

**The relationship between adrenomedullin, metabolic factors and vascular function in individuals with type 2 diabetes mellitus.**

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**Objective** - Subjects with type 2 diabetes (T2DM) are at risk for vascular injury. Several vaso-active factors (VFs) (e.g. angiotensin) have been implicated. We hypothesize that adrenomedullin, a novel VF, is deranged in subjects with T2DM.

**Research design & methods** - Using a new immunoluminometric method, plasma mid-regional proADM (MR-proADM) was measured in four groups of Chinese subjects - healthy [H, N=100, fasting plasma glucose (FPG) <5.6 mM), impaired fasting glucose (IFG) (N=60, FPG 5.6-6.9 mM), diabetic with (DN, N=100) and without (DM, N=100) nephropathy. Resting forearm cutaneous micro-circulatory perfusion (RCMP) was quantified *in vivo* using 2-Dimensional Laser Doppler flowmetry. We investigated the relationship between plasma MR-proADM concentrations, multiple metabolic factors and vascular function.

**Results** - We observed a stepwise increase in MR-proADM among the groups [mean(SD)]. H 0.27(0.09) nM/L, IFG 0.29(0.13), DM 0.42(0.13) and DN 0.81(0.54) (DM vs. H & IFG P = 0.04 and DN vs. all P<0.01). Statistical adjustment for gender, age, BMI and blood pressure did not affect the conclusions. Multiple linear regression analysis revealed that highly sensitive C-reactive protein ( $\beta=0.11$ ;P=0.01), insulin resistance index ( $\beta=0.20$ ;P=0.001), LDL-cholesterol ( $\beta=0.31$ ;P<0.001) and adiponectin ( $\beta=0.33$ ;P<0.001) were significant predictors of plasma MR-proADM concentrations among non-diabetic individuals. Among subjects with diabetes, plasma MR-proADM concentrations correlated significantly with RCMP ( $r=0.43$ , P=0.002).

**Conclusions** - Plasma MR-proADM concentration was elevated in subjects with T2DM. This was further accentuated when nephropathy set in. MR-proADM was related to multiple metabolic factors and basal micro-circulatory perfusion. Adrenomedullin might play a role in the pathogenesis of diabetic vasculopathy.

## Introduction

Individuals with type 2 diabetes (T2DM) are at risk for vascular injury. Although major advances have been made in uncovering the mechanism of diabetic vasculopathy, the exact pathophysiology remains incompletely understood (1). In recent years, the importance of endothelial dysfunction has taken central stage (2). By secreting a repertoire of finely regulated vasoactive factors (VFs) with opposing functions working in autocrine and paracrine fashion, the endothelium is pivotal in maintaining vascular homeostasis (3). Numerous VFs have been described. Among these factors, adrenomedullin – a 52-amino acid peptide – has been intensively investigated due to its vascular protective properties (e.g. vasodilatory and antiproliferative) and promising potential as a therapeutic target (4). However, understanding of the pathobiology of adrenomedullin in diabetic vasculopathy has only just begun.

In health, adrenomedullin circulates at low picomolar concentration, which increases significantly in a number of disease states including congestive heart failure, sepsis, essential hypertension and renal impairment (5). Although a few studies have investigated the relationship between adrenomedullin and type 1 diabetes (6), as far as we know, the relationship between adrenomedullin and T2DM has not been well elucidated. Preliminary studies on small number of T2DM subjects reported inconsistent results. Turk et al showed that T2DM was associated with significant elevation in plasma adrenomedullin levels (7). However, this finding was not replicated by another study, which demonstrated no difference in plasma adrenomedullin levels between patients and control subjects (8). Similarly, whether or not

the presence of diabetic microangiopathy resulted in elevation in adrenomedullin levels was also controversial. Nakamura et al (9) suggested that increment in plasma ADM was dependent on the severity of diabetic nephropathy whereas other investigators reported otherwise (7).

Metabolic factors that affect plasma adrenomedullin concentrations were also poorly understood. Animal model research revealed that hyperglycemia increased vascular adrenomedullin expression (10). Other studies however, cannot find any relationship between plasma adrenomedullin concentrations and the degree of metabolic control or risk factors traditionally associated with endothelial injury (e.g. hypertension and dyslipidemia) (7, 8). These studies were typically conducted among small numbers of subjects with potential confounders (e.g. therapeutic agents) unaccounted for. In addition, measurement of plasma adrenomedullin was technically demanding (11), subject to “noise” due to the very brief half life of mature adrenomedullin (22 min) (12) and the optimal assay format has not been well standardized (13). Therefore, part of the inconsistent observations may be attributable to un-standardized assays. A new immunoluminometric assay targeted at the mid region of pro-adrenomedullin (MR-proADM, conceptually equivalent to C-peptide from the proinsulin molecule) was recently developed in one of the investigators’ (NGM) laboratory (14). In this assay, plasma MR-proADM concentration was found to be stable at room temperature up to 72 hours, unchanged after 4 freeze-thaw cycles, unaffected by gender, timing of sampling as well as fasted or fed state. Nonetheless, MR-proADM measured by this new immunoluminometric method

showed a robust difference between healthy and disease states (ischemic heart disease or sepsis) (14). Therefore, using this improved assay, we conducted a much larger study to examine the relationship between adrenomedullin, metabolic factors and vascular function in T2DM.

## **Materials and Methods**

### *Subjects*

We recruited four groups of Chinese subjects - 100 with normal glucose tolerance (healthy, H) [fasting plasma glucose (FPG)  $\leq 5.5$  mmol/L], 60 impaired fasting glucose (IFG) (FPG between 5.6-6.9 mmol/L) and 200 with T2DM (FPG  $\geq 7.0$  mmol/L) (15). Half of the subjects with diabetes did not have any evidence of nephropathy (DM group, N =100) whereas the remaining 100 subjects had established diabetic nephropathy (DN group). Selection of these subjects was based on strict exclusion criteria. The H and IFG groups were recruited among working adults from the general populations. Subjects from the H group were not taking long term medications or had any known history of chronic medical illness such as diabetes or hypertension. The IFG subjects did not have any known history of chronic medical illness except that 12 of them self reported as having hypertension. Out of these 12 individuals, 7 of them were taking antihypertensive agents. These 7 subjects were not excluded since the number was small and the relationship between commonly used antihypertensive agents and plasma adrenomedullin is still unclear based on existing literature. The T2DM subjects were recruited from the ambulatory care diabetes centre of a secondary hospital. T2DM subjects with normal renal function (DM group) were strictly defined as early morning spot urinary albumin / creatinine ratio (ACR) as  $\leq 3.3$  mg/mM (i.e. 30 mg/g) and consistently normal serum creatinine.

DN group (N=100) was defined as the presence of proteinuria  $\geq 1.0$  g/day [equivalent to spot urinary albumin over creatinine ratio (ACR)  $\geq 113$  mg/mM (i.e. 1000 mg/g)] or persistently elevated serum creatinine with a mean Modified Diet to retard Renal Disease (MDRD) estimated glomerular filtration rate (16) of approximately 43 mls/min/1.73m<sup>2</sup>. Individuals were excluded from the DN group when renal diseases attributable to other causes were suspected. These exclusion criteria included the presence of hematuria, renal insufficiency of unexplained origin, urinary tract infection and history of rapidly progressive renal failure, glomerulonephritis and polycystic kidney disease. Such strict criteria were employed so as to better understand the effect of diabetic kidney disease *per se* (and not other form of renal impairment) on plasma adrenomedullin. To avoid mis-classification, we decided to only include subjects with well-established nephropathy in the DN group because recent data suggested that early forms of diabetic nephropathy might remit spontaneously (17). As expected, more subjects from DN group suffered from retinopathy (44% non-proliferative and 31% proliferative) compared to subjects from DM group (20% non-proliferative, 12% proliferative) (P<0.01) since diabetic nephropathy is strongly associated with retinopathy.

### *Methods*

We focused on the non-diabetic individuals (i.e. H and IFG, N = 160) to explore the relationship between major metabolic and inflammatory biomarkers and MR-proADM. This is to avoid the influence of therapeutic agents such as glucose, lipid and blood pressure lowering agents, which were used extensively among the DM and DN groups. To explore the relationship between vascular function and adrenomedullin, we randomly sampled

50 subjects from those individuals with T2DM to measure their resting forearm cutaneous micro-circulatory perfusion (RCMP) using 2-dimensional Laser Doppler Flowmetry. We made this decision based on two considerations. Firstly, we were interested in the relationship between plasma MR-proADM and vascular function in subjects with diabetes. Pooling heterogeneous subjects from all 4 study groups was therefore undesirable. Secondly, we observed from our initial results (figure 1) that subjects from the H and IFG groups had little variation in plasma MR-proADM concentrations. Therefore, to explore the relationship between RCMP and MR-proADM in these non-diabetic individuals would be statistically inefficient since the range of exposure (in this case, MR-proADM) was limited.

#### *Major metabolic indicators*

Anthropometric data were measured for all individuals. Blood pressure was measured using a sphygmomanometer according to standard procedures (18). Briefly, the subjects were rested for at least 15 minutes. Blood pressure was measured twice over the right arm, five minutes apart in a sitting position. Should the two readings differ by more than 10 mmHg (either systolic or diastolic), a third reading will be taken and the average of the closest two readings will be recorded. Venous blood samples (taken after a 10-hour fast) with EDTA as anticoagulant were kept in icebox immediately after collection and the plasma was separated from erythrocytes by centrifuging at 1500 g for 10 min at 4°C. The plasma, if not analysed, was frozen at -80°C within 30 min after collection. Together with the fasting blood specimen, an early morning urine sample was collected for the measurement of urinary albumin and creatinine using commercial assay

(Immulite, DPC, United Kingdom) with a lower detection limit of 6 mg/L. Plasma total adiponectin concentration was measured using commercial ELISA kit (R&D Systems, Inc Minneapolis, USA) with a maximum intra-assay coefficient of variance (CV) of 7.4% and inter-assay CV of 8.4%. Glucose measurements were carried out using the glucose oxidase method using the Vitros 700 Chemistry Analyser (Rochester, NY). Blood lipids [Total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL-C)] were measured by enzymatic methods using Kodak Ektachem chemistry slides, which were then read on a Vitros 700 Chemistry Analyser. HDL-C was measured after precipitation with dextran sulphate and magnesium chloride. LDL-C was calculated using Friedewald's formula. Detection of MR-proADM was performed in duplicates in blinded samples using a novel sandwich immunoassay (B.R.A.H.M.S MR-proADM LIA; B.R.A.H.M.S AG, Hennigsdorf/Berlin, Germany) as described in detail elsewhere (14). The assay has an analytical detection limit of 0.08 nmol/L, and the inter-assay coefficient of variance is <20% for values >0.12 nmol/L. The assay is linear on dilution with undisturbed recovery of the analyte. EDTA-, heparin-, and citrate-plasma samples are stable (<20% loss of analyte) for at least three days at room temperature, 14 days at 4°C, and one year at -20°C.

#### *Measurement of forearm cutaneous microcirculatory function*

All vascular reactivity measurements were performed on the same morning as the clinical evaluation while the subjects were still fasting. A single investigator who performed all the measurements (SKG) was blinded to the medical history of the subjects.

Detailed methods for the measurement of cutaneous

microcirculatory function have been reported previously by our collaborators (19). Briefly, the skin over the extensor surface of the forearm was tested by performing Laser Doppler perfusion imaging measurements at baseline and after the iontophoresis of acetylcholine (Ach, endothelium-dependent vasodilation) and sodium nitroprusside (NaNP, endothelium-independent vasodilatation) using a Laser Doppler Perfusion Imager (Lisca PIM 1.0, Lisca Development AB, Linkoping, Sweden). Baseline resting forearm cutaneous micro-circulatory perfusion (RCMP) [i.e. red blood cell flux, in volts (V)] was first measured. Perfusion over the same area was again quantified after iontophoresis. The percentage increase ( $\delta$ ) in perfusion over baseline as a reflection of magnitude of vasodilation (i.e. vascular reactivity) in response to Ach and NaNP was then calculated. The reproducibility of the technique has been previously reported (20). The coefficient of variation of the baseline measurement was 14.1% and during maximal hyperemic response after the iontophoresis 13.7%.

The study was approved by the relevant ethics committee and institution review board. Written informed consent was obtained from all the participants.

#### *Statistical analysis*

SPSS for Windows version 11.5 (SPSS Inc. Chicago, Illinois) was used for statistical analyses. Comparison of proportions was carried out using Chi-square test for independence. Body mass index (BMI) was derived using body weight (kg) divided by the square of height (meter). Formula from Homeostasis Model Assessment (HOMA) [(fasting insulin  $\times$  glucose) / 22.5] was used to derive insulin resistance index (HOMA-IR) (21).

Analysis was not stratified by gender as MR-proADM was unaffected by gender (14). Analysis of Variance

(ANOVA) with post-hoc Tukey's honestly significant difference test was used for comparison of MR-proADM between the four groups (i.e. H, IFG, DM and DN). Subsequently, Analysis of Covariance (ANCOVA) was employed to adjust for potential confounders (gender, age, BMI and blood pressure) to determine whether MR-proADM levels remained significantly different between groups. Since the conclusions were unaffected by adjustment for potential confounders, the unadjusted MR-proADM levels were reported.

Among subjects without diabetes, Pearson correlation was employed to explore the relationship between plasma MR-proADM concentrations and multiple metabolic factors [age, BMI, waist circumference, blood pressure, fasting glucose, full lipids profile, HOMA-IR, early morning spot urinary albumin over creatinine ratio, highly sensitive CRP (hsCRP) and total adiponectin]. Results were ranked according to P values (table 2). Metabolic factors (i.e. HOMA-IR, hsCRP, TG, LDL-cholesterol & adiponectin), which showed potentially important correlation ( $P \leq 0.1$ ) with plasma MR-proADM (dependent variable), were incorporated as independent variables in subsequent linear regression analysis. Waist circumference was not included in the multi-variate analysis due to strong collinearity with HOMA-IR ( $\beta=0.80$ ). In fact, waist circumference is often used as a surrogate measurement of insulin resistance in epidemiological study (22).

Among the subpopulation of subjects with diabetes who had RCMP measured, Pearson correlation was employed to explore the relationship between plasma MR-proADM concentrations, RCMP and post challenge change ( $\delta$ ) in micro-circulatory perfusion. To further quantify the strength of association between MR-proADM and RCMP,

linear regression was performed using MR-proADM as independent variable and RCMP as dependent variable. The impact of nephropathy status (i.e. as defined in DM and DN groups) on the relationship between MR-proADM and RCMP was also examined in multi-variate analysis using General Linear Model (GLM). In the GLM, MR-proADM and nephropathy status [1=nephropathy present (DN group), 0=normal renal status (DM group)] were independent variables whereas RCMP was dependent variable. A *p* value of <0.05 was considered statistically significant.

## Results

The clinical characteristics and plasma MR-proADM concentrations of 4 groups of subjects studied are shown in table 1. Difference in unadjusted plasma MR-proADM was also shown in figure 1. MR-proADM increased progressively from healthy individuals to T2DM patients with nephropathy ( $p < 0.001$  for trend, table 1). Post-hoc pair-wise comparisons revealed: DM vs. H & IFG  $P = 0.04$  and DN vs. all  $P < 0.01$  (figure 1).

Correlations between MR-proADM and multiple metabolic factors ranked according to *P* values are also shown in table 2. Multiple linear regressions (dependent variable: MR-proADM and independent variables: hsCRP & HOMA-IR, TG, LDL-cholesterol and adiponecetin) revealed that hsCRP, HOMA-IR, LDL-choelsterol and adiponecetin were significant predictors of plasma MR-proADM concentration with standardized coefficients of correlation ( $\beta$ ) of 0.11 ( $P = 0.01$ ), 0.20 ( $P = 0.001$ ), 0.31 ( $P < 0.001$ ) and 0.33 ( $P < 0.001$ ) respectively. Collectively, hsCRP, HOMA-IR, LDL-cholesterol and adiponecetin predicted approximately 84% of changes in plasma MR-proADM concentration.

Detailed results of forearm cutaneous micro-circulatory perfusion (RCMP) among a random sample of 50 subjects taken from individuals with diabetes [i.e. DM ( $n = 39$ ) and DN ( $n = 11$ ) groups] were as follows. The resting forearm skin temperature was (mean $\pm$ SD)  $30.5 \pm 0.5^\circ\text{C}$ . The RCMP measured using 2-Dimensional Laser Doppler flowmetry was  $0.80 \pm 0.25$  Volts. Pearson correlation between RCMP and MR-proADM among these 50 subjects with diabetes revealed moderately strong correlation ( $r = 0.43$ ,  $P = 0.002$ ) (figure 2). Linear regression (dependent variable: MR-proADM and independent variable: RCMP) revealed a coefficient of correlation ( $\beta$ ) of 0.80 (95% confidence interval 0.30-1.30;  $P = 0.002$ ). In other words, a unit change in plasma MR-proADM concentration will result in, on average, a 0.8 unit increase in cutaneous blood flow.

GLM analysis (to study the impact of nephropathy status on the relationship between MR-proADM and RCMP) revealed that nephropathy status had no significant influence on the observed relationship ( $\beta = 0.004$ , 95% confidence interval  $-0.16$  to  $0.15$ , *F* statistics = 0.003,  $P = 0.96$ ). Therefore, analysis of RCMP was not stratified by renal status.

Correlations between MR-proADM and indices of endothelial dependent and independent vascular reactivity [i.e. percentage increase ( $\delta$ ) in perfusion after iontophoretic transcutaneous delivery of vasoactive substances - Ach and NaNP] were unremarkable - Ach challenge  $\delta$  perfusion ( $r = -0.19$ ,  $P = 0.17$ ), NaNP challenge  $\delta$  perfusion ( $r = -0.20$ ,  $P = 0.16$ ).

## Discussion

There were three main findings in our study. Firstly, plasma MR-proADM concentration was elevated in T2DM subjects with preserved renal

function. This was further accentuated in the presence of established diabetic nephropathy. Secondly, metabolic and inflammatory factors namely insulin resistance, LDL-cholesterol, adiponectin and hsCRP appeared to be significant determinants of plasma MR-proADM concentrations. Thirdly, MR-proADM was well correlated with measurement of resting unprovoked micro-circulatory blood flow suggesting that it may be one of the determinants of basal vascular perfusion in subjects with type 2 diabetes.

The first finding was interesting because it clarified the relationship between plasma adrenomedullin concentrations and T2DM. Our data suggested that MR-proADM was mildly elevated in uncomplicated T2DM subjects (figure 1). However, in the presence of diabetic nephropathy, plasma MR-proADM became markedly deranged (consistent with Kinoshita et al (13)). The strict criteria that we adopted for the recruitment of subjects in DM group (absolutely normal renal function) and DN group (well established nephropathy) was an advantage in helping to define the relationship between diabetes and adrenomedullin i.e. adrenomedullin level increased with increasing severity of diabetes. Based on sequence homology, adrenomedullin was thought to belong to the calcitonin gene-related peptide (CGRP) superfamily (23). It was shown to be secreted from all three types of cultured vascular cells – endothelium, vascular smooth muscle cell (VSMC) and adventitial fibroblast. Previous elegant studies suggested kidney as an important source of adrenomedullin (24) whereas pulmonary vascular bed could be the main site of receptor dependent clearance of circulating adrenomedullin (25). In contrast, the exact site of clearance for MR-proADM is not known at the moment. Therefore, one

possible explanation for the elevated plasma MR-proADM concentration among T2DM with nephropathy could be reduced MR-proADM clearance. It is known that adrenomedullin could exert a wide range of vascular actions (mostly protective). These included endothelium dependent as well as independent vasodilation, anti-oxidative stress, stimulation of endothelial nitric oxide production, antiproliferation of VSMC and adventitial fibroblast (26). Taken together, the elevation of plasma MR-proADM concentration in T2DM (especially in the presence of i.e. nephropathy) could be an appropriate physiological response to on-going vascular injury (27).

Factors that up-regulate adrenomedullin production are incompletely understood. The role of hyperglycemia is controversial. *In vitro* data suggested that hyperglycemia might increase vascular adrenomedullin expression (10). However, this notion could not be substantiated *in vivo* (7,8). Other postulated mechanisms included acute hyperinsulinemia (28), increased oxidative stress (based on *in vivo* studies) (29) and proatherogenic/inflammatory factors such as angiotensin II, endothelin-1(30), interleukin-1 $\beta$  and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) (31) (based on cell culture studies). Our analysis revealed that among the multitude of metabolic factors examined (table 2), insulin resistance, hsCRP, LDL-cholesterol and adiponectin were significant determinants of plasma MR-proADM concentrations. Our observation was note-worthy given that insulin resistance had been associated with vascular injury (32) and diabetic nephropathy (33, 34). In addition, insulin resistance was found to be associated with low grade endothelial inflammation (manifesting as elevated hsCRP) (35) which had become increasingly recognized as a determinant of vasculopathy (36). The

relationship between LDL-cholesterol and adrenomedullin is poorly understood. Limited *in vitro* data from rats endothelial cell suggested that oxidized LDL might stimulate the secretion of adrenomedullin (37). Adiponectin has emerged to be one of the most important adipocytokines at the cross road of energy homeostasis, inflammation and vascular injury (38). To our knowledge, the relationship between adiponectin and adrenomedullin has not been well studied. Nevertheless, very recent report suggested that adiponectin might be associated with reduced odds of renal dysfunction in subject with type 2 diabetes (39). Therefore, the observed relationship between adiponectin and MR-proADM in our study is potentially novel and may require further investigations. Taken together, our data suggested that vasculopathic metabolic and inflammatory factors were associated with up-regulation of MR-proADM – probably a response to injury. To identify the inciting factors is important as this could lead to the discovery of novel therapeutic interventions. For instances, insulin sensitizer had been found to ameliorate renal dysfunction in individuals with T2DM (40). Lipid lowering therapy may retard the progression of renal impairment (41). Nevertheless, our study revealed that HOMA-IR, hsCRP, LDL-cholesterol and adiponectin collectively accounted for approximately 84% of variation in plasma MR-proADM concentrations. Therefore, other determinants of plasma MR-proADM un-identified in our study should be investigated in future studies.

We observed that plasma MR-proADM concentration correlated moderately well to basal cutaneous micro-circulatory blood flow among subjects with T2DM. This suggested that adrenomedullin mediated vasodilation could probably be one of

the determinants of basal micro-circulatory perfusion. A growing body of animal and human pilot studies corroborated with our present observation. These pilot studies showed that adrenomedullin induced vasodilation in isolated Canine (42) and Bovine (43) retinal arteries (another micro-circulatory bed) *in vitro*. Similarly, adrenomedullin also induced vasodilation of retinal arteries of diabetic Male Wistar rats (44) *in vivo*. In a small number of healthy human subjects, Dorner et al reported that adrenomedullin dose dependently increased choroidal blood flow and flow velocity in the ophthalmic artery(45). Therefore, the novel observation in our present observation was intriguing. One mechanism identified in the pathogenesis of diabetic nephropathy was hemodynamic mediated vascular injury (46). Sustained increase in glomerular capillary pressure driven by increase in plasma flow had been observed especially in early stage of nephropathy. The elevation in glomerular capillary pressure might be damaging to glomerular endothelial, epithelial and mesangial cells, thereby initiating and contributing to the progression of nephropathy (47). Although numerous mediators of diabetic hyperfiltration had been proposed, the exact mechanism remained unclear (48). It is therefore tempting to speculate that endothelial derived vasodilatory substances like adrenomedullin could be involved since MR-proADM was increasingly elevated from healthy to renal impaired subjects (figure 1) and well correlated to magnitude of micro-circulatory perfusion (figure 2). Should this be the case, modulating adrenomedullin action would have therapeutic potential in the prevention of diabetic nephropathy (5).

There are a few limitations in our study. It would be ideal to only recruit individuals (healthy or diabetic) not

receiving any form of pharmacological interventions (so as to observe the unbiased and unconfounded relationship between metabolic factors, ADM levels and different disease state). This however is unrealistic since individuals with diabetes and diabetic nephropathy are expected to receive intensive treatment according to current standard of care. Secondly, the safe and non-invasive measurement of micro-circulatory perfusion was based on cutaneous vasculature (instead of direct measurement of renal hemodynamics). Although experience from our group suggests that cutaneous micro-circulatory function correlates well with measurement of severity of diabetic nephropathy (49), it was only a surrogate and could not help us gain direct insights into actual changes in renal hemodynamics. Thirdly, we did not measure the serum creatinine of all the study subjects. Although we observed incremental plasma MR-proADM concentration according to

categorical diabetic and renal function status, we were unable to directly investigate the relationship between plasma MR-proADM and renal function (as estimated by serum creatinine). Having said so, the limitation of serum creatinine as an estimate of renal function (i.e. glomerular filtration rate) has been well recognized (16).

In conclusion, our study revealed that plasma MR-proADM concentration was elevated among subjects with T2DM, which was further accentuated when nephropathy set in. MR-proADM was related to multiple metabolic factors and basal micro-circulatory perfusion. Therefore, adrenomedullin might play a role in the pathogenesis of diabetic vasculopathy.

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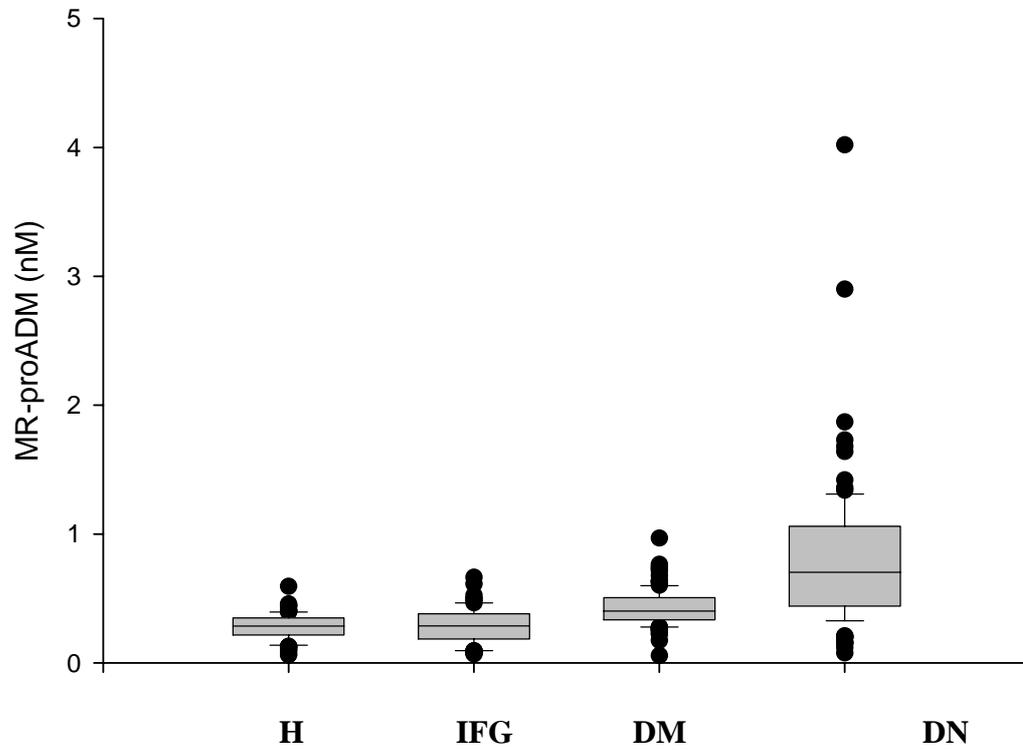
**Table 1. Clinical characteristics [mean(SD)] of healthy subjects (H), subjects with impaired fasting glucose (IFG), type 2 diabetes without nephropathy (DM) and with nephropathy (DN).**

	<b>H (n=100)</b>	<b>IFG (n=60)</b>	<b>DM (n=100)</b>	<b>DN (N=100)</b>	<b><i>p</i> value (for trend)</b>
<b>Gender (% male)</b>	50	32	58	55	0.007
<b>Age (yr)</b>	40 (14)	44 (11)	58 (10)	61 (10)	<0.001
<b>Duration of diabetes (years)</b>	-	-	16 (7)	17 (9)	0.55
<b>Body mass index (kg/m<sup>2</sup>)</b>	22.9 (3.7)	24.1 (4.2)	25.8 (4.2)	26.1 (4.5)	<0.001
<b>Systolic blood pressure (mmHg)</b>	121 (14)	126 (22)	136 (17)	147 (21)	<0.001
<b>Diastolic blood pressure (mmHg)</b>	77 (8)	78 (12)	81 (8)	81(11)	0.02
<b>Fasting plasma glucose (mmol/L)</b>	4.9 (0.3)	5.9 (0.4)	7.0 (1.2)	8.5 (3.5)	<0.001
<b>HBA1c (%)</b>	-	-	7.8(1.3)	8.1(1.6)	0.07
<b>Midregion-proadrenomedullin (nmol/L)</b>	0.27 (0.09)	0.29 (0.13)	0.42 (0.13)	0.81(0.54)	<0.001

**Table 2. Pearson coefficient of correlation between multiple metabolic factors and mid-regional pro-adrenomedullin (MR-proADM) among non-diabetic individuals**

	<b>Mean (<math>\pm</math>SD) plasma concentration</b>	<b>Pearson coefficient of correlation with MR-proADM</b>	<b>P value</b>
<b>Homeostatis Model Analysis (HOMA) – insulin resistance index</b>	1.96(1.52)	0.183	0.02
<b>Highly sensitive c-reactive protein (hsCRP) (mg/L)</b>	0.23(0.29)	0.157	0.047
<b>Triglyceride (mM)</b>	1.26(0.81)	0.13	0.08
<b>LDL-cholesterol (mM)</b>	3.35(0.88)	-0.13	0.08
<b>Waist circumference (cm)</b>	76.0(14.2)	0.14	0.08
<b>Total adiponectin (<math>\mu</math>g/ml)</b>	7.65(4.88)	0.13	0.10
<b>Body mass index (kg/m<sup>2</sup>)</b>	23.4(3.9)	0.12	0.11
<b>Age (years)</b>	40(11)	0.09	0.22
<b>HDL-cholesterol (mM)</b>	1.67(0.45)	0.08	0.27
<b>Total cholesterol (mM)</b>	5.56(0.97)	-0.06	0.44
<b>Urinary albumin / creatinine ratio (mg/g)</b>	13.4(26.2)	0.03	0.68
<b>Systolic blood pressure (mmHg)</b>	123(18)	-0.001	0.99
<b>Diastolic blood pressure (mmHg)</b>	77(10)	-0.003	0.97

**Figure 1: Box-plot of plasma midregion-proadrenomedullin (MR-proADM) concentrations among individuals with normal glucose tolerance (H), impaired fasting glucose (IFG), diabetes without nephropathy (DM) and diabetes with nephropathy (DN). (DM vs. H & IFG P = 0.04 and DN vs. all P<0.01)**



**Figure 2: Resting forearm cutaneous micro-circulatory perfusion (RCMP) vs. plasma mid-regional proadrenomedullin (MR-proADM) concentrations among 50 subjects with type 2 diabetes**

