Association between Body Mass Index, Asymmetric Dimethylarginine and Risk of Cardiovascular Events and Mortality in Norwegian Patients with Suspected Stable Angina Pectoris

Heidi Borgeraas1,2*, Jens Kristoffer Hertel1, Gard Frodahl Tveitevåg Svingen3,4, Eva Ringdal Pedersen3,4, Reinhard Seifert4, Ottar Nygård2,3,5, Jøran Hjelmesæth1,2

1 Morbid Obesity Center, Vestfold Hospital Trust, Tønsberg, Norway, 2 Department of Endocrinology, Morbid Obesity and Preventive Medicine Institute of Clinical Medicine University of Oslo, Oslo, Norway, 3 Department of Clinical Science, University of Bergen, Bergen, Norway, 4 Department of Heart Disease, Haukeland University Hospital, Bergen, Norway, 5 KG Jebsen Center for Diabetes Research, Haukeland University Hospital, Bergen, 5021, Norway

* These authors contributed equally to this work.
* heibor@siv.no

Abstract

Background

Asymmetric dimethylarginine (ADMA) is associated with increased risk of atherosclerotic cardiovascular disease and mortality through inhibition of nitrogen oxide (NO) synthesis. As positive correlations between serum concentrations of NO and body mass index (BMI) have been observed, we aimed to explore whether the potential associations between plasma ADMA levels and the risk of acute myocardial infarction (AMI) and mortality were modified by BMI.

Methods

Multivariable Cox proportional hazard models were used to estimate the hazard ratios (HR) for AMI, cardiovascular death and all-cause mortality according to baseline plasma ADMA levels in 4122 patients with suspected stable angina pectoris. Analyses were subsequently repeated in patients with BMI below (low BMI) or above (high BMI) median.

Results

A total of 2982 patients (72%) were men. Median (range) age, plasma ADMA level and BMI were 62 (21–88) years, 0.54 (0.10–1.25) μmol/L and 26.3 (18.5–54.3) kg/m², respectively. During a mean (standard deviation) follow-up time of 4.7 (1.4) years, 337 (8%) patients suffered from an AMI, 300 (7%) died, whereof 165 (55%) due to cardiovascular disease. Each 0.1 μmol/L increment in plasma ADMA level was associated with an increased risk of AMI.
(HR (95% CI) 1.21 (1.08, 1.35) and cardiovascular death 1.30 (1.13, 1.49) in participants with low BMI only. Interactions were significant for AMI ($p = 0.04$) and CV death ($p = 0.03$). BMI did not modify the association between plasma ADMA levels and all-cause mortality.

**Conclusion**

Plasma ADMA levels were associated with risk of AMI and cardiovascular death among patients with low BMI only.

**Introduction**

Plasma asymmetric dimethylarginine (ADMA) is recognized as a biomarker of atherosclerotic cardiovascular (CV) disease risk and mortality [1]. ADMA acts as a non-selective inhibitor of the nitrogen oxide synthases (NOS) and may increase the risk of CV complications through reduced synthesis of nitrogen oxide (NO).

NO, which is synthesized in small amounts by endothelial NOS (eNOS) during basal conditions, is an essential component for endothelial function and mediates endothelial vasodilatation, inhibits platelet aggregation and leukocyte adhesion to the endothelium and regulates myocardial contractility [2]. In acute and chronic inflammatory processes, NO levels might, however, become excessively high due to activation of inducible NOS (iNOS) [3]. In elevated quantities, NO reacts readily with other free radicals, increasing nitrosative and oxidative stress and potentially leading to injury of both the endothelium and myocytes [4–7].

Overweight and obesity are associated with chronic low grade inflammation [8]. The excess adipose tissue may cause accumulation of pro-inflammatory macrophages and subsequent induction of iNOS expression [9,10]. Notably, increased serum NO concentrations and markers of nitrosative and oxidative stress, have been observed in overweight and obese individuals, as compared to normal weight controls [11–14].

Due to the detrimental effects of high NO levels, an increased ADMA production might theoretically be protective in conditions associated with increased levels of iNOS-derived NO. Thus, in a large cohort of Norwegian patients with suspected stable angina pectoris we aimed to investigate whether the potential associations between plasma ADMA levels and risk of AMI, CV death and all-cause mortality were modified by BMI.

**Materials and Methods**

**Study design, setting and population**

A detailed description of the patients included in the present investigation has previously been published [15]. In short, two university hospitals in Western Norway recruited 4164 patients undergoing coronary angiography for suspected stable angina pectoris during the period from January 2000 to April 2004. Of these patients, 2573 (61.8%) were enrolled in the Western Norway B Vitamin Intervention Trial (WENBIT) (ClinicalTrials.gov Identifier: NCT00354081) which studied the effect of B-vitamin intervention on incident CV events and mortality [16].

Patients characterized as underweight (BMI $< 18.5$) ($n = 30$) or those with missing data on BMI or ADMA ($n = 11$) were excluded. One patient with an extremely high plasma ADMA level, relative to the other patients was excluded as well, resulting in a total of 4122 subjects eligible for analysis. Data on plasma symmetric dimethylarginine (SDMA) were available from the WENBIT subpopulation ($n = 2551$).
The study protocol met the mandate of the Declaration of Helsinki and was approved by the Regional Committee for Medical and Health Research Ethics and the Norwegian Data Inspectorate. Written informed consent was obtained from all participants.

Baseline variables

Each patient provided information about medical history, risk factors, and medications through a self-administered questionnaire, and all information was subsequently validated against medical records, as previously reported [16]. Height and weight were measured in light clothing at baseline by trained personnel, and BMI was calculated by dividing weight by height squared (kg/m²). Fasting referred to not having ingested food at least 6 hour prior to blood sample collection. Diabetes mellitus included type 1 and 2. Self-reported current smokers, those who quit smoking within <1 month prior to examination and patients with plasma cotinine >85 ng/mL were regarded as current smokers [17]. Left ventricular ejection fraction (LVEF) and extent of coronary artery disease (CAD) were assessed as previously described [18].

Blood samples were collected by study personnel prior to angiography and stored at -80°C until analysis. Patients were not requested to fast. Plasma ADMA and SDMA were determined by high performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) at BEV-ITAL AS, Bergen, Norway (www.bevital.no), and the within-day coefficient of variation was 5–7% for ADMA and 8–9% for SDMA. Methods for measurement of serum apolipoprotein A-I (apoA-I), apolipoprotein B (apoB), lipoprotein (a) (Lp(a)), C-reactive protein (CRP), plasma cotinine, homocysteine [19] and calculation of LDL cholesterol and estimated glomerular filtration rate (eGFR) have previously been reported[18].

End points and follow up

The patients were followed from angiography until either they experienced an AMI (fatal and non-fatal), died or throughout December 31th 2006.

Clinical events information was collected as previously described [20]. An event was classified as fatal if death occurred within 28 days after onset. AMI was classified according to the diagnostic criteria of the revised AMI definition published in 2000 [21]. CV death included causes of death coded I00-I99 or R96, according to the International Statistical Evaluation of Disease, 10th Revision System. All events were adjudicated by an endpoint committee who had no information on baseline biochemical characteristic.

Statistical methods

Continuous variables are presented as means (± standard deviation (SD)) or medians (range) and categorical variables are reported as counts (percentage). Between-group differences were tested with independent samples t-test for continuous variables and chi-square test for categorical variables. Non-normally distributed variables (diastolic blood pressure, plasma homocysteine, serum creatinine, CRP, glucose, triglycerides and Lp(a)) were log transformed before analysis. Correlation analyses between ADMA and continuous and dichotomous variables were performed by calculating the Pearson product moment correlation coefficient and the point-biserial correlation coefficient, respectively. Adjusted correlations were carried out by calculating the partial correlation coefficient.

Cox proportional hazard models were used to calculate hazard ratios (HR) and 95% confidence intervals (95% CI) for incident AMI, CV death and all-cause mortality per 0.1 μmol/L increase in plasma ADMA levels. Proportionality assumptions were tested by visual examination of log minus log plots and by calculating Schoenfeld residuals. Selection of covariates in
the multivariate adjusted model were based on clinical relevance the change in estimate method [22], and included age (years), sex, diabetes mellitus (yes/no), current smoking (yes/no), statin treatment (yes/no), homocysteine (μmol/L), hemoglobin (g/dL), apoB/apoA-I ratio and Lp(a) (mmol/L) (model 1). We considered systolic and diastolic blood pressure (mmHg), impaired LVEF (yes/no), the extent of significant CAD (0–3), eGFR (mL/min), use of beta blockers (yes/no), ACE-inhibitors (yes/no) and loop diuretics (yes/no) to be possible mediators of the effect of ADMA and did for that reason not include any of these variables in the main multivariate model (model 1) [23]. The variables were, however, added to an additional multivariate model (model 2). Vitamin B6 (yes/no) or folate/B12 (yes/no) WENBIT intervention status had no significant effect on the estimates (data not shown). Univariate, age and sex adjusted and multivariate adjusted HRs (95% CI) for incident AMI, CV death and all-cause mortality per 0.1 μmol/L increase in plasma SDMA levels were also calculated.

BMI was grouped according to the median value and patients with BMI equal to or below and above median were classified as "low BMI" and "high BMI", respectively. The possible interaction between BMI and ADMA was examined by this stratification and by including the interaction product term of BMI (low and high BMI) and plasma ADMA (continuous) in the Cox model. The possible effect modification by BMI on the associations between plasma SDMA and incident AMI, CV death and all-cause mortality was also examined.

Non-linear effects were additionally investigated with generalized additive model (GAM) plots using penalized smoothing splines for the functional form of the covariate [24]. Potential breakpoints in the loglinear proportional hazards model were investigated with segmented regression (R-package segmented version 0.5–1.1).

All probability values are 2-tailed, and considered significant when <0.05. Statistical analyses were performed with SPSS 18 (SPSS Inc, Chicago, IL) and R 2.14.2 (The R-Foundation for Statistical Computing, Vienna, Austria).

Results
Baseline characteristics
For the 4122 patients included in the cohort, the mean (SD) age was 62 (10) years and 72% were males. The median (range) BMI was 26.3 (18.5–54.3) kg/m² and plasma ADMA level was 0.54 (0.10–1.25) μmol/L.

Baseline characteristics of the study population are presented in Table 1. As compared with patients with high BMI (> 26.3 kg/m²), the low BMI patient group (≤ 26.3 kg/m²) was on average 2 years older, less often fasting, included more frequently current smokers and had higher mean plasma ADMA and arginine levels. Those with low BMI had lower levels of atherogenic lipids, blood glucose, CRP, lower blood pressure and eGFR, and made up a lower proportion with diabetes. Patients with high BMI were more often treated with statins, beta blockers, ACE-inhibitors and loop diuretics at discharge from the hospital.

Baseline plasma ADMA levels were correlated with female gender, older age, a history of peripheral vascular disease, use of loop diuretics, plasma homocysteine and serum creatinine levels, and inversely correlated with BMI, use of statins, eGFR, serum hemoglobin and Lp(a) (S1 Table). The correlation between plasma ADMA levels and BMI was no longer significant after adjustment for age and sex (data not shown).

Baseline plasma SDMA was measured in a subpopulation (n = 2551), and the mean (SD) plasma SDMA was 0.56 (0.15) μmol/L. Median BMI was 26.5kg/m², and the mean (SD) plasma SDMA was significantly (p<0.001) higher among those with low BMI (0.58 (0.15) μmol/L) compared to those with high BMI (0.54 (0.14) μmol/L). Plasma SDMA levels were correlated with BMI (r = -0.15, p<0.01) and plasma ADMA levels (r = 0.33, p<0.01).
Table 1. Baseline characteristics in the total population and according to patients with low and high BMI.

|                      | Total          | Low BMI<sup>a</sup> | High BMI<sup>b</sup> | p-value |
|----------------------|----------------|---------------------|----------------------|---------|
|                      | 26.3 (18.5–54.3)<sup>c</sup> | 24.2 (18.5–26.3)    | 29.0 (26.4–54.3)     |         |
| n = 4122             | n = 2061       | n = 2061            | p-value              |         |
| **Demographic**      |                |                     |                      |         |
| **Male sex, n (%)**  | 2982 (72.3)    | 1471 (71.4)         | 1511 (73.3)          | 0.16    |
| **Age (years), mean (SD)** | 62 (10)     | 63 (10)             | 61 (10)              | <.0001  |
| **Fasting, n(%)**    | 1097 (26.6)    | 511 (24.8)          | 586 (28.4)           | <.01    |
| **Clinical**         |                |                     |                      |         |
| **Systolic**         | 141 (21)       | 140 (21)            | 142 (21)             | <.01    |
| **Diastolic**        | 81 (10)        | 80 (10)             | 83 (10)              | <.001   |
| **Impaired LV ejection** | 534 (13.0)   | 265 (12.9)          | 269 (13.1)           | 0.86    |
| **Cardiovascular**   |                |                     |                      |         |
| **Diabetes**         | 494 (12.0)     | 175 (8.5)           | 319 (15.5)           | <.001   |
| **Current smoker**   | 1056 (25.7)    | 584 (28.4)          | 472 (23.0)           | <.001   |
| **Ex smoker**        | 1930 (46.9)    | 900 (43.7)          | 1030 (50.1)          | <.001   |
| **Never smoked**     | 1129 (27.4)    | 576 (28.0)          | 553 (26.9)           | 0.45    |
| **Cardiovascular**   |                |                     |                      |         |
| **Previous acute**   | 1668 (40.5)    | 833 (40.4)          | 835 (40.5)           | 0.95    |
| **Peripheral**       | 285 (6.9)      | 137 (6.6)           | 148 (7.2)            | 0.50    |
| **Peripheral**       | 371 (9.0)      | 203 (9.8)           | 168 (8.2)            | 0.06    |
| **Previous PCI**     | 793 (19.2)     | 375 (18.2)          | 418 (20.3)           | 0.10    |
| **Previous coronary**| 477 (11.6)     | 225 (10.9)          | 252 (12.2)           | 0.19    |
| **Extent**           |                |                     |                      |         |
| **Coronary artery**  | 1028 (24.9)    | 524 (25.4)          | 504 (24.5)           | 0.47    |
| **1 vessel**         | 957 (23.2)     | 472 (22.9)          | 485 (23.5)           | 0.63    |
| **2 vessel**         | 921 (22.3)     | 457 (22.1)          | 464 (22.5)           | 0.79    |
| **3 vessel**         | 1216 (29.5)    | 608 (29.5)          | 608 (29.5)           | 1.00    |
| **Medication**       |                |                     |                      |         |
| **Acetylsalicylic**  | 3369 (81.7)    | 1675 (81.3)         | 1693 (82.1)          | 0.47    |
| **Beta blockers**    | 3303 (80.1)    | 1610 (78.1)         | 1693 (82.1)          | <.01    |
| **ACE-inhibitors**   | 2988 (72.5)    | 1454 (70.5)         | 1534 (74.4)          | <.01    |
| **Loop diuretics**   | 886 (20.8)     | 372 (18.0)          | 484 (23.5)           | <.001   |
| **Biochemical**      |                |                     |                      | <.001   |
| **ADMA (μmol/L)**    | 0.56 (0.12)    | 0.57 (0.12)         | 0.55 (0.11)          | <.01    |
| **Arginine (μmol/L)**| 78.9 (22.7)   | 80.0 (23.1)         | 77.8 (22.2)          | <.01    |
| **Creatinine (μmol/L)** | 11.4 (4.94)  | 11.5 (4.87)         | 11.2 (5.01)          | 0.02    |
| **Creatinine (μmol/L)** | 92.6 (31.0) | 92.9 (33.5)         | 92.3 (28.2)          | 0.54    |
| **eGFR (mL/min)**    | 87.8 (17.2)    | 86.7 (17.4)         | 88.9 (17.0)          | <.001   |
| **CRP (mg/L)**       | 3.69 (7.17)    | 3.38 (7.61)         | 4.01 (6.69)          | <.01    |
| **Glucose (mmol/L)** | 6.35 (2.40)    | 6.00 (2.27)         | 6.71 (2.49)          | <.001   |
| **HbA1c (mmol/L)**   | 6.22 (1.39)    | 6.11 (1.32)         | 6.33 (1.44)          | <.001   |
| **Hemoglobin (g/dL)**| 14.3 (1.24)    | 14.1 (1.22)         | 14.4 (1.23)          | <.001   |
| **ApoA-I (g/L)**     | 1.32 (0.27)    | 1.35 (0.26)         | 1.28 (0.25)          | <.001   |
| **ApoB (g/L)**       | 0.90 (0.25)    | 0.88 (0.24)         | 0.93 (0.26)          | <.001   |
| **ApoB/ApoA ratio**  | 0.71 (0.25)    | 0.68 (0.24)         | 0.75 (0.25)          | <.001   |

(Continued)
End points and follow-up

During a mean (SD) follow-up of 4.7 (1.4) years, 337 (8%) patients experienced an AMI, of which 101 (30%) were fatal. A total of 300 (7%) patients died whereof 165 (55%) of these fatalities were due to CV disease.

Associations between ADMA, AMI and mortality

Each 0.1 μmol/L increase in plasma ADMA level was associated with an increased risk of AMI (HR (95% CI) 1.12 (1.03, 1.22)), CV death (1.20 (1.07, 1.33)) and all-cause mortality; (1.21 (1.11, 1.31)) (Table 2, model 1). The associations were linear with no significant break-points (data not shown). After further adjustments for possible mediators of ADMA, the associations between plasma ADMA and risk of AMI (1.07 (0.98, 1.17)) and CV death (1.12 (0.99, 1.26)) were attenuated (Table 2, model 2).

One patient was excluded from the study based on extreme plasma levels of ADMA relative to the other patients (10 SD above mean). Inclusion of this outlier yielded similar results (data not shown).

Among those with data on plasma SDMA (n = 2551), the associations between plasma SDMA levels and risk of AMI, CV death and all-cause mortality were comparable to the associations between ADMA and endpoints (S2 Table).

Stratification by BMI

BMI level modified the risk association between plasma ADMA and AMI (p for interaction = 0.04) and CV death (p for interaction = 0.03), but not all-cause mortality (p for interaction = 0.15). Each 0.1 μmol/L increase in plasma ADMA was associated with an increased risk of AMI and CV death in participants with low BMI only; HR (95% CI) 1.21 (1.08, 1.35) and 1.30 (1.13, 1.49), respectively (Table 2 and Fig 1). After further adjustments (model 2), the interactions between plasma ADMA and BMI with regards to risk of AMI (p for interaction = 0.05) and CV death (p for interaction = 0.031) remained significant, while the interaction between plasma ADMA and BMI with regards to all-cause mortality was still non-significant (p for interaction = 0.09). Adjustment for CRP and fasting did not significantly
### Table 2. Risk of acute myocardial infarction, cardiovascular death and all-cause mortality according to plasma ADMA levels in the total population and in patients with high or low BMI.

| Patients     | Model                  | Acute myocardial infarction | Cardiovascular death | All-cause mortality |
|--------------|------------------------|-----------------------------|----------------------|---------------------|
|              |                        | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value |
| **Total population** | **Univariate** | ADMA (per 0.1 μmol/L increase) | 1.20 (1.11, 1.30) | <0.001 | 1.36 (1.23, 1.50) | <0.001 | 1.35 (1.25, 1.45) | <0.001 |
| | Age and sex adjusted | ADMA (per 0.1 μmol/L increase) | 1.15 (1.06, 1.24) | <0.01 | 1.25 (1.13, 1.39) | <0.001 | 1.26 (1.17, 1.36) | <0.001 |
| | Multivariate adjusted | Model 1^a | ADMA (per 0.1 μmol/L increase) | 1.12 (1.03, 1.22) | <0.01 | 1.20 (1.07, 1.33) | 0.001 | 1.21 (1.11, 1.31) | <0.001 |
| | | Model 2^b | ADMA (per 0.1 μmol/L increase) | 1.07 (0.98, 1.17) | 0.15 | 1.12 (0.99, 1.26) | 0.06 | 1.16 (1.07, 1.27) | <0.01 |
| **Low BMI^c** | **Univariate** | ADMA (per 0.1 μmol/L increase) | 1.29 (1.16, 1.42) | <0.001 | 1.46 (1.29, 1.65) | <0.001 | 1.39 (1.26, 1.53) | <0.001 |
| | Age and sex adjusted | ADMA (per 0.1 μmol/L increase) | 1.23 (1.11, 1.37) | <0.001 | 1.36 (1.19, 1.54) | <0.001 | 1.31 (1.18, 1.45) | <0.001 |
| | Multivariate adjusted | Model 1^a | ADMA (per 0.1 μmol/L increase) | 1.21 (1.08, 1.35) | <0.01 | 1.30 (1.13, 1.49) | <0.001 | 1.26 (1.13, 1.40) | <0.001 |
| | | Model 2^b | ADMA (per 0.1 μmol/L increase) | 1.12 (1.00, 1.27) | 0.07 | 1.25 (1.07, 1.46) | <0.01 | 1.25 (1.11, 1.41) | <0.001 |
| **High BMI^d** | **Univariate** | ADMA (per 0.1 μmol/L increase) | 1.10 (0.97, 1.24) | 0.13 | 1.22 (1.03, 1.44) | 0.02 | 1.29 (1.15, 1.45) | <0.001 |
| | Age and sex adjusted | ADMA (per 0.1 μmol/L increase) | 1.06 (0.93, 1.20) | 0.40 | 1.13 (0.95, 1.34) | 0.18 | 1.20 (1.02, 1.35) | <0.01 |
| | Multivariate adjusted | Model 1^a | ADMA (per 0.1 μmol/L increase) | 1.02 (0.90, 1.16) | 0.72 | 1.06 (0.89, 1.27) | 0.53 | 1.14 (1.00, 1.29) | 0.04 |
| | | Model 2^b | ADMA (per 0.1 μmol/L increase) | 1.00 (0.90, 1.14) | 0.98 | 1.00 (0.82, 1.21) | 0.96 | 1.09 (0.95, 1.24) | 0.22 |

ADMA: asymmetric dimethylarginine; BMI: body mass index; CI: confidence interval; HR: hazard ratio;

^a Age (years), sex, diabetes mellitus (yes/no), current smoking (yes/no), statin treatment (yes/no), homocysteine (μmol/L), hemoglobin (g/dL), apoB/apoA-I ratio and Lp(a) (mmol/L)

^b Age (years), sex, diabetes mellitus (yes/no), current smoking (yes/no), statin treatment (yes/no), homocysteine (μmol/L), hemoglobin (g/dL), apoB/apoA-I ratio and Lp(a) (mmol/L) diastolic blood pressure (mmHg), systolic blood pressure (mmHg), treatment with beta blockers (yes/no), extent of significant CAD (0–3), estimated glomerular filtration rate (mL/min), loop diuretics (yes/no), ACE-inhibitors (yes/no) and impaired left ventricular ejection fraction (yes/no)

^c Equal to or below median (26.3 kg/m²) BMI

^d Above median (26.3 kg/m²) BMI

influence the results (data not shown). Segmented Cox regression analyses revealed a linear association between plasma ADMA levels and endpoints, with no significant break-points (data not shown).

BMI did, however, not significantly modify the association between plasma SDMA levels and risk of AMI, CV death and all-cause mortality (S2 Table).
Discussion

Principal findings

In this large prospective cohort study of patients with suspected stable angina pectoris, increasing baseline plasma levels of ADMA were associated with higher risk of incident AMI, CV death and all-cause mortality. The associations between plasma ADMA levels and incident AMI and CV death were, however, significant in patients with low BMI (equal to or below median BMI of 26.3 kg/m²).
median BMI (26.3 kg/m²)) only. The association between plasma ADMA level and all-cause mortality was not modified by BMI.

ADMA and risk prediction

High plasma ADMA levels have been associated with increased risk of incident CV events and all-cause mortality in patients with stable coronary heart disease [25–28], following PCI [26], chronic heart failure [29], and recent AMI [30], peripheral arterial disease [31], hemodialysis patients [32], renal transplant recipients with multi organ-failure [33] diabetes mellitus[34,35] as well as in the general population [36–38].

To the best of our knowledge, this is, however, the first study to investigate the possible moderating effect of BMI on the association between plasma ADMA levels and the risk of AMI and mortality in a population of patients with suspected coronary artery disease.

Possible explanations

The NO molecule can be thought of as a double edged sword; under physiological conditions NO is produced in low concentrations and participates in the maintenance of homeostasis in the cardiovascular, immune and central nervous systems [39]. However, during pathophysiologic conditions such as inflammation, iNOS may generate up to 1000 times the normal amount of NO [40] with subsequent production of reactive nitrogen and oxygen species [3]. Overweight and obesity are related to chronic low grade inflammation[8], elevated levels of NO and markers of nitrosative and oxidative stress [11–14]. As ADMA is a non-selective inhibitor of NOS, the lack of association observed between plasma ADMA levels and risk of AMI and CV death in overweight and obese patients in the current study can be put down to a possible beneficial regulatory effect of ADMA on the inflammatory processes and the elevated NO levels. We would thus expect the interaction between ADMA and BMI to be attenuated when adjusting for an inflammatory marker, but including CRP in the regression analyses did not influence this interaction. A possible explanation might be the extensive use of statins which are known to decrease CRP levels [41] and also increase NO levels [42].

In a subpopulation, increasing levels of SDMA were also associated with increased risk of AMI, CV death and all-cause mortality. In contrast to ADMA, SDMA does not directly inhibit NO synthesis, thus a beneficial regulatory effect on elevated NO levels by SDMA in overweight and obese individuals is unlikely. As expected, the associations between SDMA and endpoints were not modified by BMI.

On a similar note, a Framingham offspring study revealed that the predictive value of plasma ADMA levels for all-cause mortality was only evident in patients without diabetes, while the association appeared to disappear in those with diabetes [38]. BMI and diabetes are closely interrelated, and there were admittedly more diabetic patients in the high BMI group in the present study, yet there were no significant interactions between plasma ADMA levels and diabetes with regards to any of the end points. Furthermore, in the general male population, plasma ADMA levels have been found to be associated with increased risk of coronary events in non-smokers, but not in smokers [36]. No such interaction was observed in the current study. However, it is also worth considering that both hyperglycemia and cigarette smoke increases the formation of ROS and induces inflammation [43,44]; hence these effect modifications might potentially be explained by a similar mechanism to that suggested in this study.

Stratified analyses also revealed stronger risk associations of ADMA to all cause mortality among subjects with low BMI compared to those with high BMI. However, the interaction was not statistically relevant in relation to this endpoint.
Strengths and limitations of the study

The study included a large and well-characterised population and had a prospective design. The effect of ADMA is thought to be mediated through reduced NO availability. Elevated ADMA levels may in turn result in increased blood pressure, cardiac dysfunction [45], increased progression of atherosclerosis [46,47] and reduced renal plasma flow and GFR [48–50]. The inclusion of variables reflecting any of these parameters might thus cause an underestimation of the effect of ADMA. We did, however, include possible mediators in an additional multivariate model, and as expected, the effect of ADMA on the risk of AMI and CV death was somewhat attenuated. The interaction between ADMA and BMI was, however, still significant.

A possible limitation to our study is the single baseline measurements of ADMA, which may mean that the true risk estimates were underestimated due to regression dilution bias [51]. Also, we cannot preclude the possibility of residual confounding, an inherent limitation in all observational research.

Conclusion

We confirmed previous findings of a positive association between plasma ADMA levels and incident AMI, CV death and all-cause mortality. The associations between plasma ADMA levels and risk of AMI and CV death were, however, confined to patients with low BMI only. In contrast, BMI had no modifying effect on the relationship between plasma AMA levels and all-cause mortality. More research is warranted in order to corroborate our findings in other populations and to clarify the possible underlying mechanisms.

Supporting Information

S1 Table. Baseline characteristics and correlations with plasma ADMA levels.

S2 Table. Risk of acute myocardial infarction, cardiovascular death and all-cause mortality according to plasma SDMA levels in the total population and in patients with high or low BMI.

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Author Contributions

Conceived and designed the experiments: ON. Performed the experiments: ON GFTS ERP. Analyzed the data: HB RS. Wrote the paper: HB JKH GFTS ERP ON JH.

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