

The Relationship Between Adrenomedullin, Metabolic Factors, and Vascular Function in Individuals With Type 2 Diabetes

SU CHI LIM, MBBS¹
 NILS G. MORGENTHALER, MD, PHD²
 TAVINTHARAN SUBRAMANIAM, MBBS¹

YEW SENG WU, DIP¹
 SIEW KHENG GOH, BSC¹
 CHEE FANG SUM, MBBS¹

OBJECTIVE — Subjects with type 2 diabetes are at risk for vascular injury. Several vasoactive factors (e.g., angiotensin) have been implicated. We hypothesize that adrenomedullin, a novel vasoactive factor, is deranged in subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS — Using a new immunoluminometric method, plasma midregional proadrenomedullin (MR-proADM) was measured in four groups of Chinese subjects: healthy ($n = 100$, fasting plasma glucose [FPG] <5.6 mmol/l), impaired fasting glucose (IFG) ($n = 60$, FPG 5.6 – 6.9 mmol/l), and diabetic subjects with ($n = 100$) and without ($n = 100$) nephropathy. Resting forearm cutaneous microcirculatory 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: healthy group mean \pm SD 0.27 ± 0.09 , IFG group 0.29 ± 0.13 , diabetic group 0.42 ± 0.13 , and diabetic nephropathy group 0.81 ± 0.54 nmol/l (diabetic vs. healthy and IFG groups, $P = 0.04$; and diabetic nephropathy group vs. all, $P < 0.01$). Statistical adjustment for sex, 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 nondiabetic 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 type 2 diabetes. This was further accentuated when nephropathy set in. MR-proADM was related to multiple metabolic factors and basal microcirculatory perfusion. Adrenomedullin might play a role in the pathogenesis of diabetic vasculopathy.

Diabetes Care 30:1513–1519, 2007

Individuals with type 2 diabetes 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 with opposing functions working in autocrine and paracrine fashion, the endothelium is pivotal in maintaining vascular homeostasis (3). Numerous vasoactive factors 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 healthy states, 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 type 2 diabetes has not been well elucidated. Preliminary studies on small numbers of type 2 diabetic subjects reported inconsistent results. Turk et al. (7) showed that type 2 diabetes was associated with significant elevation in plasma adrenomedullin levels. However, this finding was not replicated by another study, which demonstrated no difference in plasma adrenomedullin levels between diabetic 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 adrenomedullin 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 in whom potential confounders (e.g., therapeutic agents) were unaccounted. In addition, measurement of plasma adrenomedullin was techni-

From the ¹Department of Medicine, Alexandra Hospital, Singapore, Republic of Singapore; and the ²Research Department, B.R.A.H.M.S. AG Biotechnology Center, Hennigsdorf/Berlin, Germany.

Address correspondence and reprint requests to Su Chi Lim, Department of Medicine, Alexandra Hospital, 378 Alexandra Rd., Singapore 159964, Republic of Singapore. E-mail: su_chi_lim@alexhosp.com.sg.

Received for publication 12 September 2006 and accepted in revised form 6 March 2007.

Published ahead of print at <http://care.diabetesjournals.org> on 23 March 2007. DOI: 10.2337/dc06-1899.

Abbreviations: ACR, albumin-to-creatinine ratio; hsCRP, highly sensitive C-reactive protein; FPG, fasting plasma glucose; GLM, general linear model; HOMA-IR, homeostasis model assessment of insulin resistance; IFG, impaired fasting glucose; MR-proADM, midregional proadrenomedullin; RCMP, resting forearm cutaneous microcirculatory perfusion.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

© 2007 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

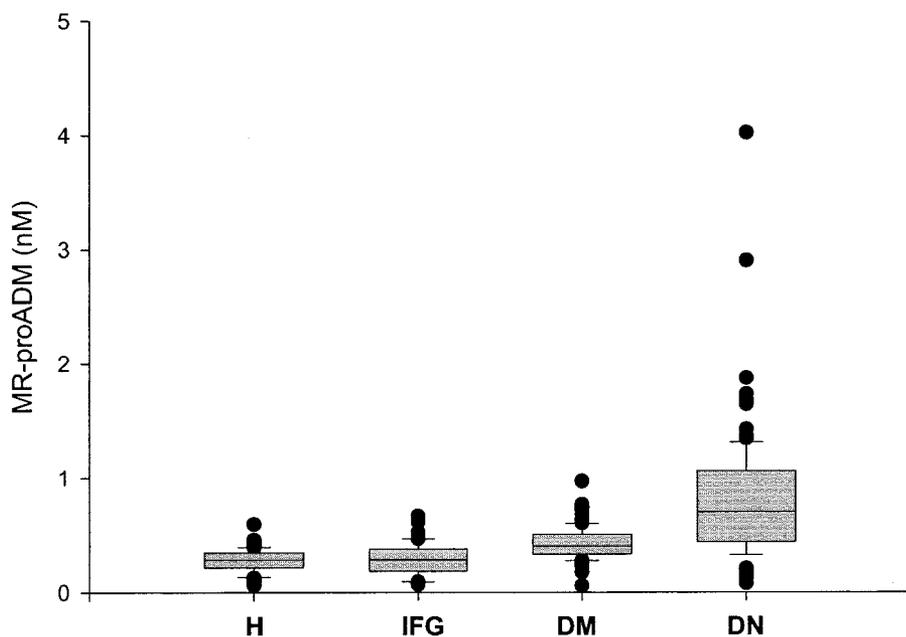


Figure 1—Box plot of plasma MR-proADM concentrations among individuals with normal glucose tolerance (H), IFG, diabetes without nephropathy (DM) and diabetes with nephropathy (DN). DM vs. H and IFG, $P = 0.04$; DN vs. all, $P < 0.01$.

cally 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 unstandardized assays. A new immunoluminometric assay targeted at midregional proadrenomedullin ([MR-proADM], conceptually equivalent to C-peptide from the proinsulin molecule), was recently developed in one of the investigators’ (N.G.M.) laboratory (14). In this assay, plasma MR-proADM concentration was found to be stable at room temperature for up to 72 h, unchanged after four freeze-thaw cycles, and unaffected by sex, timing of sampling, or fasted versus 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 type 2 diabetes.

RESEARCH DESIGN AND METHODS

Methods— We recruited four groups of Chinese subjects: 100 healthy subjects with normal glucose tolerance (fasting plasma glucose [FPG] ≤ 5.5 mmol/l), 60 with impaired fasting glucose (IFG)

(FPG between 5.6 and 6.9 mmol/l), and 200 with type 2 diabetes (FPG ≥ 7.0 mmol/l) (15), half of whom did not have any evidence of nephropathy (diabetic group, $n = 100$) whereas the remaining 100 subjects had established diabetic nephropathy. Selection of these subjects was based on strict exclusion criteria. The healthy and IFG groups were recruited among working adults from the general population. Subjects from the healthy group were not taking long-term medications or have 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 self-reported having hypertension, of whom 7 were taking antihypertensive agents. These seven 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 type 2 diabetic subjects were recruited from the ambulatory care diabetes center of a secondary hospital. Type 2 diabetic subjects with normal renal function (the diabetic group) were strictly defined as early morning spot urinary albumin-to-creatinine ratio (ACR) ≤ 3.3 mg per mmol/l (i.e., 30 mg/g) and consistently normal serum creatinine. The diabetic nephropathy group ($n = 100$) was defined according to the presence of proteinuria ≥ 1.0 g/day (equivalent to spot urinary ACR ≥ 113 mg per mmol/l [i.e., 1,000

mg/g]) or persistently elevated serum creatinine with a mean MDRD (Modified Diet in Renal Disease) formula–estimated glomerular filtration rate (16) of ~ 43 ml/min per 1.73 m² (2). Individuals were excluded from the diabetic nephropathy 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 forms of renal impairment) on plasma adrenomedullin. To avoid misclassification, we decided to only include subjects with well-established nephropathy in the diabetic nephropathy group because recent data suggested that early forms of diabetic nephropathy might remit spontaneously (17). As expected, more subjects from the diabetic nephropathy group suffered from retinopathy (44% nonproliferative and 31% proliferative) compared with subjects from the diabetic group (20% nonproliferative and 12% proliferative) ($P < 0.01$), since diabetic nephropathy is strongly associated with retinopathy.

We focused on the nondiabetic individuals (i.e., the healthy and IFG groups, $n = 160$) to explore the relationship between major metabolic and inflammatory biomarkers and MR-proADM. This was to avoid the influence of therapeutic agents such as glucose-, lipid-, and blood pressure-lowering agents, which were used extensively among the diabetic and diabetic nephropathy groups. To explore the relationship between vascular function and adrenomedullin, we randomly sampled 50 subjects from those individuals with type 2 diabetes to measure their resting forearm cutaneous microcirculatory perfusion (RCMP) using 2-dimensional laser Doppler flowmetry. We made this decision based on two considerations. First, we were interested in the relationship between plasma MR-proADM and vascular function in subjects with diabetes. Pooling heterogeneous subjects from all four study groups was therefore undesirable. Second, we observed from our initial results (Fig. 1) that subjects from the healthy and IFG groups had little variation in plasma MR-proADM concentrations. Therefore, to explore the relationship between RCMP and MR-proADM in these nondiabetic individu-

Table 1—Clinical characteristics of healthy subjects (H), subjects with IFG, and type 2 diabetic subjects without nephropathy (DM) and with nephropathy (DN)

	H	IFG	DM	DN	P for trend
n	100	60	100	100	—
Sex (% male)	50	32	58	55	0.007
Age (years)	40 ± 14	44 ± 11	58 ± 10	61 ± 10	<0.001
Duration of diabetes (years)	—	—	16 ± 7	17 ± 9	0.55
BMI (kg/m ²)	22.9 ± 3.7	24.1 ± 4.2	25.8 ± 4.2	26.1 ± 4.5	<0.001
Blood pressure (mmHg)	—	—	—	—	—
Systolic	121 ± 14	126 ± 22	136 ± 17	147 ± 21	<0.001
Diastolic	77 ± 8	78 ± 12	81 ± 8	81 ± 11	0.02
Fasting plasma glucose (mmol/l)	4.9 ± 0.3	5.9 ± 0.4	7.0 ± 1.2	8.5 ± 3.5	<0.001
A1C (%)	—	—	7.8 ± 1.3)	8.1 ± 1.6	0.07
MR-proADM (nmol/l)	0.27 ± 0.09	0.29 ± 0.13	0.42 ± 0.13)	0.81 ± 0.54	<0.001

Data are means ± SD unless otherwise indicated.

als 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 min. Blood pressure was measured twice over the right arm 5 minutes apart in a sitting position. Should the two readings differ by >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-h fast) with EDTA as anticoagulant were kept in an icebox immediately after collection, and the plasma was separated from erythrocytes by centrifuging at 1,500g for 10 min at 4°C. The plasma, if not analyzed, was frozen at -80°C within 30 min of 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 UK) with a lower detection limit of 6 mg/l. Plasma total adiponectin concentration was measured using a commercial enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN) with a maximum intra-assay coefficient of variance (CV) of 7.4% and interassay 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, triglycerides, and HDL cholesterol) were measured by enzymatic methods using Kodak Ektachem chemistry slides,

which were then read on a Vitros 700 Chemistry Analyser. HDL cholesterol was measured after precipitation with dextran sulfate and magnesium chloride. LDL cholesterol 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. Sevidil 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 interassay CV 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 3 days at room tem-

perature, 14 days at 4°C, and 1 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 (S.K.G.), was blinded to the medical history of the subjects.

Detailed methods for the measurement of cutaneous microcirculatory function have been previously reported 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

Table 2—Pearson coefficient of correlation between multiple metabolic factors and MR-proADM among nondiabetic individuals

	Mean ± SD Plasma concentration	Pearson coefficient of correlation with MR-proADM	P
HOMA-IR	1.96 ± 1.52	0.183	0.02
hsCRP (mg/l)	0.23 ± 0.29	0.157	0.047
Triglycerides (mM)	1.26 ± 0.81	0.13	0.08
LDL cholesterol (mmol/l)	3.35 ± 0.88	-0.13	0.08
Waist circumference (cm)	76.0 ± 14.2	0.14	0.08
Total adiponectin (µg/ml)	7.65 ± 4.88	0.13	0.10
BMI (kg/m ²)	23.4 ± 3.9	0.12	0.11
Age (years)	40 ± 11	0.09	0.22
HDL cholesterol (mmol/l)	1.67 ± 0.45	0.08	0.27
Total cholesterol (mmol/l)	5.56 ± 0.97	-0.06	0.44
Urinary ACR (mg/g)	13.4 ± 26.2	0.03	0.68
Systolic blood pressure (mmHg)	123 ± 18	-0.001	0.99
Diastolic blood pressure (mmHg)	77 ± 10	-0.003	0.97

and after the iontophoresis of acetylcholine (endothelium-dependent vasodilation) and sodium nitroprusside ([NaNP] endothelium-independent vasodilatation) using a laser Doppler perfusion imager (Lisca PIM 1.0; Lisca Development AB, Linköping, Sweden). Baseline RCMP (i.e., erythrocyte flux, in volts) was first measured. Perfusion over the same area was again quantified after iontophoresis. The percentage of increase (δ) in perfusion over baseline as a reflection of magnitude of vasodilation (i.e., vascular reactivity) in response to acetylcholine and NaNP was then calculated. The reproducibility of the technique has been previously reported (20). The CV 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, Chicago, IL) was used for statistical analyses. Comparison of proportions was carried out using χ^2 test for independence. BMI was calculated as weight in kilograms divided by the square of height in meters. A formula was used to derive the homeostasis model assessment of insulin resistance (HOMA-IR): (fasting insulin \times glucose)/22.5 (21).

Analysis was not stratified by sex, as MR-proADM was unaffected by sex (14). ANOVA with post hoc Tukey's honestly significant difference test was used for comparison of MR-proADM between the four groups. Subsequently, ANCOVA was employed to adjust for potential confounders (sex, 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 ACR, highly sensitive C-reactive protein [hsCRP], and total adiponectin). Results were ranked according to *P* values (Table 2). Metabolic factors (i.e., HOMA-IR, hsCRP, triglycer-

ides, LDL cholesterol, adiponectin), which showed a 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 multivariate 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 studies (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 postchallenge change (δ) in microcirculatory perfusion. To further quantify the strength of association between MR-proADM and RCMP, linear regression was performed using MR-proADM as the independent variable and RCMP as the dependent variable. The impact of nephropathy status (i.e., as defined in diabetic and diabetic nephropathy groups) on the relationship between MR-proADM and RCMP was also examined in multivariate analysis using a general linear model (GLM). In the GLM, MR-proADM and nephropathy status (1 = diabetic nephropathy group, 0 = diabetic with normal renal status group) were independent variables, whereas RCMP was the dependent variable. A *P* value of <0.05 was considered statistically significant.

RESULTS — The clinical characteristics and plasma MR-proADM concentrations of the four groups of subjects studied are shown in Table 1. The difference in unadjusted plasma MR-proADM is also shown in Fig. 1. MR-proADM increased progressively from healthy individuals to type 2 diabetic patients with nephropathy ($P < 0.001$ for trend, Table 1). Post hoc pairwise comparisons revealed diabetic versus healthy and IFG, $P = 0.04$; diabetic nephropathy versus all, $P < 0.01$ (Fig. 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; independent variables: hsCRP, HOMA-IR, triglycerides, LDL cholesterol, and adiponectin) revealed that hsCRP, HOMA-IR, LDL cholesterol, and adiponectin were significant predictors of plasma MR-proADM concentration with standardized CVs (β) 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 adiponectin predicted $\sim 84\%$ of changes in plasma MR-proADM concentration.

Detailed results of RCMP among a random sample of 50 subjects taken from individuals with diabetes (i.e., diabetic [$n = 39$] and diabetic nephropathy [$n = 11$] groups) were as follows. The resting forearm skin temperature was $30.5 \pm 0.5^\circ\text{C}$. The RCMP, measured using 2-dimensional laser Doppler flowmetry, was 0.80 ± 0.25 V. Pearson correlation between RCMP and MR-proADM among these 50 subjects with diabetes revealed moderately strong correlation ($r = 0.43$, $P = 0.002$) (Fig. 2). Linear regression (dependent variable, MR-proADM; independent variable, RCMP) revealed a CV (β) of 0.80 (95% CI 0.30–1.30; $P = 0.002$). In other words, a unit change in plasma MR-proADM concentration will, on average, result in 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% CI -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 indexes of endothelial-dependent and -independent vascular reactivity (i.e., percentage of increase [δ] in perfusion after iontophoretic transcutaneous delivery of vasoactive substances acetylcholine and NaNP) were unremarkable: acetylcholine challenge δ perfusion ($r = -0.19$, $P = 0.17$), NaNP challenge δ perfusion ($r = -0.20$, $P = 0.16$).

CONCLUSIONS — There were three main findings in our study. First, plasma MR-proADM concentration was elevated in type 2 diabetic subjects with preserved renal function. This was further accentuated in the presence of established diabetic nephropathy. Second, metabolic and inflammatory factors, namely insulin resistance, LDL cholesterol, adiponectin, and hsCRP, appeared to be significant determinants of plasma MR-proADM concentrations. Third, MR-proADM was well correlated with measurement of resting, unprovoked microcirculatory blood flow, suggesting that it may be one of the determinants of basal vascular perfusion in subjects with type 2 diabetes.

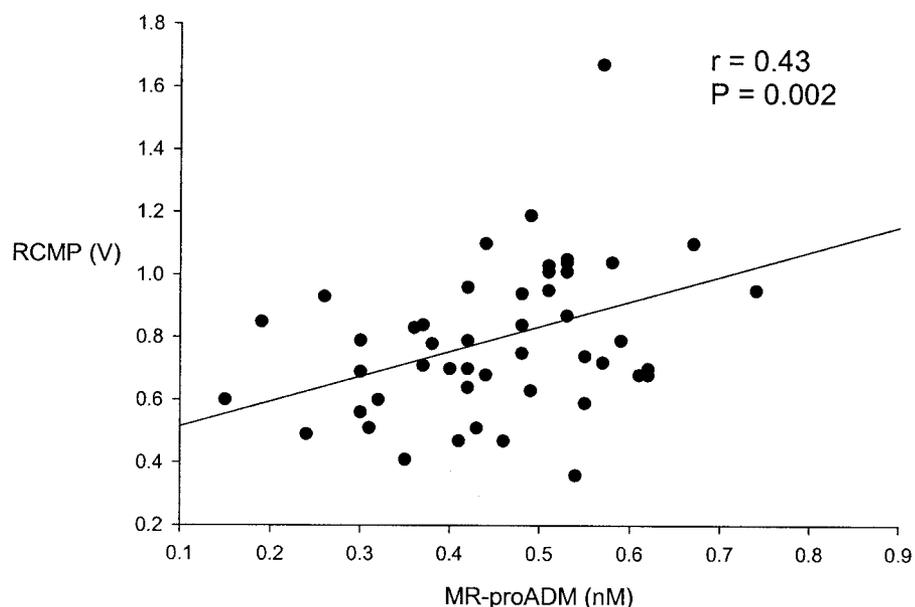


Figure 2—RCMP vs. plasma MR-proADM concentrations among 50 subjects with type 2 diabetes.

The first finding was interesting because it clarified the relationship between plasma adrenomedullin concentrations and type 2 diabetes. Our data suggested that MR-proADM was mildly elevated in subjects with uncomplicated type 2 diabetes (Fig. 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 the diabetic (normal renal function) and diabetic nephropathy (well established nephropathy) groups 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 superfamily (23). It was shown to be secreted from all three types of cultured vascular cells: endothelium, vascular smooth muscle cell, 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 type 2 diabetes with nephropathy could be reduced MR-

proADM clearance. It is known that adrenomedullin could exert a wide range of vascular actions (mostly protective). These include endothelium-dependent and -independent vasodilation, antioxidative stress, stimulation of endothelial nitric oxide production, antiproliferation of vascular smooth muscle cell, and adventitial fibroblast (26). Taken together, the elevation of plasma MR-proADM concentration in type 2 diabetes (especially in the presence of nephropathy) could be an appropriate physiological response to ongoing vascular injury (27).

Factors that upregulate 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 β , and tumor necrosis factor- α (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 noteworthy given that insulin resistance had been associated with vascular injury (32) and di-

abetic nephropathy (33,34). In addition, insulin resistance was found to be associated with low-grade endothelial inflammation (manifesting as elevated hsCRP) (35), which has become increasingly recognized as a determinant of vasculopathy (36). The relationship between LDL cholesterol and adrenomedullin is poorly understood. Limited in vitro data from endothelial cells in rats suggested that oxidized LDL might stimulate the secretion of adrenomedullin (37). Adiponectin has emerged as one of the most important adipocytokines at the crossroad 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 studies suggested that adiponectin might be associated with reduced odds of renal dysfunction in subjects 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 upregulation 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 instance, insulin sensitizer had been found to ameliorate renal dysfunction in individuals with type 2 diabetes (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 ~84% of variation in plasma MR-proADM concentrations. Therefore, other determinants of plasma MR-proADM unidentified in our study should be investigated in future studies.

We observed that plasma MR-proADM concentration correlated moderately well with basal cutaneous microcirculatory blood flow among subjects with type 2 diabetes. This suggested that adrenomedullin-mediated vasodilation could probably be one of the determinants of basal microcirculatory perfusion. A growing body of animal and human pilot studies corroborated our present observation. These pilot studies showed that adrenomedullin induced vasodilation in isolated canine (42) and bovine (43) retinal arteries (another microcirculatory bed) in vitro. Similarly, adrenomedullin also induced vasodilation of retinal arter-

ies of diabetic male Wistar rats in vivo (44). In a small number of healthy human subjects, Dorner et al. (45) reported that adrenomedullin dose dependently increased choroidal blood flow and flow velocity in the ophthalmic artery. Therefore, the novelty 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 stages 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 (Fig. 1) and well correlated to magnitude of microcirculatory perfusion (Fig. 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, adrenomedullin 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 standards of care. Secondly, the safe and noninvasive measurement of microcirculatory perfusion was based on cutaneous vasculature (instead of direct measurement of renal hemodynamics). Although experience from our group suggests that cutaneous microcirculatory 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. Third, 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). This said, 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 type 2 diabetes, which was further accentuated when nephropathy set in. MR-proADM was related to multiple metabolic factors and basal microcirculatory perfusion. Therefore, adrenomedullin might play a role in the pathogenesis of diabetic vasculopathy.

Acknowledgments—The study was funded partially by the National Medical Research Council (NMRC/0518/2001) and National Health Care Group (NHG-STP 04001; SIG 06036), Ministry of Health of Singapore, Republic of Singapore.

References

1. Brownlee M: Banting Lecture 2004: The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 54:1615–1625, 2005
2. Widlansky ME, Gokce N, Keaney JF Jr, Vita JA: The clinical implications of endothelial dysfunction. *J Am Coll Cardiol* 42: 1149–60, 2003
3. Behrendt D, Ganz P: Endothelial function: from vascular biology to clinical applications. *Am J Cardiol* 90:40L–48L, 2002
4. Bunton DC, Petrie MC, Hillier C, Johnston F, McMurray JJ: The clinical relevance of adrenomedullin: a promising profile? *Pharmacol Ther* 103:179–201, 2004
5. Julian M, Cacho M, Garcia MA, Martin-Santamaria S, de Pascual-Teresa B, Ramos A, Martinez A, Cuttitta F: Adrenomedullin: a new target for the design of small molecule modulators with promising pharmacological activities. *Eur J Med Chem* 40:737–750, 2005
6. Garcia-Unzueta MT, Montalban C, Pesquera C, Berrazueta JR, Amado JA: Plasma adrenomedullin levels in type 1 diabetes: relationship with clinical parameters. *Diabetes Care* 21:999–1003, 1998
7. Turk HM, Buyukberber S, Sevinc A, Ak G, Ates M, Sari R, Savli H, Cigli A: Relationship between plasma adrenomedullin levels and metabolic control, risk factors, and diabetic microangiopathy in patients with type 2 diabetes. *Diabetes Care* 23: 864–867, 2000
8. Kinoshita H, Kato K, Kuroki M, Nakamura S, Kitamura K, Hisanaga S, Fujimoto S, Eto T: Plasma adrenomedullin levels in patients with diabetes. *Diabetes Care* 23:253–254, 2000
9. Nakamura T, Honda K, Ishikawa S, Kitamura K, Eto T, Saito T: Plasma adrenomedullin levels in patients with non-insulin dependent diabetes mellitus: close relationships with diabetic complications. *Endocr J* 45:241–246, 1998
10. Hayashi M, Shimosawa T, Fujita T: Hyperglycemia increases vascular adrenomedullin expression. *Biochem Biophys Res Commun* 258:453–456, 1999
11. Lewis LK, Smith MW, Yandle TG, Richards AM, Nicholls MG: Adrenomedullin(1–52) measured in human plasma by radioimmunoassay: plasma concentration, adsorption, and storage. *Clin Chem* 44:571–577, 1998
12. Meeran K, O'Shea D, Upton PD, Small CJ, Ghatei MA, Byfield PH, Bloom SR: 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, 1997
13. Kinoshita H, Fujimoto S, Tokura T, Hisanaga S, Kitamura K, Eto T: Plasma mature form of adrenomedullin in diabetic nephropathy. *Intern Med* 40:841–2, 2001
14. Morgenthaler NG, Struck J, Alonso C, Bergmann A: Measurement of midregional proadrenomedullin in plasma with an immunoluminometric assay *Clin Chem* 51:1823–1829, 2005
15. American Diabetes Association: Diagnosis and classification of diabetes mellitus (Position Statement). *Diabetes Care* 29 (Suppl.1):S43–S48, 2006
16. Stevens LA, Coresh J, Greene T, Levey AS: Assessing kidney function: measured and estimated glomerular filtration rate. *N Engl J Med* 354:2473–83, 2006
17. Araki SI, Handa M, Sugimoto T, Isono M, Isshiki K, Kashiwagi A, Koya D: Factors associated with frequent remission of microalbuminuria in patients with type 2 diabetes. *Diabetes* 54:2983–2987, 2005
18. National Health and Nutrition Examination Survey III, 1988–1994. In *Manual for Medical Technicians and Laboratory Procedures Used in NHANES III*. Hyattsville, MD, U.S. Dept. of Health and Human Services, 1997
19. Caballero AE, Saouaf R, Arora S, Lim SC, Smakowski P, Park JY, King GL, LoGerfo FW, Horton ES, Veves A: Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. *Diabetes* 48:1856–1862, 1999
20. Veves A, Saouaf R, Donaghue VM, Moolooly CA, Kistler JA, Giurini JM, Horton ES, Fielding RA: Aerobic exercise capacity remains normal despite impaired endothelial function in the micro- and macrocirculation of physically active IDDM patients. *Diabetes* 46:1846–1852, 1997

21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and B-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 412–419, 1985
22. Farin HM, Abbasi F, Reaven GM: Comparison of body mass index versus waist circumference with the metabolic changes that increase the risk of cardiovascular disease in insulin-resistant individuals. *Am J Cardiol* 98:1053–1056, 2006
23. Brain SD, Grant AD: Vascular actions of calcitonin gene-related peptide and adrenomedullin. *Physiol Rev* 84:903–934, 2004
24. Eto T, Kitamura K, Kato J: Biological and clinical roles of adrenomedullin in circulation control and cardiovascular diseases. *Clin Exp Pharmacol Physiol* 26:371–380, 1999
25. Nishikimi T, Kitamura K, Saito Y, Shimada K, Ishimitsu T, Takamiya M, Kangawa K, Matsuo H, Eto T, Omae T, et al.: Clinical studies on the sites of production and clearance of circulating adrenomedullin in human subjects. *Hypertension* 24:600–604, 1994
26. Kato J, Tsuruda T, Kita T, Kitamura K, Eto T: Adrenomedullin: a protective factor for blood vessels. *Arterioscler Thromb Vasc Biol* 25:2480–2487, 2005
27. Ishimitsu T, Nishikimi T, Saito Y, Kitamura K, Eto T, Kangawa K, Matsuo H, Omae T, Matsuoka H: Plasma levels of adrenomedullin, a newly identified hypotensive peptide, in patients with hypertension and renal failure. *J Clin Invest* 94: 2158–2161, 1994
28. Katsuki A, Sumida Y, Gabazza EC, Murashima S, Urakawa H, Morioka K, Kitagawa N, Tanaka T, Araki-Sasaki R, Hori Y, Nakatani K, Yano Y, Adachi Y: Acute hyperinsulinemia is associated with increased circulating levels of adrenomedullin in patients with type 2 diabetes mellitus. *Eur J Endocrinol* 14:71–75, 2002
29. Katsuki A, Sumida Y, Urakawa H, Gabazza EC, Maruyama N, Morioka K, Kitagawa N, Hori Y, Nakatani K, Yano Y, Adachi Y: Increased oxidative stress is associated with elevated plasma levels of adrenomedullin in hypertensive patients with type 2 diabetes. *Diabetes Care* 26: 1642–1643, 2003
30. Sugo S, Minamino N, Shoji H, Kangawa K, Matsuo H: Effects of vasoactive substances and cAMP related compounds on adrenomedullin production in cultured vascular smooth muscle cells. *FEBS Lett* 369:311–314, 1995
31. Sugo S, Minamino N, Shoji H, Kangawa K, Kitamura K, Eto T, Matsuo H: Interleukin-1, tumor necrosis factor and lipopolysaccharide additively stimulate production of adrenomedullin in vascular smooth muscle cells. *Biochem Biophys Res Commun* 207:25–32, 1995
32. Yki-Jarvinen H: Insulin resistance and endothelial dysfunction. *Best Pract Res Clin Endocrinol Metab* 17:411–430, 2003
33. De Cosmo S, Trevisan R, Minenna A, Vedovato M, Viti R, Santini SA, Dodesini AR, Fioretto P, Trischitta V: Insulin resistance and the cluster of abnormalities related to the metabolic syndrome are associated with reduced glomerular filtration rate in patients with type 2 diabetes. *Diabetes Care* 29:432–434, 2006
34. Kobayashi S, Maesato K, Moriya H, Ohtake T, Ikeda T: Insulin resistance in patients with chronic kidney disease. *Am J Kidney Dis* 45:275–280, 2005
35. Sjöholm A, Nystrom T: Endothelial inflammation in insulin resistance. *Lancet* 365:610–612, 2005
36. Pepys MB, Hirschfield GM: C-reactive protein: a critical update. *J Clin Invest* 111: 1805–1812, 2003
37. Isumi Y, Shoji H, Sugo S, Tochimoto T, Yoshioka M, Kangawa K, Matsuo H, Minamino N: Regulation of adrenomedullin production in rat endothelial cells. *Endocrinology* 139:838–846, 1998
38. Rajala MW, Scherer PE: Minireview: The adipocyte: at the crossroads of energy homeostasis, inflammation, and atherosclerosis. *Endocrinology* 144:3765–3773, 2003
39. Lin J, Hu FB, Curhan G: Serum adiponectin and renal dysfunction in men with type 2 diabetes. *Diabetes Care* 30:239–244, 2007
40. Pistrosch F, Herbrigg K, Kindel B, Passauer J, Fischer S, Gross P: Rosiglitazone improves glomerular hyperfiltration, renal endothelial dysfunction, and microalbuminuria of incipient diabetic nephropathy in patients. *Diabetes* 54:2206–2211, 2005
41. Fried LF, Orchard TJ, Kasiske BL: Effect of lipid reduction on the progression of renal disease: a meta-analysis. *Kidney Int* 59:260–269, 2001
42. Okamura T, Ayajiki K, Kangawa K, Toda N: Mechanism of adrenomedullin-induced relaxation in isolated canine retinal arteries. *Invest Ophthalmol Vis Sci* 38: 56–61, 1997
43. Boussey k, Delaey c, Van de Voorde J: Influence of Adrenomedullin on Tone of Isolated Bovine Retinal Arteries. *Invest Ophthalmol Vis Sci* 45: 552–559, 2004
44. Kaneko Y, Saito M, Mori A, Sakamoto K, Nakahara T, Ishii K: Vasodilator effects of adrenomedullin on retinal arterioles in streptozotocin-induced diabetic rats. *J Ocul Pharmacol Ther* 22:317–322, 2006
45. Dorner GT, Garhöfer G, Huemer KH, Golestani E, Zawinka C, Schmetterer L, Wolzt M: Effects of adrenomedullin on ocular hemodynamic parameters in the choroid and the ophthalmic artery. *Invest Ophthalmol Vis Sci* 44:3947–3951, 2003
46. O'Bryan GT, Hostetter TH: The renal hemodynamic basis of diabetic nephropathy. *Semin Nephrol* 17:93–100, 1997
47. Hostetter TH: Diabetic nephropathy: metabolic versus hemodynamic considerations. *Diabetes Care* 15:1205–1215, 1992
48. O'Donnell MP, Kasiske BL, Keane WF: Glomerular hemodynamic and structural alterations in experimental diabetes mellitus. *FASEB J* 2:2339–2347, 1988
49. Lim SC, Caballero AE, Smakowski P, LoGerfo FW, Horton ES, Veves A: Soluble intercellular adhesion molecule, vascular cell adhesion molecule, and impaired microvascular reactivity are early markers of vasculopathy in type 2 diabetic individuals without microalbuminuria. *Diabetes Care* 22:1865–1870, 1999