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Systemic inflammation is linked to low arginine and high ADMA plasma levels resulting in an unfavorable NOS substrate-to-inhibitor ratio – the Hoorn Study

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Abstract

Inflammation is associated with a reduced availability of nitric oxide (NO) in the vasculature. We investigated the possible involvement of altered levels of substrate (arginine) and inhibitor (asymmetric dimethylarginine; ADMA) of NO synthase (NOS). Plasma concentrations of arginine and ADMA, the inflammatory markers C-reactive protein (CRP) and myeloperoxidase (MPO), and oxidized LDL (oxLDL) were measured in 369 male and 377 female participants (aged 50 to 87 years) of a population-based cohort study. The arginine/ADMA ratio decreased significantly across increasing tertiles of CRP and MPO. These negative associations remained significant in a linear regression model with both MPO ($P=0.002$) and CRP ($P<0.001$) as independent variables and adjusted for age, sex and cardiovascular risk factors. In a fully adjusted regression model, MPO was positively associated with ADMA (5.4 [95% CI 1.3 to 9.4] nmol/L change of ADMA per SD increase of MPO; $P=0.010$), whereas CRP was not ($P=0.36$). Conversely, in a fully adjusted model, CRP was negatively associated with arginine (-2.8 [95% CI -4.0 to -1.6] $\mu\text{mol/L}$ arginine per SD of CRP; $P<0.001$), without a significant contribution of MPO ($P=0.23$). The relationship between MPO and ADMA became stronger with increasing levels of oxLDL (1.8, 5.2, and 8.7 nmol/L ADMA per SD of MPO for increasing tertiles of oxLDL), consistent with the ability of MPO to amplify oxidative stress. In contrast, the relationship between CRP and arginine was not modified by levels of oxLDL. In conclusion, an unfavorable NOS substrate/inhibitor ratio may contribute to the reduced NO bioavailability associated with inflammation.

INTRODUCTION

Endothelium-derived nitric oxide (NO), which is synthesized from arginine by NO synthase (NOS), is an important regulator of vascular homeostasis. In addition to being a powerful endogenous vasodilator, NO inhibits the adhesion of inflammatory cells to the vascular wall, the aggregation of platelets, and the proliferation of smooth muscle cells [1]. Inactivation and/or reduced synthesis of NO is seen in conjunction with risk factors for cardiovascular disease (CVD) and may promote endothelial dysfunction, hypertension, thrombus formation, and atherogenesis [1-3].

Infusion or chronic oral administration of arginine has been shown to increase NO production, suggesting that under physiological conditions substrate availability affects NO production [4]. NO production can be inhibited by the endogenous NOS inhibitor asymmetric dimethylarginine (ADMA), which originates from the proteolysis of methylated proteins [5]. Normal physiological levels of ADMA probably only have a modest effect on NOS activity, but under pathological conditions intracellular ADMA is elevated and may reach levels sufficient to inhibit NOS and impair vascular function [6]. Increased plasma levels of ADMA have been found to predict future CVD events, independent of traditional risk factors [7,8]. Therefore, on top of other regulatory mechanisms, NO production is determined by both substrate availability (arginine) and the presence of inhibitor (ADMA), which may be adequately reflected by their ratio [9].

Inflammation is involved in all phases of the atherosclerotic process [10]. The inflammatory markers C-reactive protein (CRP) and myeloperoxidase (MPO) are both associated with endothelial dysfunction [11-13], possibly due to a reduced bioavailability of NO, but whether low-grade inflammation is associated with altered levels of arginine and/or ADMA has not been thoroughly investigated. We have addressed this issue by studying how concentrations of arginine and ADMA are related to concentrations of CRP and MPO in plasma samples from 746 participants of a population-based cohort study, the Hoorn Study.

MATERIALS AND METHODS

Subjects

For the present investigation we used data from the 2000 Hoorn Study follow-up examination and the Hoorn Screening Study, which are population-based studies on glucose metabolism and cardiovascular disease in a white population. This study population has been described in detail elsewhere [14]. From the 822 participants, we excluded 74 subjects with missing data on primary variables of interest and two subjects with extreme values for arginine or ADMA. In total 746 subjects (369 men and 377 women) remained, of whom 267 had a normal glucose metabolism, 190 had impaired glucose metabolism and 289 had type 2 diabetes according to WHO 1999 criteria [15]. The local ethics committee approved the study, and all participants gave their written informed consent.

Biochemical analyses

Plasma concentrations of arginine, ADMA, and symmetric dimethylarginine (SDMA) were measured by high-performance liquid chromatography (HPLC) with fluorescence detection as earlier described [16], using modified chromatographic separation conditions [17]. The intra-assay and inter-assay coefficients of variation (CV) for all analytes were <1.5% and <4.0%, respectively. Plasma CRP concentrations were determined with a highly sensitive in-house sandwich enzyme-linked immunosorbent assay (ELISA), with an intra-assay CV of 3.9% and inter-assay CV of 8.7% [18]. A sandwich ELISA (Mercodia, Uppsala, Sweden) was used to determine MPO concentrations in EDTA-plasma, with intra- and inter-assay CVs of 3.9% and 5.0%, respectively [19]. Apolipoprotein-B100 (apoB100) concentrations were determined

nephelometrically using an “Image 800” immunochemistry system (Beckman Coulter, Fullerton, CA). Circulating plasma oxidized LDL (oxLDL) was determined by competitive ELISA (Merckodia) with intra- and inter-assay CVs of 6.7% and 7.0%, respectively [20]. The 4E6-monoclonal antibody on which this assay is based is directed against a conformational epitope in the apoB100 moiety of LDL that is generated as a consequence of substitution of lysine residues of apoB100 with aldehydes [21]. HbA1c was analyzed by ion-exchange HPLC on a modular diabetes monitoring system (Bio-Rad, Veenendaal, The Netherlands). Glucose was measured enzymatically (Roche, Mannheim, Germany) and insulin was determined by a two-site immuno-radiometric assay (Medgenix, Diagnostics, Fleurus, Belgium). Total cholesterol, HDL-cholesterol and triglycerides (TG) were measured by standard enzymatic methods (Roche). LDL-cholesterol concentration was determined with a direct method with the “N-geneous” assay (GenZyme, Cambridge, MA, USA). With this method, a triglyceride concentration up to 13.5 mmol/L does not interfere with measurement of LDL-cholesterol.

Renal function and cardiovascular disease

Microalbuminuria was defined as a urinary albumin/creatinine ratio ≥ 2.0 mg/mmol. Glomerular filtration rate was estimated using the 4-variable Modification of Diet in Renal Disease (MDRD) Study equation [22]. Prior cardiovascular disease was defined as a history of angina, claudication, myocardial infarction, transient ischemic attack or ischemic stroke, abnormalities on a resting ECG (Minnesota codes 1.1-1.3, 4.1-4.3, 5.1-5.3 or 7.1), undergone coronary bypass surgery or angioplasty, peripheral arterial bypass or non-traumatic amputation and/or ankle-brachial index of <0.9 in either leg.

Statistics

Unless otherwise indicated, data are presented as mean and SD or, if skewed, median and interquartile range. Skewed variables, notably CRP and MPO, were natural log-transformed before statistical analyses. Student’s t-test was applied for comparison of variables between two groups. Linear regression analysis was used to assess the relationships of CRP and MPO with arginine, ADMA, and their ratio. To adjust for mutual confounding, both CRP and MPO were entered together as independent variables in multivariable regression models with arginine, ADMA or the arginine/ADMA ratio as dependent variable. Subsequently these models were further adjusted for age and sex alone, or additionally for glucose tolerance status and the following cardiovascular risk factors: waist circumference, LDL- and HDL-cholesterol, current smoking, and estimated glomerular filtration rate. Regression coefficients were expressed as change in the dependent variable per SD increase of CRP or MPO. Data were analyzed using SPSS software, version 17 (SPSS Inc., Chicago, IL). A two-tailed probability value <0.05 was considered to indicate statistical significance.

RESULTS

Increased levels of CRP and MPO are associated with a decreased arginine/ADMA ratio

Subject characteristics are shown in Table 1. Plasma concentrations of CRP, MPO, and arginine did not differ between men and women, but women had significantly higher ADMA levels (0.459 ± 0.053 versus 0.438 ± 0.060 $\mu\text{mol/L}$; $P < 0.001$) and a lower arginine/ADMA ratio (204.3 ± 33.2 versus 220.0 ± 41.7 ; $P < 0.001$) than men. The arginine/ADMA ratio decreased across increasing tertiles of CRP and MPO (Figure 1A and B) and these trends were highly significant after adjustment for age and sex ($P < 0.001$ for both CRP and MPO). Although CRP and MPO were significantly correlated (Figure 1C), this relation was not very strong, indicating that both inflammatory markers, at least to some extent, also carry different information. In line with this, two-dimensional stratification of the cohort by tertiles of CRP

and MPO revealed that in each tertile of CRP, increasing levels of MPO were associated with a decrease of the arginine/ADMA ratio. Likewise, in each stratum of MPO the arginine/ADMA ratio decreased with increasing levels of CRP (Figure 1D). In subjects with both CRP and MPO in the highest tertile the mean arginine/ADMA ratio was 200, 12% lower than the mean ratio of 227 in subjects with both CRP and MPO in the lowest tertile, the difference being equivalent to ~ 0.7 times the standard deviation of the arginine/ADMA ratio in the entire cohort.

Independent associations between CRP and arginine and between MPO and ADMA

To investigate whether the associations of both inflammatory markers with the arginine/ADMA ratio were independent of age, sex, and established cardiovascular risk factors we explored linear regression models (Table 2). In separate regression models, both CRP and MPO were significantly associated with the arginine/ADMA ratio and when mutual adjustment was performed by entering both CRP and MPO in the same regression model, these associations were slightly attenuated but still highly significant, also after further adjustment for age, sex, glucose tolerance status, and established cardiovascular risk factors.

Next, we separately explored the associations of CRP and MPO with numerator and denominator of the arginine/ADMA ratio by linear regression analyses (Table 2). In crude models, both CRP and MPO showed a significant negative association with arginine and a significant positive association with ADMA. Upon mutual adjustment, however, only CRP remained significantly associated with arginine, whereas the relation between MPO and arginine lost significance. Conversely, in the mutually adjusted model only MPO retained its significant association with ADMA, whereas the relation between CRP and ADMA lost significance. The negative association between CRP and arginine and the positive association between MPO and ADMA were only slightly attenuated and remained highly significant after further adjustment for age, sex, glucose tolerance status, and established cardiovascular risk factors. To investigate whether these associations were subject to modification by glucose metabolism, regression analyses were repeated after stratification for glucose tolerance status. The age- and sex-adjusted regression coefficients of the relation between CRP and arginine and the relation between MPO and ADMA did not differ significantly between subjects with normal glucose metabolism, impaired glucose metabolism, and type 2 diabetes ($P > 0.2$ for all comparisons).

We also investigated potential relationships of CRP and MPO with SDMA. In unadjusted regression models, CRP was positively associated with SDMA (10.1 [95% CI 2.2 to 18.0] nmol/L SDMA per standard deviation increase of MPO; $P = 0.012$), whereas MPO was not ($P = 0.19$). In a mutually and age- and sex-adjusted model neither CRP ($P = 0.17$) nor MPO ($P = 0.88$) were significantly related to SDMA.

Oxidative stress strengthens the relation between MPO and ADMA

Because hydrogen peroxide is an obligate cosubstrate of MPO, the activity of MPO is enhanced by increased production of reactive oxygen species. To test whether the relation between MPO and ADMA was strengthened under conditions of oxidative stress, we repeated the regression analyses after stratification of the cohort according to plasma levels of oxidized LDL. In age- and sex-adjusted regression models in which both MPO and CRP were used as independent variables, the strength of the association between MPO and ADMA was stronger at higher levels of oxLDL (1.8, 5.2, and 8.7 nmol/L ADMA per standard deviation of MPO for increasing tertiles of oxLDL; Figure 2A). In contrast, the strength of the association between plasma levels of CRP and arginine did not differ between strata of oxLDL (Figure 2B). It should be noted that the amount of oxLDL in the circulation not only depends on oxidative stress but also on the total amount of LDL particles. By using the ratio of oxLDL

over apoB100, adjustment for particle number is made and, therefore, this ratio may more accurately reflect oxidative stress. The increase of the strength of the association between MPO and ADMA across tertiles of the oxLDL/apoB100 ratio (1.4, 4.4, and 11.1 nmol/L ADMA per standard deviation of MPO for increasing tertiles of the oxLDL/apoB100 ratio) was slightly more pronounced as compared to the increasing trend across tertiles of oxLDL.

DISCUSSION

The most salient finding of this study is that low-grade systemic inflammation was associated with a decreased arginine/ADMA ratio, reflecting a low NOS substrate/inhibitor ratio. Two striking differences between the inflammatory markers CRP and MPO were apparent. First, elevated levels of both markers were associated with a low arginine/ADMA ratio, but in the case of CRP this was mainly caused by a negative association with arginine, and in the case of MPO by a positive association with ADMA. Second, oxLDL strengthened the association between MPO and ADMA, consistent with the ability of MPO to amplify oxidative stress, whereas the association between CRP and arginine was not modified by levels of oxLDL.

Arginine is one of the most versatile amino acids, serving as a building block in protein synthesis and as a precursor for the synthesis of NO, urea, polyamines, proline, glutamate, creatine and agmatine [23]. The biochemical pathways involved share, and under some circumstances compete for, the available amounts of arginine. Endothelial cells for example express arginases that compete with NOS for substrate and, if highly expressed, may “starve” endothelial NOS [3]. Evidence for a role of increased arginase activity in endothelial dysfunction has been provided in animal models of CVD [3], and endothelial arginase has been identified as a target for treatment of atherosclerosis [24]. Inflammation-induced upregulation of arginase activity seems a plausible explanation for the inverse association between plasma levels of CRP and arginine observed in this study. A similar negative association between CRP and arginine has also been reported in critically ill children [25].

Both ADMA and SDMA are generated by methylation of arginine residues in proteins and released in the cytosol as free amino acids during proteolysis. ADMA is largely cleared through intracellular metabolism by dimethylarginine dimethylaminohydrolase (DDAH), whereas SDMA is mainly cleared by renal excretion [5,26]. Theoretically, plasma levels of ADMA can rise by increased generation, decreased clearance, or altered distribution between the circulation and intracellular stores. Although the design of our study does not allow discriminating between these possibilities, the results provide some pointers to the mechanisms most likely involved. The fact that MPO was positively related to ADMA, but not to SDMA, argues against involvement of mechanisms shared by both methylarginines, such as protein methylation and proteolysis. Also a change in glomerular filtration rate is not a likely cause, because this would affect SDMA more than ADMA [27]. This leaves a change in the activity of DDAH, that degrades ADMA but not SDMA, as a likely candidate. DDAH, which has a critical sulfhydryl group in its catalytic site, is sensitive to oxidative stress that decreases its activity [26,28,29]. The fact that MPO, but not CRP, was associated with ADMA is compatible with oxidative stress-induced inactivation of DDAH, because only MPO is directly linked to oxidative stress. The microenvironment of the subendothelial space of the vascular wall is especially conducive to MPO activity. Mitochondrial respiration, NAD(P)H oxidases, xanthine oxidase, and uncoupled NOS are major sources of the highly-reactive superoxide radical, that is actively converted into hydrogen peroxide by superoxide dismutase. Although less reactive than the superoxide radical, hydrogen peroxide is the cosubstrate for all MPO-catalyzed reactions. MPO amplifies the oxidative potential of hydrogen peroxide by producing a variety of reactive oxidants, including hypochlorous acid [30,31]. In the present study we measured circulatory oxLDL to gauge oxidative stress in the

vascular wall. Other markers of oxidative stress, such as isoprostanes, may better than oxLDL reflect oxidative stress at the whole body level. A unique feature of oxLDL, however, is that it is formed exclusively by oxidative processes in the vasculature, because in the circulation LDL is effectively protected against oxidation [32]. We have previously observed a positive association between plasma levels of MPO and blood pressure that was most prominent at high levels of oxLDL, in line with enhancement of MPO activity by increased vascular production of reactive oxygen species [33]. We hypothesized that vascular oxidative stress may also enhance MPO-mediated oxidative inactivation of DDAH. Indeed, we observed that the relation between MPO and ADMA was strongest in individuals with a high plasma level of oxLDL. The impact of oxidative stress on the relation between MPO and ADMA was slightly more prominent if stratification was performed on the basis of the oxLDL/apoB100 ratio, consistent with our previous finding that this ratio may better reflect oxidative stress [20].

All in all, it is likely that the inflammation-associated decreased levels of arginine and increased levels of ADMA are caused by upregulation of arginase and inhibition of DDAH activity, respectively. Both pathways result in lowering of the NOS substrate/inhibitor ratio, leading to a diminished NO production and endothelial dysfunction. The existence of these separate pathways is consistent with CRP being mainly produced by the liver, whereas MPO is locally produced at sites of inflammation including the vasculature. It should be noted, however, that there may be extensive crosstalk between both pathways. CRP has for instance been shown to stimulate MPO release from polymorphonuclear cells and monocytes [34], which may in part explain the association between both markers observed in the present study. Furthermore, both CRP and MPO may negatively affect the bioavailability of NO in ways that are independent of the arginine/ADMA ratio, such as direct scavenging of NO and NOS uncoupling [31,35].

The present study has some limitations that deserve mention. First, arginine and ADMA were measured in the circulation, but both NO synthesis and ADMA generation are intracellular events. It is therefore not clear whether the circulating levels of arginine and ADMA appropriately reflect their levels at the site of NOS. On a parallel note, it is not clear to what extent circulatory levels of MPO, CRP, and oxLDL reflect local levels in the vasculature. However, the circulating level of ADMA has been shown to predict future cardiovascular events in several prospective cohort studies [7,8], and inverse associations of circulatory levels of CRP, MPO, and oxLDL with vascular function have been demonstrated [11-13,20], suggesting a biological relevance of circulating levels. Second, the present study was conducted in white elderly subjects and therefore the results may be different in other ethnic and age groups. Third, the cross-sectional design of the study limits the possibility to draw definitive conclusions on causality. Major strengths of our study include the large number of subjects and the availability of a wide array of clinical and biochemical variables to control for potential confounding.

Endothelial production of adequate amounts of NO is essential for maintaining proper vascular function. Shortage of NO leads to endothelial dysfunction and accelerates atherogenesis, whereas an excess of NO may cause circulatory shock. The importance of control of NO availability is underlined by the fact that NOS activity is regulated at many levels, including transcription, translation, phosphorylation and other posttranslational modifications, interactions with other proteins and the plasma membrane, binding of cofactors, and the local concentrations of substrate (arginine) and endogenous inhibitor (ADMA). Although the reduced availability of NO that is associated with systemic inflammation most likely cannot be ascribed to a single regulatory level, the results of the present study show that a decrease of arginine and an increase of ADMA concentrations may be contributing factors. In addition, oxidative stress seems to enhance the association between

inflammation and increased ADMA levels. Taken together, these findings suggest that improvement of NO production by an increase of the substrate/inhibitor ratio may be best achieved by combined management of inflammation and oxidative stress.

AUTHOR CONTRIBUTIONS

Leonard van der Zwan contributed to data acquisition, data analysis and writing of the manuscript. Peter Scheffer and Tom Teerlink contributed to experimental design, data acquisition, data analysis and manuscript revision. Jacqueline Dekker, Coen Stehouwer and Robert Heine designed, initiated and managed the Hoorn Study and contributed to revision of the manuscript.

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FIGURE LEGENDS

Figure 1. Mean (SEM) arginine/ADMA ratio in plasma of subjects stratified by tertiles of CRP (A), or tertiles of MPO (B), the relation between plasma levels of CRP and MPO (C), and the mean Arginine/ADMA ratio after two-dimensional stratification of the cohort by tertiles of CRP and MPO (D). *P* for trend values (A and B) were derived from age- and sex-adjusted linear regression analyses and the strength of the association (C) as indicated by Pearson's correlation coefficient was calculated after logarithmic transformation.

Figure 2. Influence of oxidative stress, assessed by stratification based on plasma levels of oxidized LDL, on the relationship between plasma levels of MPO and ADMA (A) and the relationship between plasma levels of CRP and arginine (B). The strengths of the relations was derived from mutually-, age- and sex-adjusted linear regression models and expressed as change in concentration of the dependent variable (ADMA or arginine) per 1 standard deviation of the independent variable (MPO or CRP). MPO and CRP were natural log-transformed prior to analysis. Error bars represent SE.

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Table 1. Subject characteristics

| Variable | Unit | |
|------------------------------|--------------------------------|------------------|
| <i>N</i> | | 746 |
| Age | years | 68.8 (7.2) |
| Sex, male | % | 49 |
| Arginine | µmol/L | 94.1 (15.6) |
| ADMA | µmol/L | 0.449 (0.058) |
| Arginine/ADMA ratio | µmol/µmol | 212.1 (38.5) |
| SDMA | µmol/L | 0.498 (0.110) |
| Myeloperoxidase | µg/L | 56.1 (47.4-67.0) |
| C-reactive protein | mg/L | 2.20 (1.06-4.68) |
| HbA1c | % | 6.1 (0.8) |
| Fasting glucose | mmol/L | 6.1 (5.5-7.0) |
| Insulin | pmol/L | 62 (43-90) |
| LDL-cholesterol | mmol/L | 3.6 (0.9) |
| HDL-cholesterol | mmol/L | 1.38 (0.40) |
| Triglycerides | mmol/L | 1.4 (1.0-1.9) |
| Apolipoprotein-B100 | g/L | 1.02 (0.23) |
| Oxidized LDL | U/L | 64.8 (15.2) |
| OxLDL/apoB100 ratio | U/g | 64.0 (9.7) |
| Lipid lowering medication | % | 18 |
| Body mass index | kg/m ² | 27.7 (4.2) |
| Waist circumference | cm | 96.5 (12.2) |
| Systolic blood pressure | mm Hg | 142 (20) |
| Diastolic blood pressure | mm Hg | 83 (11) |
| Antihypertensive medication | % | 39 |
| Current smoking | % | 15 |
| (Micro-)albuminuria | % | 15 |
| Glomerular filtration rate | ml/min per 1.73 m ² | 60.5 (10.6) |
| Prior cardiovascular disease | % | 56 |

Mean (standard deviation) or for skewed variables median (interquartile range) are shown.

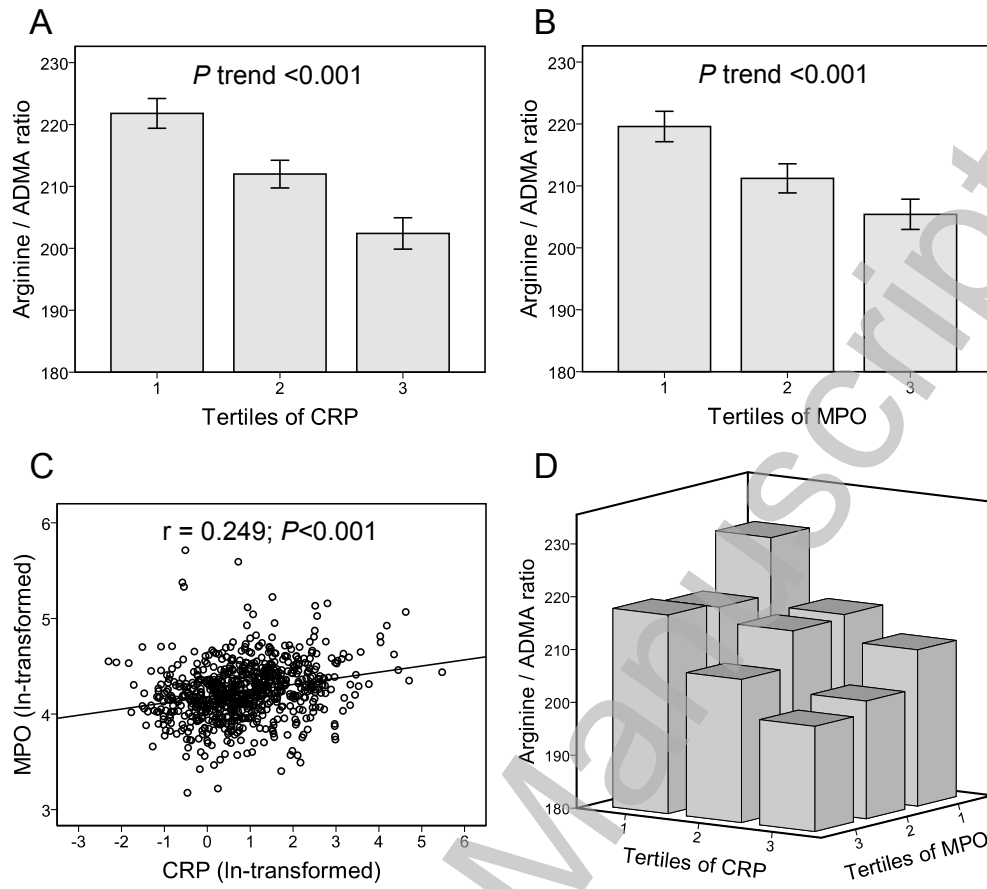
ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine

Table 2. Linear regression models for the relations of CRP and MPO with arginine, ADMA, and their ratio

| Model | | Arginine / ADMA ratio | | Arginine | | ADMA | |
|---------------------------------------|-----|-----------------------|---------|---------------------|---------|-------------------|---------|
| | | Beta (95% CI) | P-value | Beta (95% CI) | P-value | Beta (95% CI) | P-value |
| 1. Crude | CRP | -9.9 (-12.5 to -7.2) | <0.001 | -3.2 (-4.3 to -2.2) | <0.001 | 4.9 (0.8 to 9.0) | 0.020 |
| | MPO | -7.0 (-9.7 to -4.3) | <0.001 | -1.4 (-2.5 to -0.2) | 0.017 | 7.5 (3.4 to 11.6) | <0.001 |
| 2. Mutually adjusted | CRP | -8.7 (-11.4 to -5.9) | <0.001 | -3.1 (-4.2 to -2.0) | <0.001 | 3.2 (-1.0 to 7.5) | 0.13 |
| | MPO | -4.8 (-7.6 to -2.1) | <0.001 | -0.6 (-1.7 to 0.5) | 0.31 | 6.7 (2.4 to 10.9) | 0.002 |
| 3. Mutually and age- and sex-adjusted | CRP | -8.5 (-11.1 to -5.8) | <0.001 | -3.2 (-4.3 to -2.0) | <0.001 | 2.3 (-1.7 to 6.4) | 0.26 |
| | MPO | -4.1 (-6.7 to -1.4) | 0.003 | -0.6 (-1.7 to 0.5) | 0.31 | 5.1 (1.0 to 9.2) | 0.014 |
| 4. 3 + glucose tolerance status | CRP | -8.5 (-11.2 to -5.8) | <0.001 | -3.0 (-4.2 to -1.9) | <0.001 | 3.2 (-1.0 to 7.3) | 0.13 |
| | MPO | -4.0 (-6.7 to -1.4) | 0.003 | -0.5 (-1.7 to 0.6) | 0.34 | 5.3 (1.2 to 9.3) | 0.011 |
| 5. 3 + waist circumference | CRP | -7.5 (-10.3 to -4.7) | <0.001 | -2.7 (-3.9 to -1.5) | <0.001 | 2.8 (-1.6 to 7.1) | 0.21 |
| | MPO | -4.0 (-6.7 to -1.3) | 0.004 | -0.6 (-1.7 to 0.6) | 0.34 | 5.1 (1.0 to 9.3) | 0.015 |
| 6. 3 + LDL- and HDL-cholesterol | CRP | -7.8 (-10.5 to -5.0) | <0.001 | -3.0 (-4.2 to -1.8) | <0.001 | 1.7 (-2.6 to 5.9) | 0.44 |
| | MPO | -4.2 (-6.8 to -1.5) | 0.002 | -0.6 (-1.8 to 0.5) | 0.29 | 5.2 (1.1 to 9.3) | 0.013 |
| 7. 3 + current smoking | CRP | -8.8 (-11.4 to -6.1) | <0.001 | -3.4 (-4.5 to -2.3) | <0.001 | 1.8 (-2.3 to 5.9) | 0.39 |
| | MPO | -4.3 (-7.0 to -1.6) | 0.002 | -0.8 (-1.9 to 0.4) | 0.19 | 4.7 (0.6 to 8.8) | 0.023 |
| 8. 3 + glomerular filtration rate | CRP | -8.5 (-11.1 to -5.8) | <0.001 | -3.2 (-4.3 to -2.1) | <0.001 | 2.3 (-1.8 to 6.3) | 0.27 |
| | MPO | -4.1 (-6.8 to -1.4) | 0.003 | -0.6 (-1.7 to 0.6) | 0.32 | 5.2 (1.1 to 9.2) | 0.012 |
| 9. Fully adjusted* | CRP | -7.5 (-10.4 to -4.6) | <0.001 | -2.8 (-4.0 to -1.6) | <0.001 | 2.0 (-2.3 to 6.3) | 0.36 |
| | MPO | -4.4 (-7.1 to -1.7) | 0.002 | -0.7 (-1.8 to 0.4) | 0.23 | 5.4 (1.3 to 9.4) | 0.010 |

Beta values represent increase in the arginine/ADMA ratio, arginine concentration ($\mu\text{mol/L}$) or ADMA concentration (nmol/L) per 1-SD increment of CRP or MPO. *Mutually adjusted model, additionally adjusted for age, sex, glucose tolerance status, LDL- and HDL-cholesterol, smoking, and glomerular filtration rate.

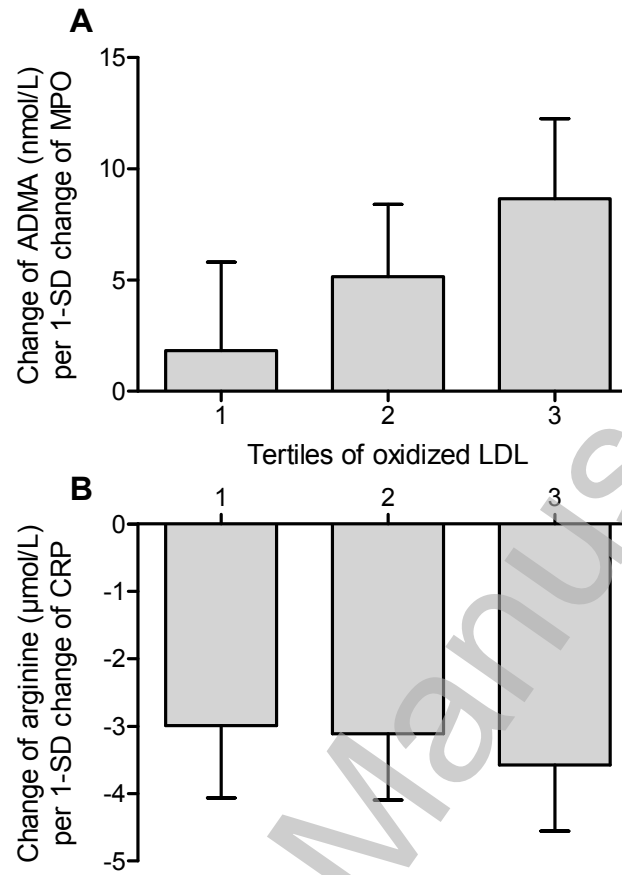
Figure 1



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Figure 2



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