Paper

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Effects of ACE inhibition and AT_1-receptor antagonism on endothelial function and insulin sensitivity in essential hypertensive patients

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Abstract

Objective

Disturbed endothelial function is closely associated with hyperinsulinaemia and insulin resistance in essential hypertension. The aims of this study were:
1) to evaluate whether the two alternative drugs, angiotensin-converting enzyme (ACE) inhibitors and Angiotensin II (Ang II) antagonists, had comparable effects on glucose metabolism and endothelial function.
2) to determine whether they improve endothelial dysfunction through modulating insulin resistance and oxidative stress.

Study design and methods

Essential hypertensive patients were randomised into two groups: Twelve (nine patients in final analysis) patients were given enalapril (enalapril group), and twelve (nine patients in final analysis) were given losartan (losartan group). Twelve sex- and age-matched normotensive volunteers were included as controls. Before and after six months of treatment, endothelial function, insulin sensitivity and lipid peroxidation (TBARs) and NO metabolites (NOx) were evaluated.

Results

Endothelial function, measured as flow mediated dilatation (FMD), was improved in both of the treatment groups (p=0.0001). Calculated insulin sensitivity index also improved in the enalapril-treated group (p=0.05) but not in the losartan-treated group, compared with baseline levels. TBARS values decreased significantly in the enalapril group compared with baseline levels (p<0.001). FMD was positively correlated with insulin sensitivity index (r=0.32, p<0.05) and NOx levels (r=0.39, p=0.01) and negatively correlated with TBARS levels (r=-0.53, p=0.0002) in hypertensive patients.

Conclusion

Inhibition of the renin-angiotensin system, either with ACE inhibitors or AT_1-receptor blockers improves endothelial dysfunction. ACE inhibition has prominent effects on improving insulin sensitivity and decreasing oxidative stress in essential hypertensive patients.

Introduction

Endothelial dysfunction, which is considered as an early marker of atherosclerosis, is common in patients with essential hypertension.[1,2] Hyperinsulinaemia and insulin resistance, which is also common in essential hypertension, is accompanied by endothelial dysfunction.[3-6]

Some antihypertensive drugs were demonstrated to modulate insulin resistance and endothelial function.[7-9] While commonly used drugs, such as thiazide diuretics and β-receptor antagonists are effective in the treatment of hypertension, they have been reported to impair insulin sensitivity.[7] On the other hand, alpha-receptor antagonists, calcium channel blockers and angiotensin-converting enzyme (ACE) inhibitors have either neutral or favourable effects on glucose metabolism and insulin sensitivity.[7-9] Furthermore, in a study comparing different antihypertensive regimens, among ACE inhibitors (ACE-Is), calcium channel blocker, β-blockers and diuretics, ACE-Is were the only drugs which successfully increased post-ischaemic vasodilatation.[10] Angiotensin II (Ang II) receptor blockers (ARBs) are known to be as effective as ACE-Is in the treatment of hypertension, but their actions on glucose metabolism and endothelial function are controversial.[9,11-13]

The aims of this study were:

1. to evaluate whether ACE-Is and ARBs had comparable effects on glucose metabolism and endothelial function, and
2. to determine whether they improve endothelial dysfunction through modulating insulin resistance and oxidative stress in essential hypertensive patients.

Design and methods

Patient selection

Twenty-four patients with stage 1 hypertension, according to JNC VI criteria,[14] were included in the study. Previous antihypertensive medications were discontinued for three weeks during the wash-out period to ensure that blood pressure (BP) levels were stable. BP was measured from the right upper extremity using a mercury sphygmomanometer with the patient in a sitting position. After 10 minutes of rest, the average of three consecutive measurements, performed five minutes apart, was calculated as the BP value. The first and the fifth sounds of Korotkoff to the nearest even number

were regarded as the systolic and diastolic pressures, respectively. The measurements were carried out by the same physician throughout the study. Patients were excluded if they were smokers or had secondary hypertension, renovascular disease, connective tissue disorders, a history of malignant hypertension, myocardial infarction, cerebrovascular disease, heart failure, diabetes, obesity (body mass index >27 kg/m^2), other systemic diseases, or renal failure (serum creatinine >1.2 mg/dl). None of the subjects was taking lipid-lowering agents, hormone replacement therapy, antioxidant therapy, antiaggregants, steroids or other drugs which might affect BP. Patients were included in the study after exclusion of secondary hypertension. All of the participants underwent initial evaluation by means of medical history, physical examination, haematological and biochemical profile, including measurement of blood glucose, serum electrolytes (sodium, potassium), urea, creatinine, lipids, thyroid function tests and urine analysis. Diabetes was excluded according to American Diabetes Association criteria. Renal ultrasound and Doppler examinations were normal in all hypertensive patients.

All patients had been hypertensive for at least six months, according to the hospital records. Ten patients were on antihypertensive medication and none of them were on ACE-Is or ARBs. Previous antihypertensive medications were discontinued for three weeks during the wash-out period. Patients were randomised into two groups: Twelve patients were given enalapril (Renitec, Merck Sharp Dohme, Turkey), and twelve were given losartan (Cozaar, Merck Sharp Dohme, Turkey). Patients were randomised according to a pre-prepared randomisation list and followed in an unblinded fashion. After a six-week titration period, patients were followed monthly for six months. The drug dosages ranged from 5–40 mg/day for enalapril and from 50–100 mg/day for losartan. Antihypertensive effects of the study medications were evaluated by clinic BP measurements. Patient's diets were unchanged, with no restriction on sodium or protein intake. BP was measured at each visit between 8 to 10 a.m. (before the study medication was given) and 24 hours after the ingestion of the last dose.

Compliance and tolerability to study drugs were assessed by pill count and monitoring of spontaneous reports of adverse experiences at each visit. Blood samples and 24-hour urine collections were obtained, endothelial function was measured, and an oral glucose tolerance test (OGTT) was done at the beginning and end of the six-month treatment period.

Twelve sex- and age-matched normotensive volunteers were also evaluated at baseline and after six months for the same parameters.

Informed consent was obtained from all participants and the study was performed in accordance with the declaration of Helsinki and after the approval of Marmara University Medical School Ethics Committee.

Insulin sensitivity

Insulin sensitivity was determined by OGTT, based on the formula described by Matsuda and De Fronzo, and named as the insulin sensitivity index composite (ISI Composite).[15] Whole-body insulin sensitivity during an oral glucose tolerance test was calculated by the following formula:

$$10000/\sqrt{(\text{FPG} \times \text{FPI}) \times (\text{mean OGTTG} \times \text{mean OGTTI})}$$

Where FPG = fasting plasma glucose
FPI = fasting plasma insulin
OGTTG = OGTT plasma glucose
OGTTI = OGTT plasma insulin

After an overnight fast, an OGTT (75 g glucose) was performed between 8 and 9 a.m. Blood samples were drawn at 0, 30, 60, 90 and 120 minutes after the administration of glucose for the measurement of serum glucose and insulin concentrations. The homeostasis model assessment for estimating insulin resistance (HOMA IR) was used for assessment of insulin resistance.[16]

Endothelial function

Endothelial function was determined by a non-invasive method, which was described by Celermajer *et al*.[17] This method evaluates endothelial function by using post-ischaemic (forearm) vasodilatation.

Endothelium-dependent (flow mediated) vasodilatation was measured using a high-resolution ultrasound (General Electric, Logic 700) with a 8.5-MHz linear-array transducer. The ECG was monitored continuously. Vessel diameters were analysed on frozen images over the length of an artery of >1 cm (brachial artery) according to operator judgement. Three measurements were taken for three cardiac cycles at the end of diastole (R wave on the ECG) and the mean was calculated. Subjects rested for at least 10 minutes before the first record. Increased flow was induced by deflating a pneumatic tourniquet after a 5-minute suprasystolic forearm arterial compression. The post-ischaemic measurement was performed 45–60 seconds after cuff deflation. The percentage change of vessel diameter was expressed as flow mediated dilatation (FMD).

To test endothelium-independent dilatation (GTN), further scans were performed 4 minutes after sublingual administration of 0.4 mg glyceryl trinitrate, as a direct nitric oxide (NO) donor. The time interval between the first and second measurements was at least 20 minutes to allow vessel recovery.

Ultrasonography was performed in a blinded fashion by the same investigator post-ischaemia and GTN application. Intra-observer variability in image acquisition and analysis was below 2%.

Assays

Serum insulin levels were determined by an immunometric assay (Immulite, DPC, CA). Within-run precision ranged between 3.8% and 4.8%, for a mean range of 10.7–439 mIU/ml. The total precision ranged between 4.8–5.8% for the same mean range. Serum glucose levels were measured

Table 1 Demographic data of all study groups

	Enalapril group (n=9)	Losartan group (n=9)	Control group (n=12)
Age (yrs)	38.6±7.9	42.2±12.8	42.9±4.5
Gender (M/F)	4/8	5/7	5/7
BMI (kg/m^2)	24.7±4.9	24.4±4.5	24.0±4.8
Duration of hypertension (yrs)	3.1±3.7	3.3±3.0	-

Data are presented as mean ± SD. BMI = body mass index

Table 2 Blood pressure measurements and lipid levels of study groups

	Enalapril group		Losartan group		Control group	
	Before treatment	After treatment	Before treatment	After treatment	Baseline	Final
SBP (mmHg)	149±11	126±11[b]	150±21	126±14[b]	113±12[a]	112.9±12[a]
DBP (mmHg)	98±7	79±3[c]	100±5	80±2[c]	81±4[a]	80.6±4[a]
Cholesterol (mg/dl)	208±34	206±42	207±36	207±43	176±19	174.5±19
Triglyceride (mg/dl)	116±27	118±37	114±53	115±32	108±25	103±25
HDL (mg/dl)	56±13	52±17	49±9	43±6	44±10	43±11
LDL (mg/dl)	128±25	129±41	135±28	127±34	110±31	108±33

Data are presented as mean±SD. [a] = p<0.001 *vs.* before treatment measurements of enalapril and losartan groups; [b] = p<0.05 *vs.* before treatment; [c] = p<0.001 *vs.* before treatment; SBP = systolic blood pressure; DBP = diastolic blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein

by the glucose oxidase method. Serum total cholesterol, uric acid and triglyceride levels were measured by enzymatic colorimetric assays (Roche Diagnostics GmbH, Mannheim, Germany). The within-run and between-day CV values were 0.8% and 1.7% for total cholesterol, 0.5%–1.7% for uric acid and 1.5–1.8% for triglyceride assays. Serum high density lipoprotein (HDL) cholesterol levels were determined by a direct automated method using PEG-modified enzymes, sulphated a-cyclodextrin and dextran sulphate (Roche Diagnostics GmbH, Mannheim, Germany). The within-run CV was 1.3% and between-day CV was 2.6% for a mean HDL cholesterol level of 23±0.6 mg/dl. Lipid peroxidation was measured by the formation of thiobarbituric acid reactive substances (TBARS), as described previously.[18] Serum nitrate and nitrite determinations (NOx) were made by a colorimetric assay (Boehringer Mannheim, Germany) based on nitrogen monoxide determination via nitrate on microtitre plates. The intra-test variance is ≤10% and the inter-test variance is <20%.

Statistical analysis

The analysis of the data was performed with an IBM-compatible Instat 3 programme. Paired *t*-test and ANOVA were used where appropriate. Tukey Kramer multiple comparison tests were applied as post hoc tests for inter-group comparisons following ANOVA. Correlation analysis was performed with Pearson test. The areas under the curves of glucose and insulin during OGTT were calculated according to the trapezoid rule. The results were given as mean±SD. The differences were considered significant at p<0.05.

Results

Two patients from the enalapril group and one patient from the losartan group withdrew from the study because of adverse events and two patients from the losartan and one from the enalapril group requested to leave the study. The remaining 18 hypertensive patients and 12 normotensive controls were evaluated at baseline and final analysis. The demographic characteristics of hypertensive patients and normotensive controls are presented in Table 1. There were no significant differences in age, gender, BMI and duration of hypertension.

Office systolic and diastolic BPs were significantly higher in the hypertensive patients compared with controls. Both of the hypertensive groups had lower levels of BPs at the end of the study period compared with their baseline values (Table 2). All patients achieved the goal of DBP < 90 mmHg at the end of the study period. Serum lipid levels were similar between the hypertensive groups and the control subjects.

Endothelial function, measured as FMD, was significantly lower in hypertensive patients than healthy controls at baseline (p<0.01). FMD measurements increased significantly in both of the treatment groups at the end of six months' treatment (8.4±4.5% *vs.* 14.0±4.0%, p<0.01 in enalapril group and 7.9±3.9% *vs.* 12.4±1.9%, p<0.05 in the losartan group) (Figure 1). Endothelium-independent dilatation values were not different between the enalapril, losartan and control groups at baseline and at the end of the treatment period. These values did not change at the end of the treatment period (18.3±2.4% *vs.* 18.6±1.6%, 18.2±5.3% *vs.*

Figure 1 Changes in flow-mediated dilatation (FMD) before and after enalapril and losartan treatment in hypertensive patients. FMD measurements were significantly improved after six months of enalapril ($p<0.01$) and losartan ($p<0.05$) treatment

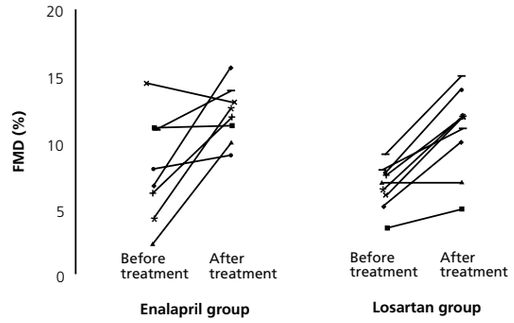


Table 3 Parameters based on serum insulin and glucose in all study groups

	Enalapril group (n=9)		Losartan group (n=9)		Control group (n=12)	
	Before treatment	After treatment	Before treatment	After treatment	Baseline	Final
ISI Composite	0.9±0.3	1.9±0.6[b]	1.1±0.3	1.3±0.4	2.0±1[a]	2.1±1[a]
HOMA IR	2.9±1.7	1.2±0.6[d]	2.3±0.6	1.5±0.7	1.2±0.4[c]	1.1±0.4[c]
Glucose AUC (mg.h/ml)	105±25	62±48	101±48	75±44	106±94	112±97
Insulin AUC (mIU.h/ml)	114±91	69±45	133±72	132±90	68±34	69±36

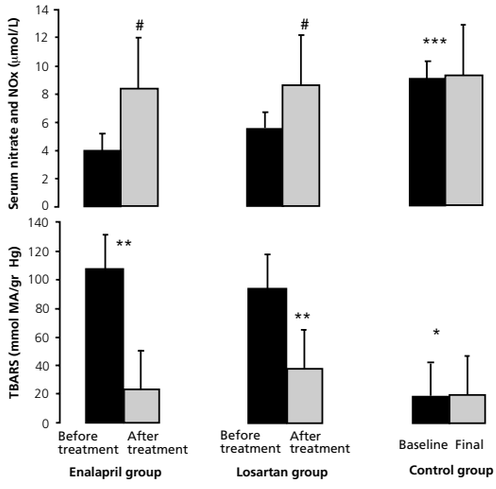
Data are presented as mean±SD. [a] = $p<0.005$ *vs.* before treatment measurements in enalapril and losartan groups; [b] = $p<0.001$ *vs.* before treatment; [c] = $p<0.0001$ *vs.* before treatment measurements in enalapril and losartan groups; [d] = $p<0.01$ *vs.* before treatment; ISI composite = insulin sensitivity index composite; HOMA IR = homeostasis model assessment for estimating insulin resistance; AUC = area under the curve

19.6±5.4%, 18.1±3% *vs.* 18.7±2% in enalapril, losartan groups and healthy controls respectively baseline and final measurements).

The ISI Composite index was significantly lower in both of the hypertensive groups compared with healthy controls at baseline and after treatment ($p<0.005$). The ISI Composite index improved in enalapril-treated hypertensive patients (0.9±0.3 *vs.* 1.9±0.6, $p<0.05$) at the end of the six-month period, while ISI Composite index calculations in losartan-treated hypertensive patients did not change significantly (1.1±0.3 *vs.* 1.3±0.4, $p>0.05$) (Table 3). Baseline calculations of HOMA IR were significantly higher in hypertensive patients compared with healthy controls ($p<0.0001$). Although enalapril-treated hypertensive patients had lower levels of HOMA after treatment ($p<0.01$), calculations in the losartan group did not reach statistical significance at the end of the six-month treatment period (see Table 3). Insulin and glucose areas under the OGTT curve were not different between the hypertensive groups and healthy controls.

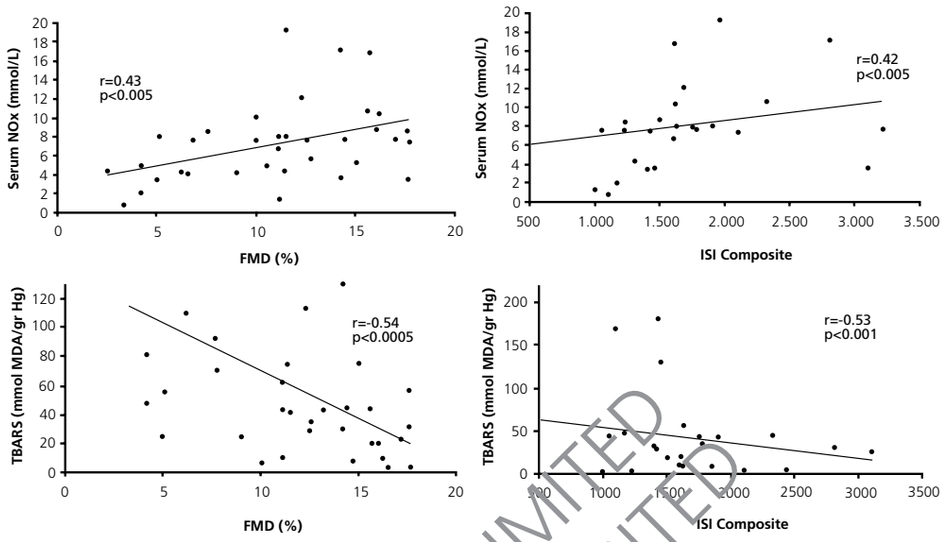
Serum NO metabolite levels were significantly lower at baseline in untreated hypertensive patients compared with healthy controls (4.0±1.8 mmol/L, 5.5±2.8 mmol/L and 9.1±3.2 mmol/L in enalapril, losartan and control groups, respectively, $p=0.02$). After treatment, NOx measurement showed a significant increase in the enalapril group (4.0±1.8 mmol/L *vs.* 8.4±3.9 mmol/L, $p<0.05$) and losartan group (5.5±2.8 mmol/L *vs.* 8.6±4.1 mmol/L, $p<0.05$) compared with baseline values (Figure 2).

Figure 2 Serum nitric oxide metabolites (NOx) and thiobarbituric acid reactive substance (TBARs) measurements of all study groups. *$p=0.02$ *vs.* baseline levels of hypertensive groups, **$p<0.0001$ *vs.* baseline levels of hypertensive groups, ***$p<0.001$ *vs.* before treatment measurements, #$p<0.05$ *vs.* pretreatment measurements. $p<0.01$ *vs.* before treatment values



TBARs levels were significantly higher at

Figure 3 FMD was positively correlated with serum NOx levels (r=0.39, p=0.01) and negatively correlated with erythrocyte TBARs levels (r=-0.53, p=0.0002). Insulin sensitivity index was found to be positively correlated with serum NOx levels (r=0.30, p=0.04) and negatively correlated with erythrocyte TBARs levels (r=-0.38, p=0.01) in hypertensive patients



baseline in hypertensive patients than in controls (107.1±50.6, 93.3±62.6 and 18.1±8.5 MDA/g Hg in enalapril, losartan and control groups respectively p=0.0001). Both enalapril and losartan groups had lower levels of TBARs at the end of the treatment period compared with their baseline levels (107.1±50.6 *vs.* 22.9±16.3 mmol MDA/g Hg, p<0.001 in enalapril and 93.3±62.6 *vs.* 36.3±8.5 mmol MDA/g Hg, p<0.01 in losartan groups) (see Figure 2).

TBARs levels were inversely correlated with ISI calculations (r=–0.38 p=0.01) and FMD (r=–0.53, p=0.0002) in hypertensive patients. Serum NOx levels showed a positive correlation with ISI Composite index calculations (r=0.30, p=0.04) and FMD measurements (r=0.39, p=0.01) at baseline and at the end of the study period (Figure 3).

FMD was positively correlated with ISI Composite index (r=0.32, p<0.05). ISI was found to be negatively correlated with HOMA IR calculations (r =–0.50, p=0.0003).

Discussion

The present study confirms previous findings that impaired endothelial function and insulin sensitivity, accompanied by increased oxidative stress, are present in patients with essential hypertension.[1-4] Enalapril and losartan treatment for six months improved FMD, but only enalapril treatment had a significant effect on insulin sensitivity in hypertensive patients in this study.

Insulin resistance, with resulting hyperinsulinaemia, is common in patients with hypertension.[5] ACE-Is are known to improve insulin sensitivity.[8,13] Although the effects of ARBs on insulin sensitivity have been less extensively studied, conflicting results have been reported.[9,19] To our knowledge, this is the first study comparing the effects of an ACE-I and an ARB on endothelial function in relation to insulin sensitivity.

Our study is in agreement with previous reports, which failed to show beneficial effects of losartan treatment on insulin sensitivity.[9,19,20] Fogari *et al.*[9] compared the effects of losartan with that of lisinopril. In this study, BP control was better in the lisinopril group, which might be a factor contributing to the effects on insulin sensitivity. However, in our study, the degree of BP decline was similar in both enalapril and losartan groups, indicating that ACE-Is might theoretically have some additional benefits on insulin sensitivity compared with losartan.

Bradykinin might be an additional factor in improvement of glucose metabolism, since it enhances muscle blood flow and consequently the rate of insulin and glucose delivery to target tissues, thereby facilitating glucose transport proteins and accelerating oxidation of plasma glucose.[21,22]

FMD, which is considered to be a measure of endothelial function, correlated positively with calculated insulin sensitivity index in hypertensive patients. Since insulin sensitivity was not improved significantly in the losartan-treated group, insulin resistance couldn't be implicated as the primary factor in impaired endothelial function in essential hypertension.

Improvement in endothelial function was comparable between the enalapril and losartan groups in this study. ACE is positioned on the endothelium to influence the activity of Ang II, bradykinin and NO synthase.[2] ACE inhibition has two major actions: prevention of Ang II formation and prevention of bradykinin breakdown.[21,23] The beneficial effects of ACE-Is on endothelial function may depend on both of these mechanisms. The

bradykinin pathway could not be the only route via which renin angiotensin system (RAS) inhibition improves endothelial function, since ARBs have also beneficial effects on endothelial function.

One of the mechanisms leading to endothelial dysfunction is the disturbance of NO metabolism in patients with hypertension.[24] In this study, we found increased NOx levels in both of the treatment groups at the end of the treatment period and a positive correlation between NOx levels and FMD. Similar to our results, Girolama *et al.*, demonstrated that enalapril increases nitrate/nitrite levels in essential hypertensive patients.[25] Recently, AT_1-receptor blockade with valsartan was shown to improve basal NO production and release. This effect seems to be BP-independent in essential hypertensive patients.[26] Treatment with ACE-Is increases the level of bradykinin, which is a stimulator of NO synthesis through B2 receptor stimulation.[27] Moreover, in chronic L-NAME-treated male Sprague-Dawley rats, administration of quinapril also completely restored eNOS mRNA levels in aortic tissue.[28] On the other hand, losartan increases NO synthesis by activating AT_2-receptors.[29]

Oxidative stress also appears to be involved in endothelial dysfunction.[30] Direct evidence comes from a recent study, in which vitamin C reversed endothelial dysfunction induced by insulin infusion.[4] In this study, we used lipid peroxidation as a marker of oxidative stress and found that TBARS levels were higher in hypertensive patients than in healthy controls at baseline. Both enalapril and losartan treatment had comparable and beneficial effects in this study. Erythrocyte TBARs levels were inversely correlated with FMD in hypertensive groups. Recent studies demonstrated the influence of ACE-Is and ARBs on oxidative stress. Ang II has a specific role in inducing oxidative stress in essential hypertensive patients.[31] Decreased vascular NADP(H) oxidase or increased endothelial superoxide dismutase activity might explain the effects of ACE inhibition on lipid peroxidation.[31,32]

Although we controlled for factors such as obesity, hypertriglyceridaemia and low low density lipoprotein (LDL) cholesterol, which are all known to affect insulin sensitivity and endothelial function, this study has several limitations. The number of patients involved in the final analyses was small and this study could not exclude the effect of BP control by RAS inhibition on ISI Composite index. To determine this, the addition of a hypotensive group, in which BP is controlled by using a calcium channel blocker, would clarify the issue. Studies with a larger population are needed to clarify long-term effects of ACE-Is and ARBs on endothelial function and insulin sensitivity in essential hypertensive patients.

In conclusion, inhibition of the RAS, either with ACE-Is or ARBs, improved endothelial dysfunction, decreased oxidative stress and increased NOx levels in essential hypertensive patients. ACE-Is had a greater effect on improving insulin sensitivity than ARBs in these patients.

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