Original Communication

Effects of Combined Lipoic Acid and Pyridoxine on Albuminuria, Advanced Glycation End-Products, and Blood Pressure in Diabetic Nephropathy

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Abstract: This study was designed to investigate the effects of combined administration of lipoic acid and pyridoxine on albuminuria, oxidative stress, blood pressure, serum advanced glycation end-products, nitric oxide (NO), and endothelin-1 in patients with diabetic nephropathy. Thirty-four patients were randomly assigned to either a supplement group or a placebo group. The patients in the supplement group received 800 mg lipoic acid and 80 mg pyridoxine daily for 12 weeks, whereas the placebo group received corresponding placebos. Urinary albumin, serum malondialdehyde (MDA), and systolic blood pressure decreased significantly in the supplement group compared to the placebo group ($p < 0.05$). Serum NO increased in the supplement group compared to the placebo group ($p < 0.05$). Serum pentosidine and carboxymethyl lysine decreased significantly in the supplement group at the end of week 12 compared to baseline ($p < 0.05$). No statistically significant differences were observed between the two groups in mean changes of serum endothelin-1, glucose, and diastolic blood pressure. The present study indicates that combined administration of lipoic acid and pyridoxine improves albuminuria in patients with diabetic nephropathy by reducing oxidative stress, advanced glycation end-products, and systolic blood pressure. The reduction in microalbuminuria may be of benefit in retarding the progression of diabetic nephropathy.

Key words: diabetic nephropathy, lipoic acid, pyridoxine, advanced glycation end-products, oxidative stress, blood pressure

Introduction

The earliest sign of diabetic nephropathy, a chronic, progressive kidney disease that develops in about 20–50 % of all people with diabetes, is microalbuminuria [1–4]. Without specific treatment, levels of microalbuminuria slowly increase, resulting in macroalbuminuria in these patients, who eventually reach end-stage renal disease. This is why diabetic nephropathy is the most common cause of renal failure in most countries in the world [1–4]. Also, the risk of developing cardiovascular disease is 2–3 times higher in diabetic patients with microalbuminuria compared to persons with normal albumin excretion, whilst in diabetic patients with macroalbuminuria, the risk is increased at least 10-fold [1]. High serum concentrations of advanced glycation end-products (AGEs), oxidative stress, and hypertension are three important risk factors for diabetic nephropathy [5–7].

At present, administration of antihypertensive drugs, preferably angiotensin-converting enzyme inhibitors (ACEI) and/or angiotensin receptor-blockers (ARB), coupled with sodium- and protein-restricted diets, are used as common treatments for diabetic nephropathy. In spite of these renoprotective treatments, the prevalence of diabetic nephropathy and eventually, renal failure, is still high in diabetic patients [1, 8].

Two studies have indicated that various forms of vitamin B6, including pyridoxamine and pyridoxal phosphate, could reduce AGE formation and prevent progression of albuminuria in diabetic rats [9,10]. Simultaneously, other investigations showed that lipoic acid, a powerful antioxidant with water- and lipid-soluble characteristics [11], considerably reduced albuminuria in diabetic rats [12, 13]. However, in human studies, the administration of vitamin B6 (as pyridoxamine) or lipoic acid alone did not significantly decrease the albuminuria in diabetic patients [14, 15]. Therefore, in the present study, we investigated the effects of the combined administration of lipoic acid and vitamin B6 (as pyridoxine) on albuminuria in patients with diabetic nephropathy. In addition, to determine the mechanisms of the effects of this combination on albuminuria, we studied the effects of the combined administration of lipoic acid and pyridoxine on serum AGEs (including pentosidine and carboxymethyl lysine), NO and endothelin-1, blood pressure, and oxidative stress.

Materials and Methods

This study was a randomized, double-blind, placebo-controlled trial.

Subjects and Ethical Aspects

The minimum sample size estimation for each group was 15 at a power (1-β) of 90 % and $\alpha = 0.05$ for a two-arm parallel study with two-tailed testing to detect a difference of 55 mg/L in urinary microalbumin concentration with a pooled standard deviation of 47 mg/L, obtained from the study of Morcos [15]. Thirty-four patients (13 men and 21 women) with type II diabetes in the age range of 34 to 78 years were selected using convenience sampling from the outpatient clinic of the Endocrinology Division at Taleghani hospital in Tehran, Iran. Patients were selected according to the following criteria: Urinary albumin excretion of 30–1000 mg/g creatinine, confirmed at least twice with a one-month interval, serum creatinine ≤1.2 mg/dL, absence of hematuria, urinary tract infection, other renal diseases, symptomatic autonomic neuropathy, previous organ transplant, and heart failure. We excluded subjects that had received pyridoxine, lipoic acid, and vitamin E and/or C supplements within one month prior to the beginning of the study.

The study protocol was approved by the Ethics Committee of the National Nutrition and Food Technology Research Institute of Iran (Reference Number: P/25/47/6253). The study was in adherence with the Declaration of Helsinki. Written informed consent was obtained from all patients.

Protocol

Patients, after stratification according to their baseline level of albuminuria, were randomly allocated to either a lipoic acid-plus-pyridoxine-treated group (LA+PY) or placebo group by blocked randomization.

Subjects in the LA+PY group received 800 mg lipoic acid as 2 capsules (each containing 400 mg pure racemic (R/S) lipoic acid) and 80 mg vitamin B6 as 2 tablets (each containing 40 mg pure pyridoxine hydrochloride) daily for 12 weeks, whereas the placebo group received corresponding placebos containing lactose. The capsules and tablets were taken as two doses in the morning and evening.

Vitamin B6 tablet and corresponding placebos were produced by the Iran Hormone Company, Tehran,

Iran. Lipoic acid capsules and corresponding placebos were provided by the research unit of the 13-Aban drug store, Tehran, Iran.

Subjects were advised not to change their dietary habits, physical activities, and drug regimens. At baseline and the end of week 12, an early morning urine sample and 8 mL of blood were collected from each patient after a 12- to 14-hour fasting period. Blood samples were kept at room temperature (20–25°C) for 20–30 minutes. After clotting, the samples were centrifuged at 2000 rpm for 10 minutes. The samples of serum and urine were separated into small aliquots and were frozen at -70°C, until they were used. All assays were carried out in duplicate on samples thawed only once.

Measurements

Serum glucose and urinary creatinine were assessed using various colorimetry methods by commercial kits (Pars Azemoon, Tehran, Iran) with the aid of a Selectra 2 Autoanalyzer (Vital Scientific, Spankeren, The Netherlands). Intra-assay coefficients of variation (CV) for serum glucose and urinary creatinine were 2.8 % and 1.2 %, respectively. Urinary albumin concentration was measured using enzyme-linked immunosorbent assay (ELISA) kits (Orgentec Diagnostika, Mainz, Germany), with an intra-assay CV of 2.6 %. Serum concentration of MDA was assessed using colorimetry method by commercial kits (Cayman Chemical, Ann Arbor, MI, USA), with an intra-assay CV of 6.2 %. Serum concentrations of pentosidine and carboxymethyl lysine were assessed using ELISA kits (USCN Life Science & Technology, Wuhan, China). Intra-assay CVs for serum concentrations of pentosidine and carboxymethyl lysine were 7 % and 6.9 %, respectively. Serum NO concentration was determined using the colorimetry method by commercial kits (Active Motif, Tokyo, Japan), with an intra-assay CV of 7 %. Serum endothelin-1 concentration was measured using ELISA kits (Biomedica, Vienna, Austria), with an intra-assay CV of 7.8 %.

Patients were weighed at baseline and the end of weeks 6 and 12. In addition, the dietary intakes of subjects were assessed using a 3-day dietary recall (2 weekdays and 1 weekend day) at baseline and the end of weeks 6 and 12. Patients' diets were analyzed by Nutritionist IV software (N Squared Computing, San Bruno, CA, USA).

For blood pressure measurements at baseline and after three months' supplementation, participants were first asked to rest for 15 minutes, when a trained physician measured the blood pressure twice in seated participants, with a 10-minute interval, by using a standard mercury sphygmomanometer, and thereafter the mean of 2 measurements was considered as the participant's blood pressure.

Compliance

To ascertain patients' compliance, we provided each patient with a fixed number of capsules and tablets and instructions to return the unused capsules and tablets at the end of the study. Based on the number of returned capsules and tablets by each patient, their degree of compliance was determined, and the degree of compliance for our patients was over 90 %. In this study, no adverse events were reported.

Statistical Analysis

Statistical analysis of data was performed using the Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL, USA) for Windows version 16.0. A χ^2 test was used to compare qualitative variables between the two groups. Since all quantitative parameters according to the Kolmogorov-Smirnov test had normal distributions, we used a *t*-test and paired *t*-test to compare parameters between and within groups, respectively. In addition, because dietary and anthropometric parameters were measured 3 times during the study, analysis of variance for repeated measurements was used to compare data between these time points. The results are expressed as mean ± standard error (SE), and differences were considered statistically significant at $p \leq 0.05$.

Results

The baseline characteristics of patients did not differ significantly between the two groups (Table I).

The anthropometric and the dietary factors showed no statistically significant differences between the two groups at the baseline and the end of weeks 6 and 12. In addition, these factors did not significantly change within each group during the study (Table II).

The biochemical parameters showed no statistically significant differences between the two groups at the baseline (Table III).

Urinary albumin concentration was reduced significantly in the LA+PY group at the end of week 12

compared to baseline ($p < 0.05$), whereas no statistically significant change was observed in the placebo group. The decrease of urinary albumin concentration in the LA+PY group was statistically significant in comparison with the placebo group ($p < 0.05$; Table III).

Serum MDA concentration decreased significantly in the LA+PY group at the end of week 12 compared to baseline ($p < 0.05$), whereas no statistically significant change was observed in the placebo group. The reduction of serum MDA concentration in the LA+PY group was statistically significant in comparison with the placebo group ($p < 0.05$; Table III).

Serum pentosidine and carboxymethyl lysine decreased significantly in the LA+PY group ($p < 0.05$) at the end of week 12 compared to baseline, whereas no statistically significant change was observed in the placebo group. The reductions in serum pentosidine and carboxymethyl lysine in the LA+PY group were not statistically significant in comparison with the placebo group (Table III).

No statistically significant change was observed in fasting serum glucose within each group during the study (Table III).

Serum endothelin-1 did not significantly change within each group during the study (Table III). Serum NO concentration increased significantly in the LA+PY group at the end of week 12 compared to baseline ($p < 0.05$), whereas no statistically significant change was observed in the placebo group. The increase of serum NO concentration in the LA+PY group was statistically significant, compared to the placebo group ($p < 0.05$; Table III).

Diastolic blood pressure showed no statistically significant change within each group during the study, whereas systolic blood pressure decreased significantly in the LA+PY group, compared to the placebo group ($p < 0.05$; Table III).

Serum creatinine in LA+PY group and placebo group at the baseline were 1.01 ± 0.03 mg/dL and 0.95 ± 0.04 mg/dL, respectively. At the end of study, these values were 1.01 ± 0.04 mg/dL in the LA+PY group and 0.93 ± 0.06 mg/dL in the placebo group. There were no statistically significant differences between the two groups in serum creatinine at the baseline and the end of study.

Discussion

In our study, urinary albumin concentration decreased significantly to 74 mg/g creatinine in the LA+PY group. We have found no study to date investigating the effect of combined administration of lipoic acid and pyridoxine on urinary albumin concentration in diabetic nephropathy, to compare with the results of our study. The reduction of urinary albumin in our study may be partly due to administration of pyridoxine. This finding is in agreement with those of Degenhardt *et al.* [10] and Nakamura *et al.* [9], who showed that urinary albumin concentration in diabetic rats treated with the various forms of vitamin B6, including pyridoxamine and pyridoxal phosphate, was significantly lower compared to non-treated diabetic rats. In a study of Williams *et al.*, however, daily administration of 100 mg pyridoxamine for 24 weeks to pa-

Table I: Baseline characteristics of patients in the lipoic acid plus pyridoxine- treated group and the placebo group.

Characteristics	LA + PY (n = 17)	Placebo (n = 17)
Age (years)[1]	60.0 ± 2.0	61.0 ± 3.0
Duration of diabetes (years)[1]	13.0 ± 1.5	14.0 ± 1.5
Sex		
Men	7.0 (41.0 %)	6.0 (35.0 %)
Women	10.0 (59.0 %)	11.0 (65.0 %)
Smokers	0.0 (0.0 %)	1.0 (6.0 %)
Type of treatment for diabetes		
Insulin	7.0 (41.0 %)	9.0 (53.0 %)
Oral hypoglycemic agents	10.0 (59.0 %)	8.0 (47.0 %)
Anti-hypertensive drugs (ACE-I or ARB)	7.0 (41.0 %)	8.0 (47.0 %)

[1]Age and duration of diabetes are presented as mean ± SE.
ACEI: angiotensin converting enzyme inhibitors
ARB: angiotensin receptor blockers

Table II: Anthropometric and dietary factors in the lipoic acid plus pyridoxine-treated group and the placebo group.[1]

Factors	Groups	Baseline	Week 6	Week 12
Weight (kg)	LA + PY	75.0 ± 3.5	75.0 ± 3.5	75.0 ± 3.5
	Placebo	70.0 ± 3.0	70.0 ± 3.0	70.0 ± 3.0
BMI (kg/m^2)	LA + PY	28.0 ± 1.3	28.0 ± 1.3	28.0 ± 1.3
	Placebo	27.0 ± 1.2	27.0 ± 1.2	27.0 ± 1.2
Energy (kcal/d)	LA + PY	1639.0 ± 125.0	1640.0 ± 124.0	1634.0 ± 123.0
	Placebo	1628.0 ± 77.0	1628.0 ± 76.0	1620.0 ± 75.0
Protein (g/d)	LA + PY	69.0 ± 6.0	71.0 ± 6.0	68.0 ± 6.0
	Placebo	62.0 ± 5.5	64.0 ± 5.0	62.0 ± 5.0
Carbohydrate (g/d)	LA + PY	223.0 ± 16.0	222.0 ± 15.0	222.0 ± 16.0
	Placebo	226.0 ± 13.0	226.0 ± 12.0	227.0 ± 13.0
Fiber (g/d)	LA + PY	11.0 ± 0.8	11.0 ± 1.0	11.0 ± 1.0
	Placebo	12.0 ± 1.1	12.0 ± 1.1	12.0 ± 1.0
Fat (g/d)	LA + PY	52.0 ± 6.0	52.0 ± 5.0	52.0 ± 5.0
	Placebo	52.0 ± 3.0	52.0 ± 3.0	51.0 ± 3.0
SAFA (g/d)	LA + PY	16.0 ± 1.8	16.0 ± 1.8	16.0 ± 1.6
	Placebo	15.0 ± 0.9	15.0 ± 0.8	15.0 ± 0.7
MUFA (g/d)	LA + PY	17.0 ± 2.6	17.0 ± 2.5	17.0 ± 2.5
	Placebo	16.5 ± 1.8	16.0 ± 1.7	16.0 ± 1.8
PUFA (g/d)	LA + PY	15.0 ± 1.3	15.0 ± 1.2	15.0 ± 1.3
	Placebo	18.0 ± 1.3	18.0 ± 1.3	17.0 ± 1.1
Cholesterol (mg/d)	LA + PY	163.0 ± 20.0	167.0 ± 18.0	162.0 ± 19.0
	Placebo	179.0 ± 32.0	179.0 ± 33.0	179.0 ± 33.0
Vitamin E (mg/d)	LA + PY	8.5 ± 1.6	9.0 ± 1.4	9.0 ± 1.5
	Placebo	7.7 ± 1.3	8.0 ± 1.2	7.9 ± 1.1
Vitamin C (mg/d)	LA + PY	77.0 ± 11.0	77.0 ± 11.0	78.0 ± 11.0
	Placebo	78.0 ± 8.0	77.0 ± 8.0	77.0 ± 8.0
Vitamin B6 (mg/d)	LA + PY	1.1 ± 0.09	1.1 ± 0.10	1.1 ± 0.09
	Placebo	1.1 ± 0.15	1.0 ± 0.14	1.0 ± 0.14

All values are presented as mean ± SE.
[1]n = 17 for all values.
BMI: body mass index; MUFA: monounsaturated fatty acids
SAFA: saturated fatty acids; PUFA: polyunsaturated fatty acids

tients with diabetic nephropathy did not significantly decrease urinary albumin concentrations compared to a placebo group [14].

The decrease of urinary albumin in our study may also be partly due to the administration of lipoic acid, as reported by Melhem *et al.* [13] and Winiarska *et al.* [16], who found that urinary albumin concentration in diabetic animals receiving lipoic acid was significantly lower compared to untreated diabetic animals. Also, in the Morcos *et al.* study, daily administration of 600 mg lipoic acid to diabetic patients for 18 months decreased urinary albumin concentration from 29 mg/L at baseline to 15 mg/L at the end of the study, a reduction that was not statistically significant [15]. Therefore, previous studies showed that the administration of vitamin B6 or lipoic acid alone significantly decreased albuminuria in diabetic animals, but not in diabetic patients. However, the present study showed that combined administration of lipoic acid and vitamin B6 (as pyridoxine) could reduce albuminuria in patients with diabetic nephropathy.

In the present study, the reduction of albuminuria in the LA+PY group may be due to a decrease in oxidative stress, AGE formation, systolic blood pressure, and a rise in serum NO concentration. Oxidative stress and AGE compounds, by various mechanisms, play roles in the development of diabetic nephropathy. Firstly, AGEs stimulate gene expression of various growth factors, including transforming growth factor-β, connective tissue growth factor, and platelet-derived growth factor in the kidney [17, 18], which mediate production of extracellular matrix proteins such as collagens I, III, IV, V, VI, laminin, and fibronectin [6, 7, 17, 19, 20]. Accumulation of extracellular matrix proteins causes thickening of the glomerular basement membrane, glo-

Table III: Serum concentrations of fasting glucose, MDA, pentosidine, carboxymethyl lysine, NO, endothelin-1, systolic and diastolic blood pressures, and urinary albumin in the lipoic acid plus pyridoxine-treated group and the placebo group.[1]

Parameters	Groups	Baseline	Week 12	Changes[2]
Fasting serum glucose (mmol/L)	LA + PY	8.5 ± 0.3	8.7 ± 0.4	0.2 ± 0.3
	Placebo	8.8 ± 0.3	8.5 ± 0.3	−0.3 ± 0.4
MDA (μmol/L)	LA + PY	4.8 ± 0.6	3.6 ± 0.5 *	−1.2 ± 0.5 ‡
	Placebo	4.1 ± 0.3	4.1 ± 0.3	0.0 ± 0.3
Pentosidine (ng/mL)	LA + PY	18.2 ± 4.6	11.3 ± 2.0 *	−6.8 ± 2.8
	Placebo	10.4 ± 1.3	9.3 ± 1.4	−1.1 ± 1.2
Carboxymethyl lysine (ng/mL)	LA + PY	13.8 ± 1.1	11.5 ± 0.9 *	−2.3 ± 1.0
	Placebo	11.5 ± 0.8	10.9 ± 0.8	−0.6 ± 0.7
NO (μmol/L)	LA + PY	30.6 ± 0.4	31.6 ± 0.6 *	1.0 ± 0.5 ‡
	Placebo	30.9 ± 0.7	30.6 ± 0.4	−0.3 ± 0.4
Endothelin-1(fmol/mL)	LA + PY	1.1 ± 0.2	0.9 ± 0.1	−0.2 ± 0.1
	Placebo	1.1 ± 0.3	0.9 ± 0.2	−0.2 ± 0.1
Systolic blood pressure (mmHg)	LA + PY	142.0 ± 4.5	140.0 ± 5.0	−2.0 ± 1.0 ‡
	Placebo	133.0 ± 4.5	135.0 ± 4.5	1.5 ± 1.0
Diastolic blood pressure (mmHg)	LA + PY	77.0 ± 2.8	77.0 ± 2.8	0.0 ± 0.3
	Placebo	72.0 ± 2.2	72.0 ± 2.1	0.0 ± 0.4
Urinary microalbumin ($mg/g_{creatinine}$)	LA + PY	236.0 ± 75.0	162.0 ± 44.0 *	−74.0 ± 34.0 ‡
	Placebo	181.0 ± 52.0	208.0 ± 61.0	27.0 ± 16.0

All values are presented as mean ± SE.
[1] n = 17 for all values.
[2] Changes reflect week 12 – baseline values.
*) $p < 0.05$ vs. baseline
‡) $p < 0.05$ vs. the placebo group
NO: nitric oxide; MDA: malondialdehyde

merulosclerosis, hyperfiltration, increased glomerular blood pressure, and finally microalbuminuria [17]. Secondly, AGE formation on extracellular matrix proteins reduces their ability to interact with negatively charged proteoglycans and increases vascular permeability to albumin [7]. Thirdly, AGEs induce gene expression of vascular endothelial growth factor, which increases glomerular permeability and microalbuminuria [7, 19]. Finally, oxidative stress causes a decrease in NO bioavailability [21, 22] and an elevation in angiotensinogen expression [21], both of which increase glomerular blood pressure and microalbuminuria.

Increased tissue or serum AGE concentration is an important cause of diabetes complications [7] and it has been shown that accumulation of carboxymethyl lysine and pentosidine in glomeruli is correlated with the severity of diabetic nephropathy [5]. In the present study, serum concentrations of carboxymethyl lysine and pentosidine were significantly reduced by 17 % and 39 % in the LA+PY group, respectively. These reductions in the LA+PY group were not statistically significant in comparison with the placebo group. This may be due to the short duration of the study; in a longer study, these reductions may become statistically significant in comparison with the placebo group. We found no study on the effects of combined administration of lipoic acid and pyridoxine on serum AGEs, especially carboxymethyl lysine and pentosidine, in diabetic nephropathy, to compare with the results of our study. However, the reductions of serum carboxymethyl lysine and pentosidine in the LA+PY group may be due principally to the administration of pyridoxine. Few studies in diabetic rats have indicated that various forms of vitamin B6, including pyridoxamine and pyridoxal phosphate, could reduce AGE formation [9,10]. In addition, Williams *et al.* showed that daily administration of 500 mg pyridoxamine to patients with diabetic nephropathy for 20 weeks significantly reduced plasma carboxymethyl lysine concentration [14]. Various forms of vitamin B6 reduce AGE formation by three possible mechanisms [23]. Firstly, they prevent conversion of protein-Amadori to AGEs via complexing with catalytic metal ions; secondly, various forms of vitamin B6 inhibit protein modifications by scavenging reactive carbonyl compounds of glucose and lipid degradation and thirdly; they may inhibit metal-catalyzed oxidative steps in protein modifications by reactive carbonyl compounds [23].

In our study, the reduction of serum AGEs in the LA+PY group may also be partly due to the administration of lipoic acid. In the oxidative stress state, reactive oxygen species (ROS) react with carbohydrates, polyunsaturated fatty acids, or amino acids and yield reactive carbonyl compounds, which subsequently react with proteins and form AGEs [20, 24]. Therefore, the administration of lipoic acid reduces oxidative stress and consequently causes a decrease in AGE formation. In agreement with our study, Thirunavukkarasu *et al.* reported that the administration of lipoic acid decreased AGE formation in rats [25].

Oxidative stress is increased in diabetic patients and it is considered as a major cause of diabetes complications [7, 26, 27]. In our study, compared to the placebo group, serum MDA in the LA+PY group decreased significantly by 25 %, which may be due principally to the administration of lipoic acid, which is a powerful antioxidant. This result is in agreement with the findings of other studies [16, 28–30].

The combined administration of lipoic acid and pyridoxine did not reduce fasting serum glucose in diabetic nephropathy in our study, and we found no research in this field in diabetic nephropathy, to compare with the results of our study. However, Muellenbach *et al.* showed that combined administration of lipoic acid (92 mg/kg) and pyridoxamine (60 mg/kg) to obese Zucker rats, resulted in the reduction of fasting plasma glucose [31]. The disagreement of Muellenbach *et al.*'s finding with that of our study may be due to the administration of very high doses of lipoic acid and pyridoxamine to rats, whereas these doses are not allowed in humans. Few studies on the effect of lipoic acid alone on fasting plasma glucose are available, and their results are in agreement with that of our study [32–34].

High blood pressure significantly increases the risk of the progression of nephropathy in diabetic patients [21]. In our study, serum concentration of endothelin-1, as a vasoconstrictor agent, did not significantly change in the LA+PY group, whereas serum concentration of NO, as a vasodilator agent, increased significantly in the LA+PY group compared to the placebo group. The elevation of serum NO in the LA+PY group may be due principally to administration of lipoic acid and the reduction of oxidative stress. There are several interactions between ROS and NO leading to a decrease in NO bioavailability. In oxidative stress, ROS including free radical superoxide anion ($\cdot O_2^-$) rapidly reacts with NO and yields reactive nitrogen species such as peroxynitrite ($ONOO^-$), associated with a decrease in NO bioavailability [21, 22].

During the present study, diastolic blood pressure did not significantly change within each group, whereas systolic blood pressure in the LA+PY group was reduced by 2 mmHg; the reduction that was statistically significant compared to the placebo group may be because of the decrease of oxidative stress by the lipoic acid supplement and the consequent increase in serum NO. In agreement with our results, Thirunavukkarasu *et al.* showed that the administration of lipoic acid decreased blood pressure in male Wistar rats [35].

We did not measure the serum concentrations of pyridoxine and lipoic acid, and this was a limitation of our study.

In conclusion, the present study indicates that combined administration of lipoic acid and pyridoxine improves albuminuria in patients with diabetic nephropathy by reducing oxidative stress, advanced glycation end-products, and systolic blood pressure. The reduction in microalbuminuria may be of benefit in retarding the progression of diabetic nephropathy, but further studies would be needed to determine that benefit.

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