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# Circulating angiotensin II is associated with body fat accumulation and insulin resistance in obese subjects with type 2 diabetes mellitus

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**Abstract**

Adipocytes express all components of the renin-angiotensin system, and the renin-angiotensin system is involved in obesity and insulin resistance. Circulating angiotensin II (Ang II) is detectable in blood, but its significance in human obesity remains unknown. The aim of this study was to investigate plasma Ang II in obese patients with type 2 diabetes mellitus (T2D) and the change during weight loss. Fifty Japanese obese subjects with T2D (body weight, 75.0 ± 14.1 kg; body mass index, 29.1 ± 3.7 kg/m$^2$; visceral fat area [VFA], 169.3 ± 54.3 cm$^2$; hemoglobin $A_{1c}$, 7.6% ± 1.5%) were enrolled. The subjects were prescribed a diet of daily caloric intake of 20 kcal/kg for 24 weeks. Plasma Ang II was measured by radioimmunoassay. Leptin, adiponectin, and lipoprotein lipase mass in preheparin serum were also measured as adipocyte-derived factors. After 24 weeks of weight reduction diet, the mean body weight, VFA, and hemoglobin $A_{1c}$ decreased significantly by 2.3%, 7.0%, and 8.3%, respectively. The mean plasma Ang II decreased by 24% ($P < .0001$) and correlated with body weight both at baseline ($r = 0.425$, $P = .0018$) and at 24 weeks ($r = 0.332$, $P = .0181$). The change in Ang II correlated with changes in body weight ($r = 0.335$, $P = .0167$) and VFA ($r = 0.329$, $P = .0191$). The change in Ang II also correlated positively with change in leptin ($r = 0.348$, $P = .0127$) and tended to correlate negatively with change in lipoprotein lipase mass in preheparin serum ($r = -0.260$, $P = .0683$), which is a marker of insulin sensitivity. Plasma Ang II is associated with body weight, decreases during weight loss, and is associated with markers of insulin resistance in obese subjects with T2D.


## 1. Introduction

During the last decade, it has become evident that adipocytes are extremely active endocrine cells that secrete important hormones, cytokines, vasoactive substances, and other peptides. Adipocyte-specific or -enriched hormones are now known as *adipokines* and are often actively involved in the regulation of diverse physiologic functions such as energy homeostasis, insulin sensitivity, inflammation, appetite, angiogenesis, and blood pressure. Obesity is a common underlying condition for cardiovascular diseases and coexists with insulin resistance caused by the accumulated adipose tissue [1,2]. Currently, tumor necrosis factor–α, resistin, adiponectin, and leptin are considered to be key adipokines related to insulin resistance in obesity [3-6].

Angiotensin II (Ang II) is an important regulator of blood pressure. Therefore, many angiotensin-converting enzyme (ACE) inhibitors and angiotensin type 1 ($AT_1$) receptor blockers are widely used as antihypertensive drugs. Interestingly, recent clinical trials have revealed that these agents provide other metabolic benefits such as improvement of serum triglycerides level and reduction in new onset of type 2 diabetes mellitus (T2D) [7-11]. We have also reported that the $AT_1$ receptor blocker valsartan enhances a marker of insulin sensitivity, lipoprotein lipase mass in preheparin serum (LPL mass), in diabetic patients with hypertension [11]. In vitro studies showed that inhibition of adipogenic differentiation by Ang II decreased the gene expression of proliferator-activated receptor γ and fatty acid synthase in human preadipocytes [12]. We also reported that the expression of LPL, which is a key regulator of serum triglycerides, was suppressed by inhibition of adipogenic differentiation through activation of the renin-angiotensin

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system (RAS) in 3T3-L1 cells [13]. Therefore, Ang II may have a powerful metabolic effect on adipose tissue in human obesity. On the other hand, it is known that adipocytes express angiotensinogen, the sole precursor of Ang II, as well as renin, ACE, and chymase, which are required for its conversion to Ang II [12,14]. We observed that angiotensinogen expression in 3T3-L1 cells was strongly enhanced with increase in incubation time [13]. Other investigators also verified that adipogenic differentiation was associated with a marked increase in RNA expression of angiotensinogen, renin, and ACE in human preadipocytes [12]. These findings suggest that enlarged adipocytes may consequently produce more Ang II and that the increased Ang II may influence glucose and lipid metabolism in insulin target organs including adipocytes.

Circulating angiotensinogen, renin, aldosterone, and ACE activity have been reported to be elevated in obese human subjects and down-regulated during weight loss [15-19]. Circulating Ang II is also detectable in blood; however, its role in human obesity and insulin resistance is unknown. The aim of this study was to investigate circulating Ang II in obese patients with T2D and the change during weight loss.

Table 1
Various parameters at baseline and end of study in 50 obese patients with T2D

| | Baseline | Wk 24 | *P* value |
|---|---|---|---|
| Age (y) | 51.9 ± 12.3 | – | – |
| Male/female (n) | 16/34 | – | – |
| Body weight (kg) | 75.0 ± 14.1 | 73.3 ± 14.7 | .0100 |
| BMI (kg/m$^2$) | 29.1 ± 3.7 | 28.4 ± 3.9 | .0088 |
| VFA (cm$^2$) | 169.3 ± 54.3 | 157.4 ± 57.8 | .0108 |
| SFA (cm$^2$) | 260.8 ± 95.2 | 246.6 ± 89.6 | .0438 |
| Systolic blood pressure (mm Hg) | 137.0 ± 17.1 | 130.9 ± 15.9 | .0036 |
| Diastolic blood pressure (mm Hg) | 81.6 ± 11.5 | 78.6 ± 9.6 | .0372 |
| Fasting blood glucose (mg/dL) | 165.5 ± 58.8 | 157.1 ± 59.0 | .1773 |
| $HbA_{1c}$ (%) | 7.60 ± 1.55 | 6.97 ± 1.36 | <.0001 |
| Total cholesterol (mg/dL) | 216.0 ± 49.1 | 207.4 ± 35.5 | .2249 |
| Triglyceride (mg/dL) | 159.8 ± 109.2 | 160.2 ± 97.8 | .9715 |
| HDL cholesterol (mg/dL) | 53.3 ± 12.7 | 52.2 ± 11.0 | .4463 |
| LDL cholesterol (mg/dL) | 130.7 ± 40.3 | 123.1 ± 32.4 | .2265 |
| LPL mass in preheparin serum (ng/mL) | 48.7 ± 17.8 | 51.2 ± 18.7 | .1234 |
| Leptin (ng/mL) | 11.2 ± 6.6 | 10.7 ± 4.8 | .3832 |
| Adiponectin (μg/mL) | 6.72 ± 4.14 | 6.80 ± 3.66 | .8347 |
| Plasma Ang II (pg/dL) | 7.92 ± 3.37 | 6.02 ± 2.23 | <.0001 |

Values are expressed as mean ± SD. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.

## 2. Materials and methods

### 2.1. Subjects

The study group comprised 50 (16 men and 34 women) Japanese adult obese subjects (body mass index [BMI], 25.1-42.9 kg/m$^2$) with T2D who attended the Center of Diabetes, Endocrine, and Metabolism, Toho University Sakura Medical Center. In this study, *obesity* was defined as a BMI greater than 25 kg/m$^2$ according to the criteria for obesity in Japan [20]. Patients with diabetic retinopathy, renal dysfunction (including diabetic nephropathy), congestive heart failure, coronary heart disease, and abnormal liver dysfunction (transaminases greater than twice the upper limit of normal) were excluded. The other exclusion criterion was serious systemic diseases such as acute/chronic inflammations and malignancies. Baseline characteristics of these patients are shown in Table 1. The patients had been prescribed a diet of daily caloric intake of 25 to 30 kcal/kg at least 3 months before the study. At the start of the study, the subjects were prescribed a diet of daily caloric intake of 20 kcal/kg for 24 weeks. With the aim to achieve and maintain the above energy intake, clinical nutritionists gave detailed individual counseling based on an interview and body weight/food records kept by the subjects. Among 50 subjects, 18, 31, and 25 were under treatment with antihypertensive, antihyperlipidemic, and/or antidiabetic agent, respectively. Drugs that had been taken for more than 2 months before the study were continued during this study, subjected to the condition that the doses remained unchanged. However, patients treated with ACE inhibitor and/or $AT_1$ receptor blocker were excluded because plasma Ang II is highly sensitive to these drugs. Drugs for diabetes mellitus such as insulin and sulfonylureas were reduced or discontinued when hypoglycemia occurred. The study was approved by the institutional review board, and all patients provided written informed consent before participation in the study.

### 2.2. Body weight, body fat distribution, blood pressure, and blood sampling

Body weight was measured after an overnight fast. Visceral fat area (VFA) was determined using computed tomography. The computed tomographic scan was performed at the umbilical level with the subject resting in supine position. The subcutaneous fat area (SFA) was calculated by subtracting VFA from total fat area. Blood pressure was measured in a sitting position after 5-minute rest in the morning. Blood samples were taken in the morning after 12 hours of fasting and 30 minutes of rest in supine position. For plasma Ang II and hemoglobin $A_{1c}$ ($HbA_{1c}$) measurements, blood was collected in an EDTA tube. Serum was separated within 1 hour for LPL mass measurement, blood glucose, serum lipoprotein, and other analyses. The samples for plasma Ang II and LPL mass measurement were frozen at −80°C.

### 2.3. Plasma Ang II assay

Plasma Ang II was measured by a radioimmunoassay (RIA) (Mitsubishi Chemical Medience, Tokyo, Japan) [21]. In brief, 0.5-mL plasma samples were collected into syringes containing 2.5 mL EDTA and prechilled ethanol. The samples were centrifuged and extracted. After purification of the samples, immunoreactive Ang II was measured in

duplicate by RIA using anti–Ang II antiserum followed by iodinated Ang II ($^{125}$I-Ang II) and goat anti-rabbit antiserum as the second antibody.

### 2.4. LPL mass assay

The LPL mass was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using a specific monoclonal antibody against bovine milk LPL, as described by Kobayashi et al [22]. A commercial kit from Daiichi Pure Chemicals (Tokyo, Japan) was used in this study. In this assay system, linearity was observed from 5 to 400 ng/mL. Within-run coefficient of variation was 2.8%, and between-day coefficient of variation was 4.3%.

### 2.5. Measurement of leptin and adiponectin

Plasma leptin was measured by RIA using the Human Leptin RIA Kit (Linco Research, St Charles, MO). The RIA method was developed at Linco Research to measure leptin concentrations in human plasma, as reported previously [23]. Plasma adiponectin was measured by an ELISA system (adiponectin ELISA kit, Otsuka Pharmaceutical, Tokushima, Japan), as reported previously [24].

### 2.6. Statistical analysis

The results are expressed as mean ± SD. The SPSS 15.0 software (SPSS, Chicago, IL) was used for all statistical analyses. Paired *t* test was performed to determine whether the differences in levels between baseline and week 24 were statistically significant. A *P* value less than .05 was considered to be significant.

## 3. Results

Table 1 shows the clinical variables of the 50 obese subjects in the study. After the 24-week weight reduction diet, the mean body weight, BMI, VFA, and SFA decreased significantly, although the mean weight reduction (2.3%) was less than expected. The decrease in mean VFA (7.0%, $P = .0108$) was greater than that of mean SFA (5.4%, $P = .0438$). The mean systolic blood pressure (4.5%, $P = .0036$), diastolic blood pressure (3.7%, $P = .0372$), and $HbA_{1c}$ (8.3%, $P < .0001$) also decreased significantly during weight loss; however, other glucose and lipid parameters showed no remarkable changes.

### 3.1. Relationship between plasma Ang II and body fat accumulation

The mean plasma Ang II was dramatically reduced by 24.0% accompanying weight loss ($P < .0001$) (Table 1). The change in plasma Ang II correlated with the changes in body weight ($r = 0.335$, $P = .0167$) (Fig. 1A) and VFA ($r = 0.329$, $P = .0191$) (Fig. 1B), but not with change in SFA (Fig. 1C). In a cross-sectional analysis, the mean plasma Ang II correlated with the mean body weight at both baseline ($r = 0.425$, $P = .0018$) (Fig. 2A) and week 24 ($r = 0.332$, $P = .0181$) (Fig. 2B). However, the mean plasma Ang II did not correlate with body fat distribution (data not shown).

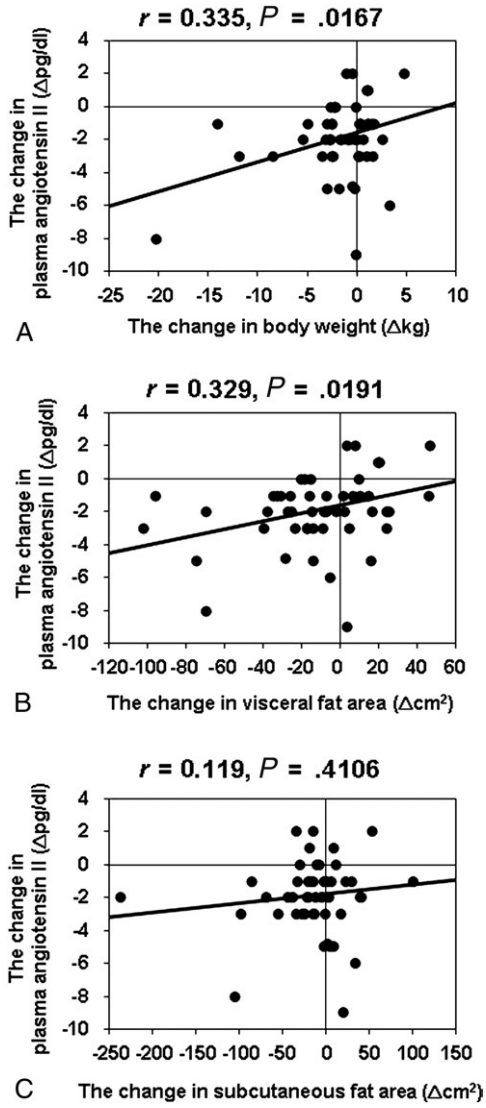


Fig. 1. The relationship between the change in body weight, VFA, SFA, and plasma Ang II in 50 obese subjects with T2D. The scatter plots with regression lines show the data between the change in plasma Ang II and (A) body weight, (B) VFA, and (C) SFA during weight reduction diet.

### 3.2. Relationship between plasma Ang II and blood pressure and blood parameters

No significant correlation was observed between plasma Ang II and blood pressure both at baseline and after the 24-week weight reduction diet (data not shown), and no positive correlation was observed between the change in plasma Ang II and blood pressure during weight loss (Table 2). Although the subjects who were treated with antihypertensive drugs (n = 18) were excluded in this study, there was no correlation between plasma Ang II and blood pressure and also no correlation between the change in plasma Ang II and blood

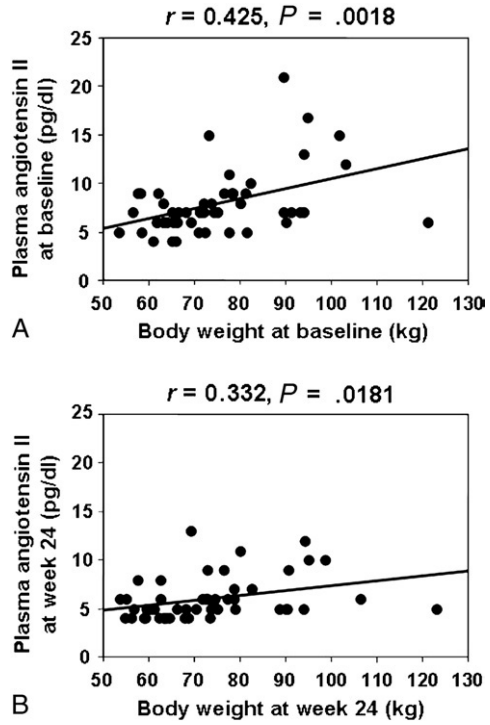


Fig. 2. The relationship between mean body weight and plasma Ang II in 50 obese subjects with T2D. The scatter plots with regression lines show the data at (A) baseline and (B) week 24 of low-calorie weight reduction diet.

pressure (data not shown). Glucose and lipid parameters also did not show any correlation with plasma Ang II.

### 3.3. Relationship between plasma Ang II and adipocyte-derived factors

To investigate the relationship between plasma Ang II and adipocyte function, we also measured the adipocyte-derived factors LPL mass, leptin, and adiponectin in this study. We and other investigators have reported that LPL mass reflects LPL production in the whole body, particularly in adipocytes, and that low LPL mass reflects an increase in fat accumulation and insulin resistance [25-31].

As described in Table 1, the mean LPL mass and adiponectin increased by 5.1% and 1.2%, respectively, and mean leptin decreased by 4.5% while on weight reduction diet, although these changes ware not significant. In the cross-sectional analysis, all 3 parameters did not correlate with plasma Ang II (data not shown). However, during weight loss, plasma Ang II correlated positively with the change in leptin ($r$ = 0.348, $P$ = .0127) and tended to correlate negatively with the change in LPL mass ($r$ = −0.260, $P$ = .0683) (Table 2). No correlation was observed between the changes in plasma Ang II and adiponectin.

## 4. Discussion

In this study, plasma Ang II was associated with body weight and decreased after the subjects had been on a 24-week weight reduction diet. In addition, the close relationship between plasma Ang II and adipocyte-derived factors LPL mass and leptin suggests that circulating Ang II is associated with adipocyte metabolism. The other components of RAS such as angiotensinogen, renin, aldosterone, and ACE in the blood are regarded as good markers in obese subjects [15-19]. However, circulating Ang II has not been fully investigated in obese subjects. Only 1 study group in Germany has examined the relationship between serum Ang II measured by enzyme immunoassay and weight loss in obese subjects; however, there was no association between them [18]. Therefore, this is the first report that verifies the relationship between circulating Ang II and obesity. In this study, Ang II in plasma was measured by RIA; and it might be one of the reasons why we could verify the relationship. On the other hand, it is clear that the other components of RAS in the blood are good markers in obese subjects as well as plasma Ang II. In future studies, we should examine the relationship between plasma Ang II and the other components of RAS to understand the RAS in obese subjects.

Several lines of evidence support the notion that plasma Ang II reflects insulin resistance. Plasma Ang II correlated with VFA accompanying weight loss in this study. Many studies have referred to the relationship between visceral adiposity and insulin resistance [1,2,29]. The LPL mass tended to correlate negatively with plasma Ang II during weight loss in this study, and the LPL mass has been reported to decrease with increase in VFA and considered to be a marker of insulin sensitivity [22,27-29]. In addition, in vitro studies also support our findings. We reported that Ang II suppressed adipogenic differentiation, leading to reduced LPL expression in 3T3-L1 cells [13]. The German group has also concluded that the antiadipogenic actions of Ang II inhibit further recruitment of preadipocytes and formation of new, insulin-sensitive adipocytes [12]. Interestingly, this study and the German investigation in 3T3-L1 cells and human adipocytes, respectively, both found that angiotensinogen expression was enhanced with increase in cell size. These findings suggest that RAS derived from large

Table 2
Correlation between change in plasma Ang II and changes in levels of various parameters

| | ΔPlasma Ang II | |
|---|---|---|
| | $r$ | $P$ value |
| ΔSystolic blood pressure | −0.187 | .1951 |
| ΔDiastolic blood pressure | −0.304 | .0316 |
| ΔFasting blood glucose | −0.230 | .1080 |
| Δ$HbA_{1c}$ | −0.242 | .0902 |
| ΔTotal cholesterol | 0.063 | .6648 |
| ΔTriglyceride | 0.174 | .2279 |
| ΔHDL cholesterol | −0.180 | .2109 |
| ΔLDL cholesterol | 0.060 | .6810 |
| ΔLPL mass in preheparin serum | −0.260 | .0683 |
| ΔLeptin | 0.348 | .0127 |
| ΔAdiponectin | −0.043 | .7695 |

adipocytes in obese persons may regulate adipocyte-derived factors and insulin resistance. From this point of view, plasma Ang II may be a good marker of the RAS, which is involved in body fat accumulation and insulin resistance–associated obesity.

Plasma Ang II did not correlate with adiponectin despite showing a good relationship with both LPL mass and leptin in this study. Adiponectin is a well-known adipokine and a marker of insulin sensitivity [5]. Furuhashi et al [32] observed that RAS blockade increased adiponectin concentration and improved insulin sensitivity in humans. Our result cannot rule out a relationship between RAS and adiponectin in adipocytes. On the other hand, LPL mass is enhanced by an insulin sensitizer, troglitazone [27]; and low LPL mass correlates with visceral fat accumulation [28], is a biomarker of metabolic syndrome [29], and reflects homeostasis model assessment of insulin resistance [30,31]. These reports clearly suggest that low LPL mass reflects insulin resistance, and we can conclude that plasma Ang II is associated with markers of insulin resistance in obesity.

Angiotensin II has been perceived as an important regulator of blood pressure, and both plasma Ang II and blood pressure decreased significantly in this study. However, no significant correlation between plasma Ang II and blood pressure both at baseline and end of this study and during weight reduction diet was observed. Although the negative correlation was observed between the change in plasma Ang II and diastolic blood pressure, it should be of no significance because the diastolic blood pressure decreased significantly together with the decrease in plasma Ang II during weight loss diet. On the other hand, it is obvious that hypertension is tightly involved in obesity; and hypertension caused by obesity is thought to be related with several factors such as RAS, sympathetic nervous system, renal structural and functional changes, and adipokines [33]. Therefore, we cannot exclude the possibility that blood pressure reduction is associated with suppression of RAS by weight loss; however, we could not detect the relationship between plasma Ang II and blood pressure. There are several articles that have indicated the association between plasma Ang II and blood pressure. Angiotensin-converting enzyme inhibitors reduce blood pressure together with plasma Ang II [34,35], and restricted sodium diet potentiates the decrease in plasma Ang II [36,37]. On the other hand, recent studies have shown that intrarenal Ang II content is much higher than circulating Ang II level and that high intrarenal Ang II levels may contribute to the development of hypertension [38]. Furthermore, a Mexican group has reported that blood pressure correlates positively with intrarenal Ang II, but not with plasma Ang II, in a rat model of salt-sensitive hypertension [39]. These findings suggest that Ang II in the kidney, rather than that in circulating blood, may be vasoactive with local effects.

In this study, the mean body weight was reduced by 2.3% after 24 weeks of low-calorie weight reduction diet. According to a review of 12 studies of medical weight loss intervention using low-calorie diet for 10 to 56 weeks, 5.9% to 25.5% reduction in body weight was achieved [40]. One reason for the less than satisfactory weight reduction in this study may be poor compliance with the low-calorie diet. Another possible reason is that the mean initial BMI of this study population was 29.1 $kg/m^2$, in contrast to 28.7 to 36.9 $kg/m^2$ in the above-mentioned studies. Therefore, we suppose that this insufficient weight reduction is the reason for no drastic changes in adipocyte-derived factors and parameters of glucose and lipid metabolism. In other words, we can emphasize that plasma Ang II is a very sensitive marker during weight loss.

In summary, to investigate whether circulating Ang II reflects body fat accumulation and insulin resistance in an obese state, we studied plasma Ang II in obese patients with T2D on a low-calorie weight reduction diet. Plasma Ang II was associated with body weight and was decreased accompanying mild weight loss, and the change in plasma Ang II correlated positively with change in VFA and negatively with change in leptin. These results suggest that plasma Ang II is associated with body fat accumulation and markers of insulin resistance in obesity.

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