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ORIGINAL ARTICLE

HYPERINSULINEMIA LEADS THE ELEVATION OF PLASMA ALDOSTERONE CONCENTRATION INDEPENDENTLY WITH OBESITY, DYSLIPIDEMIA, AND INSULIN RESISTANCE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Abstract The study was to define plasma insulin level with the association of renin-angiotensin-aldosterone system activity in patients with type 2 diabetes. One hundred fifty type 2 diabetic patients and 24 non-diabetic subjects were studied. There was no statistical difference between the two groups in age, gender, the prevalence of hypertension, and serum potassium concentration. Plasma aldosterone concentration (PAC) was significantly higher in type 2 diabetics than that in non-diabetic subjects (10.4 ± 4.9 vs. 7.4 ± 3.7 ng/dl ; p=0.004) ; however plasma renin activity (PRA) did not differ significantly between the two groups. In diabetic patients, PAC correlated significantly with body mass index (BMI), fasting plasma insulin (F-IRI), homeostasis model assessment insulin resistance (HOMA-R), urinary C-peptide excretion (U-CPR), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C). PRA correlated significantly with F-IRI and HOMA-R, but did not correlate with BMI, U-CPR, TG, and HDL-C. The additional contribution of U-CPR in predicting PAC was significant after adjustment for age, BMI, F-IRI, TG, HDL-C, and PRA (β=0.204, p=0.016). These findings indicate that hyperinsulinemia may affect the increase in PAC unrelated with obesity, dyslipidemia, insulin resistance that are components of metabolic syndrome in patients with type 2 diabetes.

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Key words: type 2 diabetes mellitus; hyperinsulinemia; insulin resistance; plasma renin activity; plasma aldosterone concentration.

原　著

2 型糖尿病患者において高インスリン血症は肥満，脂質異常症，インスリン抵抗性とは独立して血漿アルドステロン濃度上昇に関与している

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抄録　2 型糖尿病 (DM) 患者におけるレニン－アンジオテンシン－アルドステロン系と高インスリン血症の関係ついて検討した．2 型 DM 患者において，血漿アルドステロン濃度 (PAC) は body mass index (BMI)，空腹時インスリン濃度 (F-IRI)，HOMA 指数，尿中 C ペプチド排泄量 (U-CPR)，中性脂肪 (TG) 値，高比重リポ蛋白－コレステロール (HDL-C) 値との間に有意な相関関係が認められた．血漿レニン活性 (PRA) については，F-IRI，HOMA 指数との間にのみ有意な相関関係が認められた．さらに，目的変数を PAC，説明変数を年齢，BMI，F-IRI，U-CPR，TG，HDL-C，PRA として多変量解析を行うと，U-CPR は PAC の独立した説明変数であることが示された．以上から，2 型 DM 患者において，高インスリン血症は肥満，脂質異常症，インスリン抵抗性とは独立して PAC 上昇に関与していると考えられた．

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キーワード： 2 型糖尿病；高インスリン血症；インスリン抵抗性；血漿レニン活性；血漿アルドステロン濃度．

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Introduction

The renin-angiotensin-aldosterone system (RAAS) plays an important role in the onset of hypertension, as well as in the progression of cardiovascular[1] and renal diseases[2]. Recent studies have suggested that the disorder of RAAS is closely associated with the metabolic syndrome (MetS)[3-5], with insulin resistance. Angiotensinogen (AGT) is the substrate of renin in the RAAS and is converted into angiotensin I (Ang I) which is the precursor of angiotensin II (Ang II). Moreover, AGT has been shown to be expressed in adipose tissue and has a possible pathophysiological role in the onset of obesity-related hypertension[6]. Ang II, which is a well-known physiological regulator of aldosterone biosynthesis, is the main hypertensive substance in RAAS and has been strongly implicated in the pathogenesis of micro- and macro-vascular diseases[7-8].

The Framingham Offspring Study[9] examined the relation of the biomarker panel to incidence of MetS. After adjustment for clinical risk factors, plasma levels of aldosterone and plasminogen activator inhibitor-1 were associated with the incidence of MetS. Engeli et al.[10] reported that levels of AGT, renin, and aldosterone were elevated in obese subjects, and that weight loss reduced these substances. Massiera et al. [11] has shown that overexpression of AGT in adipose tissue in transgenic mice results in significant elevation of blood pressure (BP), and suggested it contributed to obesity-related hypertension. These facts suggest that in obese subjects, including MetS, adipocyte-derived AGT plays an important role in the onset of hypertension. Hyperinsulinemia is frequently associated with insulin resistance, and also causes excessive action in kidney, arterial wall, adipose tissue, and sympathetic nervous system. Furthermore, Rocchini et al.[12] demonstrated that hyperinsulinemia increased the Ang II-mediated secretion of aldosterone in dogs. These results suggest that insulin plays an important role in the regulation of aldosterone secretion.

The onset of type 2 diabetes requires a deficiency in insulin secretion[13] and deterioration in insulin resistance. The association of insulin action with RAAS has not been fully elucidated in patients with type 2 diabetes. In the present study, we examined RAAS activity in patients with type 2 diabetes with corresponding assessment of insulin secretion and resistance.

Materials and Methods

One hundred fifty patients with type 2 diabetes (93 males, 57 females) and 24 non-diabetic subjects (15 males, 9 females) who have no endocrine diseases, such as Cushing syndrome, pheochromocytoma, and primary aldosteronism, were studied. All subjects were hospitalized in Hirosaki University Hospital and put on a regular salt containing diet (NaCl 9 g/day), and dietary therapy administered provided less than 30 kcal/kg of ideal body weight.

After hospitalization, all subjects were assessed for weight, height and body mass index (BMI). Patients taking Ang I-converting-enzyme inhibitor (ACE-I), Ang II type 1 receptor (AT1R) blocker (ARB), diuretics, β-blocker, aldosterone antagonist, oral hypoglycemic agents, insulin, and incretin related agents were excluded. Patients with serum creatinine ≧ 1.1 mg/dl were excluded. After an overnight fast, we measured systolic blood pressure (SBP) and diastolic BP (DBP), and blood samples were obtained from the subjects in a quiet room after lying on the bed for over 30 min. PRA, plasma aldosterone concentration (PAC) and plasma insulin level (IRI) were measured by radioimmunoassay. Homeostasis model assessment insulin resistance (HOMA-R)

Table 1 Clinical parameters of type 2 diabetics and non-diabetic subjects

	DM	non-DM	*p* value
n(male/female)	150(93/57)	24(15/9)	NS
Age(years old)	56.9±13.1	51.3±15.9	NS
BMI(kg/m^2)	27.6±5.7	24.9±4.6	0.037
Hypertension(%)	43.3	38.2	NS
K(mEq/l)	3.96±0.35	3.97±0.28	NS
PRA(ng/ml/h)	1.6±1.6	1.3±0.7	NS
PAC(ng/dl)	10.4±4.9	7.4±3.7	0.004
ARR	11.7±10.8	7.3±5.8	NS

Results are expressed as mean±SD.
Abbreviations : BMI=body mass index, PRA=plasma renin activity, PAC=plasma aldosterone concentration, ARR=PAC/PRA ratio.

was calculated from fasting plasma glucose (FPG, mg/dl) and IRI(μU/ml) using the following formula : [FPG×F-IRI／405]. HbA1c was determined by high performance liquid chromatography. Plasma triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) concentrations were determined by using enzymatic colorimetric kits. Low-density lipoprotein(LDL)-C concentration was calculated by Friedewald's expression. The initial designation of hypertension was based on a screening SBP≧140 mmHg and/or DBP≧90 mmHg or taking antihypertensive medications. C-peptide in urine has been used to evaluate endogenous insulin secretion[14]. We measured 24-hour urinary C-peptide excretion(U-CPR) repeated for 3 days. C-peptide was measured by immunocheminometric assay method.

Diabetic nephropathy was classed according to 24-hour urinary albumin excretion(UAE) monitored for 3 days(stage 1, UAE<30 mg/day ; stage 2, UAE=30～300 mg/day ; stage 3, UAE>300 mg/day). UAE was analyzed by immunoturbidimetric assay method.

Values were expressed as the mean±SD. Differences in numerical data among two groups were evaluated by Mann-Whitney's U test. Correlations between the covariates for RAAS and the parameters in diabetes were calculated by Spearman's rank correlations. Multiple variable regression analysis was used to estimate the association between PAC and U-CPR after adjustment for age, BMI, F-IRI, HOMA-R, TG, HDL-C, and PRA in subsequent models.

Results

1. Clinical parameters in type 2 diabetes and non-diabetic subjects

The clinical parameters of type 2 diabetic patients and non-diabetic subjects in the present study are outlined in Table 1 and Table 2, respectively. There was no statistical difference between these two groups in age, gender, the prevalence of hypertension, and serum potassium concentration. PAC in type 2 diabetics(10.4±4.9 ng/dl) was significantly higher than in the non-diabetic subjects(7.4±3.7 ng/dl)(p=0.004) ; however, PRA and PAC/PRA ratio(ARR) did not differ significantly between the groups.

2. Correlation between the covariates of RAAS and parameters in patients with type 2 diabetes(Table 3)

PAC demonstrated significant correlation with age(rs=−0.289, $p<0.01$), BMI(rs=0.168, $p<0.05$), F-IRI(rs=0.217, $p<0.05$), HOMA-R (rs=0.184, $p<0.05$), U-CPR(rs=0.295, $p<0.01$,

Table 2 Clinical characteristics of patients with type 2 diabetes

N(Male／Female)	150(93／57)
Age(years old)	51.3 ± 15.9
Duration(years)	4.5 ± 5.7
BMI(kg/m^2)	27.6 ± 5.7
HbA1c(%)	8.6 ± 2.1
F-IRI(μU/ml)	9.0 ± 6.4
HOMA-R	3.1 ± 2.2
U-CPR(μg/day)	83.3 ± 46.6
Hypertension(%)	43.3
Retinopathy(%)	27.3
Nephropathy(%)	24.7
Neuropathy(%)	38.0
TG(mg/dl)	155.4 ± 98.8
HDL-C(mg/dl)	46.0 ± 10.0
LDL-C(mg/dl)	113.7 ± 36.0

Abbreviations : BMI=body mass index, F-IRI=fasting plasma insulin, HOMA-R=homeostasis model assessment insulin resistance, U-CPR=24hr urinary C-peptide excretion, TG=triglyceride, HDL-C=high density lipoprotein cholesterol, LDL-C=low density lipoprotein cholesterol.

Table 3 Correlations between the covariates of renin-angiotensin-aldosterone system and the parameters in type 2 diabetics

	PRA	PAC
Age	−0.326*	−0.289*
BMI	0.176**	0.168**
HbA1c	−0.033	−0.152
F-IRI	0.180**	0.217**
HOMA-R	0.202**	0.184**
U-CPR	0.130	0.295*
Triglyceride	0.074	0.221*
HDL-cholesterol	0.030	−0.197**

*$p<0.01$, **$p<0.05$.
Abbreviations : PRA=plasma renin activity, PAC=plasma aldosterone concentration, ARR=PAC/PRA ratio, BMI=body mass index, F-IRI=fasting plasma insulin, HOMA-R=homeostasis model assessment insulin resistance, U-CPR=24hr urinary C-peptide excretion, HDL=high density lipoprotein.

Fig. 1), TG(rs=0.221, $p<0.01$) and HDL-C(rs=−0.197, $p<0.05$). On the other hand, PRA had significant correlations with age(rs=−0.326, $p<0.01$), BMI(rs=0.176, $p<0.05$), F-IRI(rs=0.180, $p<0.05$) and HOMA-R(rs=0.202, $p<0.05$). However, PRA had no significant correlation with U-CPR, TG, and HDL-C. In non-diabetic subjects, there was no significant correlation between PRA and BMI, PAC and BMI, and ARR and BMI(data not shown).

3. β coefficients for the association between PAC and U-CPR in type 2 diabetics

Table 4 presents the results of regression models that were constructed to assess the

Table 4 β coefficients for the association between PAC and U-CPR in type 2 diabetics

Linear-regression covariates	β coefficient	*p* value
Age, BMI, TG, HDL-C, and F-IRI	0.225	0.008
Age, BMI, TG, HDL-C, F-IRI, and PRA	0.204	0.017
Age, BMI, TG, HDL-C, and HOMA-R	0.215	0.016
Age, BMI, TG, HDL-C, HOMA-R, and PRA	0.200	0.029

β= standardized partial regression coefficient.
Abbreviations : PRA=plasma renin activity, PAC=plasma aldosterone concentration, BMI=body mass index, F-IRI=fasting plasma insulin, U-CPR=24hr urinary C-peptide excretion, TG=triglyceride, HDL-C=high density lipoprotein cholesterol, HOMA-R=homeostasis model assessment insulin resistance.

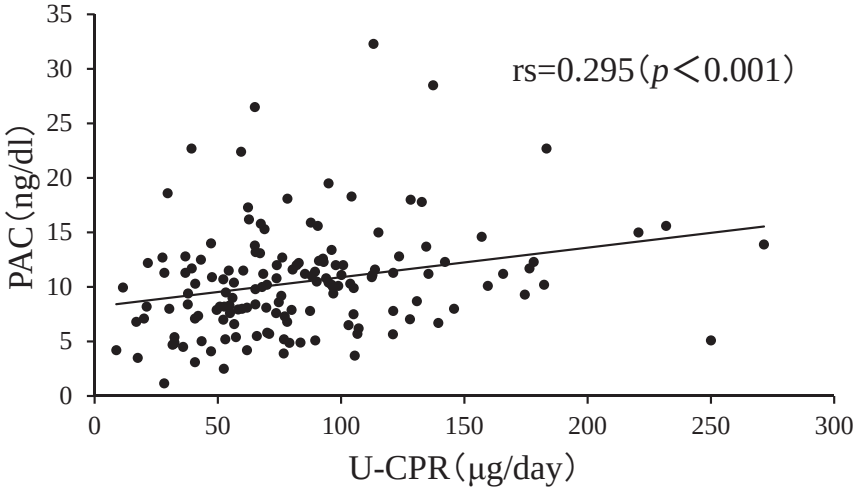

Figure 1 Correlation between PAC and U-CPR in type 2 diabetics
Abbreviations : PAC=plasma aldosterone concentration, U-CPR=24hr urinary C-peptide excretion.

independent contribution of U-CPR in predicting PAC after adjustment for BMI, F-IRI, HOMA-R, TG, HDL-C, and PRA. In each model, the additional contribution of U-CPR in predicting PAC was illustrated after adjustment for the prior parameters. There was significant correlation between PAC and U-CPR after adjustment for age, BMI, TG, HDL-C, and F-IRI (β=0.225, *p*=0.008). This relationship remained robust after the addition of PRA as a covariate (β=0.204, p=0.017). After adjustment for age, BMI, TG, HDL-C, and HOMA-R, there was significant correlation between PAC and U-CPR (β=0.215, *p*=0.016). This relationship remained robust after the addition of PRA as a covariate (β=0.200, *p*=0.029).

Discussion

Many clinical studies have demonstrated that the clustering of risk factors, such as hypertension, dyslipidemia, hyperglycemia, and insulin resistance, could cause and accelerate macrovascular events in patients with type 2 diabetes[15-17]. The prevalence of hypertension in patients with type 2 diabetes was higher than that in non-diabetic subjects, and hypertension was shown as an important risk factor of cardiovascular events in type 2 diabetics in the United Kingdom Prospective Diabetes

Study (UKPDS)[17]. RAAS has been firmly established as a major determinant of BP, and recent studies have suggested that the disorder of RAAS is closely associated with MetS[3-5]. Colussi et al.[18] showed that hyperinsulinemia, evaluated by fasting IRI and plasma C-peptide but not 24-hour urinary C-peptide excretion for endogenous insulin secretion, was related to PAC in hypertensive patients. However, they did not clarify the mechanism of hyperinsulinemia-induced aldosterone secretion in hypertensive patients. The Framingham Offspring Study demonstrated that higher PAC was associated with the development of MetS and with longitudinal changes of its components[9]. The present study was aimed at patients with type 2 diabetes but not patients with MetS or hypertension, and used 24-hour urinary C-peptide excretion to evaluate endogenous insulin secretion. We revealed that there was a significant correlation between PAC and U-CPR after adjustment for age, BMI, TG, HDL-C, HOMA-R, and PRA. This suggests that hyperinsulinemia affects the elevation of PAC unrelated to obesity, dyslipidemia and insulin resistance, which are MetS components in patients with type 2 diabetes. Activation of RAAS is linked to insulin signaling in tissues such as skeletal muscle and liver, as well as in other target tissues including arterial wall, kidney, and sympathetic nerve system. The secretion of aldosterone in humans is mainly regulated through changes in concentration of adrenocorticotropic hormone (ACTH) and AngⅡ. Rocchini et al.[12] demonstrated that euglycemic hyperinsulinemia resulted in significantly greater changes in AngⅡ-stimulated increments of PAC than was observed when AngⅡ was administered alone in dogs. This fact suggests that insulin plays an important role in the regulation of aldosterone secretion. Aldosterone influences epithelial fluid and electrolyte excretion, and elevated aldosterone levels might result in substantial damage of the heart and blood vesseles[1]. Thus, a smaller increase in PAC in patients with type 2 diabetes with hyperinsulinemia might be an important factor that contributes to the increased cardiovascular events.

AGT, converted to AngⅠ and then AngⅡ, serves as unique substance of renin and initiates RAAS activation[6]. AGT is primarily synthesized by the liver, although AGT mRNA is present in several tissues including adipose tissue. Several studies demonstrated that there was a significant correlation between plasma AGT concentrations and BMI in some obese hypertensives, especially patients with excess fat deposit in their abdomen, so called visceral fat obesity[19, 20]. These facts suggest that AGT is an important adipocyte-derived hypertensive substance in obese subjects. In the present study, we demonstrated that PRA significantly correlated with BMI, F-IRI and HOMA-R. However, PRA had no significant correlation with BMI after adjustment of F-IRI (β=0.077, p=0.378) or HOMA-R (β=0.054, p=0.383). Further studies are necessary to elucidate the precise mechanism for the effect of adipose-derived AGT on RAAS activity in type 2 diabetes.

Type 2 diabetes is associated with abnormal structure and metabolisms of circulating lipoproteins, which normally serve as a major source of cholesterol for adrenocortical steroidogenesis. VLDLs, composed of endogenous triglycerides, were demonstrated to have stimulatory effect of aldosterone production in human adrenocortical cells[21]. Moreover, it was demonstrated that modified HDLs, such as glycoxidized HDL, induced a significant increase in scavenger receptor expression and employed protein kinase C as well as extracellular single-regulated kinase as downstream effectors of aldosterone release[22]. In the present study, we revealed that PAC had significant correlations

with TG and HDL-C. These facts suggest that abnormal composition and metabolism of circulating lipoproteins in diabetes may promote adrenocortical aldosterone synthesis and secretion.

In conclusion, the present study demonstrated that PAC in patients with type 2 diabetes was significantly higher than that in the non-diabetic subjects. We revealed the possibility that insulin directly stimulates aldosterone production independently of obesity, dyslipidemia, and insulin resistance, which are MetS components in patients with type 2 diabetes. Thus, hyperinsulinemia may play a certain role in increasing PAC that contributes to cardiovascular events in patients with type 2 diabetes.

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