Biomarkers, 2011; 16(3): 281–287

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.3109/1354750X.2011.553750

informa
healthcare

RESEARCH ARTICLE

Influence of confounding factors on plasma Mid-Regional pro-Adrenomedullin and Mid-Regional pro-A-type Natriuretic Peptide concentrations in healthy individuals

Sanjay S. Bhandari[1], J.E. Davies[1], J. Struck[2], and L.L. Ng[1]

[1]*Department of Cardiovascular Sciences, University of Leicester and NIHR Cardiovascular Biomedical Research Unit, Leicester, UK and* [2]*B.R.A.H.M.S. AG, Hennigsdorf, Berlin, Germany*

ABSTRACT

Background: MRproADM and MRproANP can be used as diagnostic and prognostic markers in heart failure.

Aim: The objective was to identify confounding factors for the interpretation of plasma MRproADM and MRproANP concentrations.

Methods: A total of 518 healthy volunteers with a mean age of 60.84 ± 7.41 years were analyzed. We evaluated the influence of demographic factors, renal function and echocardiographic indices on the candidate peptides.

Results: Multivariate analysis revealed that age ($P < 0.001$), BMI ($P < 0.001$) and eGFR ($P < 0.001$) were independent predictors for MRproADM concentrations in healthy subjects. The independent predictors for MRproANP were age ($P < 0.001$), female gender ($P < 0.001$), heart rate ($P < 0.001$) and eGFR ($P = 0.039$).

Conclusion: The interpretation of both peptides is multifaceted due to confounders. Knowledge of these factors will further our understanding of how these peptides behave in health and in disease.

Keywords: Mid-regional pro-adrenomedullin, mid-regional pro-A-type natriuretic peptide, heart failure, body mass index, renal function

Introduction

The application of cardiac biomarkers is widespread, ranging from the diagnosis of myocardial infarction (MI) with the gold standard cardiac troponins (Thygesen et al., 2007) to the diagnosis of acute heart failure (HF) with the natriuretic peptides (Maisel et al., 2002), and their use in prognostication in an array of disease processes (Panteghini, 2006; Panteghini, 2010; Dolci and Panteghini, 2006). New peptides are being identified to complement existing tools in improving the diagnostic and prognostic accuracy of cardiovascular disease. Among these are novel peptides such as mid-regional pro-adrenomedullin (MRproADM) and mid-regional pro-A-type natriuretic peptide (MRproANP), which have been shown to be comparable to B-type natriuretic peptide (BNP) in the prognosis of HF (Gegenhuber et al., 2007) and in the diagnosis of acute HF (Maisel et al., 2010).

Adrenomedullin (ADM) is a 52-amino-acid peptide, which was first discovered from human pheochromocytoma (Kitamura et al., 1993a) and has later been detected in a variety of tissues such as brain, lungs, heart, kidneys, and gastrointestinal organs (Ichiki et al., 1994; Kitamura et al., 1993b; Washimine et al., 1995). It is expressed in endothelial cells, vascular smooth muscle cells, fibroblasts and adipocytes, mediating vasorelaxation, natriuresis, diuresis and increasing cardiac output (Ishimitsu et al., 1998; Nakamura et al., 1997; Sugo et al., 1994). It is derived from a precursor preproadrenomedullin (preproADM), consisting of a 185-amino-acid chain (Kitamura et al., 1993b). Concerns exist regarding the accuracy and reliability of ADM quantification, largely in part due to its short half-life of 22 min and its partial binding to complement factor H (Meeran et al., 1997). In

Address for Correspondence: Dr. Sanjay S. Bhandari, Department of Cardiovascular Sciences, Pharmacology and Therapeutics Group, University of Leicester, Robert Kilpatrick Clinical Sciences Building, Leicester Royal Infirmary, Leicester LE2 7LX, Tel.: (0116) 2523125; Fax: (0116) 2423108, E-mail: sanjay1bhandari@hotmail.com

(Received 14 November 2010; revised 07 January 2011; accepted 08 January 2011)

Abbreviations:

MRproADM, mid-regional pro-adrenomedullin
MRproANP, mid-regional pro-A-type natriuretic peptide
NTproBNP, N-terminal pro-B-type natriuretic peptide
BNP, B-type natriuretic peptide
HF, heart failure
AMI, acute myocardial infarction
NYHA, New York heart association classification
BMI, body mass index
eGFR, estimated glomerular filtration rate
BP, blood pressure
ANP, atrial natriuretic peptide
MDRD, modification of diet in renal disease
DT, deceleration time
IVRT, isovolumetric relaxation time
LV, left ventricular
LVEF, LV ejection fraction
LVH, Left ventricular hypertrophy
LVWMI, LV wall motion index
EDTA, ethylenediaminetetraacetic acid
MAE, methyl-acridinium ester.

comparison to ADM, the mid-regional portion of proADM (45–92 amino acid of preproADM) is more stable. The stoichiometric generation of MRproADM allows it to be used as a surrogate marker for the ADM system.

The BACH study showed that MRproADM was prognostically more accurate than BNP or N-terminal pro-BNP (NTproBNP) at predicting 90-day mortality in patients presenting to hospital with acute shortness of breath and HF. The investigators suggested that MRproADM should be adopted in preference to NTproBNP or BNP in risk-stratifying patients with acute HF (Maisel et al., 2010).

MRproANP is strongly related with prognosis in patients with chronic HF, independent of parameters such as left ventricular ejection fraction (LVEF), New York Heart Association (NYHA) classification, age, gender, plasma creatinine and NTproBNP (von Haehling et al., 2007). Both MRproADM and MRproANP have also shown promise as prognostic biomarkers after acute myocardial infarction (AMI), independent of established risk-stratifying markers such as NTproBNP (Khan et al., 2007; Khan et al., 2008).

ANP is a member of the natriuretic peptide family, possessing natriuretic, diuretic and vasodilatory actions (Vesely, 2001). ANP is secreted from the myocardium, predominantly from the atria, in response to wall stretch (Ruskoaho, 1992). The bioactive ANP is derived from the enzymatic cleavage of its precursor proANP. The N-terminal and C-terminal epiptopes of the proANP are prone to further degradation in circulation (Ala-Kopsala et al., 2004). MRproANP represents the 53–90 amino acid fragment of the N-terminal of proANP. This portion is biochemically inactive and more stable than other precursor fragments, which has led to its adoption as a surrogate marker for the ANP system (Ala-Kopsala et al., 2004).

With the introduction of MRproADM and MRproANP as potential diagnostic and prognostic factors in HF and acute coronary syndromes (ACS), there is a need to understand variables, which may influence their concentrations in healthy individuals, in order to derive a working reference range for diagnosis or prognosis of heart disease. The aims of this population-based study was to identify these confounding factors for the interpretation of plasma MRproADM and MRproANP concentrations, which may lead to improved utility of these markers.

Materials and methods

Study population

A total of 518 healthy volunteers were derived from a screening study performed in the local community. A set of study definitions were used. Information regarding history of ischemic heart disease (myocardial infarction or angina), hypertension, diabetes mellitus, smoking and cardiovascular medications were collected from patient's records. This study complied with the Declaration of Helsinki and was approved by the local ethics committee. All healthy volunteers gave written informed consent for physical examination, echocardiography and phlebotomy. Participants with a history of ischemic heart disease, hypertension, diabetes mellitus, those with ECG or echocardiographic abnormalities [including segmental wall motion abnormalities, valvular disease, left ventricular hypertrophy (LVH)] and those on cardiovascular medications were excluded from the present study.

Blood sampling

Phlebotomy was performed in volunteers. Samples for measuring the plasma concentrations of the peptides were collected in prechilled tubes containing ethylenediaminetetraacetic acid (EDTA) and aprotinin. Plasma was stored at–70 °C until assay and all analyses were done in a single batch.

Creatinine assay

The estimated glomerular filtration rate (eGFR) of these subjects was derived using the modification of diet in renal disease (MDRD) formula (Stevens et al., 2007). Concentrations of creatinine were determined by the compensated kinetic Jaffe method (Siemens Advia 2400, USA). The creatinine method is traceable to a High Pressure Liquid Chromatography (HPLC) candidate reference method, which uses reference materials from the National Institute of Standards and Technology (NIST), via patient sample correlation.

MRproADM assay

A novel sandwich immunoluminometric assay was used to determine MRproADM concentrations (Morgenthaler et al., 2005). Tubes were coated with sheep polyclonal antisera directed against amino acid

sequence 83–94 of preproADM as the capture antibody. Sheep antibody raised against the amino acid sequence 68–86 of preproADM was used as a tracer labeled with methyl-acridinium ester (MAE). Dilution of peptide representing 45–92 of preproADM in horse serum was used as calibrators. The immunoassay was conducted by incubating 10 µL of sample/standard and 200 µL of tracer in the coated tubes for 2 h at room temperature. Test tubes were washed four times with 1 ml of wash solution, and bound chemiluminescence was measured on a LB952T luminometer (Berthold, Germany). The limit of detection was 0.08 nmol/L and the inter-assay coefficient of variation was <20% for values >0.12 nmol/L. The intra-assay coefficient of variation was <8% for samples containing the range 0.08–14.7 nmol/L (Morgenthaler et al., 2005).

MRproANP assay

MRproANP concentrations were determined by a novel sandwich immunoluminometric assay, which has been reported previously (Morgenthaler et al., 2004). Tubes were coated with sheep capture antibody (polyclonal antisera directed against amino acid sequence 73–90 of proANP). Sheep antibody raised against the amino acid sequence 53–72 of proANP was used as a tracer. The immunoassay was conducted by incubating 50 µL of sample/standard and 200 µL of tracer in the coated tubes for 30 min at room temperature. Test tubes were washed three times with 1 ml of wash solution, and bound chemiluminescence was measured on a LB952T luminometer (Berthold, Germany). The limit of detection was 6.0 pmol/L, and the intra-assay coefficient of variation was <10% for samples containing 23–3000 pmol/L. The inter-assay coefficient of variation was 10% for a concentration of 65 pmol/L (Morgenthaler et al., 2004).

Echocardiography

Transthoracic echocardiography was performed on volunteers using a Sonos 5500 instrument (Philips Medical Systems, Reigate, Surrey, UK). A 16-segment left ventricular wall motion index (LVWMI) based on the American Society of Echocardiography model was derived by scoring each LV segment (1 = normal, 2 = hypokinesis, 3 = akinesis) and dividing the total by the number of segments scored. LVEF was calculated using the biplane method of discs formula (Schiller et al., 1989). All the healthy volunteers in this study had a LVWMI = 1 (i.e., no segmental wall motion abnormalities), and no evidence of valvular disease or LVH. Left ventricular (LV) mass was calculated using the Devereux et al. (1993) formula and indexed for body surface area to obtain LV mass index. LVH is diagnosed when the LV mass index is greater than 134 g/m^2, 110 g/m^2 in males and females, respectively (World Health Organization, 1999).

The transmitral peak flow during early (E) and the atrial (A) filling phase was determined using pulsed wave Doppler examination at the tips of the mitral valve leaflets. The E/A ratio, left ventricular isovolumetric relaxation time (IVRT) and deceleration time (DT) were calculated from these traces.

Statistical analysis

Statistical analysis was performed using Statistics Package for Social Sciences version 16.0 (SPSS Inc, Chicago, IL). Variables that did not follow a Gaussian distribution were log transformed prior to statistical analysis. Concentrations of MRproADM, MRproANP and plasma creatinine had a non-Gaussian distribution and were log transformed. For continuous variables in two independent groups, the Student's *t*-test was used. Pearson's correlation coefficients were used to investigate the influence of patient characteristics on log MRproADM and log MRproANP concentrations in univariate analyses. To analyze the interaction of multiple independent variables on MRproADM and MRproANP concentrations, linear regression was used. A *P* value below 0.05 was deemed to be statistically significant.

Results

The clinical and demographic features together with the plasma biomarker concentrations and echocardiographic parameters are presented in Table 1. The mean age of the study population was 60.84 ± 7.41 years. The cohort consisted of 58% of males ($n = 300$).

Univariate and multivariate analyses for MRproADM

Median plasma MRproADM concentrations were significantly higher in females compared to males (0.48 [0.30–0.92] vs. 0.45 nmol/L [0.27–1.06]; $P = 0.001$, respectively). In univariate analysis MRproADM was significantly positively correlated with older age ($r_s = 0.400$;

Table 1. Baseline characteristics of the 518 healthy subjects.

Clinical characteristics	Sample population ($n = 518$)
Age (y), mean (SD)	60.84 (7.41)
eGFR (ml min^{-1} 1.73 m^2 surface area), median (range)	75 [40–111]
BMI (kg/m^2), mean (SD)	25.74 (3.78)
Heart rate (min^{-1}), mean (SD)	71.56 (11.42)
Systolic BP (mmHg), mean (SD)	123.51 (12.73)
Diastolic BP (mmHg), mean (SD)	73.0 (9.74)
Biochemical assays	
Plasma Creatinine (µmol/L), median [range]	85.0 [58.0–156.0]
Plasma MRproADM (nmol/L), median [range]	0.46 [0.27–1.06]
Plasma MRproANP (pmol/L), Median [range]	49.80 [4.08–173.00]
Echocardiographic characteristics	
Left Atrium (cm), mean (SD)	3.26 (0.50)
E/A ratio, mean (SD)	0.89 (0.21)
DT (ms), mean (SD)	232.35 (51.87)
IVRT (ms), mean (SD)	110.85 (43.12)
LV mass (g), mean (SD)	145.79 (44.73)
LV mass index (g/m^2), mean (SD)	78.98 (19.75)
LVEF (%), mean (SD)	61.97 (5.07)

$P<0.001$). (Figure 1A) MRproADM was significantly correlated with increasing BMI ($r_s=0.205$; $P<0.001$) and systolic BP ($r_s=0.139$; $P=0.002$) (Table 2). An inverse relationship was observed between MRproADM and eGFR ($r_s=-0.338$; $P<0.001$). (Figure 2A) E/A ratio as an index of diastolic dysfunction was negatively associated with plasma MRproADM concentrations, ($r_s=-0.182$; $P<0.001$).

Significant clinical and echocardiographic variables in univariate analyses were used as covariates in multivariate analysis. Multivariate analysis revealed that age ($P<0.001$), BMI ($P<0.001$) and eGFR ($P<0.001$) were independent predictors of plasma MRproADM concentrations in healthy subjects (Table 3).

Univariate and multivariate analyses for MRproANP

Median plasma MRproANP concentrations were significantly elevated in the female cohort compared to the males (56.10 [24.00–173.00] vs. 46.00 pmol/L [4.08–136.00]; $P<0.001$, respectively). Plasma MRproANP concentrations were positively correlated with age ($r_s=0.392$; $P<0.001$). (Figure 1B) An inverse relationship was observed between MRproANP concentrations and heart rate ($r_s=-0.119$; $P=0.007$) and eGFR ($r_s=-0.244$; $P<0.001$). (Figure 2B) No significant relationships were identified between plasma MRproANP concentrations and the echocardiographic parameters.

In multivariate analysis, the independent predictors of plasma MRproANP concentrations were age ($P<0.001$), female gender ($P<0.001$), heart rate ($P<0.001$) and eGFR ($P=0.039$).

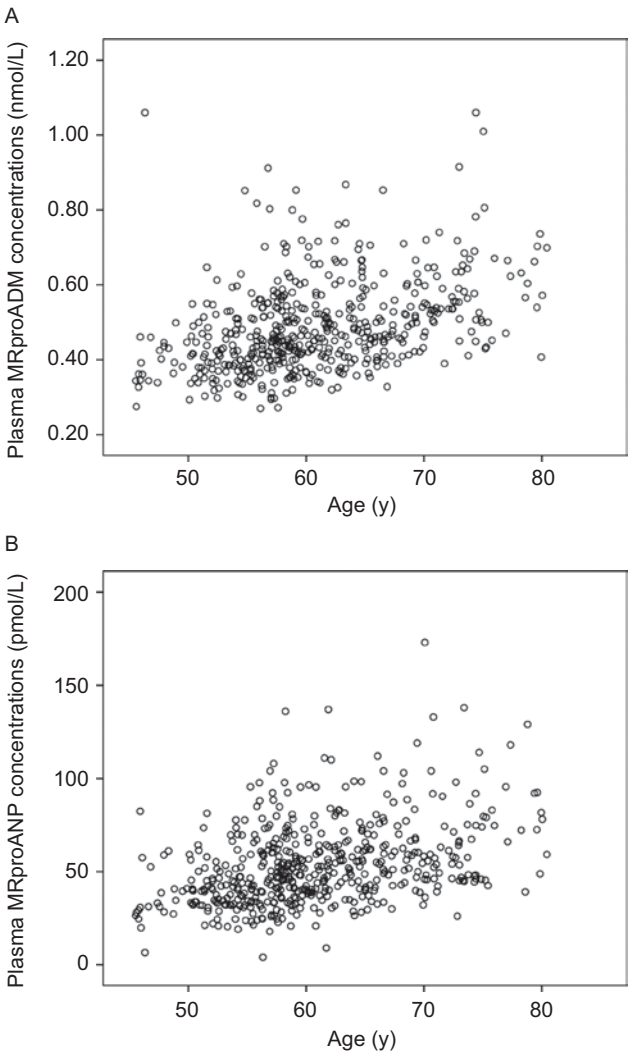

Figure 1. Relationship between plasma MRproADM and MRproANP with age. (A) Relationship between plasma MRproADM and age. $r_s=0.400$, $P<0.001$. (B) Relationship between plasma MRproANP and age. $r_s=0.392$, $P<0.001$.

Discussion

Novel peptides such as MRproADM and MRproANP have been shown to be comparable with the natriuretic peptides in the prognosis of ACS and HF (Gegenhuber et al., 2007; Khan et al., 2007; Khan et al., 2008).

The reference range, and factors that could influence plasma MRproADM and MRproANP in individuals without cardiovascular disease, has not been established. The magnitude of effects of variables, such as age and gender and their potential importance in the interpretation

Table 2. Pearson's correlations between the clinical parameters and the candidate peptides.

Clinical parameters	Log MRproADM	Log MRproANP
Age	$r_s=0.400$	$r_s=0.392$
	$P<0.001$	$P<0.001$
Male Gender	$r_s=-0.119$	$r_s=-0.281$
	$P=0.007$	$P<0.001$
BMI	$r_s=0.205$	$r_s=-0.072$
	$P<0.001$	$P=0.104$
Heart Rate	$r_s=0.080$	$r_s=-0.119$
	$P=0.069$	$P=0.007$
Systolic BP	$r_s=0.139$	$r_s=0.080$
	$P=0.002$	$P=0.074$
Diastolic BP	$r_s=-0.070$	$r_s=-0.040$
	$P=0.121$	$P=0.372$
eGFR	$r_s=-0.338$	$r_s=-0.244$
	$P<0.001$	$P<0.001$
LA size	$r_s=-0.008$	$r_s=0.015$
	$P=0.853$	$P=0.743$
DT	$r_s=0.044$	$r_s=0.022$
	$P=0.338$	$P=0.629$
E/A ratio	$r_s=-0.182$	$r_s=-0.047$
	$P<0.001$	$P=0.293$
IVRT	$r_s=0.033$	$r_s=-0.039$
	$P=0.475$	$P=0.400$
LV Mass	$r_s=-0.024$	$r_s=-0.049$
	$P=0.617$	$P=0.309$
LV mass index	$r_s=-0.055$	$r_s=0.017$
	$P=0.255$	$P=0.729$
LVEF	$r_s=-0.044$	$r_s=0.035$
	$P=0.640$	$P=0.710$

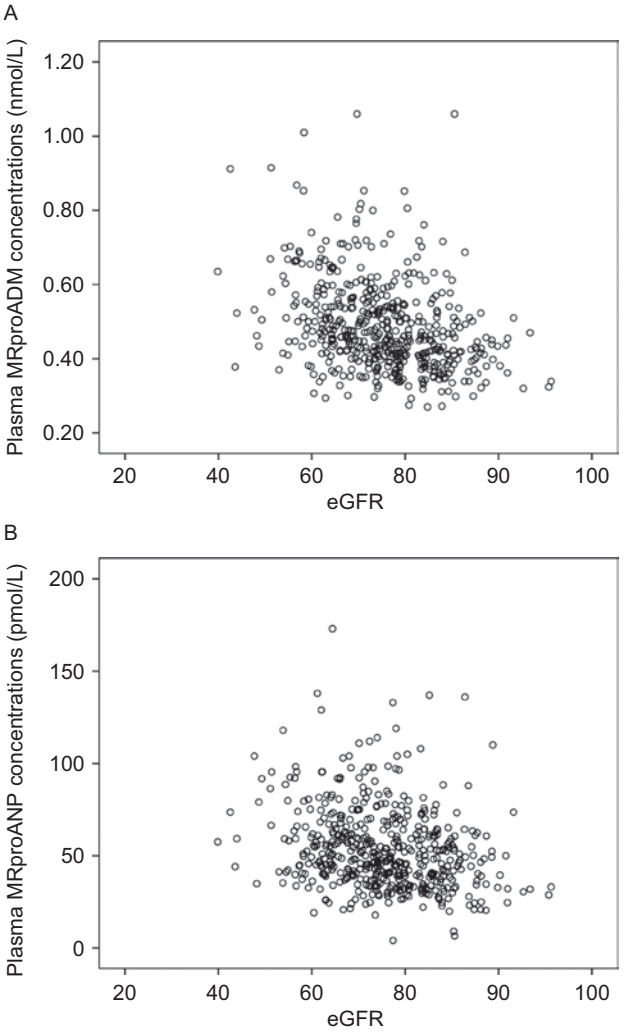

Figure 2. Relationship between plasma MRproADM and MRproANP with eGFR. (A) Relationship between plasma MRproADM and eGFR. $r_s = -0.338$, $P < 0.001$. (B) Relationship between plasma MRproANP and eGFR. $r_s = -0.244$, $P < 0.001$.

Table 3. Multivariate analyses for the prediction of plasma Log MRproADM and Log MRproANP concentrations in healthy subjects.

	Standardized regression coefficients (beta)	Significance
Independent predictors of plasma Log MRproADM		
Age	0.397	$P < 0.001$
Male gender	0.017	$P = 0.686$
BMI	0.233	$P < 0.001$
eGFR	−0.198	$P < 0.001$
SBP	0.021	$P = 0.608$
E/A	0.059	$P = 0.185$
Independent predictors of plasma Log MRproANP		
Age	0.335	$P < 0.001$
Male gender	−0.205	$P < 0.001$
Heart rate	−0.196	$P < 0.001$
eGFR	−0.089	$P = 0.039$

of plasma MRproADM and MRproANP concentrations, remains unclear.

In this study, MRproADM concentrations increased with age and were higher in female subjects without cardiovascular disease and detectable structural heart disease. These findings are mirrored in patients with AMI (Khan et al., 2007). In this present study, age and renal function were independently predictive of plasma MRproADM concentrations in healthy individuals. The increase in plasma concentrations of MRproADM with age is independent of age-related changes in renal function. This relationship cannot be explained by age-related changes in cardiac structure, as the effect of age was independent of left atrial (LA) size, LV mass, E/A ratio and IVRT.

The effect of increasing BMI on plasma MRproADM concentrations was significant. MRproADM concentrations were positively correlated with increasing BMI, which corroborates findings by Nomura et al. (2009). A possible explanation for this finding is that ADM has been shown to be expressed in a number of tissues particularly adipose tissue (Li et al., 2007). ADM may serve to protect against the deleterious effects of increasing BMI and the subsequent development of metabolic syndrome by promoting vasodilatation and insulin sensitivity via paracrine and autocrine actions (Shimosawa et al., 2003). Its effects on insulin resistance and its secretion by adipose tissue may represent a counter regulatory endocrine response to increasing obesity.

In this study, both MRproADM and MRproANP were influenced by eGFR in the healthy individuals. A similar finding was reported in disease, whereby an increase in the plasma concentrations of both peptides, across the GFR stages in nondiabetic patients with chronic kidney disease was observed, hence inferring a correlation between both biomarkers and renal disease severity (Dieplinger et al., 2009). The natriuretic and vasodilator properties of both peptides may have a protective role in the preservation of renal function (Dieplinger et al., 2009). However, other possibilities remain to be investigated, such as the retention of mid-regional peptides in renal impairment if these peptides are primarily eliminated by renal excretion.

Plasma concentrations of MRproANP were significantly related to female gender and increasing age. These findings corroborate findings by Khan et al. (2008) who investigated the prognostic utility of plasma MRproANP concentrations in context of AMI. The trend for elevated concentrations of MRproANP in females is similar to that observed for other natriuretic peptides, inferring a possible relationship with estrogen status (Redfield et al., 2002).

Heart rate was inversely correlated with MRproANP concentrations. Multivariate analyses revealed that heart rate was predictive of plasma MRproANP concentrations in the healthy subjects. This connection may reflect that synthesis and secretion of MRproANP from the atria are dependent upon diastolic duration or filling pressure.

Investigating the interaction between the ADM/ANP system and clinical/echocardiographic variables in a healthy population as opposed to patients with cardiovascular disease furthers our understanding of how these neuro-hormonal systems behave in health. This present study should be used to highlight the possible confounding variables in future studies that examine these biomarkers in the diseased states.

A limitation of the current study is that the study population consisted entirely of White Caucasians. Hence, these findings cannot be extrapolated to other ethnic groups without further studies. Although the relationship of MRproADM and MRproANP with the MDRD formula derived eGFR was documented, a relationship with renal function may necessitate direct measurement of GFR. This study was only conducted in healthy individuals, thus no comparison with diseased states can be made objectively. This was a cross-sectional study, and the remit of this study was not to establish causality between the trends identified. Further studies are required to elucidate the underlying mechanisms for these connections.

Conclusion

This study suggests that MRproADM is influenced by age, BMI and renal function in healthy individuals. In addition, the interpretation of plasma MRproANP concentrations is multifaceted due to potential confounders such as age, female gender, heart rate and renal function. Knowledge of these factors will further our understanding of how these peptides behave in health and in disease.

Declaration of interest

This study was supported by research grants from the Brandenburg Ministry of Economics, Germany, and the European Regional Development Fund (EFRE/ERDF). L.L.N. is supported by the Leicester NIHR Cardiovascular Biomedical Research Unit.

References

Ala-Kopsala M, Magga J, Peuhkurinen K, Leipälä J, Ruskoaho H, Leppäluoto J, Vuolteenaho O. (2004). Molecular heterogeneity has a major impact on the measurement of circulating N-terminal fragments of A- and B-type natriuretic peptides. *Clin Chem* 50:1576–1588.

Devereux RB, Koren MJ, de Simone G, Okin PM, Kligfield P. (1993). Methods for detection of left ventricular hypertrophy: application to hypertensive heart disease. *Eur Heart J* 14 Suppl D:8–15.

Dieplinger B, Mueller T, Kollerits B, Struck J, Ritz E, von Eckardstein A, Haltmayer M, Kronenberg F; MMKD Study Group. (2009). Pro-A-type natriuretic peptide and pro-adrenomedullin predict progression of chronic kidney disease: the MMKD Study. *Kidney Int* 75:408–414.

Dolci A, Panteghini M. (2006). The exciting story of cardiac biomarkers: from retrospective detection to gold diagnostic standard for acute myocardial infarction and more. *Clin Chim Acta* 369:179–187.

Gegenhuber A, Struck J, Dieplinger B, Poelz W, Pacher R, Morgenthaler NG, Bergmann A, Haltmayer M, Mueller T. (2007). Comparative evaluation of B-type natriuretic peptide, mid-regional pro-A-type natriuretic peptide, mid-regional pro-adrenomedullin, and Copeptin to predict 1-year mortality in patients with acute destabilized heart failure. *J Card Fail* 13:42–49.

Ichiki Y, Kitamura K, Kangawa K, Kawamoto M, Matsuo H, Eto T. (1994). Distribution and characterization of immunoreactive adrenomedullin in human tissue and plasma. *FEBS Lett* 338:6–10.

Ishimitsu T, Miyata A, Matsuoka H, Kangawa K. (1998). Transcriptional regulation of human adrenomedullin gene in vascular endothelial cells. *Biochem Biophys Res Commun* 243:463–470.

Khan SQ, Dhillon O, Kelly D, Squire IB, Struck J, Quinn P, Morgenthaler NG, Bergmann A, Davies JE, Ng LL. (2008). Plasma N-terminal B-Type natriuretic peptide as an indicator of long-term survival after acute myocardial infarction: comparison with plasma midregional pro-atrial natriuretic peptide: the LAMP (Leicester Acute Myocardial Infarction Peptide) study. *J Am Coll Cardiol* 51:1857–1864.

Khan SQ, O'Brien RJ, Struck J, Quinn P, Morgenthaler N, Squire I, Davies J, Bergmann A, Ng LL. (2007). Prognostic value of midregional pro-adrenomedullin in patients with acute myocardial infarction: the LAMP (Leicester Acute Myocardial Infarction Peptide) study. *J Am Coll Cardiol* 49:1525–1532.

Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, Eto T. (1993a). Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem Biophys Res Commun* 192:553–560.

Kitamura K, Sakata J, Kangawa K, Kojima M, Matsuo H, Eto T. (1993b). Cloning and characterization of cDNA encoding a precursor for human adrenomedullin. *Biochem Biophys Res Commun* 194:720–725.

Li Y, Jiang C, Wang X, Zhang Y, Shibahara S, Takahashi K. (2007). Adrenomedullin is a novel adipokine: adrenomedullin in adipocytes and adipose tissues. *Peptides* 28:1129–1143.

Maisel A, Mueller C, Nowak R, Peacock WF, Landsberg JW, Ponikowski P, Mockel M, Hogan C, Wu AH, Richards M, Clopton P, Filippatos GS, Di Somma S, Anand I, Ng L, Daniels LB, Neath SX, Christenson R, Potocki M, McCord J, Terracciano G, Kremastinos D, Hartmann O, von Haehling S, Bergmann A, Morgenthaler NG, Anker SD. (2010). Mid-region pro-hormone markers for diagnosis and prognosis in acute dyspnea: results from the BACH (Biomarkers in Acute Heart Failure) trial. *J Am Coll Cardiol* 55:2062–2076.

Maisel AS, Krishnaswamy P, Nowak RM, McCord J, Hollander JE, Duc P, Omland T, Storrow AB, Abraham WT, Wu AH, Clopton P, Steg PG, Westheim A, Knudsen CW, Perez A, Kazanegra R, Herrmann HC, McCullough PA; Breathing Not Properly Multinational Study Investigators. (2002). Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. *N Engl J Med* 347:161–167.

Meeran K, O'Shea D, Upton PD, Small CJ, Ghatei MA, Byfield PH, Bloom SR. (1997). Circulating adrenomedullin does not regulate systemic blood pressure but increases plasma prolactin after intravenous infusion in humans: a pharmacokinetic study. *J Clin Endocrinol Metab* 82:95–100.

Morgenthaler NG, Struck J, Alonso C, Bergmann A. (2005). Measurement of midregional proadrenomedullin in plasma with an immunoluminometric assay. *Clin Chem* 51:1823–1829.

Morgenthaler NG, Struck J, Thomas B, Bergmann A. (2004). Immunoluminometric assay for the midregion of pro-atrial natriuretic peptide in human plasma. *Clin Chem* 50:234–236.

Nakamura M, Yoshida H, Makita S, Arakawa N, Niinuma H, Hiramori K. (1997). Potent and long-lasting vasodilatory effects of adrenomedullin in humans. Comparisons between normal subjects and patients with chronic heart failure. *Circulation* 95:1214–1221.

Nomura I, Kato J, Tokashiki M, Kitamura K. (2009). Increased plasma levels of the mature and intermediate forms of adrenomedullin in obesity. *Regul Pept* 158:127–131.

Panteghini M. (2010). Cardiac: is this biomarker ready for the prime time? *Scand J Clin Lab Invest Suppl* 242:66–72.

Panteghini M. (2006). The new definition of myocardial infarction and the impact of troponin determination on clinical practice. *Int J Cardiol* 106:298–306.

Redfield MM, Rodeheffer RJ, Jacobsen SJ, Mahoney DW, Bailey KR, Burnett JC Jr. (2002). Plasma brain natriuretic peptide concentration: impact of age and gender. *J Am Coll Cardiol* 40:976–982.

Ruskoaho H. (1992). Atrial natriuretic peptide: synthesis, release, and metabolism. *Pharmacol Rev* 44:479–602.

Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I. (1989). Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr* 2:358–367.

Shimosawa T, Ogihara T, Matsui H, Asano T, Ando K, Fujita T. (2003). Deficiency of adrenomedullin induces insulin resistance by increasing oxidative stress. *Hypertension* 41:1080–1085.

Stevens LA, Coresh J, Feldman HI, Greene T, Lash JP, Nelson RG, Rahman M, Deysher AE, Zhang YL, Schmid CH, Levey AS. (2007). Evaluation of the modification of diet in renal disease study equation in a large diverse population. *J Am Soc Nephrol* 18:2749–2757.

Sugo S, Minamino N, Kangawa K, Miyamoto K, Kitamura K, Sakata J, Eto T, Matsuo H. (1994). Endothelial cells actively synthesize and secrete adrenomedullin. *Biochem Biophys Res Commun* 201:1160–1166.

Thygesen K, Alpert JS, White HD, Jaffe AS, Apple FS, Galvani M, Katus HA, Newby LK, Ravkilde J, Chaitman B, Clemmensen PM, Dellborg M, Hod H, Porela P, Underwood R, Bax JJ, Beller GA, Bonow R, Van der Wall EE, Bassand JP, Wijns W, Ferguson TB, Steg PG, Uretsky BF, Williams DO, Armstrong PW, Antman EM, Fox KA, Hamm CW, Ohman EM, Simoons ML, Poole-Wilson PA, Gurfinkel EP, Lopez-Sendon JL, Pais P, Mendis S, Zhu JR, Wallentin LC, Fernández-Avilés F, Fox KM, Parkhomenko AN, Priori SG, Tendera M, Voipio-Pulkki LM, Vahanian A, Camm AJ, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Morais J, Brener S, Harrington R, Morrow D, Lim M, Martinez-Rios MA, Steinhubl S, Levine GN, Gibler WB, Goff D, Tubaro M, Dudek D, Al-Attar N; Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. (2007). Universal definition of myocardial infarction. *Circulation* 116:2634–2653.

Vesely DL. (2001). Atrial natriuretic peptides in pathophysiological diseases. *Cardiovasc Res* 51:647–658.

von Haehling S, Jankowska EA, Morgenthaler NG, Vassanelli C, Zanolla L, Rozentryt P, Filippatos GS, Doehner W, Koehler F, Papassotiriou J, Kremastinos DT, Banasiak W, Struck J, Ponikowski P, Bergmann A, Anker SD. (2007). Comparison of midregional pro-atrial natriuretic peptide with N-terminal pro-B-type natriuretic peptide in predicting survival in patients with chronic heart failure. *J Am Coll Cardiol* 50:1973–1980.

Washimine H, Asada Y, Kitamura K, Ichiki Y, Hara S, Yamamoto Y, Kangawa K, Sumiyoshi A, Eto T. (1995). Immunohistochemical identification of adrenomedullin in human, rat, and porcine tissue. *Histochem Cell Biol* 103:251–254.