasis*, vol. 113, no. 5, pp. 916–930, 2015.

[81] Y. Bai, L. Sun, L. Du et al., "Association of circulating levels of asymmetric dimethylarginine (ADMA) with carotid intima-media thickness: evidence from 6168 participants," *Ageing Research Reviews*, vol. 12, no. 2, pp. 699–707, 2013.

[82] M. Turiel, L. Tomasoni, S. Sitia et al., "Effects of long-term disease-modifying antirheumatic drugs on endothelial function in patients with early rheumatoid arthritis," *Cardiovascular Therapeutics*, vol. 28, no. 5, pp. e53–e64, 2010.

[83] A. Sandoo, T. Dimitroulas, T. E. Toms et al., "Clinical remission following treatment with tumour necrosis factor-alpha antagonists is not accompanied by changes in asymmetric dimethylarginine in patients with rheumatoid arthritis," *Clinical Biochemistry*, vol. 45, no. 16-17, pp. 1399–1403, 2012.

[84] K. Angel, S. A. Provan, P. Mowinckel, I. Seljeflot, T. K. Kvien, and D. Atar, "The l-arginine/asymmetric dimethylarginine ratio is improved by anti-tumor necrosis factor–α therapy in inflammatory arthropathies. Associations with aortic stiffness," *Atherosclerosis*, vol. 225, no. 1, pp. 160–165, 2012.

[85] T. Dimitroulas, J. Hodson, A. Sandoo, J. Smith, and G. D. Kitas, "Endothelial injury in rheumatoid arthritis: a crosstalk between dimethylarginines and systemic inflammation," *Arthritis Research & Therapy*, vol. 19, no. 1, p. 32, 2017.

[86] B. Kwaśny-Krochin, P. Głuszko, and A. Undas, "Plasma asymmetric dimethylarginine in active rheumatoid arthritis: links with oxidative stress and inflammation," *Polish Archives of Internal Medicine*, vol. 122, no. 6, pp. 270–276, 2012.

[87] A. Surdacki, J. Martens-Lobenhoffer, A. Wloch et al., "Plasma asymmetric dimethylarginine is related to anticitrullinated protein antibodies in rheumatoid arthritis of short duration," *Metabolism*, vol. 58, no. 3, pp. 316–318, 2009.

[88] T. Şentürk, N. Yılmaz, G. Sargın, K. Köseoğlu, and Ç. Yenisey, "Relationship between asymmetric dimethylarginine and endothelial dysfunction in patients with rheumatoid arthritis," *European Journal of Rheumatology*, vol. 3, no. 3, pp. 106–108, 2016.

[89] A. Sandoo, T. Dimitroulas, J. Hodson, J. P. Smith, K. M. Douglas, and G. D. Kitas, "Cumulative inflammation associates with asymmetric dimethylarginine in rheumatoid arthritis: a 6 year follow-up study," *Rheumatology*, vol. 54, pp. 1145–1152, 2014.

[90] M. Turiel, F. Atzeni, L. Tomasoni et al., "Non-invasive assessment of coronary flow reserve and ADMA levels: a case–control study of early rheumatoid arthritis patients," *Rheumatology*, vol. 48, no. 7, pp. 834–839, 2009.

[91] D. Spasovski and T. Sotirova, "Link between dimethyl arginine derivats and Acpa antibodies in patients with rheumatoid arthritis," *Interdisciplinary Journal of Microinflammation*, vol. 1, no. 2, 2014.

[92] T. Dimitroulas, A. Sandoo, J. Hodson, J. Smith, K. M. Douglas, and G. D. Kitas, "Associations between asymmetric dimethylarginine, homocysteine, and the methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism (rs1801133) in rheumatoid arthritis," *Scandinavian Journal of Rheumatology*, vol. 45, no. 4, pp. 267–273, 2016.

[93] T. Dimitroulas, A. Sandoo, J. J. J. C. S. V. van Zanten et al., "Predictors of asymmetric dimethylarginine levels in patients with rheumatoid arthritis: the role of insulin resistance," *Scandinavian Journal of Rheumatology*, vol. 42, no. 3, pp. 176–181, 2013.

[94] P. Anyfanti, A. Triantafyllou, E. Gkaliagkousi et al., "Subendocardial viability ratio in patients with rheumatoid arthritis: comparison with healthy controls and identification of prognostic factors," *Clinical Rheumatology*, vol. 36, no. 6, pp. 1229–1236, 2017.

[95] T. Dimitroulas, J. Hodson, V. F. Panoulas, A. Sandoo, J. Smith, and G. Kitas, "Genetic variations in the alanine–glyoxylate aminotransferase 2 (AGXT2) gene and dimethylarginines levels in rheumatoid arthritis," *Amino Acids*, vol. 49, no. 6, pp. 1133–1141, 2017.

[96] G. L. Erre, A. Piras, S. Mura et al., "Asymmetric dimethylarginine and arterial stiffness in patients with rheumatoid arthritis: a case–control study," *Journal of International Medical Research*, vol. 44, Supplement 1, pp. 76–80, 2016.

[97] T. Dimitroulas, A. Sandoo, J. Hodson, J. Smith, V. F. Panoulas, and G. D. Kitas, "Relationship between dimethylarginine dimethylaminohydrolase gene variants and

asymmetric dimethylarginine in patients with rheumatoid arthritis," *Atherosclerosis*, vol. 237, no. 1, pp. 38–44, 2014.

[98] A. A. Kayacelebi, V. V. Pham, J. Willers et al., "Plasma homoarginine (hArg) and asymmetric dimethylarginine (ADMA) in patients with rheumatoid arthritis: is homoarginine a cardiovascular corrective in rheumatoid arthritis, an anti-ADMA?," *International Journal of Cardiology*, vol. 176, no. 3, pp. 1129–1131, 2014.

[99] D. Tousoulis, C. Antoniades, C. Vasiliadou et al., "Effects of atorvastatin and vitamin C on forearm hyperaemic blood flow, asymmentrical dimethylarginine levels and the inflammatory process in patients with type 2 diabetes mellitus," *Heart*, vol. 93, no. 2, pp. 244–246, 2007.

[100] T. M. Lu, Y. A. Ding, H. B. Leu, W. H. Yin, W. H. H. Sheu, and K. M. Chu, "Effect of *rosuvastatin* on plasma levels of asymmetric dimethylarginine in patients with hypercholesterolemia," *The American Journal of Cardiology*, vol. 94, no. 2, pp. 157–161, 2004.

[101] Y. Nishiyama, M. Ueda, T. Otsuka et al., "Statin treatment decreased serum asymmetric dimethylarginine (ADMA) levels in ischemic stroke patients," *Journal of Atherosclerosis and Thrombosis*, vol. 18, no. 2, pp. 131–137, 2011.

[102] A. S. Antonopoulos, M. Margaritis, R. Lee, K. Channon, and C. Antoniades, "Statins as anti-inflammatory agents in atherogenesis: molecular mechanisms and lessons from the recent clinical trials," *Current Pharmaceutical Design*, vol. 18, no. 11, pp. 1519–1530, 2012.

[103] K. M. Mäki-Petäjä, L. Day, J. Cheriyan et al., "Tetrahydrobiopterin supplementation improves endothelial function but does not alter aortic stiffness in patients with rheumatoid arthritis," *Journal of the American Heart Association*, vol. 5, no. 2, article e002762, 2016.

[104] S. Van Doornum, G. McColl, and I. P. Wicks, "Atorvastatin reduces arterial stiffness in patients with rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 63, no. 12, pp. 1571–1575, 2004.

[105] T. Nakamura, E. Sato, N. Fujiwara et al., "Ezetimibe decreases serum levels of asymmetric dimethylarginine (ADMA) and ameliorates renal injury in non-diabetic chronic kidney disease patients in a cholesterol-independent manner," *Pharmacological Research*, vol. 60, no. 6, pp. 525–528, 2009.

[106] K. M. Mäki-Petäjä, A. D. Booth, F. C. Hall et al., "Ezetimibe and simvastatin reduce inflammation, disease activity, and aortic stiffness and improve endothelial function in rheumatoid arthritis," *Journal of the American College of Cardiology*, vol. 50, no. 9, pp. 852–858, 2007.

[107] F. Ursini, C. Leporini, F. Bene et al., "Anti-TNF-alpha agents and endothelial function in rheumatoid arthritis: a systematic review and meta-analysis," *Scientific Reports*, vol. 7, no. 1, p. 5346, 2017.

[108] R. Palomino-Morales, C. Gonzalez-Juanatey, T. R. Vazquez-Rodriguez et al., "A1298C polymorphism in the *MTHFR* gene predisposes to cardiovascular risk in rheumatoid arthritis," *Arthritis Research & Therapy*, vol. 12, no. 2, article R71, 2010.

[109] A. Radhakutty, B. L. Mangelsdorf, S. M. Drake et al., "Opposing effects of rheumatoid arthritis and low dose prednisolone on arginine metabolomics," *Atherosclerosis*, vol. 266, pp. 190–195, 2017.

[110] R. Agca, S. C. Heslinga, S. Rollefstad et al., "EULAR recommendations for cardiovascular disease risk management in patients with rheumatoid arthritis and other forms of inflammatory joint disorders: 2015/2016 update," *Annals of the Rheumatic Diseases*, vol. 76, no. 1, pp. 17–28, 2017.

[111] J. M. Young, C. H. Strey, P. M. George et al., "Effect of atorvastatin on plasma levels of asymmetric dimethylarginine in patients with non-ischaemic heart failure," *European Journal of Heart Failure*, vol. 10, no. 5, pp. 463–466, 2008.

[112] T. L. Yang, M. F. Chen, X. Xia, B. L. Luo, and Y. J. Li, "Effect of fenofibrate on the level of asymmetric dimethylarginine in individuals with hypertriglyceridemia," *European Journal of Clinical Pharmacology*, vol. 62, no. 3, pp. 179–184, 2006.

[113] S. Westphal, K. Borucki, C. Luley, J. Martens-Lobenhoffer, and S. M. Bode-Böger, "Treatment with niacin lowers ADMA," *Atherosclerosis*, vol. 184, no. 2, pp. 448–450, 2006.

[114] H. Fujii, K. Kono, K. Nakai et al., "Renin-angiotensin system inhibitors reduce serum asymmetric dimethylarginine levels and oxidative stress in normotensive patients with chronic kidney disease," *Nephron Extra*, vol. 4, no. 1, pp. 18–25, 2014.

[115] C. Delles, M. Schneider, S. John, M. Gekle, and R. Schmieder, "Angiotensin converting enzyme inhibition and angiotensin II AT1-receptor blockade reduce the levels of asymmetrical N^G, N^G-dimethylarginine in human essential hypertension," *American Journal of Hypertension*, vol. 15, no. 7, pp. 590–593, 2002.

[116] T. D. Wang, W. J. Chen, W. C. Cheng, J. W. Lin, M. F. Chen, and Y. T. Lee, "Relation of improvement in endothelium-dependent flow-mediated vasodilation after *rosiglitazone* to changes in asymmetric dimethylarginine, endothelin-1, and C-reactive protein in nondiabetic patients with the metabolic syndrome," *The American Journal of Cardiology*, vol. 98, no. 8, pp. 1057–1062, 2006.

[117] T. Asagami, F. Abbasi, M. Stuelinger et al., "Metformin treatment lowers asymmetric dimethylarginine concentrations in patients with type 2 diabetes," *Metabolism*, vol. 51, no. 7, pp. 843–846, 2002.

[118] B. V. Khan, S. T. Rahman, T. Haque et al., "Vascular effects of nebivolol added to hydrochlorothiazide in African Americans with hypertension and echocardiographic evidence of diastolic dysfunction: the NASAA study," *Journal of Cardiovascular Pharmacology and Therapeutics*, vol. 17, no. 3, pp. 291–297, 2012.

[119] A. Oguz, M. Uzunlulu, E. Yorulmaz, Y. Yalcin, N. Hekim, and F. Fici, "Effect of nebivolol and metoprolol treatments on serum asymmetric dimethylarginine levels in hypertensive patients with type 2 diabetes mellitus," *The Anatolian Journal of Cardiology*, vol. 7, pp. 383–388, 2007.

[120] S. Hetzel, D. DeMets, R. Schneider et al., "Aspirin increases nitric oxide formation in chronic stable coronary disease," *Journal of Cardiovascular Pharmacology and Therapeutics*, vol. 18, no. 3, pp. 217–221, 2013.

[121] D. P. Holden, J. E. Cartwright, S. S. Nussey, and G. S. Whitley, "Estrogen stimulates dimethylarginine dimethylaminohydrolase activity and the metabolism of asymmetric dimethylarginine," *Circulation*, vol. 108, no. 13, pp. 1575–1580, 2003.

[122] M. S. Post, M. O. Verhoeven, M. J. van der Mooren, P. Kenemans, C. D. A. Stehouwer, and T. Teerlink, "Effect of hormone replacement therapy on plasma levels of the cardiovascular risk factor asymmetric dimethylarginine: a randomized, placebo-controlled 12-week study in healthy early postmenopausal women," *The Journal of Clinical Endocrinology & Metabolism*, vol. 88, no. 9, pp. 4221–4226, 2003.

[123] C. J. Wu, L. Wang, X. Li, C. X. Wang, J. P. Ma, and X. S. Xia, "Impact of adding folic acid, vitamin B(12) and probucol to

standard antihypertensive medication on plasma homocysteine and asymmetric dimethylarginine levels of essential hypertension patients," *Zhonghua Xin Xue Guan Bing Za Zhi*, vol. 40, no. 12, pp. 1003–1008, 2012.

[124] S. Ziegler, F. Mittermayer, C. Plank, E. Minar, M. Wolzt, and G. H. Schernthaner, "Homocyst(e)ine-lowering therapy does not affect plasma asymmetrical dimethylarginine concentrations in patients with peripheral artery disease," *The Journal of Clinical Endocrinology & Metabolism*, vol. 90, no. 4, pp. 2175–2178, 2005.

[125] F. Mittermayer, J. Pleiner, M. Francesconi, and M. Wolzt, "Treatment with α-lipoic acid reduces asymmetric dimethylarginine in patients with type 2 diabetes mellitus," *Translational Research*, vol. 155, no. 1, pp. 6–9, 2010.