Regulatory Peptides 158 (2009) 127–131

Contents lists available at ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep

# Increased plasma levels of the mature and intermediate forms of adrenomedullin in obesity

Ikuo Nomura [a], Johji Kato [b,*], Mariko Tokashiki [a], Kazuo Kitamura [a]

[a] Department of Internal Medicine, Circulatory and Body Fluid Regulation, Faculty of Medicine, University of Miyazaki, Kiyotake, Miyazaki 889-1692, Japan
[b] Frontier Science Research Center, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan

ARTICLE INFO

*Article history:*
Received 23 April 2009
Received in revised form 20 June 2009
Accepted 16 August 2009
Available online 23 August 2009

*Keywords:*
Body mass index
Insulin sensitivity
Metabolic disorder

ABSTRACT

Adrenomedullin (AM) is a cardiovascular protective peptide produced in various organs and tissues including adipose tissue. In the present study, we measured the plasma AM levels of subjects with or without obesity by two assay methods to separately evaluate the biologically active AM–$NH_2$ and the intermediate form of AM–glycine (AM–Gly). We measured the total AM and AM–$NH_2$ levels of plasma in 52 obese and 172 non-obese residents of a Japanese community, who received regular health check-ups and had no overt cardiovascular disease. AM–Gly values were obtained by subtracting AM–$NH_2$ levels from those of total AM. Both the AM–$NH_2$ and AM–Gly levels of the subjects with obesity were higher than those without obesity, and significant relationships were noted between body mass index (BMI) and the plasma levels of the two molecular forms of AM in a simple regression analysis. Moreover, the significant factors identified by multivariate analyses were BMI and serum triglyceride for AM–$NH_2$ and diastolic blood pressure, insulin, high-density lipoprotein-cholesterol, and plasma renin activity for AM–Gly. These results suggest active roles for the two molecular forms of AM in metabolic disorders associated with obesity in subjects without overt cardiovascular disease.



## 1. Introduction

Consisting of 52 amino-acid residues, adrenomedullin (AM) is a biologically active peptide that exerts a wide range of actions including vasodilatation, improvement of vascular endothelial function, inhibition of cardiovascular remodeling, modulation of adipogenesis, and alleviation of insulin resistance [1–7]. Although it was initially isolated from pheochromocytoma tissue, the AM peptide is produced in various organs such as the adrenal medulla, heart, blood vessels, and adipose tissue [1–9]. AM also circulates in human blood, and plasma AM levels were found to be elevated in patients with hypertension, obesity, heart failure, acute myocardial infarction, and atherosclerotic vascular diseases [2–4,10–13]. Based on its biological actions, AM is assumed to participate in mechanisms that act against the development or progression of metabolic or cardiovascular diseases. In the biosynthesis of AM peptide, the intermediate form AM–glycine (AM–Gly) is processed from the AM precursor proAM, a 126 amino-acid peptide, and then the biologically active mature form of AM–$NH_2$ is produced by the action of amidation enzymes [2–4]. We previously reported that AM in the human blood consists of two molecular forms, the mature and intermediate AM peptides [14]. There have been a number of reports on AM measurement in human plasma; however, the assay methods used in most of these studies were unable to measure the mature and intermediate AM levels separately [2–4,14]. In the present study, we measured plasma AM levels with two types of immunoreactive radiometric assays (IRMA) to detect AM–$NH_2$ and AM–Gly in non-obese and obese residents of a Japanese community without overt cardiovascular disease. We then compared these AM levels with other clinical parameters, to study the pathophysiological role of AM in obesity-related metabolic disorders.

## 2. Materials and methods

### 2.1. Study subjects and protocol

Local residents of the Kiyotake area, Miyazaki, Japan, who underwent an annual regular health check-up in 2007 were examined for this study (81 males and 143 females; 60.8 ± 9.8 years, mean ± SD). Upon visiting the community center of Kiyotake town, the medical history of the subjects was taken by nurses and confirmed by physicians. The subjects enrolled were determined to have no overt cardiovascular diseases such as ischemic heart disease, congestive heart failure, or stroke from their medical history and physical examination, and those given glucose-lowering agents for diabetes mellitus were excluded from this study to allow the precise evaluation of insulin-sensitivity. Obesity was defined as a body mass index (BMI) of 25 $Kg/m^2$ or higher, according to the criteria of the Japan Society for the Study of Obesity [15]. Blood pressure was measured with an oscillometric automatic device (BP-103iII, Colin, Japan) in a sitting

* Corresponding author. Tel.: +81 985 85 9718; fax: +81 985 85 6596.
*E-mail address:* jkjpn@med.miyazaki-u.ac.jp (J. Kato).

0167-0115/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2009.08.003

**Table 1**
The basal profile of the subjects examined in this study.

| | Non-obese | Obese |
|---|---|---|
| Male/female (*n*) | 57/115 | 24/28 |
| Age (year) | 60.5 ± 9.7 | 61.5 ± 10.3 |
| SBP (mmHg) | 123 ± 18 | 134 ± 17** |
| DBP (mmHg) | 74 ± 11 | 79 ± 10** |
| BMI (kg/m$^2$) | 21.4 ± 2.0 | 27.5 ± 3.3** |
| Serum creatinine (mg/dl) | 0.71 ± 0.14 | 0.76 ± 0.19 |

SBP and DBP: systolic and diastolic blood pressure; BMI: body mass index; mean ± SD; **$P<0.01$.

**Table 2**
Metabolic and humoral factors.

| | Non-obese | Obese |
|---|---|---|
| Fasting blood glucose (mg/dl) | 96 ± 16 | 106 ± 26** |
| Insulin (μIU/ml) | 4.3 ± 2.0 | 8.9 ± 5.4** |
| HOMA index | 1.04 ± 0.52 | 2.47 ± 2.40** |
| Cholesterol (mg/dl) | 211 ± 32 | 213 ± 32 |
| HDL-cholesterol (mg/dl) | 64 ± 13 | 56 ± 13** |
| Triglyceride (mg/dl) | 97 ± 54 | 123 ± 86** |
| Plasma renin activity (ng/ml/h) | 0.87 ± 0.64 | 0.88 ± 0.54 |
| Plasma aldosterone (pg/ml) | 87 ± 45 | 92 ± 40 |

HOMA: homeostasis model assessment; HDL: high-density lipoprotein; mean ± SD; **$P<0.01$.

position by experienced nurses, and then blood was drawn from an antecubital vein after overnight fasting. To measure the total and mature AM levels in plasma, blood was collected in tubes with 1.0 mg/mL of EDTA-2Na and 500 kallikrein inhibitory units (KIU)/mL of aprotinin. Plasma was obtained by centrifugation at 3000 rpm for 10 min at 4 °C and stored at −40 °C until the assay.

This study was approved by the Review Committee for Cooperative and Commissioned Research and the Ethics Committee of the University of Miyazaki Faculty of Medicine. All subjects examined gave their written informed consent before participating in this study.

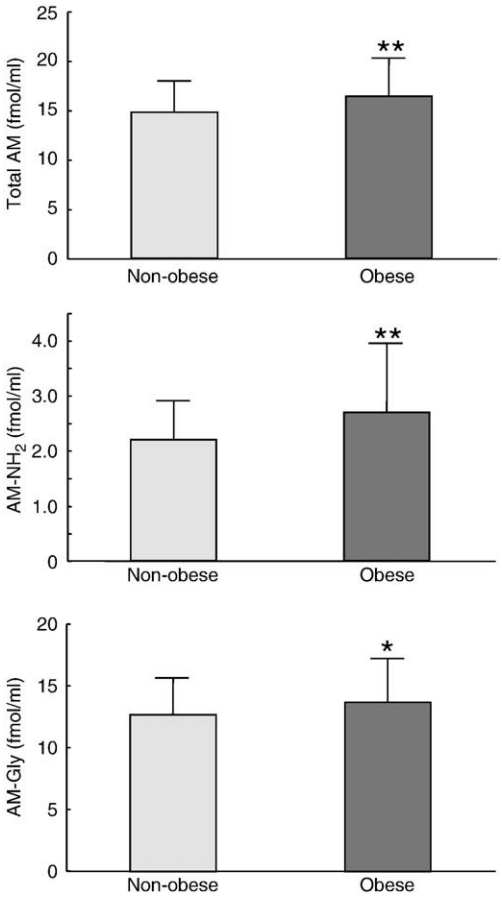


**Fig. 1.** Plasma levels of total AM, AM–$NH_2$, and AM–Gly in non-obese and obese subjects. The data are shown as means ± SD. *$P<0.05$, **$P<0.01$, vs. non-obese subjects.

**Table 3**
Coefficients of correlation obtained by simple regression analysis.

| | AM–$NH_2$ | AM–Gly |
|---|---|---|
| Basal parameters | | |
| Age | 0.142* | n.s. |
| BMI | 0.222** | 0.211** |
| SBP | 0.152* | 0.259** |
| DBP | 0.149* | 0.296** |
| Serum creatinine | n.s. | 0.181** |
| Metabolic or humoral parameters | | |
| Insulin | n.s. | 0.313** |
| HOMA index | n.s. | 0.293** |
| HDL-cholesterol | n.s. | −0.266** |
| Triglycerides | 0.198** | 0.273** |
| Plasma renin activity | n.s. | 0.199** |

The abbreviations are listed in Tables 1 and 2. *$P<0.05$, **$P<0.01$, n.s. = not significant.

## 2.2. Assay procedures

Total and mature AM levels were measured by AM RIA and AM mature RIA (Shionogi Pharmaceutical Co., Ltd., Osaka, Japan), respectively [16,17], and values for the intermediate form AM–Gly were obtained by subtracting the plasma levels of AM–$NH_2$ from those of total AM. The details of these assays are described in the original reports by Ohta et al. and those of others [7,16,17]. Plasma

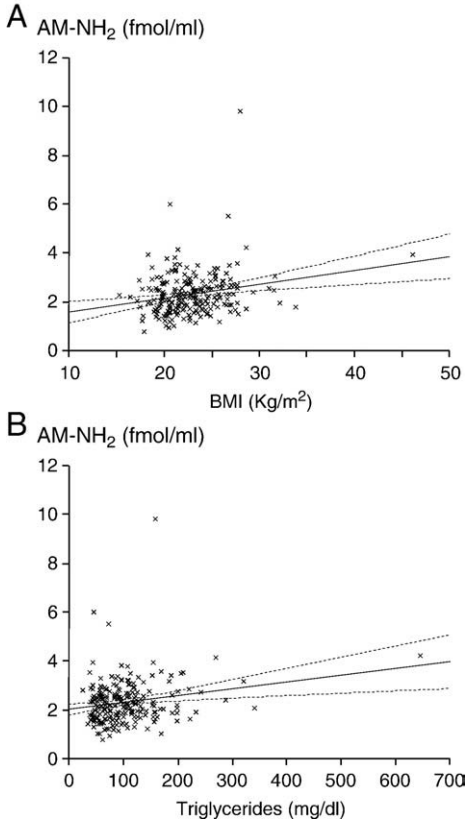


**Fig. 2.** Relationships between the AM–$NH_2$ and BMI (A) or serum triglyceride level (B). Regression lines and 95% confidence limits are shown on each graph.

renin activity and aldosterone concentration were determined by radioimmunoassays as previously reported [11], and serum insulin level was measured by an enzyme immunoassay. Serum lipid and glucose levels were measured with an automatic analyzer (OLYMPUS AU2700, OLYMPUS, Tokyo, Japan), and serum creatinine levels were determined by an enzymatic method.

### 2.3. Statistical analysis

All the data were analyzed with SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). Comparisons between two groups were assessed by the unpaired *t*-test or chi-squared test, and correlations between two parameters were tested by simple regression analysis. The relationships were further analyzed by multiple regression analysis with a step-wise method to identify the factor(s) independently associated with the plasma levels of AM–$NH_2$ and AM–Gly. In this multivariate analysis, the basal or metabolic factors that were found to be significant by simple regression were used as independent covariates. All the data are expressed as means ± S.D., and *P* values of less than 0.05 were considered to be significant.

## 3. Results

In a comparison of the basal clinical data from the non-obese and obese residents (Table 1), no significant differences were noted in gender, age, or serum creatinine levels, while both the systolic and diastolic blood pressure (SBP and DBP) levels of the obese subjects were higher than those of the non-obese subjects. The results of the measurement of metabolic and humoral factors are shown in Table 2, where obesity-related metabolic parameters such as the blood levels of fasting glucose, insulin, high-density lipoprotein-cholesterol (HDL-cholesterol), triglycerides, and the homeostasis model assessment (HOMA) index of the subjects with obesity are shown to be higher than those without obesity, but this was not the case for serum total cholesterol level, plasma renin activity, or aldosterone concentration.

As shown in Fig. 1, the plasma levels of total AM, AM–$NH_2$, and AM–Gly were all significantly elevated in the obese subjects compared with the non-obese subjects. Table 3 shows the coefficients of correlation obtained by simple regression analyses of the relationships between two AM values and other basal, metabolic, or humoral parameters in the subjects. Both the AM–$NH_2$ and AM–Gly level were significantly correlated with BMI, SBP, and DBP, and significant relationships were observed between the AM–$NH_2$ level and age and between the AM–Gly and serum creatinine levels. In the analyses of the metabolic and humoral data, we found that the AM–$NH_2$ level was significantly correlated with serum triglyceride levels, while the AM–Gly level was correlated with all the parameters listed in Table 3. These significant relationships between the two AM values and BMI or metabolic or humoral factors are shown in Figs. 2 and 3 by scattered plotting.

We further analyzed the relationships by means of multivariate analysis with a step-wise method in order to identify the independently significant factors for the two AM values. As shown in Table 4, BMI and the serum triglyceride levels were found to be independently significant for the AM–$NH_2$ level, while DBP, insulin and HDL-cholesterol levels, and plasma renin activity were significant for the AM–Gly level. To look at gender differences in plasma AM levels, we also carried out multivariate analysis including gender in addition to the significant parameters presented in Table 3, but gender was not extracted as a significant factor of the plasma levels of AM–$NH_2$ or AM–Gly.

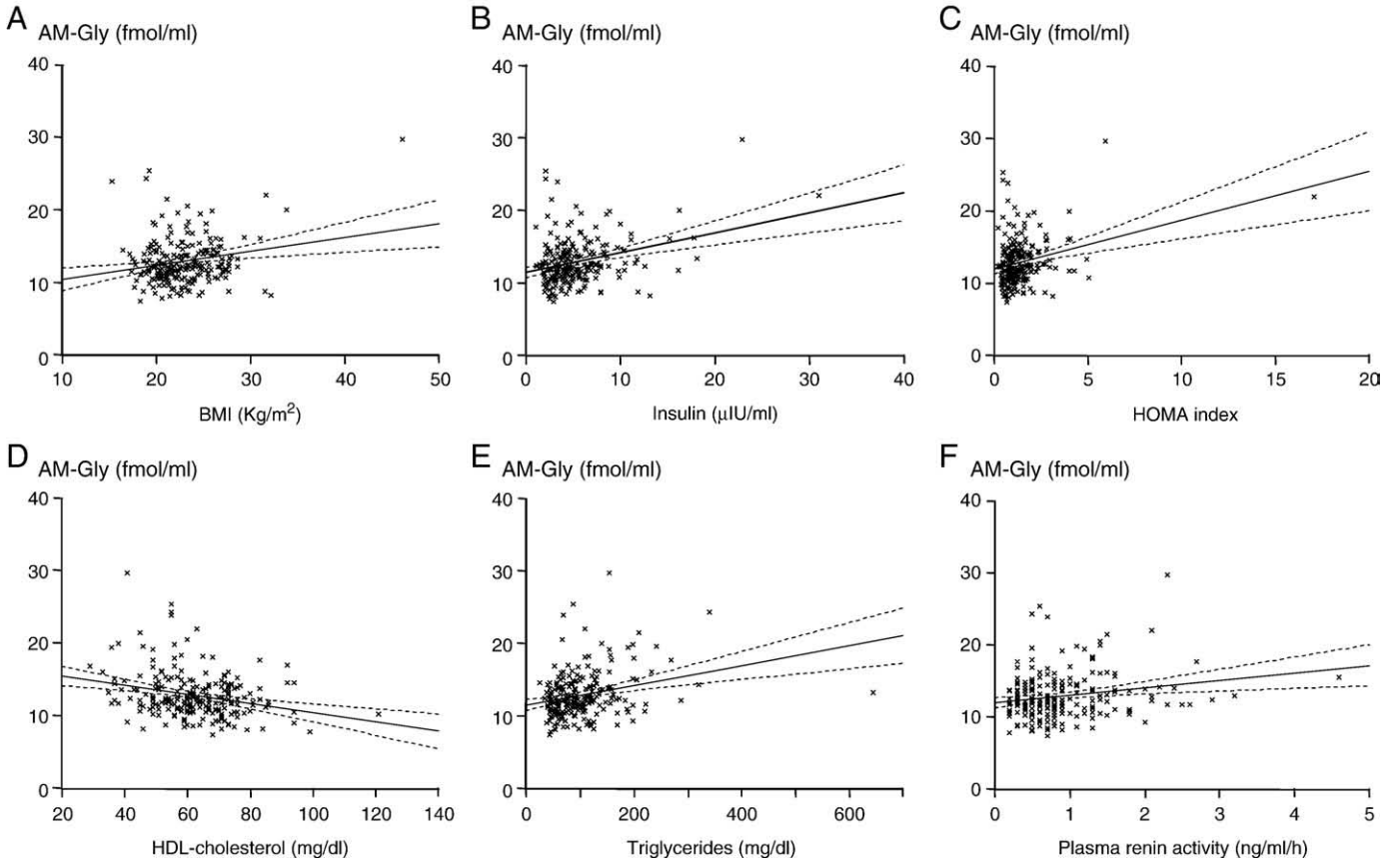


**Fig. 3.** Relationships between the AM–Gly and BMI (A), insulin (B), HOMA index (C), HDL-cholesterol (D), triglyceride (E) levels, or plasma renin activity (F). Regression lines and 95% confidence limits are shown on each graph.

**Table 4**
Multiple regression analysis with a step-wise method.

| Dependent variables | Independent covariates | β | P |
|---|---|---|---|
| AM–$NH_2$ | BMI | .185 | .006 |
| | Triglycerides | .154 | .022 |
| AM–Gly | DBP | .210 | .001 |
| | Insulin | .177 | .008 |
| | HDL-cholesterol | −.178 | .006 |
| | Plasma renin activity | .177 | .005 |

The independent covariates included in this analysis were those judged to be significant by the simple regression analysis (Table 3).
The abbreviations are listed in Tables 1 and 2.

## 4. Discussion

Two molecular forms of AM circulate in the blood of humans and rats; one is a mature form of AM with an amidated C-terminal (AM–$NH_2$), and the other is an intermediate form with a non-amidated C-terminal glycine (AM–Gly) [14,18]. There are a number of reports on AM measurements in plasma and tissue, but in most of these cases, the immunoreactive AM detected was the total AM level consisting of both AM–$NH_2$ and AM–Gly [2–4,14]. Using a radioimmunoassay to detect total AM levels, we have previously reported a significant relationship between plasma AM levels and BMI in subjects who had undergone a regular medical check-up [12], and an obesity-related increase in the plasma AM level has also been reported in an animal model of obesity [8]. In the present study, we measured the plasma levels of AM with two types of immunoreactive radiometric assays that detect total and mature AM, respectively, and found that both the AM–$NH_2$ and AM–Gly levels were elevated in subjects with obesity according to their BMI, as compared to those who were not obese.

Cultured cells isolated from the blood vessels or myocardium have been shown to produce and secrete AM [3,4]. We have previously shown significant increases in the plasma levels of AM–$NH_2$ between the femoral artery and vein and between the aortic root and coronary sinus in patients with ischemic heart disease, suggesting the active secretion of AM–$NH_2$ from the vasculature of the lower extremities and from the heart in humans [19]. In patients with heart failure, the plasma AM–$NH_2$ levels are correlated with the severity of the disease, but not with BMI or obesity [20]. It has been shown that AM–$NH_2$ and AM–Gly are produced and secreted from adipose tissue and cultured adipocytes [7,8,21], a finding that suggests the contribution of adipose tissue to the obesity/BMI-related increase in plasma AM levels. The organs or tissues that contribute to the AM peptides circulating in the human blood may depend on the presence or absence of cardiovascular disease. For example, in patients with heart failure, the cardiac tissue or vasculature appears to be a source of plasma AM, while adipose tissue may contribute to the mature or intermediate forms of AM circulating in the blood of humans without overt cardiovascular disease. It has been reported that the plasma levels of total AM or the mid-regional fragment of the AM precursor peptide are reduced following body weight reduction in obese patients [22,23], supporting the notion of the contribution of adipose tissue to the level of circulating AM peptides. However, further studies are necessary to prove this hypothesis, because the present study is simply a cross-sectional observation.

The mechanism involved in the obesity/BMI-related increase in plasma AM levels remains to be clarified but may be discussed based on previous findings obtained by in vitro studies and on the statistical correlations observed in the present study. According to previous studies using cultured cells, tumor necrosis factor-α (TNF-α) is an important factor for the stimulation of AM production in adipocytes [21]. TNF-α has been shown to be involved in the development of insulin resistance [24,25]. In the present study, the AM–Gly level was related to metabolic parameters of insulin resistance such as the HOMA index, HDL-cholesterol, and triglycerides, suggesting a role for TNF-α in the BMI/obesity-related increase. We may also need to note the significant correlation between the AM–Gly level and plasma renin activity, because the renin–angiotensin system has been shown to be an important factor for stimulating AM production and secretion [2–4]. In the present study, the findings of interest include the differences in the correlation coefficients obtained by simple regression and in the significant factors demonstrated by the multivariate analysis between AM–$NH_2$ and AM–Gly. According to our previous studies [14,26,27], it is possible that the AM–$NH_2$ levels in plasma depend on at least two factors: enzymatic amidation activity and peptide clearance by receptor binding. These factors may have been involved in producing the different results of the simple and multiple regression analyses between AM–$NH_2$ and AM–Gly.

Lastly, we need to discuss the role(s) of AM–$NH_2$ and AM–Gly in plasma, the levels of which were elevated in relation to increased BMI and/or obesity. AM has been shown to have a wide range of actions including blood pressure reduction, modulation of adipogenesis, and alleviation of insulin resistance [2–7]. AM–Gly itself is biologically inactive, but we showed that AM–Gly exerts vasodilatory action following conversion into AM–$NH_2$ in isolated rat aortas ex vivo [27]. In this experiment, AM–Gly took a much longer time to reach the maximal relaxation than did AM–$NH_2$, suggesting a role for AM–Gly as a hormone reservoir. Based on the correlations between the two molecular forms of AM and the other clinical parameters, we speculate that AM acts against the development of a number of obesity-induced disorders such as insulin resistance and elevated blood pressure.

In conclusion, the present study showed that both the AM–$NH_2$ and AM–Gly levels in plasma are elevated in subjects with increased BMI or obesity without overt cardiovascular disease. The correlations between the plasma AM levels and the other clinical parameters suggest a possible, active role for this bioactive peptide in metabolic disorders associated with obesity.

## References

[1] Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, Eto T. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun 1993;192:553–60.
[2] Eto T, Kitamura K, Kato J. Biological and clinical roles of adrenomedullin in circulation control and cardiovascular diseases. Clin Exp Pharmacol Physiol 1999;26:371–80.
[3] Kato J, Tsuruda T, Kitamura K, Eto T. Adrenomedullin: a possible autocrine or paracrine hormone in the cardiac ventricles. Hypertens Res 2003;26(Suppl): S113–9.
[4] Kato J, Tsuruda T, Kita T, Kitamura K, Eto T. Adrenomedullin: a protective factor for blood vessels. Arterioscler Thromb Vasc Biol 2005;25:2480–7.
[5] Shimosawa T, Ogihara T, Matsui H, Asano T, Ando K, Fujita T. Deficiency of adrenomedullin induces insulin resistance by increasing oxidative stress. Hypertension 2003;41:1080–5.
[6] Harmancey R, Senard JM, Rouet P, Pathak A, Smih F. Adrenomedullin inhibits adipogenesis under transcriptional control of insulin. Diabetes 2007;56:553–63.
[7] Iemura-Inaba C, Nishikimi T, Akimoto K, Yoshihara F, Minamino N, Matsuoka H. Role of adrenomedullin system in lipid metabolism and its signaling mechanism in cultured adipocytes. Am J Physiol Regul Integr Comp Physiol 2008;295:R1376–84.
[8] Nambu T, Arai H, Komatsu Y, Yasoda A, Moriyama K, Kanamoto N, Itoh H, Nakao K. Expression of the adrenomedullin gene in adipose tissue. Regul Pept 2005;132: 17–22.
[9] Paulmyer-Lacroix O, Desbriere R, Poggi M, Achard V, Alessi MC, Boudouresque F, Ouafik L, Vuaroqueaux V, Labuhn M, Dutourand A, Grino M. Expression of adrenomedullin in adipose tissue of lean and obese women. Eur J Endocrinol 2006;155:177–85.
[10] Kitamura K, Ichiki Y, Tanaka M, Kawamoto M, Emura J, Sakakibara S, Kangawa K, Matsuo H, Eto T. Immunoreactive adrenomedullin in human plasma. FEBS Lett 1994;341:288–90.
[11] Kato J, Kobayashi K, Etoh T, Tanaka M, Kitamura K, Imamura T, Koiwaya Y, Kangawa K, Eto T. Plasma adrenomedullin concentration in patients with heart failure. J Clin Endocrinol Metab 1996;81:180–3.
[12] Kato J, Kitamura K, Uemura T, Kuwasako K, Kita T, Kangawa K, Eto T. Plasma levels of adrenomedullin and atrial and brain natriuretic peptides in the general population: their relations to age and pulse pressure. Hypertens Res 2002;25: 887–92.
[13] 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 1994;94: 2158–61.

[14] Kitamura K, Kato J, Kawamoto M, Tanaka M, Chino N, Kangawa K, Eto T. The intermediate form of glycine-extended adrenomedullin is the major circulating molecular form in human plasma. Biochem Biophys Res Commun 1998;244: 551–5.
[15] Examination Committee of Criteria for 'Obesity Disease' in Japan. Japan Society for the Study of Obesity: new criteria for 'obesity disease' in Japan. Circ J 2002;66: 987–92.
[16] Ohta H, Tsuji T, Asai S, Sasakura K, Teraoka H, Kitamura K, Kangawa K. One-step direct assay for mature-type adrenomedullin with monoclonal antibodies. Clin Chem 1999;45:244–51.
[17] Ohta H, Tsuji T, Asai S, Tanizaki S, Sasakura K, Teraoka H, Kitamura K, Kangawa K. A simple immunoradiometric assay for measuring the entire molecules of adrenomedullin in human plasma. Clin Chim Acta 1999;287:131–43.
[18] Yamaga J, Hashida S, Kitamura K, Tokashiki M, Aoki T, Inatsu H, Ishikawa N, Kangawa K, Morishita K, Eto T. Direct measurement of glycine-extended adrenomedullin in plasma and tissue using an ultrasensitive immune complex transfer enzyme immunoassay in rats. Hypertens Res 2003;26(Suppl):S45–53.
[19] Hirayama N, Kitamura K, Imamura T, Kato J, Koiwaya Y, Eto T. Secretion and clearance of the mature form of adrenomedullin in humans. Life Sci 1999;64: 2505–9.
[20] Hirayama N, Kitamura K, Imamura T, Kato J, Koiwaya Y, Tsuji T, Kangawa K, Eto T. Molecular forms of circulating adrenomedullin in patients with congestive heart failure. J Endocrinol 1999;160:297–303.
[21] Li Y, Totsune K, Takeda K, Furuyama K, Shibahara S, Takahashi K. Differential expression of adrenomedullin and resistin in 3T3-L1 adipocytes treated with tumor necrosis factor-alpha. Eur J Endocrinol 2003;149:231–8.
[22] Minami J, Nishikimi T, Ishimitsu T, Makino Y, Kawano Y, Takishita S, Kangawa K, Matsuoka H. Effect of a hypocaloric diet on adrenomedullin and natriuretic peptides in obese patients with essential hypertension. J Cardiovasc Pharmacol 2000;36(Suppl 2):S83–6.
[23] Vila G, Riedl M, Maier C, Struck J, Morgenthaler NG, Handisurya A, Prager G, Ludvik B, Clodi M, Luger A. Plasma MR-proADM correlates to BMI and decreases in relation to leptin after gastric bypass surgery. Obesity 2009;17:1184–8.
[24] Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science 1996;271:665–8.
[25] Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 1997;389: 610–4.
[26] Ishiyama Y, Kitamura K, Ichiki Y, Sakata J, Kida O, Kangawa K, Eto T. Haemodynamic responses to rat adrenomedullin in anaesthetized spontaneously hypertensive rats. Clin Exp Pharmacol Physiol 1995;22:614–8.
[27] Cao YN, Kitamura K, Ito K, Kato J, Hashida S, Morishita K, Eto T. Glycine-extended adrenomedullin exerts vasodilator effect through amidation in the rat aorta. Regul Pept 2003;113:109–14.