

## Original Article

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# Asymmetric dimethylarginine (ADMA) levels display a morning peak in patients with acute myocardial infarction

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**Abstract.** High Asymmetric Dimethylarginine (ADMA) levels are associated with increased platelet activity, elevated blood pressure, vasoconstriction and impaired vascular relaxation. We hypothesized that the myocardial infarction morning peak of occurrence is closely related to a morning peak of ADMA levels. We performed a cross-sectional study among patients with documented myocardial infarction who had been enrolled in the prospective MISSION! Intervention Study. In total, serum ADMA levels were measured in their acute setting of myocardial infarction in 120 patients. The frequency of myocardial infarction onset of symptoms and emergency coronary catheterization and the ADMA levels displayed a similar daily pattern with a morning peak between 06:00–11:59 h. The absolute ADMA levels peak was between 06:00–07:59 h with a median (interquartile range) peak value of 1.01 (0.84–1.21)  $\mu\text{mol/L}$  for the  $n = 9$  patients vs. 0.75 (0.61–0.89)  $\mu\text{mol/L}$  for the remaining 111 patients admitted throughout the rest of the 24-hour interval ( $p = 0.003$  for between groups comparison). The amplitude (95% confidence interval) of the circadian variation of ADMA levels was 0.08  $\mu\text{mol/L}$  (0.004–0.16) with  $p = 0.042$  for statistic model significance.

In conclusion, ADMA levels display a 24-hour variation with a significant morning peak in patients with acute myocardial infarction. These findings may relate ADMA levels to the acute onset of thrombotic cardiovascular events.

**Keywords:** Asymmetric dimethylarginine, myocardial infarction, circadian rhythm, platelets, thrombosis

## 1. Introduction

The incidence of acute thrombotic events such as myocardial infarction and ischemic stroke display a daily

variation with a well documented morning peak [1–5]. The typical morning myocardial infarction is generally considered to be triggered by factors that mechanically disrupt the vulnerable plaque, such as transiently increased blood pressure [6,7] and viscosity, coupled with increased platelet aggregability [8,9] and decreased coronary blood flow [10,11].

Asymmetric dimethylarginine (ADMA) is an endogenous amino-acid produced by virtually all human

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cells as a result of methylation of arginine residues in proteins. After protein degradation ADMA is liberated leading to low reference levels of ADMA in serum. Because it is structurally similar to L-arginine (the substrate of nitric oxide (NO) synthase for the formation of NO), ADMA competitively inhibits the NO synthases in cells [12–16].

Decreased NO availability determines enhanced platelet activation and aggregation [14,16,17]. High ADMA levels are also associated with elevated blood pressure, vasoconstriction, impaired endothelium-dependent relaxation and increased endothelial adhesiveness for monocytes [18–20]. We therefore hypothesized that the myocardial infarction morning peak of occurrence is closely related to a morning peak of ADMA levels.

The aim of the present study was to investigate the 24-hour ADMA levels variation in patients with acute myocardial infarction.

## 2. Subjects, materials and methods

### 2.1. Study design and population

We performed a cross-sectional study among patients who had been enrolled in the prospective MISSION! Intervention Study. Details about the MISSION! Intervention Study have been published elsewhere [21, 22]. In brief, this was a single center, single blind, randomized prospective non-inferiority study to evaluate clinical, angiographic and intravascular ultrasonography (IVUS) results in ST-elevation myocardial infarction (STEMI) patients treated with either bare-metal stents (BMS) or sirolimus-eluting stents (SES). The study was approved by the ethics committee and all patients gave written and informed consent. The study was conducted from February 2004 until October 2006. Post-procedure and follow-up IVUS data were available in 184/310 patients (60%; 104 SES; 80 BMS) included in the MISSION! Intervention Study [22]. For the present study we chose to measure ADMA levels in the 184-patient cohort with complete IVUS data. Among these, ADMA levels were successfully determined in 120 patients. These patients were not selected in any way other than blood sample availability and fulfillment of specified ADMA measurement conditions. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

### 2.2. Patient selection

Patients were eligible if STEMI symptoms started < 9 hours before the procedure and the ECG demonstrated STEMI (ST-segment elevation  $\geq 0.2$  mV in  $\geq 2$  contiguous leads in V1 through V3 or  $\geq 0.1$  mV in other leads, or (presumed) new left bundle branch block).

Exclusion criteria were: 1) age < 18 years or > 80 years; 2) left main stenosis of  $\geq 50\%$ ; 3) triple vessel disease, defined as  $\geq 50\%$  stenosis in  $\geq 3$  major epicardial branches; 4) previous percutaneous coronary intervention (PCI) or coronary artery by-pass graft (CABG) of the infarct related artery; 5) thrombolytic therapy for the index infarction; 6) target vessel reference diameter < 2.25 mm or > 3.75 mm; 7) target lesion length > 24 mm; 8) need for mechanical ventilation; 9) contraindication to the use of aspirin, clopidogrel, heparin or abciximab; 10) known renal failure; or 11) a life expectancy < 12 months.

### 2.3. Measurements

In MISSION! Intervention Study data are collected from each patient on medical history and medications, symptoms on arrival to the hospital, ECG examination, index times (time of onset of symptoms, time of call for medical help, time of first medical contact, time of hospital arrival, time of emergency percutaneous coronary intervention (PCI) start, and in-hospital and follow-up records. The time of onset of acute myocardial infarction symptoms was reported by the patient and recorded by the first examining physician. Patients were asked again about the first symptoms of myocardial infarction during their hospital stay. When the time of symptom onset was in doubt, a consensus time was established together with the patient, relatives, and physicians involved in the case. Myocardial infarction was documented on the basis of troponin T > 0.1  $\mu\text{g/L}$  and at least one of the following: clinical symptoms  $\pm$  relevant electrocardiogram (ECG) and  $\pm$  angiographic evidence. Patients were considered to have dyslipidemia, hypertension, and diabetes if they had been diagnosed with such by a physician previous to the present hospital admission. First blood collection for routine measurements (including baseline creatinine) was performed either in the hospital emergency department or in the catheterization room at the beginning of the procedure (in case the patient was referred directly to the coronary care unit). All times of blood collection were automatically recorded in the patient's electronic file.

Table 1  
Baseline characteristics for  $n = 120$  patients based upon ADMA levels

Patient characteristics	ADMA			P-value
	Normal ( $n = 57$ ) [0.43–0.75] $\mu\text{mol/L}$	High ( $n = 42$ ) [0.76–0.99] $\mu\text{mol/L}$	Very high ( $n = 21$ ) [1.00–1.66] $\mu\text{mol/L}$	
Age (yrs)	57 (49–66)	60 (49–70)	66 (48–72)	0.35
Men (%)	46 (81)	31 (74)	16 (76)	0.71
BMI ( $\text{kg/m}^2$ )*	27 (25–29)	26 (24–28)	26 (24–29)	0.63
Current smokers (%)	35 (61)	21 (50)	11 (52)	0.20
Diabetes mellitus (%)	4 (7)	1 (2)	1 (5)	0.60
Systolic blood pressure (mmHg)	140 (126–160)	125 (119–150)	140 (132–155)	0.04
Diastolic blood pressure (mmHg)	81 (76–90)	80 (70–86)	80 (80–89)	0.17
Heart rate (beats/min)	70 (60–80)	72 (58–83)	70 (60–91)	0.91
Hypertension (%)	18 (32)	12 (29)	7 (33)	0.91
Hyperlipidemia (%)	10 (18)	7 (17)	5 (24)	0.77
Positive familial history of CAD (%)	29 (51)	14 (33)	5 (24)	0.03
Previous MI (%)	2 (4)	1 (2)	2 (10)	0.38
Previous PCI (%)	0 (0)	1 (2.4)	1 (4.8)	0.31
Previous CABG (%)	0 (0)	0 (0)	0 (0)	–
Beta-blockers (%)	11 (19)	3 (7)	4 (19)	0.20
Aspirin (%)	4 (7)	4 (10)	2 (10)	0.88
Ca-antagonists (%)	4 (7)	3 (7)	4 (19)	0.22
Clopidogrel (%)	0 (0)	0 (0)	0 (0)	–
Statins (%)	8 (14)	3 (7)	1 (5)	0.35
ACE-inhibitors (%)	3 (5)	1 (2)	0 (0)	0.47
Coumarins (%)	0 (0)	0 (0)	0 (0)	–
Insulin (%)	1 (2)	1 (2)	0 (0)	0.78
Oral anti-diabetics (%)	3 (5)	0 (0)	1 (5)	0.17

Rounded values are median (interquartile range) or number (percentage). \* BMI was available for  $n = 99$  patients. BMI = body mass index ( $\text{kg/m}^2$ ), MI = myocardial infarction, PCI = percutaneous coronary intervention, CABG = coronary artery by-pass graft, ACE = angiotensin converting enzyme, CAD = coronary artery disease.

#### 2.4. ADMA determination

Unlike routine measurements blood collection, the study blood was collected identically for every patient at the beginning of the emergency PCI procedure through the arterial sheath immediately after its positioning. Time of study blood collection corresponds therefore to the emergency PCI start time (automatically recorded in the patient's electronic file). Blood was processed and stored at  $-80^\circ\text{C}$ . Serum ADMA concentrations have been quantified [23] by ELISA (DLD Diagnostica GMBH, Hamburg, Germany). Serum samples were thawed minutes before assay. According to the manufacturer, reference range of serum ADMA concentration is  $0.4\text{--}0.75 \mu\text{mol/L}$ . Detection limit is  $0.05 \mu\text{mol/L}$ , intra-assay variation is 7.5% at an ADMA concentration of  $0.81 \mu\text{mol/L}$  and inter-assay variation is 9.8% at an ADMA concentration of  $1.01 \mu\text{mol/L}$ .

#### 2.5. Statistical analyses

Baseline characteristics are presented as median (interquartile range) or number (percentage). Baseline

characteristics are presented according to 3 increasing ADMA categories in Table 1. The reference range for ADMA concentration, as provided by the manufacturer is  $0.4\text{--}0.75 \mu\text{mol/L}$  and these values were categorized "normal". The remaining values were arbitrarily categorized into "high" ( $0.76\text{--}0.99 \mu\text{mol/L}$ ) and "very high" ( $1.00\text{--}1.66 \mu\text{mol/L}$ ). Differences among the 3 ADMA baseline categories were estimated with Kruskal Wallis non-parametric test for k-independent samples (for continuous variables) or Pearson Chi-Square test (for categorical variables).

The median ADMA level of the patients within the highest two-hour interval was compared with the median ADMA level of the rest of the patients using a Mann-Whitney non-parametric test for two independent samples. A p-value  $< 0.05$  was considered statistically significant.

We used linear regression to model the individual ADMA levels according to the time of blood collection (emergency PCI start time). The circadian variation was expressed by the sinusoidal functions,  $\text{Sin}(2\pi t/12)$  and  $\text{Cos}(2\pi t/12)$ , where  $t$  is the time of blood collection expressed in hours. The combined functions allow the

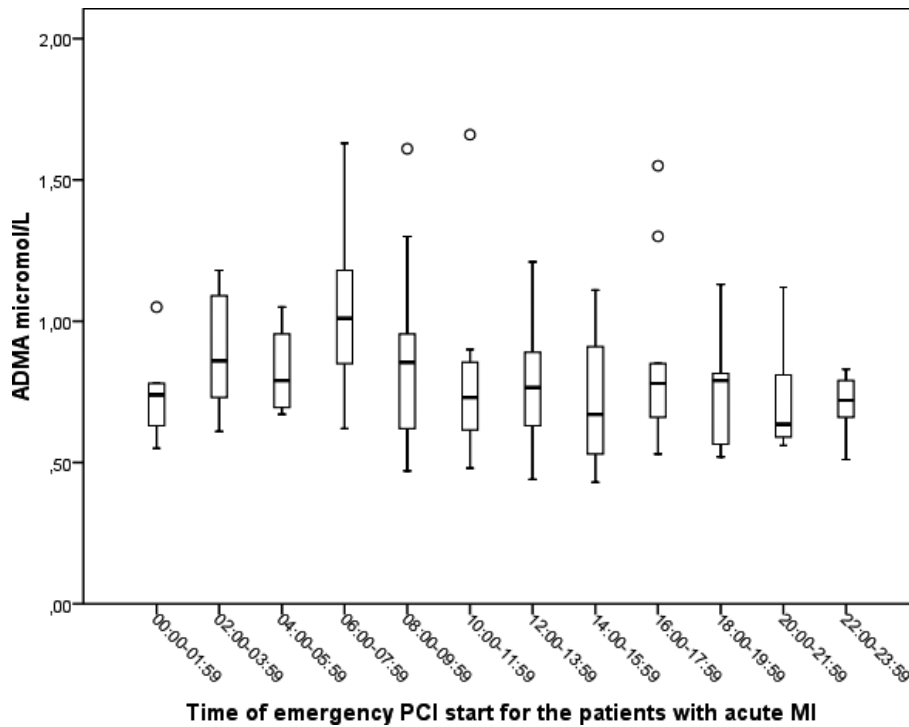


Fig. 1. Box and whisker plot showing median values, interquartile ranges (box), and extreme values (whiskers) of plasma ADMA levels among all 120 patients according to the 12 two-hour intervals of emergency PCI starting at midnight. The time of emergency PCI start is the time of blood drawing.

peak size to occur at any time of the day. The amplitude of the 24 h ADMA levels variation and its standard error were calculated using the regression coefficients ( $\beta_1$ , and  $\beta_2$ ) and variances ( $s_1$ ,  $s_2$ , and  $s_{12}$ ) obtained from the regression model in the following formulas:

$$\text{Amplitude} = \sqrt{\beta_1^2 + \beta_2^2};$$

$$\text{Standard error}(\sqrt{\beta_1^2 + \beta_2^2}) =$$

$$\sqrt{\frac{s_1^2\beta_1^2 + 2s_{12}\beta_1\beta_2 + s_2^2\beta_2^2}{\beta_1^2 + \beta_2^2}}.$$

In the present analysis we defined “amplitude” of 24 h ADMA levels variation as the difference between the highest and the lowest points in a modeled 24 h ADMA levels curve. In parts of the chronobiologic literature “amplitude” may be defined as a difference between the mean and the peak or trough of the fitted model. Therefore, the excursion of peak to trough defined in our analysis as “amplitude” may be elsewhere referred as “double amplitude”.

The formula for the standard error of the amplitude was derived using the delta method [24].

### 3. Results

ADMA levels were measured in 120 patients. Patients’ characteristics are presented according to 3 increasing ADMA categories in Table 1. When computed upon 6-hour intervals, the frequency of myocardial infarction onset of symptoms and the frequency of emergency PCIs showed a similar daily pattern with a morning peak between 06:00 h and 11:59 h (40.8% and 33.3%, respectively, of all cases). The frequencies of myocardial infarction onset of symptoms and emergency PCIs for the other 3 six-hour intervals were: 15.8% and 13.3% between 00:00–05:59 h, 25% and 30% between 12:00–17:59 h and 18.3% and 23.3%, respectively, between 18:00–23:59 h. ADMA levels displayed the same trend with a morning peak represented by the patients undergoing emergency PCI during the 06:00–11:59 h interval with a median (interquartile range) ADMA value of 0.84 (0.63–0.98)  $\mu\text{mol/L}$ . For the 00:00–05:59 h, 12:00–17:59 h and the 18:00–23:59 h intervals the median (interquartile range) ADMA levels were 0.77 (0.68–1.01), 0.74 (0.59–0.90) and 0.66 (0.58–0.81)  $\mu\text{mol/L}$ , respectively.

When depicted upon two-hour intervals, the ADMA peak levels presented between 06:00–07:59 h (Fig. 1).

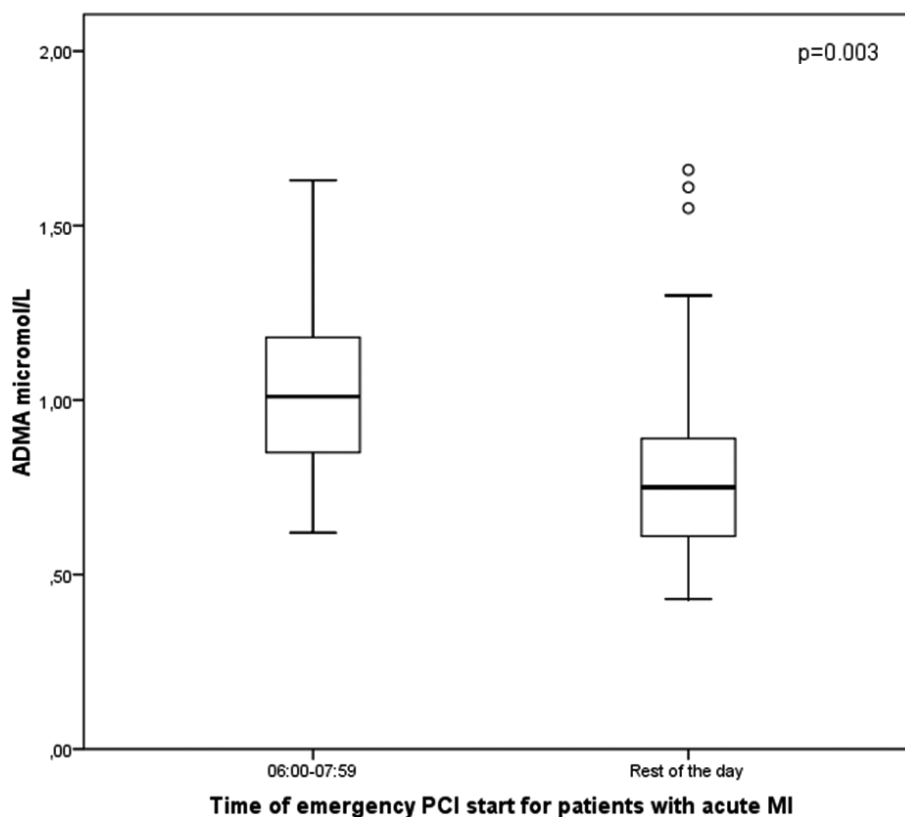


Fig. 2. Box and whisker plot showing median values, interquartile ranges (box), and extreme values (whiskers) of plasma ADMA levels among all 120 patients according to the 06:00 a.m.–07:59 a.m versus the rest of day intervals of emergency PCI. The time of emergency PCI start is the time of blood drawing.

In this 2-hour peak interval, the median (interquartile range) ADMA level was 1.01 (0.84–1.21)  $\mu\text{mol/L}$  for the  $n = 9$  patients. In contrast, the median (interquartile range) ADMA level was 0.75 (0.61–0.89)  $\mu\text{mol/L}$  for the remaining 111 patients admitted throughout the rest of the 24-hour interval ( $p = 0.003$  for comparison) (Fig. 2). In order to relate the ADMA peak levels to the acute myocardial infarction occurrence peak we analyzed the frequency of symptoms onset on basis of 2-hour intervals (Fig. 3). The Figs 1 and 3 show that the ADMA peak levels (between 06:00–07:59 h) precede the acute myocardial infarction peak of symptoms onset (between 10:00–11:59 h) suggesting the possibility of a causal relation.

The amplitude (95% CI) of the circadian variation of ADMA levels was 0.08  $\mu\text{mol/L}$  (0.004–0.16) with  $p = 0.042$  for statistic model significance.

#### 4. Discussion

In our cohort of acute myocardial infarction patients we found: 1) a clear morning peak for the onset of

symptoms, emergency PCI and ADMA levels between 06:00 h and 11:59 h; 2) absolute peak ADMA levels between 06:00 h and 07:59 h, significantly higher than the measurements during the remainder of the day, and 3) a significant amplitude of 0.08  $\mu\text{mol/L}$  for daily ADMA variation.

ADMA is an endogenous analogue of L-arginine that competitively inhibits NO synthase in all human cells including endothelium [15] and platelets [14]. It decreases, as a result, the NO production of cells such as endothelial cells and platelets. Since NO normally inhibits platelet aggregation and adhesion [14,16], any decrease in NO levels may trigger thrombus formation and development on a ruptured plaque. It is documented that NO generated from activated platelets inhibits further platelet recruitment to the developing thrombus [17]. This may explain why platelets from patients with acute myocardial infarction and unstable angina produce significantly less NO than those from patients with stable angina [25].

In the light of our present findings, we may hypothesize that elevated ADMA levels increase platelet

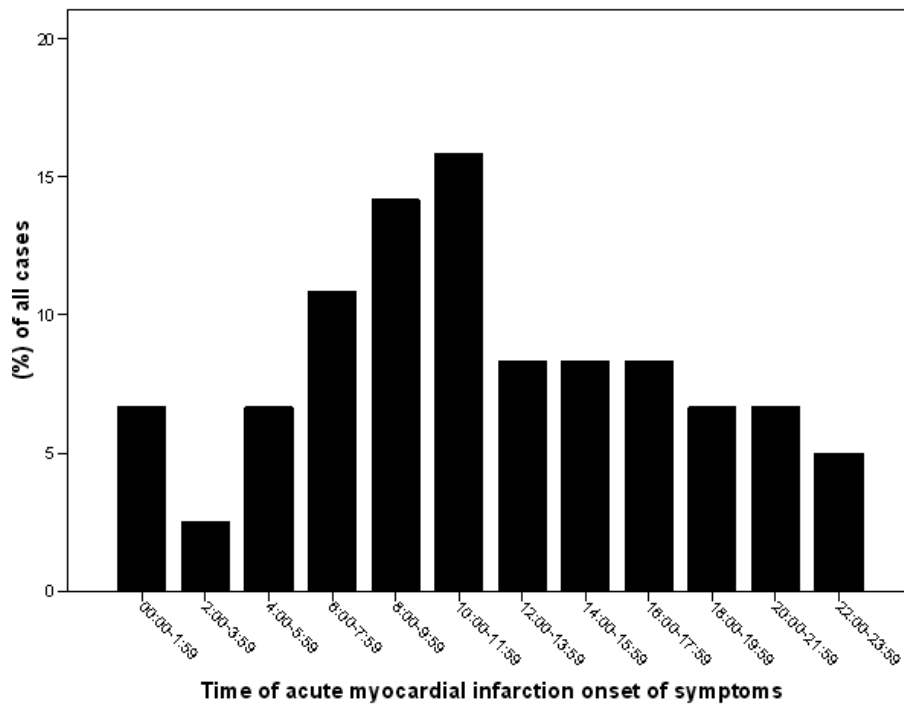


Fig. 3. The percentage frequency of myocardial infarction onset of symptoms among all 120 patients is displayed according to 12 two-hour intervals starting at midnight.

activity via suppressed NO bioavailability. This may contribute to the morning excess of myocardial infarction. In fact, most investigators report an increased platelet adhesiveness and activation during the morning hours [9,26–29] but the exact mechanism of morning platelet over-activation is still under debate.

Besides platelet activation, high ADMA levels are also associated with elevated blood pressure, vasoconstriction and impaired relaxation [19,30,31], mechanisms that may well be incriminated in the morning occurrence of myocardial infarction [6–11].

The remaining question is what determines ADMA levels 24-hour variation with a morning peak. One study showed a direct relation between renal function and ADMA levels [32]. However, 2 studies that investigated the circadian rhythm in creatinine in different populations and in different age groups [33,34] found a creatinine peak in the late afternoon or early evening, between 18:00 h and 19:00 h. It is thus out of phase with our reported rhythm in ADMA.

ADMA production-degradation pathway may play an important role. ADMA is synthesized through the methylation of protein arginine residues by protein arginine methyltransferase (PRMTs) [30,35,36]. In endothelial cells, the expression of PRMTs is upregulated

in the presence of native or oxidized LDL [37] but no evidence exists at this level that might explain a circadian variation. The clearance of ADMA from plasma occurs in both the liver and the kidneys, that show a high net uptake of ADMA [38]. For the degradation of ADMA the enzyme dimethylarginine dimethylaminohydrolase (DDAH) is primarily responsible [39]. In mice overexpressing human DDAH, plasma ADMA levels were reduced by about 50% and associated with approximately 2-fold increases in NO synthase activity in heart and aortic wall compared to wild-type mice [40]. Although DDAH activity is extremely sensitive to factors as oxidative stress and inflammation [41] there is no evidence for a daily variation in DDAH activity that may explain an ADMA morning peak. A possible existence of polymorphisms in the ADMA synthesis-degradation pathways that may determine daily variation via BMAL1–CLOCK (heterodimer that drives and maintains circadian oscillations) [42].

Our study was not designed to determine whether ADMA displays a circadian rhythm in individual patients or healthy subjects. Instead, the purpose of the present manuscript was to report observed differences in ADMA levels during the 24-hour period (with a peak during morning hours) in a cohort of consecutive acute myocardial infarction patients.

The association between the ADMA levels morning peak that precedes the acute myocardial infarction incidence morning peak is hypothesis-generating and should not be interpreted as a direct causal relation. Besides the ADMA-related changes in platelet activity and cardiovascular parameters, other important circadian rhythms with morning peaks have been described. Various coagulation factors and endogenous inhibitors of fibrinolysis are especially active during morning hours [28,43–45] and even a higher susceptibility for plaque rupture was demonstrated by means of intravascular ultrasonography [46]. The robustness of our findings is limited by the low number of patients within the two-hour ADMA morning peak. However, we have observed a clear morning peak in acute myocardial incidence which suggests that our group follows the trend reported in previous studies. To our knowledge, this is the first study to document a 24-hour variation of ADMA levels with a morning peak. Investigators may need to take into account the 24-hour ADMA levels variation when collecting blood and performing ADMA determinations.

Future studies, especially designed, may demonstrate a causal relation between higher morning ADMA levels and the morning increase in myocardial infarction incidence.

In conclusion, ADMA levels display a 24-hour variation with a significant morning peak in patients with acute myocardial infarction. These findings may relate ADMA levels to the acute onset of thrombotic cardiovascular events.

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Leiden University Medical Center, Leiden, The Netherlands.

### Conflict of interests

Authors have no disclosure to report in relation to the present work.

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