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Plasma endothelin-1 and adrenomedullin are associated with coronary artery function and cardiovascular outcomes in humans☆,☆☆

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ABSTRACT

Background: Endothelin-1 (ET-1) is a vasoconstrictor associated with cardiovascular disease, whereas adrenomedullin (ADM) is a vasorelaxant with cardioprotective properties. We sought to determine the relationship between plasma ET-1 and ADM with coronary circulatory function and long-term major adverse cardiovascular events (MACE).

Methods: Thirty-two patients undergoing coronary angiography for chest pain were recruited. Baseline plasma ET-1 and ADM levels were measured. The index of microcirculatory resistance (IMR), coronary flow mediated dilatation (cFMD) and coronary flow reserve (CFR) were measured in a non-obstructed coronary artery. Patients were assessed for MACE over a median period of 8.8 years.

Results: Plasma ET-1 levels correlated with IMR ($r = 0.57$; $p < 0.01$) and ADM levels correlated with CFR ($r = 0.50$; $p = 0.04$) and cFMD ($r = 0.62$; $p = 0.01$). After adjustment for age, gender and cardiovascular risk factors, the association between ADM and cFMD ($\beta = 0.79$; $p < 0.01$) and between ET-1 and IMR ($\beta = 5.7$; $p = 0.01$) remained significant. IMR was higher, although not statistically significant, in patients with long-term MACE (17.9 ± 5.3 vs. 13.1 ± 6.0 units; $p = 0.14$). In patients free of MACE, cFMD (9.3 ± 7.6 vs. 2.8 ± 5.0%; $p = 0.01$) and plasma ADM levels (7.6 ± 5.3 vs. 4.0 ± 1.9 pmol/L; $p = 0.07$) were higher.

Conclusions: Plasma ET-1 and ADM were associated with measures of coronary microvascular and coronary conduit vessel function, respectively. Increased cFMD and elevated plasma ADM were associated with a cardioprotective effect.

1. Introduction

The endothelium is a critical mediator of vascular function, participating in numerous physiological processes including barrier function, cellular proliferation, haemostasis, inflammation and regulation of vascular tone. Dysregulation of this system may precipitate vascular endothelial dysfunction, an early finding in the development of cardiovascular disease [1]. Abnormal coronary endothelial function is mediated by the reduced bioavailability and impaired vasodilatory effects of endothelium-derived relaxing factors, such as nitric oxide [2]. One additional mechanism arises from the increased production and corresponding biological activity of the potent vasoconstrictor endothelin-1 (ET-1). ET-1 is an endothelium-derived, 21-amino acid peptide released in response to chemical and mechanical stimuli, and may play an important role in the pathophysiology of atherosclerosis [3]. Elevated plasma levels of ET-1 are associated with multiple cardiovascular risk factors. In the peripheral circulation, ET-1 concentrations are inversely related to brachial artery flow-mediated dilatation (FMD) and correlate positively with carotid intima-media thickness, suggesting a role for ET-1 in peripheral endothelial dysfunction [4,5]. Interestingly, coronary endothelial dysfunction has been shown to precede the development of angiographically-significant atherosclerotic plaques and is associated with increased cardiac events [6].

Adrenomedullin (ADM) is a 52-amino acid peptide hormone, belonging to the calcitonin gene-related peptide family. ADM is predominantly produced by endothelial and smooth muscle cells, functioning as a potent vasodilator that elicits a long-lasting hypotensive effect [7]. ADM has been shown to reduce systemic and pulmonary vascular resistance, induce renal vasodilation and increase glomerular filtration rate [8]. Plasma levels are increased in patients with acute coronary

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syndromes, hypertension, and heart failure [9]. Under hypoxic conditions, the production of ADM from coronary endothelial cells might be enhanced in order to improve coronary blood flow [10]. In concert, these findings suggest a cardioprotective role for ADM through its vasodilatory, anti-hypertrophic and anti-fibrotic effects.

Both ET-1 and ADM are biomarkers linked to peripheral endothelial function and vascular tone, but their influence on human coronary arteries remains underexplored. Furthermore, limited data exists regarding the prognostic ability of these biomarkers to predict future cardiovascular events, particularly in high risk patients referred for coronary angiography. We sought to determine whether plasma ET-1 and ADM are associated with coronary microcirculatory and coronary conduit function. We also evaluated the relationship between these biomarkers and coronary vascular reactivity measures in predicting long-term major adverse cardiovascular events.

2. Methods

The study was approved by the Austin Health Human Research Ethics Committee in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from all patients. Patients were recruited if they had at least two cardiovascular risk factors and required a clinically indicated coronary angiogram for chest pain and/or a positive functional study. Patients who presented with an acute coronary syndrome were excluded. Thirty-two consecutive patients with either angiographically normal coronary arteries, near normal (<20% diameter stenosis) coronary arteries or obstructive coronary artery disease (CAD) in one vessel (>50% stenosis) with another normal or near normal coronary artery were studied.

2.1. Coronary vascular reactivity measures

All coronary studies were performed in the cardiac catheterisation laboratories of Austin Health, Melbourne, Australia. Vasoactive medications were withheld for 48 h before the study and intracoronary nitrates were avoided. A coronary pressure wire (RADI Medical Systems, Uppsala, Sweden) was placed in the mid to distal segment of an angiographically normal or near normal coronary artery to assess the coronary microcirculation by measuring the thermodilution derived coronary flow reserve (CFR) and index of microcirculatory resistance (IMR) [11,12]. Coronary flow mediated dilatation (cFMD) was assessed in the same coronary artery.

IMR and CFR were calculated as previously described [13]. In summary, a 3 mL bolus of room-temperature saline was injected into the study vessel three times at rest. The resting mean transit time (T_{mn}) was recorded and averaged. T_{mn} was defined as the time required for saline to travel from the injection site at the tip of the coronary guide catheter to the distal sensor on the coronary pressure wire. Maximal hyperaemia was achieved using intravenous adenosine 140 μg/kg/min administered via a femoral venous catheter. During hyperaemia, a 3 mL bolus of room temperature saline was again injected into the chosen vessel and the hyperaemic T_{mn} recorded and averaged. The mean aortic and distal coronary pressures were recorded at baseline and during peak hyperaemia (Supplementary Fig. 1). IMR was defined as the distal coronary pressure at maximum hyperaemia multiplied by the hyperaemic T_{mn}. Coronary flow reserve was defined as the resting T_{mn} divided by the hyperaemic T_{mn}.

Coronary FMD was further measured to evaluate the ability of coronary conduit vessels to dilate in response to adenosine [14]. Baseline and adenosine-induced hyperaemic angiographic acquisitions were obtained and stored to allow offline digital quantitative coronary angiography (QCA) utilising automated edge-detection software (Supplementary Fig. 2; QAngio XA v 7.1, MEDIS Medical Imaging Systems, Leiden, Netherlands). Coronary FMD in a proximal coronary segment was calculated as the maximum percentage increase in arterial diameter during reactive hyperaemia compared to the resting baseline diameter.

2.2. Endothelin-1 and adrenomedullin assays

Forty millilitres of venous blood was collected into pre-chilled tubes (EDTA, lithium heparin and no additive) from all fasted participants between 8 am and 10 am on the day of the procedure. Blood samples were immediately placed on ice and centrifuged at 3000 rpm for 10 min at 4 °C. Plasma was collected and stored at −80 °C until extraction. Plasma ET-1 was measured using a previously described radioimmunoassay [15]. The intra-assay coefficient of variation was 7%. Plasma ADM was analysed by radioimmunoassay as previously described [16]. The intra-assay coefficient of variation ranged between 5.7% and 8.2%. Plasma biomarkers were measured independently and without knowledge of clinical parameters by the Christchurch Cardioendocrine Research Group (CCERG, University of Otago, Christchurch, NZ).

2.3. Follow-up

The median follow-up period was 8.8 years with an interquartile range of 7.9 to 9.2 years. Follow-up was completed for 30 of 32 patients. The primary endpoint was major adverse cardiovascular events (MACE) defined as a combination of unstable angina, myocardial infarction (MI), cerebrovascular accident (CVA), congestive cardiac failure (CCF) and all-cause mortality. Clinical follow-up was performed by an investigator (JT) blinded to plasma biomarker concentrations and coronary reactivity measurements. Unstable angina was defined as symptoms suggestive of an acute coronary syndrome and absent elevation in serum troponin, with or without electrocardiographic changes indicative of ischaemia. CVA was defined as ≥24-h loss in neurologic function due to either an ischaemic or hemorrhagic event, confirmed on cerebral imaging. CCF was defined as either radiographic evidence of pulmonary venous congestion, or signs and symptoms of heart failure requiring treatment, or newly documented left ventricular systolic impairment on echocardiogram.

2.4. Statistical analyses

Data was analysed using SPSS version 23 for Windows (SPSS Inc., Chicago, IL, USA). Normality of data was assessed with the Shapiro-Wilk statistic. All continuous variables are expressed as mean ± SD. The association between coronary reactivity measures and plasma vascular biomarkers was assessed using the Pearson correlation coefficient. A multivariate, linear regression analysis was further utilized to determine the independent association between plasma biomarker concentrations and coronary reactivity measures. Variables accounted for in the multivariate model included age, gender, hypertension, diabetes, dyslipidaemia, body mass index, serum glucose and total cholesterol. Plasma vascular biomarkers and coronary reactivity measures, stratified by MACE, were assessed using the unpaired *t*-test and Mann-Whitney *U* test, as appropriate. A p value of ≤0.05 was considered statistically significant.

3. Results

A total of 32 patients requiring a coronary angiogram for chest pain or a positive functional study were enrolled. Demographic and baseline characteristics are shown in Table 1. The cohort was predominantly male (69%) with a mean age of 66 ± 9 years. Dyslipidaemia (91%), hypertension (81%), a family history of ischaemic heart disease (72%) and diabetes mellitus (38%) were the most predominant risk factors. The mean plasma ET-1 and ADM levels were 2.4 ± 0.5 and 5.5 ± 4.0 pmol/L, respectively.

Pearson's correlation coefficient was calculated to elicit a relationship between both plasma ET-1 and ADM with markers of coronary circulatory function (Table 2). Plasma ET-1 was proportional to IMR ($r = 0.57$, $p < 0.01$) and ADM proportional to both CFR ($r = 0.50$, $p = 0.04$) and cFMD ($r = 0.62$, $p = 0.01$). Linear regression analysis further demonstrated, that for every 1 pmol/L increase in ET-1, IMR increased by 7 units (95% CI 2.8, 10.5; $p < 0.01$). Additionally, for each 1 pmol/L increase in ADM, CFR increased by 0.20 units (95% CI 0.01, 0.40; $p = 0.04$) and cFMD increased by 0.92% (95% CI 0.29, 1.55; $p = 0.01$). After adjustment for cardiovascular risk factors, the relationship between ADM and CFR was no longer significant. However, the association

Table 1
Patient demographic and baseline clinical characteristics.

	n = 32
Age (years)	66 ± 9.1
Male	22 (69)
Diabetes mellitus	12 (38)
Hypertension	26 (81)
Smoking	4 (13)
Family history of IHD	23 (72)
Dyslipidaemia	29 (91)
History of coronary artery disease	21 (66)
Systolic blood pressure (mm Hg)	144 ± 16
Body mass index (kg/m^2)	32.8 ± 6.1
Glucose (mmol/L)	6.0 ± 3.0
Creatinine (μmol/L)	74.3 ± 17.3
Total cholesterol (mmol/L)	4.1 ± 0.9
Triglyceride (mmol/L)	1.7 ± 0.8
Plasma endothelin-1 (pmol/L)	2.4 ± 0.5
Plasma adrenomedullin (pmol/L)	5.5 ± 4.0
Coronary flow reserve	3.3 ± 1.5
Index of microcirculatory resistance (units)	15.6 ± 5.9
Coronary flow mediated dilation (%)	5.3 ± 7.5

Data are presented as mean ± SD or number (percentage). IHD = ischaemic heart disease.

Table 2
Pearson's correlation of coronary circulatory markers and plasma biomarkers.

Coronary circulatory marker	Adrenomedullin	Endothelin-1
CFR	$r = 0.50$ (0.04)	$r = -0.05$ (0.78)
IMR	$r = -0.04$ (0.89)	$r = 0.57$ (<0.01)
cFMD	$r = 0.62$ (0.01)	$r = 0.12$ (0.55)

Data are expressed as correlation coefficient r (p value). CFR = coronary flow reserve. IMR = index of microcirculatory resistance. cFMD = coronary flow mediated dilatation.

between ET-1 and IMR ($\beta = 5.7$; 95% CI 1.4, 10.0; $p = 0.01$), and ADM with cFMD ($\beta = 0.79$; 95% CI 0.45, 1.13; $p < 0.01$), remained significant.

A MACE rate of 50% was observed in the cohort (n = 30) during long-term clinical observation, with two patients lost to follow-up. The median time to first MACE was 3.1 years. The details of individual MACE are highlighted in Table 3. The cohort was dichotomised according to MACE, with comparative analyses performed between groups with respect to biomarker concentration and coronary vascular reactivity measurements (Table 4). Patients without MACE, had a higher mean cFMD (9.3 ± 7.6 vs. 2.8 ± 5.0%; $p = 0.01$), along with a trend towards increased plasma ADM levels (7.6 ± 5.3 vs. 4.0 ± 1.9 pmol/L; $p = 0.07$). Patient with MACE, had a trend towards increased IMR (17.9 ± 5.3 vs. 13.1 ± 6.0 units; $p = 0.14$). No significant relationship was demonstrated amongst MACE subgroups for either plasma ET-1 concentration or CFR (Table 4).

4. Discussion

Two important conclusions can be drawn from this study regarding the relationship between plasma vascular biomarkers, coronary circulatory function and long-term major adverse cardiovascular events. Firstly, plasma ET-1 and ADM function as in vivo mediators of coronary vascular tone and exert their vascular effects via differential regulation of microvascular and conduit vessels. Elevated plasma levels of ET-1 correlated with increased coronary microcirculatory resistance, whereas increased ADM was associated with increased coronary flow mediated dilatation (cFMD). Secondly, elevated IMR was associated with increased long-term MACE, whereas elevated plasma ADM levels and cFMD were associated with reduced MACE.

Endothelin-1 induces coronary vasoconstriction via stimulation of ET_A receptors and activation of protein kinase C-alpha signalling pathways [17]. It has been further demonstrated that coronary artery vasodilatation by ET_A receptor antagonism is enhanced in atherosclerotic vessels in comparison to normal coronary arteries [18]. Our study demonstrated a positive correlation between plasma ET-1 levels and IMR, suggesting that coronary microcirculatory resistance is modulated by elevated levels of ET-1. These observations are supported by previous studies, which concluded that both short- and medium-term blockade of ET_A receptors promotes coronary vasodilation and improves coronary microvascular endothelial function [19]. Interestingly, progressive endothelial dysfunction and increased circulating concentrations of ET-1 have been observed in patients with symptomatic atherosclerosis [20]. In patients with non-obstructive coronary artery disease, ET-1 was not a significant regulator of coronary microcirculatory tone [19]. This discordance likely reflects the relative absence of significant coronary atherosclerosis and reduced cardiac risk factors in other studied cohorts. We further documented no significant correlation between plasma ET-1 levels and CFR, strengthening the assertion that ET-1 predominantly regulates microvascular tone. Previous clinical trials have observed a reduction in coronary CFR in the presence of an ET_A receptor antagonist, however these studies involved acetylcholine administration at the time of CFR calculation [19].

Table 3
Major adverse cardiovascular events throughout the study.

Event	Number of events
Unstable angina	8 (36.3)
MI	8 (36.3)
CCF	4 (18.2)
CVA	1 (4.5)
All-cause mortality	1 (4.5)
Total events	22

Data are presented as number (percentage). MI = myocardial infarction. CCF = congestive cardiac failure. CVA = cerebrovascular accident.

Table 4
Plasma biomarker concentrations and coronary reactivity measurements in patients with and without major cardiovascular events.

	MACE (n = 15)	No MACE (n = 15)	p-Value
Age (years)	64.3 ± 10.2	67.2 ± 8.3	0.41
Male	12 (80)	9 (60)	0.23
Diabetes mellitus	6 (40)	4 (27)	0.52
Hypertension	14 (93)	9 (60)	0.03
Smoking	2 (13)	2 (13)	0.94
Family history of IHD	9 (60)	12 (80)	0.20
Dyslipidaemia	13 (87)	12 (80)	0.96
Systolic blood pressure (mm Hg)	151 ± 15	138 ± 16	0.04
Body mass index (kg/m^2)	33.0 ± 6.7	33.0 ± 5.9	0.94
Glucose (mmol/L)	6.4 ± 2.5	5.6 ± 0.6	0.88
Creatinine (μmol/L)	79.2 ± 20.2	69.1 ± 14.5	0.15
Total cholesterol (mmol/L)	3.99 ± 0.8	3.67 ± 0.9	0.33
Triglyceride (mmol/L)	1.4 ± 1.0	0.7 ± 0.4	0.04
Plasma ET-1 (pmol/L)	2.4 ± 0.6	2.3 ± 0.4	0.61
Plasma ADM (pmol/L)	4.0 ± 1.9	7.6 ± 5.3	0.07
IMR (units)	17.9 ± 5.3	13.1 ± 6.0	0.14
CFR	15.73 ± 1.6	15.27 ± 2.2	0.88
cFMD (%)	2.8 ± 5.0	9.3 ± 7.6	0.01

Data are presented as mean ± SD or number (percentage). ET-1 = endothelin-1. ADM = adrenomedullin. IMR = index of microcirculatory resistance. CFR = coronary flow reserve. cFMD = coronary flow mediated dilatation. MACE = major adverse cardiovascular events.

In our subjects who experienced a major adverse cardiovascular event during 8.8 years of follow-up, we observed a trend towards increased IMR, with no appreciable elevation in baseline ET-1 level. These data coincide with prior reports that IMR serves as a marker of long-term clinical outcomes and prognosis in patients with coronary artery disease [21]. Although previous work has correlated elevated ET-1 levels with shorter time to incident heart failure, myocardial infarction and all-cause mortality, ET-1 measurements were not predictive of either the presence or severity of coronary artery disease [22]. Our lack of correlation between ET-1 and long-term MACE are most reasonably explained by the acute rise and fall of ET-1 levels with disease acuity [23]. Whilst plasma ET-1 predicts acute manifestations of disease and functions as a surrogate for microvascular endothelial dysfunction, its ability to predict subclinical but progressive coronary artery disease remains limited [22,23].

ADM is a potent peptide hormone with vasodilatory, natriuretic and hypotensive effects, predominantly mediated by nitric oxide and cyclic adenosine monophosphate [24]. Infusion of ADM directly into the coronary circulation produces a vasodilatory response, enhancing coronary blood flow via increased nitric oxide production [25]. We observed a positive association between plasma ADM levels and cFMD, but no association with IMR. These findings support an ADM-mediated vasodilatory effect by regulation of coronary macrovascular structures. In addition to increased nitric oxide production, this relationship may be regulated by activation of potassium channels and adenosine receptors, resulting in increased coronary flow [26]. Indeed, Ueda and colleagues [25] demonstrated ADM-induced coronary vasodilation at the level of both conduit and resistance vessels. In contrast, our study demonstrated a statistically significant increase in CFR in response to elevated ADM concentrations, however following adjustment for cardiac risk factors, the relationship was no longer significant. These apparent discrepancies are likely explained by the subjects in the present study possessing a minor degree of coronary artery disease in the studied vessel in comparison to Ueda's cohort with angiographically normal vessels [25]. Additionally, CFR accounts for both microvascular and epicardial resistance and therefore, only reflects microvascular function in the absence of epicardial disease.

Given the high-risk nature of our cohort with a tendency towards mild concomitant coronary artery disease, CFR measurements may have been negatively influenced.

Our data further advocates a cardioprotective role for plasma ADM, with an observed trend towards increased plasma levels in patients free from long-term MACE (7.6 ± 5.3 vs. 4.0 ± 1.9 pmol/L; $p = 0.07$). Mechanistically, we observed a significant elevation in cFMD in this same cohort (9.3 ± 7.6 vs. 2.8 ± 5.0%; $p = 0.01$), suggesting that at least in part, ADM may facilitate a protective vasodilatory effect on the coronary circulation. Previously, Nagaya and colleagues [27] have shown that intravenous ADM increases coronary sinus blood flow, enhances left ventricular myocardial contraction and improves left ventricular relaxation in patients with a prior myocardial infarction. In our study, plasma ADM was measured at the time of initial cardiac catheterisation, not at the time of MACE. Correlation between these timepoints and ADM levels would be helpful in affirming a direct causal effect. However, the vasodilatory effects of ADM have been shown to be short-lived and accordingly, it is conceivable that patients with MACE possessed lower baseline plasma levels, as observed in our cohort [25].

4.1. Clinical implications

Reriani and colleagues [19] demonstrated improved coronary blood flow in patients with non-obstructive CAD treated with 6 months of the ET_A receptor antagonist, atrasentan. Furthermore, coronary artery diameter was similar between the atrasentan and placebo groups, suggesting ET-1 participates in microvascular endothelial dysfunction and early atherosclerosis. In patients 24 h following primary percutaneous coronary intervention for an acute ST-elevation myocardial infarction (STEMI), CFR was lower in the cohort with reduced left ventricular ejection fraction (EF) compared to those with preserved EF. Interestingly, circulating endothelin levels were significantly higher in the low EF group at 6 and 24 h post-percutaneous coronary intervention. These observations highlight the potential importance of ET-1 as a biomarker for microvascular dysfunction and suggest that prolonged release may be associated with adverse outcomes post-STEMI [23]. Overall, our study strengthens the assertion that ET-1 may serve as a non-invasive marker and therapeutic target for microvascular endothelial dysfunction and coronary artery disease prevention.

Recent work has demonstrated that ADM is elevated in chronic heart failure, with levels proportional to NYHA class and degree of left ventricular impairment [28]. A recent systematic review further established ADM as an independent predictor of death in patients with heart failure and of MACE and death following a myocardial infarction [29]. Of note, the recently introduced neprilysin inhibitors function by attenuating neprilysin, a metallopeptidase that catalyses the degradation of vasodilator peptides including the natriuretic peptides, bradykinin and ADM. In animal models, neprilysin inhibition potentiated ADM-induced natriuresis and diuresis and enhanced the vasodilator response to intravenous ADM [30,31]. Following administration of heart failure therapy, ADM levels were slower to decline than both atrial natriuretic peptide and brain natriuretic peptide [32]. In acute myocardial infarction, plasma ADM increases at onset and declines over a 3 week period to a level above the population baseline [33]. Furthermore, direct infusion of ADM into the coronary circulation in angiographically normal arteries causes dilatation at both the level of conduit and resistance vessels [25]. However, whether infusion of ADM in patients with an acute myocardial infarction reduces the ischaemic penumbra remains unknown. In concert, these findings support the utility of ADM as a useful biomarker and prognostic indicator in heart failure and as a potential therapeutic intervention in acute coronary syndromes.

Our study has several limitations that warrant discussion. First and most importantly, a small cohort size may have impacted several results and thus our observations are hypothesis generating rather than conclusive. Within the cohort, a degree of heterogeneity likely existed with respect to the presence of microvascular and epicardial coronary artery disease. Given the differential effects of ET-1 and ADM on endothelial function, it is possible that such heterogeneity affected coronary vascular reactivity measures. Secondly, patients were enrolled based on the clinical requirement for a coronary angiogram. Accordingly, these results cannot be extrapolated to lower risk patients and therefore, caution must be exercised when interpreting the relevance of these biomarkers and vascular reactivity measurements in the general population. Thirdly, plasma biomarker concentrations were deduced from peripheral venous blood samples, not the coronary circulation. Accordingly, the direct source of these biomarkers cannot be elicited and it is therefore unclear if their secretion and effect reflects a localised or more global physiologic process. Fourthly, although acetylcholine is an established endothelium-dependent vasodilator [2], its effect on coronary vascular reactivity was not assessed in our study. Finally, we cannot account for any disease states or therapies within the cohort that may have influenced baseline plasma levels of ET-1 or ADM. However, our cohort was relatively uniform, making it unlikely that confounding variables significantly skewed the data.

5. Conclusion

The present study confirms that ET-1 is associated with increased coronary microvascular resistance and shares a proportional relationship with IMR. Correspondingly, elevated IMR was found to be associated with increased long-term major adverse cardiovascular events. In contrast, elevated ADM correlated with increased cFMD, suggesting a regulatory mechanism for coronary conduit vessel function. Together, increased plasma ADM levels and elevated cFMD were found to be protective against long-term major adverse cardiovascular events. Overall, our results yield important information regarding the regulation of coronary circulatory function and highlight multiple potential therapeutic targets in the management of coronary artery disease.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2019.04.008.

Conflicts of interest

None to report.

Author contributions

AA and OF conceived the presented idea and were responsible for the study design. AA, OF and DC performed invasive coronary angiograms and coronary vascular reactivity measurements. LB and SP contributed to sample preparation. Data analysis and interpretation was carried out by JT, AA and OF. Clinical follow-up was conducted by JT and SV. The manuscript was prepared by JT and editorial support was provided by all authors. All authors discussed the results and provided critical feedback to help shape the final manuscript.

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