Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 1278 – 1285
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Effects of losartan on serum total and high–molecular weight adiponectin concentrations in hypertensive patients with metabolic syndrome

Toshihiko Uchida[a], Minoru Shimizu[a], Yoshihiko Sakai[a], Tomoki Nakano[b], Kenji Hara[b], Kohzo Takebayashi[b], Teruo Inoue[c], Koichi Node[c], Toshihiko Inukai[b], Kan Takayanagi[a], Yoshimasa Aso[b,*]

[a]*Department of Cardiology, Koshigaya Hospital, Dokkyo Medical University, Saitama 343-8555, Japan*
[b]*Department of Internal Medicine, Koshigaya Hospital, Dokkyo Medical University, Saitama 343-8555, Japan*
[c]*Department of Cardiovascular and Renal Medicine, Saga University Faculty of Medicine, Saga 849-8501, Japan*

Received 27 December 2007; accepted 22 April 2008

Abstract

High–molecular weight (HMW) adiponectin may have the most biologic activity among several isoforms. We investigated long-term effects of losartan on serum concentrations of total and HMW adiponectin in hypertensive patients with metabolic syndrome (MS) by serial measurements over 6 months. Forty hypertensive patients first received 50 mg of losartan. Upward titration of the losartan dose was implemented to reach a target blood pressure of less than 140/90 mm Hg. Serum total adiponectin and HMW adiponectin were measured at study entry (baseline), the 3-month treatment time point, and the end of the 6-month period. Non-HMW adiponectin (ie, medium– and low–molecular weight adiponectin) was calculated as total adiponectin − HMW adiponectin. Diagnosis of MS was done by current standard criteria. In hypertensive patients without MS (n = 21), the serum total adiponectin increased from 9.8 ± 5.4 μg/mL at baseline to 11.1 ± 6.2 μg/mL at 6 months ($P < .01$). Furthermore, the serum total adiponectin was significantly higher at 6 months than at 3 months ($P < .01$). Serum HMW adiponectin also increased from 5.7 ± 3.9 μg/mL at baseline to 6.6 ± 4.4 μg/mL at 6 months ($P < .01$). In hypertensive patients with MS, the serum total adiponectin increased from 6.0 ± 2.7 μg/mL at baseline to 6.7 ± 3.3 μg/mL at 3 months and to 7.0 ± 3.1 μg/mL at 6 months ($P < .01$ for both). Furthermore, the serum HMW adiponectin concentration was significantly higher at 6 months than at 3 months ($P < .001$). However, the serum non-HMW adiponectin concentration did not change during treatment in either group. In conclusion, serum total and HMW adiponectin concentrations increase after 6 months of losartan treatment in hypertensive patients, irrespective of the presence or absence of MS.

1. Introduction

Essential hypertension is associated with insulin resistance, resulting in a high incidence of type 2 diabetes mellitus [1,2]. Furthermore, obese patients with hypertension are in a more insulin-resistant state and have an exaggerated tendency to develop diabetes [3,4]. Several large prospective clinical trials have demonstrated that the angiotensin II receptor blockers (ARBs), including losartan and valsartan, significantly decrease the onset of diabetes in hypertensive patients at high risk for cardiovascular disease (CVD), compared with patients treated with β-blocker or Ca channel blocker [5-7]. Because angiotensin II is associated with impaired insulin signaling, resulting in insulin resistance [8], the blockade of the renin-angiotensin system with ARBs may reduce the incidence of new-onset diabetes in hypertensive patients. Several studies have demonstrated that certain ARBs increase the serum concentrations of total adiponectin in patients with essential hypertension [9,10]. This increased circulating adiponectin may also be associated with improvements in insulin sensitivity after ARB treatment, leading to a reduction in the onset of diabetes in hypertensive obese patients. Based on these findings, ARBs can ameliorate insulin resistance in patients with essential

* Corresponding author. Tel.: +81 48 965 1111; fax: +81 48 965 1127.
E-mail address: yaso@dokkyomed.ac.jp (Y. Aso).

0026-0495/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2008.04.024

hypertension via increasing serum adiponectin or directly blocking angiotensin II signaling [8-10]. However, most studies investigated the short-term effects of ARBs on the serum total adiponectin concentrations after 2 weeks to 3 months of treatment. Thus, no reports have examined the long-term effects of ARB on serum adiponectin concentration in patients with hypertension.

Adiponectin is an adipocyte-specific protein that enhances both insulin sensitivity and lipid metabolism [11,12]. Serum adiponectin concentrations are reduced in patients with type 2 diabetes mellitus [13], metabolic syndrome (MS) [14], or coronary artery disease (CAD) [15]. Thiazolidinediones (TZDs), which are full agonists for peroxisome proliferator–activated receptors (PPARs), increase the gene expression and serum concentrations of adiponectin in humans [16]. Adiponectin circulates in plasma as 3 forms: a trimer (low molecular weight; LMW); a hexamer (trimer-dimer) of medium molecular weight (MMW); and a larger, multimeric, high–molecular weight form (HMW) [17]. Previous studies have reported that HMW adiponectin may be the active form of this protein because changes in the ratio of HMW to total adiponectin after TZD treatment, but not the total adiponectin concentration, were associated with improvement in hepatic insulin sensitivity [18]. Recently, we reported and confirmed that serum concentrations of HMW adiponectin can be measured quantitatively by our novel sandwich enzyme-linked immunosorbent assay (ELISA) system with a monoclonal antibody against human HMW adiponectin, IH7 [19,20]. Moreover, using our novel ELISA, we found that pioglitazone, a TZD, increases predominantly HMW adiponectin as evidenced by an increase in the HMW to total adiponectin ratio, contributing to the insulin-sensitizing effects of pioglitazone treatment [21].

Recently, EXP3179, an active metabolite of losartan, was identified as a partial agonist of PPAR-γ [22]. Peroxisome proliferator–activated receptor γ is a ligand-activated transcription factor that regulates the expression of gene regulating lipid and glucose metabolism [23]. Thiazolidinediones, which are full agonists for PPAR-γ, are powerful insulin sensitizers [23]. Thus, we hypothesize that losartan may increase serum HMW adiponectin via PPAR-γ activation by EXP3179.

We investigated the long-term effects of losartan on serum concentrations of total and HMW adiponectin in hypertensive patients with MS by serial measurements over 6 months. To our knowledge, this is the first study to measure serum concentrations of both total and HMW adiponectin simultaneously, using a novel ELISA assay that detects only HMW adiponectin, after long-term treatment with losartan.

2. Subjects and methods

2.1. Patients

We studied 40 consecutive patients with essential hypertension (16 women and 24 men). We selected hypertensive patients with systolic blood pressure (SBP) exceeding 140 mm Hg and/or diastolic BP (DBP) exceeding 90 mm Hg, irrespective of the use of antihypertensive agents. The patients first received 50 mg of losartan. Upward titration of losartan was implemented to reach a target BP of less than 140/90 mm Hg. Only 2 patients received 100 mg of losartan. We also excluded patients with frank diabetes. None of the patients had any liver disease or severe renal impairment. Patients were followed for 6 months with visits every month.

The patients were divided into 2 groups based on the presence or absence of MS. A diagnosis of MS was based on the modified criteria in the recent "Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)" [24]. *Metabolic syndrome* was defined as the presence of 3 or more of the following 5 components: obesity (waist circumference $\geq$85 cm [men] or $\geq$90 cm [women] or body mass index [BMI] $\geq$25.0); triglyceride >150 mg/dL and/or treatment with fibrates; high-density lipoprotein (HDL) cholesterol <50 mg/DL for women and <40 mg/dL for men; SBP >130 mm Hg, DBP>85 mm Hg, and/or antihypertensive medication; and fasting plasma glucose (FPG) >110 mg/dL.

All patients gave informed consent. The study was approved by the local ethics committee.

2.2. Methods

Blood samples were obtained upon study entry (baseline), at the 3-month treatment period, and at the end of the 6-month period. Venous blood was collected between 8 AM and 9 AM after an overnight fast. Serum and plasma samples were centrifuged at 2500 rpm for 15 minutes, after which the supernatant was stored at −70°C until use.

The serum HMW adiponectin concentration was measured using our novel sandwich ELISA assay based on a monoclonal antibody raised against human HMW adiponectin (Fujirebio, Tokyo, Japan). GBP28, the target antigen, is an HMW adiponectin purified from human serum with gelatin-Cellulofine (Seikagaku, Tokyo, Japan) [19]. The monoclonal antibody IH7, raised against human GBP28, was used as the capture antibody, whereas horseradish peroxidase–conjugated IH7 Fab (POD-IH7) was used as the detecting antibody; and GBP28 was used as the standard [19,20]. In brief, 96 wells of a microtiter plate were coated with anti-HMW adiponectin monoclonal antibody (IH7). One hundred microliters of serum that was diluted 1:441 was placed in each of the 96 wells. IH7 conjugated with horseradish peroxidase was used as the detecting antibody. The contents of the wells were incubated for 30 minutes with tetramethylbenzipine. After the reaction was stopped, the absorbance was measured at 450 nm. Using the same antibody as both the capturing and detecting antibodies, this sandwich ELISA system specifically measures HMW adiponectin in serum [19,20]. The HMW adiponectin

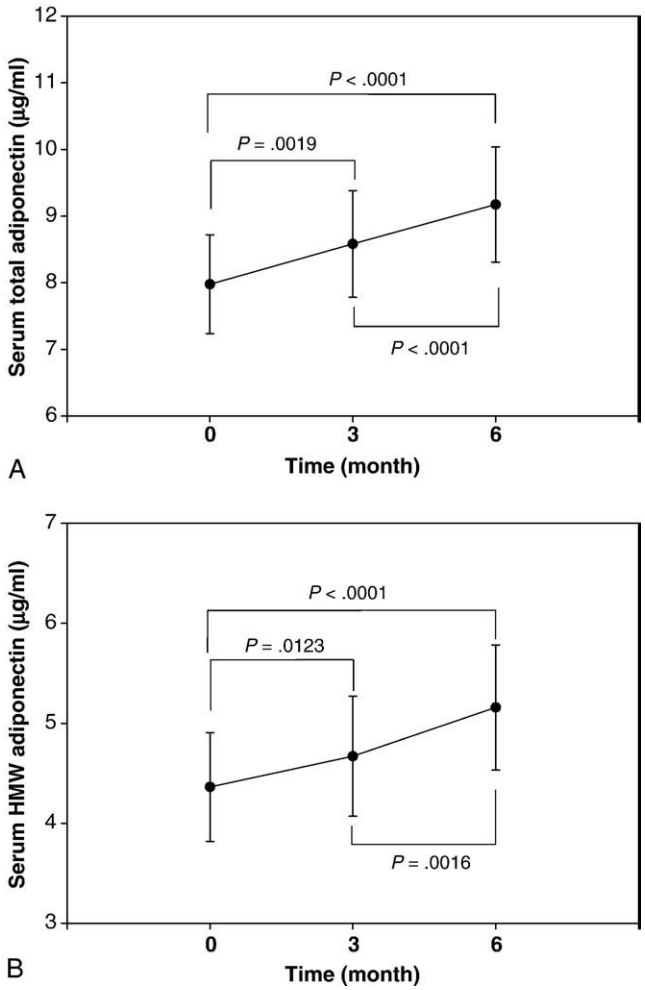

Fig. 1. Changes in serum concentrations of total adiponectin (A) and HMW adiponectin (B) after treatment with losartan in patients with essential hypertension. Data are expressed as the mean ± SEM.

concentrations in a working standard were determined by human HMW adiponectin purified by affinity chromatography using gelatin-Cellulofine [19]. Intra- and interassay coefficients of variation were 2.4% to 3.0% and 4.2% to 5.1%, respectively.

The total serum adiponectin concentration was measured by sandwich ELISA (Otsuka Pharmaceuticals, Tokyo, Japan) as previously described [13]. In brief, after boiling serum samples in sodium dodecyl sulphate buffer for 5 minutes to convert all adiponectin to a monomeric form, samples were analyzed with the ELISA system to determine the total adiponectin concentration in the serum. *Non-HMW adiponectin* (ie, MMW + LMW adiponectin) was defined as total adiponectin − HMW adiponectin.

The plasma concentrations of B-type natriuretic peptide (BNP) were measured by chemiluminescent enzyme immunoassay in EDTA-anticoagulated blood samples (Kyowa Medics, Tokyo, Japan). Serum insulin concentrations were determined by radioimmunoassay. Insulin resistance was evaluated by homeostasis model assessment (HOMA-IR), which is calculated as fasting serum insulin (in microunits per milliliter) × FPG/405.

2.3. Statistical analysis

Data are expressed as the mean ± SD or the median and interquartile ranges unless indicated otherwise. Differences between groups were analyzed by Student paired *t* test or unpaired *t* test. Differences between groups based on nonparametric data were analyzed by Wilcoxon matched-pairs test or the Mann-Whitney *U* test. Differences in normally distributed data were assessed by a 1-way analysis of variance using the Newman-Keuls multiple comparison test. Correlations were determined by linear regression analysis. A P value < .05 was accepted as indicating statistical significance. Statistical analyses were performed using SPSS 8.0 J software (SPSS, Tokyo, Japan).

3. Results

In all of the patients with essential hypertension (N = 40), the serum total adiponectin increased from 8.0 ± 4.7 μg/mL at baseline to 8.6 ± 5.1 μg/mL at 3 months and to 9.2 ± 5.3 μg/mL at 6 months after losartan treatment (P = .0019 and P < .0001, respectively; Fig. 1A). Furthermore, the serum total adiponectin concentration was significantly higher at 6 months than at 3 months (P < .0001). The serum total adiponectin concentration increased 9.1% at 3 months and 16.2% at 6 months. The serum HMW adiponectin concentration also increased from 4.4 ± 3.4 μg/mL

Table 1
Demographic, clinical, and laboratory data for hypertensive patients with or without MS

	No MS	MS	*P* value
n (female/male)	21 (9/12)	19 (7/12)	NS
Age (y)	63.8 ± 8.5	59.6 ± 10.6	.1738
BMI (kg/m^2)	22.9 ± 2.5	25.6 ± 2.6	.0016
SBP (mm Hg)	156.2 ± 22.1	164.3 ± 14.9	.1850
DBP (mm Hg)	89.5 ± 6.5	94.2 ± 9.0	.0657
FPG (mg/dL)	105.7 ± 10.4	111.6 ± 19.3	.2345
HbA_{1c} (%)	5.2 ± 0.3	5.6 ± 0.5	.0122
LDL cholesterol (mg/dL)	124.1 ± 39.1	135.5 ± 34.1	.3328
Triglyceride (mg/dL)	102.0 ± 38.3	245.7 ± 119.9	<.0001
HDL cholesterol (mg/dL)	57.2 ± 11.2	48.8 ± 12.7	.0323
Uric acid (mg/dL)	4.8 ± 1.1	5.2 ± 1.3	.2916
hs-CRP (g/L)	0.047 (0.024, 0.094)	0.092 (0.054, 0.197)	.0255
BNP (pg/mL)	22.5 (12.0, 37.7)	24.9 (15.9, 41.9)	.9784
Total adiponectin (μg/mL)	9.8 ± 5.4	6.0 ± 2.7	.0092
HMW adiponectin (μg/mL)	5.7 ± 3.9	2.9 ± 1.8	.0077
Non-HMW adiponectin (μg/mL)	4.1 ± 2.0	3.1 ± 1.1	.0763
HMW to total adiponectin ratio	0.56 ± 0.15	0.45 ± 0.13	.0170
CCB/D/βB	7/0/1	5/1/2	NS

Data are the mean ± SD or the median and interquartile ranges. NS indicates not significant; CCB, calcium channel blockers; D, diuretics; βB, β-blockers.

Table 2
Patient characteristics and laboratory data in hypertensive patients with or without MS before and after treatment with losartan

	Time (mo)		
	0	3	6
Without MS (n = 21)			
SBP (mm Hg)	156.2 ± 22.1	139.4 ± 13.9†	139.7 ± 14.8†
DBP (mm Hg)	89.5 ± 6.5	81.1 ± 11.9†	84.2 ± 7.5†
FPG (mg/dL)	105.7 ± 10.4	101.4 ± 11.3	98.8 ± 12.5
Insulin (μU/mL)	7.3 (4.6, 15.2)	7.3 (5.4, 12.1)	10.7 (7.2, 16.0)
HOMA-IR	1.6 (1.2, 4.0)	1.7 (1.1, 3.4)	2.4 (1.4, 3.2)
HbA_{1c} (%)	5.4 ± 0.7	5.4 ± 0.8	5.4 ± 0.7
LDL-C (mg/dL)	124.1 ± 39.1	118.8 ± 41.6†	115.7 ± 43.8*
Triglyceride (mg/dL)	102.0 ± 38.3	121.1 ± 67.7	112.1 ± 45.1
HDL-C (mg/dL)	57.2 ± 11.2	56.5 ± 11.4	57.3 ± 11.0
Uric acid (mg/dL)	4.8 ± 1.1	4.8 ± 1.3	4.8 ± 1.2
hs-CRP (mg/L)	0.047 (0.024, 0.094)	0.030 (0.017, 0.072)	0.056 (0.031, 0.116)
BNP (pg/mL)	22.5 (12.0, 37.7)	18.4 (13.6, 43.4)	25.6 (10.0, 45.0)
Non-HMW adiponectin	4.1 ± 2.0	4.2 ± 1.8	4.3 ± 2.0
HMW to total adiponectin ratio	0.56 ± 0.15	0.55 ± 0.14	0.57 ± 0.13
With MS (n = 19)			
SBP (mm Hg)	164.3 ± 14.9	145.7 ± 17.8‡	138.9 ± 13.8‡
DBP (mm Hg)	94.2 ± 9.0	83.3 ± 10.1§	80.9 ± 11.5‡
FPG (mg/dL)	111.6 ± 19.3	109.2 ± 10.2	105.8 ± 17.8
Insulin (μU/mL)	13.2 (8.0, 20.9)	13.5 (8.5, 24.2)	15.7 (13.5, 22.6)
HOMA-IR	3.7 (2.1, 6.6)	3.9 (2.2, 6.6)	4.1 (3.4, 6.6)
HbA_{1c} (%)	5.8 ± 0.8	5.8 ± 0.8	5.9 ± 1.0
LDL-C (mg/dL)	135.5 ± 34.1	129.8 ± 32.1	132.6 ± 38.3
Triglyceride (mg/dL)	245.7 ± 119.9	217.4 ± 96.7	216.7 ± 104.9
HDL-C (mg/dL)	48.8 ± 12.7	49.5 ± 9.8	48.5 ± 10.4
Uric acid (mg/dL)	5.2 ± 1.3	5.1 ± 1.4	5.0 ± 1.3
hs-CRP (mg/L)	0.092 (0.054, 0.197)	0.075 (0.049, 0.186)	0.138 (0.071, 0.172)
BNP (pg/mL)	24.9 (15.9, 41.9)	30.4 (10.3, 46.0)	25.0 (16.8, 32.2)
Non-HMW adiponectin	3.1 ± 1.1	3.5 ± 1.4	3.5 ± 1.1
HMW to total adiponectin ratio	0.45 ± 0.13	0.43 ± 0.14	0.46 ± 0.14

Data are expressed as mean ± SD or median and interquartile range.
* $P < .05$, † $P < .01$, ‡ $P < .001$, and § $P < .0001$ vs 0 month.

at baseline to 4.7 ± 3.8 μg/mL at 3 months and to 5.2 ± 3.9 μg/mL ($P = .0123$ and $P < .0001$, respectively; Fig. 1B) after beginning treatment. Furthermore, the serum HMW adiponectin concentration was significantly higher at 6 months than at 3 months ($P = .0016$). The serum HMW adiponectin concentration increased 7.5% at 3 months and 21.9% at 6 months. However, we found no significant differences in the HMW to total adiponectin ratio between baseline (0.50 ± 0.15) and the values at 3 months (0.49 ± 0.15) and 6 months (0.52 ± 0.14) after treatment initiation.

As shown in Table 1, BMI was higher in hypertensive patients with MS than in those without MS. Hemoglobin A_{1c} (HbA_{1c}) was significantly higher in hypertensive patients with MS than in those without MS. The serum triglyceride and high-sensitivity C-reactive protein (hs-CRP) concentrations were higher in hypertensive patients with MS than in those without MS, whereas the serum HDL cholesterol was lower in hypertensive patients with MS than in those without MS. Both the serum total and HMW adiponectin concentrations were significantly higher in those with MS than in those without MS ($P = .0092$ and $P = .0077$, respectively). Furthermore, the HMW to total adiponectin ratio was higher in those with MS than in those without MS ($P = .0170$).

In hypertensive patients without MS, the SBP decreased from 156.2 ± 22.1 mm Hg at baseline to 139.4 ± 13.9 mm Hg at 3 months and to 139.7 ± 14.8 mm Hg at 6 months ($P < .001$ for both; Table 2). The DBP decreased from 89.5 ± 6.5 mm Hg at baseline to 81.1 ± 11.9 mm Hg at 3 months and to 84.2 ± 7.5 mm Hg at 6 months ($P < .001$ for both). We found no significant changes in FPG, serum insulin, or HOMA-IR after treatment. The serum total and HMW adiponectin concentrations were significantly higher at 6 months after treatment than at baseline or at 3 months after treatment

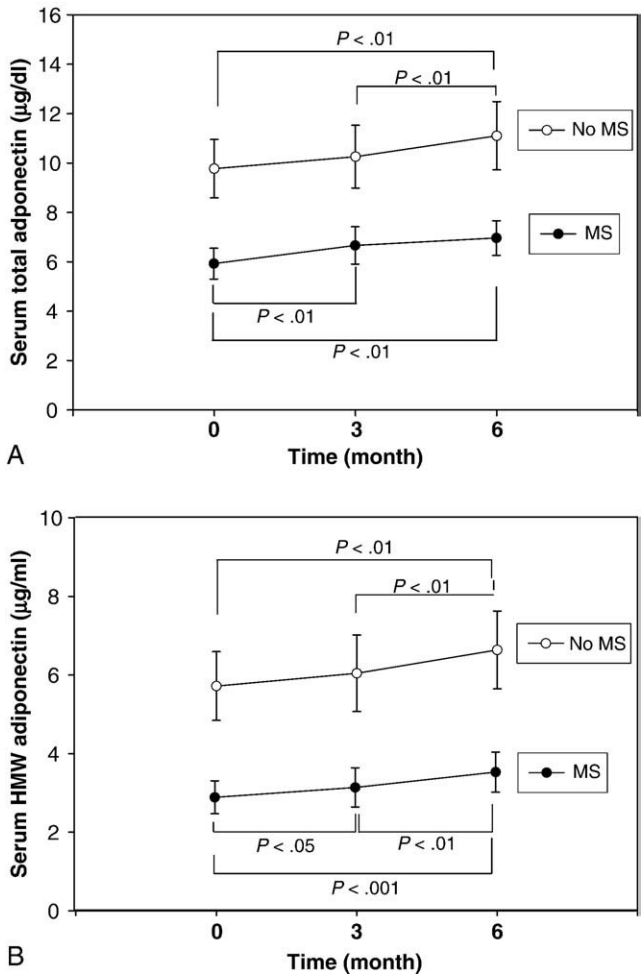

Fig. 2. Changes in the serum concentration of total adiponectin (A) and HMW adiponectin (B) after treatment with losartan in hypertensive patients with (closed circles) and without MS (open circles). Data are expressed as the mean ± SEM.

($P < .01$ for both; Fig. 2A and B). However, the HMW to total adiponectin ratio did not change. On the other hand, in hypertensive patients with MS, the SBP decreased from 163.4 ± 14.9 mm Hg at baseline to 145.7 ± 17.8 mm Hg at 3 months and to 138.9 ± 13.8 mm Hg at 6 months ($P < .001$ for both). The DBP decreased from 94.9 ± 9.0 mm Hg at baseline to 83.3 ± 10.1 mm Hg at 3 months and to 80.9 ± 11.5 mm Hg at 6 months ($P < .0001$ and $P < .001$, respectively). Similar to hypertensive patients without MS, we found no significant changes in FPG, serum insulin, or HOMA-IR after treatment. The serum total and HMW adiponectin concentrations were increased at 3 and 6 months after losartan treatment compared with baseline ($P < .05$ and $P < .01$, respectively; Fig. 2A and B). Furthermore, the serum HMW adiponectin concentration was significantly higher at 6 months than at 3 months after treatment ($P < .001$). However, the serum non-HMW adiponectin concentration did not change during the treatment in both groups. The HMW to total adiponectin ratio also did not change.

The percentage changes in the HMW adiponectin concentration were significantly higher at 6 months and at 3 months than the baseline values in both the group with MS (24.7% vs 9.2%, $P < .05$) and the group without MS (19.1% vs 6.0%, $P < .01$). However, the percentage changes in total and HMW adiponectin after 6 months of treatment with losartan did not differ significantly between the 2 groups (Fig. 3A and B).

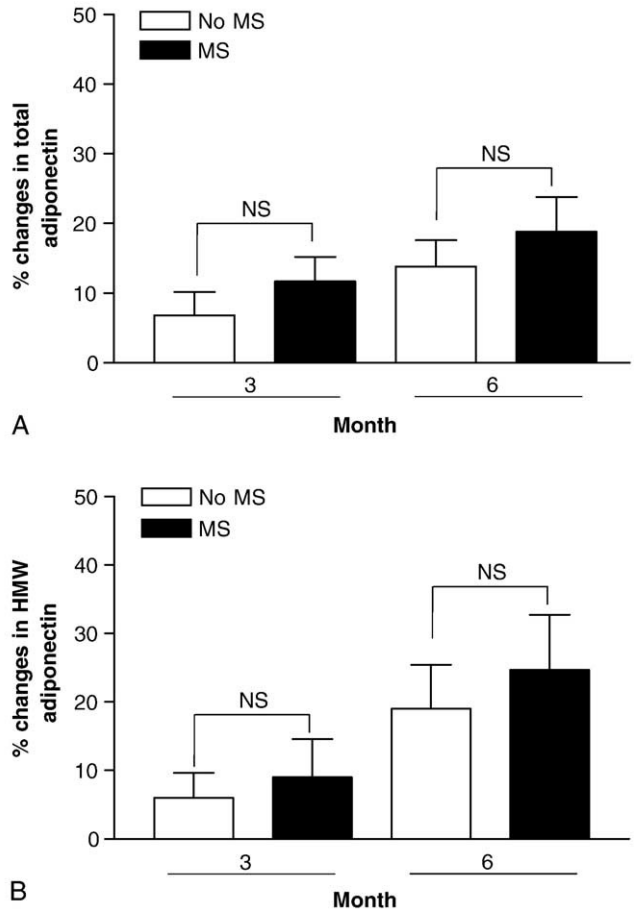

Fig. 3. A, Percentage changes in the serum total adiponectin concentration after treatment with losartan between hypertensive patients with or without MS. B, Percentage changes in the serum HMW adiponectin concentration after treatment with losartan between hypertensive patients with and without MS. Data are expressed as the mean ± SEM.

4. Discussion

The present study confirmed that losartan treatment significantly elevated the serum concentrations of total adiponectin in patients with essential hypertension (8.0 ± 4.7 μg/mL at baseline to 9.2 ± 5.3 μg/mL at 6 months), as has been reported in previous studies [9,10]. Koh et al [10] reported that the plasma total adiponectin concentration increased from 4.19 μg/mL at baseline to 5.27 μg/mL after 2 months of treatment with losartan 100 mg in hypertensive patients with hypercholesterolemia. Unlike their study, 94% of the subjects in the present study were treated with 50 mg of losartan. This suggests that the relatively low dose of 50 mg of losartan can be enough to increase the serum total adiponectin concentration in patients with essential hypertension. Moreover, the present study demonstrated that serum total adiponectin concentration was significantly higher at 6 months after losartan treatment than at baseline or at 3 months, suggesting that the serum total adiponectin concentration maintained the increase up to 6 months after losartan treatment. Although most previous studies have investigated the short-term effects of ARBs including losartan on the serum total adiponectin concentration by 2 weeks to 3 months of treatment [9,10], the present study demonstrated that the serum total adiponectin concentration continued to increase after losartan treatment in patients with essential hypertension. Thus, we can expect the long-term effects of losartan on serum adiponectin in patients with essential hypertension.

Similar to total adiponectin, we found that the serum HMW adiponectin concentration was significantly increased after treatment with losartan using our novel quantitative ELISA for HMW adiponectin. The present study demonstrated for the first time that losartan significantly increased serum concentrations of HMW in patients with essential hypertension. Previous studies have reported that HMW adiponectin may be the active form of this protein because changes in the ratio of HMW to total adiponectin concentrations after rosiglitazone treatment (a TZD), but not the total adiponectin concentration, were associated with improvement in hepatic insulin sensitivity [17].

Losartan, a prodrug, is metabolized in the liver primarily by the cytochrome P450 2C enzymes [25]. Its main metabolite EXP3174 exerts an antihypertensive action by blocking potently the AT1 receptor [25]. EXP3179, an intermediate metabolite during the hepatic metabolism, has a significant molecular homology with indomethacin, an anti-inflammatory cyclooxygenase inhibitor, which has been identified as an activator of PPAR-γ [26,27]. In vitro studies

also identified the active metabolite EXP3179 of losartan as a partial PPAR-γ agonist [22]. Thiazolidinediones, which act as full agonists for PPAR-γ, are powerful insulin sensitizers and are commonly used as antidiabetic agents. Thiazolidinediones potently increase gene expression of adiponectin in adipocytes and increase serum concentrations of total adiponectin [16]. In a recent study using pioglitazone, we found that, in patients with type 2 diabetes mellitus, not only the serum HMW adiponectin concentration but also the HMW to total adiponectin ratio increased significantly after treatment with pioglitazone, suggesting that pioglitazone increases mostly the HMW adiponectin concentration as opposed to MMW or LMW adiponectin [21]. Increased HMW adiponectin concentration may contribute to the insulin-sensitizing effects of pioglitazone treatment. Thus, we hypothesized that losartan can increase HMW adiponectin predominantly in the serum by activating PPAR-γ by EXP3179, although we could not evaluate directly the impact of EXP3179 on serum HMW adiponectin in this study. The present study showed that losartan increased both the total and HMW adiponectin in the serum by 15% and 18%, respectively, whereas the serum concentrations of non-HMW adiponectin (ie, MMW + LMW adiponectin) did not change during the treatment. This suggests that losartan predominantly increases HMW adiponectin rather than MMW or LMW adiponectin.

Several large prospective clinical trials have demonstrated that the ARBs, including losartan and valsartan, significantly decrease the onset of diabetes in hypertensive patients at high risk for CVD, compared with patients treated with β-blocker or Ca channel blocker [5-7]. In the Losartan Intervention For Endpoint Reduction in Hypertension Study, losartan treatment was associated less with the development of new-onset diabetes than atenolol treatment in hypertensive patients with left ventricular hypertrophy [5]. The mechanisms responsible for the reduction in the development of new-onset diabetes in hypertensive patients by ARBs remain to be unclear. One possible explanation is that ARBs could ameliorate insulin resistance in hypertensive patients by blocking the inhibitory effects of angiotensin II on insulin signaling in skeletal muscles at multiple sites of the insulin signaling cascade such as the insulin receptor, insulin receptor substrate–1, and phosphatidylinositol 3-kinase [8]. Another explanation is that the increased adiponectin levels induced by ARBs may contribute to an improvement in insulin sensitivity in these patients [28]. Although the present study showed no significant improvement in insulin sensitivity as evidenced by HOMA-IR after losartan treatment in hypertensive patients with or without MS, several studies have demonstrated that losartan treatment improves insulin sensitivity in patients with hypertension or impaired fasting glucose [10,29]. Taken together, an elevation in the HMW adiponectin induced by losartan may be associated with an improvement in insulin sensitivity in patients with essential hypertension.

The present study showed that both the serum total and HMW adiponectin concentrations were significantly lower in hypertensive patients with MS than in those without MS. Furthermore, we found that the HMW to total adiponectin ratio was significantly lower in hypertensive patients with MS than in those without MS. This suggests that a predominant decrease in the serum HMW adiponectin concentration may contribute to the decrease in the serum total adiponectin concentration in subjects with MS. *Metabolic syndrome* is defined by the clustering of several cardiovascular risk factors in an individual patient, including impaired glucose tolerance (diabetes), hypertension, dyslipidemia, and visceral obesity [30,31]. Several studies have demonstrated that this syndrome strongly predicts CVD, especially CAD [32,33], independently of the low-density lipoprotein (LDL) cholesterol concentration. A recent study also demonstrated that the HMW to total adiponectin ratio has better predictive power for the prediction of insulin resistance and MS than the plasma total adiponectin concentration [34]. Thus, serum HMW adiponectin concentration is more useful clinically for evaluating the diagnosis of MS than simply measuring the total serum adiponectin concentration.

The present study showed that the serum concentrations of total and HMW adiponectin increased significantly after losartan treatment in hypertensive patients with or without MS. We also found no significant differences in the changes in serum total (13.8% vs 18.8% vs of baseline) or HMW adiponectin (19.1% vs 24.7% of baseline) concentrations after treatment for 6 months between hypertensive patients with MS and those without MS. These results suggest that losartan can increase the serum HMW adiponectin in patients with essential hypertension, irrespective of the presence or absence of MS. Considering that the serum HMW concentration is profoundly reduced in people with MS, we hypothesized that losartan can increase the serum HMW adiponectin concentration in hypertensive patients with MS to a much greater extent than in those without MS. However, both groups showed similar increases in the serum HMW adiponectin concentration after losartan treatment because the present study showed no significant differences in the percentage changes in the serum total or HMW adiponectin concentrations after 6 months of treatment with losartan between hypertensive patients with MS and those without MS. One possible explanation for the lack of statistical significance is the relatively small number of subjects studied (ie, a type II error), which could be minimized by increasing the sample sizes. We conclude that losartan can increase the serum HMW adiponectin concentration in patients with essential hypertension, irrespective of the presence or absence of MS.

The present study showed that LDL cholesterol concentration was significantly decreased after losartan treatment in hypertensive patients without MS. In contrast, losartan treatment did not affect LDL cholesterol in those with MS. Because LDL cholesterol is a strong risk factor for CAD, this

LDL cholesterol–lowering effect by losartan could help prevent the development of CAD in patients with essential hypertension. In fact, in a post hoc analysis of the Reduction of Endpoints in Type 2 Diabetes With the Angiotensin II Antagonist Losartan data, losartan-treated diabetic patients had lower total and LDL cholesterol compared with placebo groups [35]. However, the mechanisms responsible for the different effects of losartan on LDL cholesterol concentration between hypertensive patients with and without MS remain to be determined.

Because hypertension is associated with insulin resistance, hypertensive subjects tend to develop type 2 diabetes mellitus [1-3]. Diabetes is a major risk factor for cardiovascular morbidity and mortality. People with type 2 diabetes mellitus have a 2- to 4-fold higher risk of CVD than nondiabetic individuals [36,37]. Furthermore, diabetes is associated with poorer outcomes after acute coronary syndromes than nondiabetes [38,39]. In a recent observational study, people who developed new-onset diabetes had the same high cardiovascular risk as patients with diabetes [40]. Thus, the prevention of the development of new diabetes is very important in patients with essential hypertension at high risk to improve the prognosis. Serum adiponectin concentration is the most useful predictor for the development of type 2 diabetes mellitus in subjects with MS [41,42]. The present study demonstrated that both the serum total and HMW adiponectin concentrations continued to increase after losartan treatment in essential hypertension for 6 months. Furthermore, losartan may specifically increase HMW adiponectin rather than MMW or LMW adiponectin because the serum non-HMW adiponectin did not change after treatment with losartan. This increased circulating HMW adiponectin with treatment of losartan may be associated with a reduction in the development of diabetes in hypertensive patients. Although, in the present study, we could not observe an improvement in insulin sensitivity as evidenced by HOMA-IR, it is possible that losartan ameliorates insulin resistance in patients with essential hypertension via increasing the serum HMW adiponectin concentration or directly blocking angiotensin II signaling, irrespective of the presence or absence of MS. Thus, losartan may be effective for preventing the development of diabetes in patients with essential hypertension.

References

[1] Kannel WB, Wilson PW, Zhang TJ. The epidemiology of impaired glucose tolerance and hypertension. Am Heart J 1991;121:1268-73.

[2] Lind L, Berne C, Lithell H. Prevalence of insulin resistance in essential hypertension. J Hypertens 1995;13:1457-62.

[3] Segura J, Campo C, Ruilope LM, Rodicio JL. Do we need to target 'prediabetic' hypertensive patients? J Hypertens 2005;23:2119-25.

[4] Sharma AM. Is there a rationale for angiotensin blockade in the management of obesity hypertension? Hypertension 2004;44:12-9.

[5] Lindholm LH, Ibsen H, Borch-Johnsen K, Olsen MH, Wachtell K, Dahlof B, et al. Risk of new-onset diabetes in the Losartan Intervention For Endpoint reduction in hypertension study. J Hypertens 2002;20: 1879-86.

[6] Kjeldsen SE, Julius S, Mancia G, McInnes GT, Hua T, Weber MA, et al. Effects of valsartan compared to amlodipine on preventing type 2 diabetes in high-risk hypertensive patients: the VALUE trial. J Hypertens 2006;2:1405-12.

[7] Burnier M, Zanchi A. Blockade of the renin-angiotensin-aldosterone system: a key therapeutic strategy to reduce renal and cardiovascular events in patients with diabetes. J Hypertens 2006;24:11-25.

[8] Folli F, Kahn CR, Hansen H, Bouchie JL, Feener EP. Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels. A potential role for serine phosphorylation in insulin/angiotensin II crosstalk. J Clin Invest 1997;100:2158-69.

[9] Furuhashi M, Ura N, Higashiura K, Murakami H, Tanaka M, Moniwa N, et al. Blockade of the renin-angiotensin system increases adiponectin concentrations in patients with essential hypertension. Hypertension 2003;42:76-81.

[10] Koh KK, Quon MJ, Han SH, Chung WJ, Ahn JY, Seo YH, et al. Additive beneficial effects of losartan combined with simvastatin in the treatment of hypercholesterolemic, hypertensive patients. Circulation 2004;110:3687-92.

[11] Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 2001;7:947-53.

[12] Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001;7:941-6.

[13] Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999;257:79-83.

[14] Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000;20:1595-9.

[15] Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, et al. Coronary artery disease. Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler Thromb Vasc Biol 2003;23:85-9.

[16] Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, et al. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes 2001;50:2094-9.

[17] Schraw T, Wang ZV, Halberg N, Hawkins M, Scherer PE. Plasma adiponectin complexes have distinct biochemical characteristics. Endocrinology 2008 [Epub ahead of print].

[18] Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, et al. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. J Biol Chem 2004;279:12152-62.

[19] Nakano Y, Tajima S, Yoshimi A, Akiyama H, Tsushima M, Tanioka T, et al. A novel enzyme-linked immunosorbent assay specific for high-molecular-weight adiponectin. J Lipid Res 2006;47:1572-82.

[20] Aso Y, Yamamoto R, Wakabayashi S, Uchida T, Takayanagi K, Takebayashi K, et al. Comparison of serum high–molecular weight (HMW) adiponectin with total adiponectin concentrations in type 2 diabetic patients with coronary artery disease using a novel enzyme-linked immunosorbent assay to detect HMW adiponectin. Diabetes 2006;55:1954-60.

[21] Aso Y, Yamamoto R, Suetusgu M, Matsumoto S, Wakabayashi S, Matsutomo R, et al. Comparison of the effects of pioglitazone and voglibose on circulating total and high–molecular-weight adiponectin, and on two fibrinolysis inhibitors, in patients with type 2 diabetes. Diabet Med 2007;24:962-8.

[22] Schupp M, Lee LD, Frost N, Umbreen S, Schmidt B, Unger T, et al. Regulation of peroxisome proliferator–activated receptor gamma activity by losartan metabolites. Hypertension 2006;47:586-9.

[23] Yki-Jarvinen H. Thiazolidinediones. N Engl J Med 2004;351:1106-18.

[24] Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the

National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.

[25] Diez J. Review of the molecular pharmacology of losartan and its possible relevance to stroke prevention in patients with hypertension. Clin Ther 2006;28:832-48.

[26] Kramer C, Sunkomat J, Witte J, Luchtefeld M, Walden M, Schmidt B, et al. Angiotensin II receptor-independent antiinflammatory and antiaggregatory properties of losartan: role of the active metabolite EXP3179. Circ Res 2002;90:770-6.

[27] Lehmann JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA. Peroxisome proliferator–activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs. J Biol Chem 1997;272:3406-10.

[28] Wang ZV, Scherer PE. Adiponectin, cardiovascular function, and hypertension. Hypertension 2008;51:8-14.

[29] Zandbergen AA, Lamberts SW, Janssen JA, Bootsma AH. Short-term administration of an angiotensin-receptor antagonist in patients with impaired fasting glucose improves insulin sensitivity and increases free IGF-I. Eur J Endocrinol 2006;155:293-6.

[30] Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988;37:1595-607.

[31] Grundy SM, Brewer Jr HB, Cleeman JI, Smith Jr SC, Lenfant C, American Heart Association, et al. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004;109:433-8.

[32] Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 2002;288:2709-16.

[33] Sattar N, Gaw A, Scherbakova O, Ford I, O'Reilly DS, Haffner SM, et al. Metabolic syndrome with and without C-reactive protein as a predictor of coronary heart disease and diabetes in the West of Scotland Coronary Prevention Study. Circulation 2003;108:414-9.

[34] Hara K, Horikoshi M, Yamauchi T, Yago H, Miyazaki O, Ebinuma H, et al. Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. Diabetes Care 2006;29:1357-62.

[35] Appel GB, Radhakrishnan J, Avram MM, DeFronzo RA, Escobar-Jimenez F, Campos MM, et al. Analysis of metabolic parameters as predictors of risk in the RENAAL study. Diabetes Care 2003;26:1402-7.

[36] Kannel WB, MacGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. Diabetes Care 1979;2:120-6.

[37] Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes Care 1993;16:434-44.

[38] Mukamal KJ, Nesto RW, Cohen MC, Muller JE, Maclure M, Sherwood JB, et al. Impact of diabetes on long-term survival after acute myocardial infarction: comparability of risk with prior myocardial infarction. Diabetes Care 2001;24:1422-7.

[39] Aguilar D, Solomon SD, Kober L, Rouleau JL, Skali H, McMurray JJ, et al. Newly diagnosed and previously known diabetes mellitus and 1-year outcomes of acute myocardial infarction: the VALsartan In Acute myocardial iNfarcTion (VALIANT) trial. Circulation 2004;110:1572-8.

[40] Verdecchia P, Reboldi G, Angeli F, Borgioni C, Gattobigio R, Filippucci L, et al. Adverse prognostic significance of new diabetes in treated hypertensive subjects. Hypertension 2004;43:963-9.

[41] Krakoff J, Funahashi T, Stehouwer CD, Schalkwijk CG, Tanaka S, Matsuzawa Y, et al. Inflammatory markers, adiponectin, and risk of type 2 diabetes in the Pima Indian. Diabetes Care 2003;26:1745-51.

[42] Daimon M, Oizumi T, Saitoh T, Kameda W, Hirata A, Yamaguchi H, et al. Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in the Japanese Population: the Funagata study. Diabetes Care 2003;26:2015-20.