

# Comparison of the Effects of Vitamins and/or Mineral Supplementation on Glomerular and Tubular Dysfunction in Type 2 Diabetes

MARYAM SADAT FARVID, PHD<sup>1</sup>  
MAHMOUD JALALI, PHD<sup>2</sup>

FEREYDOUN SIASSI, PHD<sup>2</sup>  
MOSTAFA HOSSEINI, PHD<sup>3</sup>

**OBJECTIVE** — The present study was designed to assess the effect of magnesium plus zinc, vitamins C plus E, and a combination of these micronutrients on nephropathy indexes in type 2 diabetic patients.

**RESEARCH DESIGN AND METHODS** — In a randomized, double-blind, placebo-controlled clinical trial, 69 type 2 diabetic patients were randomly divided into four groups, each group receiving one of the following daily supplement for 3 months: group M ( $n = 16$ ), 200 mg Mg and 30 mg Zn; group V ( $n = 18$ ), 200 mg vitamin C and 100 IU vitamin E; group MV ( $n = 17$ ), minerals plus vitamins; and group P ( $n = 18$ ), placebo. Urinary albumin excretion and N-acetyl- $\beta$ -D-glucosaminidase activity (NAG) in urine were determined at the beginning and at the end of the trial. Treatment effects were analyzed by general linear modeling.

**RESULTS** — Results indicate that after 3 months of supplementation, levels of urinary albumin excretion decreased in the V and MV groups ( $P = 0.034$  and  $P = 0.005$ , respectively). Urinary NAG activity did not significantly change in any treatment groups. Levels of systolic, diastolic, and mean blood pressure significantly decreased in the MV group ( $P = 0.008$ ,  $P = 0.017$ , and  $P = 0.009$ , respectively). Also, combination of vitamin and mineral supplementation had significant effects in decreasing fasting serum glucose ( $P = 0.035$ ) and malondialdehyde concentrations ( $P = 0.004$ ) and in increasing HDL cholesterol and apolipoprotein A1 levels ( $P = 0.019$ ). There was no significant change in the levels of these parameters in the other three groups.

**CONCLUSIONS** — In conclusion, the results of the present study provide evidence for the effects of vitamins C and E and also combination of magnesium, zinc, and vitamins C and E supplementation on improvement of glomerular but not tubular renal function in type 2 diabetic patients.

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**D**iabetic nephropathy is a serious complication of diabetes and a major cause of mortality and morbidity in these patients (1,2). Results of studies in animal models and humans have demonstrated that diabetes is associated with oxidative stress (3–5) and reduced levels of antioxidants (6). It has

been well established that plasma magnesium levels in patients with diabetes is somewhat decreased (7,8). Numerous studies have found decreases in physiological measures of zinc status, hyperzincuria, and indications of zinc malabsorption in diabetic individuals (7,9). Levels of plasma ascorbic acid were

found to be significantly lower in diabetic patients compared with nondiabetic control subjects in most studies (10–12). Based on their results, the researchers observed that individuals with diabetes have significant defects of antioxidant protection, which may enhance their susceptibility to oxidative stress (12).

Oxidative stress is hypothesized to play an important part in the development of late diabetes complications (6). Chronic hyperglycemia increases oxidative stress and considerably modifies the structure and function of proteins and lipids, due to glycooxidation and peroxidation (6). These modified products could contribute to the morphological and functional abnormalities seen in the kidney of patients with diabetes (4–6,13). For these reasons, there has been interest in the use of dietary antioxidants as an intervention to attenuate diabetic nephropathy. Clinical studies (14–16) suggest that the use of antioxidants indirectly helps in the prevention or improvement of diabetic nephropathy. Also, association between microalbuminuria and magnesium depletion was shown (17).

Because of the known synergistic action between vitamins E and C (18), vitamin E and zinc (19,20), and vitamin E and magnesium (21,22), a further important question is whether a combination of antioxidants provides better protection, the purpose of the study was to determine the effects of supplementation with magnesium and zinc, vitamins E and C, and a combination of these micronutrients on parameters of early nephropathy in type 2 diabetic patients.

## RESEARCH DESIGN AND METHODS

A randomized, double-blind, placebo-controlled clinical trial was conducted on 76 type 2 diabetic patients (36 men and 40 women) aged 30–69 years who have had diabetes for at least 1 year, with a bias toward those who were not macroalbuminuric (urine albumin excretion  $>300$  mg/g creatinine) and hypertensive (blood pressure  $>160/100$

From the <sup>1</sup>Department of Community Nutrition, School of Nutrition and Food Technology, Shaheed Beheshti University of Medical Sciences, Tehran, Iran; the <sup>2</sup>Department of Nutrition and Biochemistry, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; and the <sup>3</sup>Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

Address correspondence and reprint requests to Maryam Sadat Farvid, Department of Community Nutrition, School of Nutrition and Food Technology, Shaheed Beheshti University of Medical Sciences, P.O. Box 19395-4741, Tehran, Iran. E-mail: farvidm@yahoo.ca.

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**Abbreviations:** Apo, apolipoprotein; MDA, malondialdehyde; NAG, N-acetyl- $\beta$ -D-glucosaminidase.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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mmHg). The calculated sample size is 18 patients in each group in order to have 80% power to detect the postulated differences in urine albumin excretion with an  $\alpha$ -error of 5%.

Data on dietary habits, BMI, smoking, medication, and dietary supplements were obtained from careful personal interview. All subjects had to meet the following criteria to be included in the study: not taking vitamin and/or mineral supplements, thyroid hormones, estrogen, progesterone, diuretics, or antihypertensive agents; having normal hepatic function; having no history of myocardial infarction; and, in women, not being pregnant.

The subjects were fully informed of the purpose, procedures, and hazards of the trial and were free to leave the trial at any time. Written informed consent was obtained from all participants. The research protocol was approved by the ethics committee on human experimentation of Tehran University of Medical Sciences.

Dosage levels of vitamin C and magnesium were lower than those used in previous studies, which were estimated to be attainable through diet without any side effects, especially for magnesium, such as excessive gas and bloating, loose stools, and diarrhea, as reported by other studies. Zinc dose was used as tolerable upper intake level (40 mg/day). Since the mean daily intake of zinc in our diabetic patients was 8.3 mg, we increased the level of intake near to tolerable upper intake levels by giving 30 mg/day zinc supplementation.

Diabetic patients were stratified by sex and randomly assigned to one of four treatment groups using the block randomization procedure. Depending upon the treatment groups, each subject received two capsules per day for a period of 3 months. Patients were instructed to take the capsules with breakfast and dinner meals. Each capsule contained one of the following preparations and hence determined the corresponding groups: group M, zinc sulfate and magnesium oxide (providing 15 mg Zn and 100 mg Mg); group V, vitamin C (100 mg) and vitamin E (50 IU); group MV, both of the above mineral and vitamin supplements; and group P, lactose (placebo). The supplement and placebo capsules looked identical and were specially prepared for this study by Darou-Pakhsh (Tehran, Iran) and stored at 20–25°C.

After 12- to 14-h overnight fasting, between 8 and 10 A.M. and before taking any oral hypoglycemic agent(s), 20 ml

blood and morning urine samples were collected from each subject at the beginning and at the end of the 3-month trial in trace element-free tubes. Aliquots of serum and urine were transferred to polystyrene tubes that were immediately stored at  $-70^{\circ}\text{C}$  until analysis. Plasma ascorbic acid was measured with a colorimetric method (23), serum  $\alpha$ -tocopherol was determined by high-performance liquid chromatography (24), and lipid-standardized  $\alpha$ -tocopherol was calculated as serum  $\alpha$ -tocopherol concentration expressed per milligram triglyceride plus cholesterol ( $\mu\text{g}/\text{mg}$ ). Zinc and magnesium were measured in serum and urine samples by colorimetric methods using commercial kits (Randox, Crumlin, U.K., and Pars-Azmoon, Tehran, Iran, respectively). Urine microalbumin concentration was measured by immunoturbidimetric assay using commercial kit (Randox). Urine total protein was determined by the Lowry method (25). Urine *N*-acetyl- $\beta$ -D-glucosaminidase (NAG) activity was determined by colorimetric method (26). Urine creatinine was measured using Jaffe reaction (27), and all urine results were expressed in relation to gram creatinine excretion. Serum malondialdehyde (MDA) was determined by a colorimetric method (28), and lipid-standardized MDA was calculated as plasma MDA concentration expressed per milligram triglyceride plus cholesterol ( $\mu\text{mol}/\text{mg}$ ).

Fasting serum glucose was enzymatically measured. Serum fructosamine concentration was determined by commercial kit (Randox). HbA1c was measured by chromatography method using commercial kit (Sigma), and the normal range of HbA1c was 5.5–8.5% and for uncontrolled diabetic subjects was 12–20%. Serum triglycerides and total cholesterol were enzymatically measured. HDL cholesterol was determined after precipitation with phosphotungstate/magnesium and LDL cholesterol after precipitation with heparin/sodium citrate. Apolipoproteins (apos) A1 and B were measured by immunoturbidimetric method. Intra- and interassay coefficient of variation was <5% for all laboratory tests. The systolic (SBP) and diastolic (DBP) blood pressure (5 min seated rest, mean of two readings) were measured at baseline and after 3 months of supplementation. Mean arterial pressure was calculated using the following formula:  $(\text{SBP} + 2\text{DBP})/3$  (29).

Nutrient intakes were estimated using two 24-h dietary recall questionnaires at the beginning and at the end of the

3-month trial and analyzed by Food Processor software. The subjects were asked not to alter their usual diets and physical activity throughout the study, and any changes in their medication were avoided whenever possible. Compliance with the supplementation was checked by capsule counts at weeks 6 and 12 and were also confirmed through measurement of changes in the serum and/or urine levels of magnesium, zinc, and vitamins C and E. To enhance compliance, individual sessions with a skilled counselor were conducted at following visits (6 weeks and 3 months). Also, phone contacts between counseling sessions were done. Subjects who had used <90% of capsules were excluded from the statistical analysis.

### Statistical analysis

All data are expressed as means  $\pm$  SD. Kolmogorov-Smirnov normality tests were performed on independent and dependent variables before further statistical analysis. Skewed data (serum triglyceride levels and urinary concentration of  $\beta$ NAG and albumin) were log transformed for analysis. Differences between the four groups were determined by one-way ANOVA for continuous data and the  $\chi^2$  test for group data. Post hoc comparisons were performed with the Tukey test. Adjustment for differences in baseline covariates and changes in variables during study were performed by ANCOVA using general linear models. A value of  $P < 0.05$  was considered to be statistically significant. All data were analyzed using SPSS software.

**RESULTS** — Sixty-nine of 76 subjects completed the study. Two patients withdrew because of side effects in the 1st week of study, and five were excluded from statistical analysis because three had used <90% of capsules and two changed their hypoglycemic agents by the 3rd month. Table 1 lists the clinical and biochemical characteristics of the 76 patients at baseline. Four patients were diet controlled only, and the others were treated with metformin and/or sulfonylurea. None of the patients took insulin or antihypertensive agents. As shown in Table 1, at the beginning of the study, the groups were similar with respect to age, sex, duration of diabetes, BMI, smoking, and daily intake of vitamins C and E and magnesium and zinc. There were no significant changes in BMI, physical activity, dietary intake, hypoglycemic agents, or

**Table 1—Demographic, anthropometric, and biological data for the four study groups before supplementation**

	Group P	Group M	Group V	Group MV
n	19	18	20	19
Men/women	9/10	8/10	9/11	9/10
Age (years)	50 ± 9	52 ± 8	50 ± 9	50 ± 9
Duration of diabetes (years)	8.3 ± 4.3	9.0 ± 6.2	9.2 ± 5.3	7.7 ± 4.7
BMI (kg/m <sup>2</sup> )	27.4 ± 3.7	27.7 ± 4.7	27.5 ± 4.7	29.2 ± 4.0
Smokers (n)	3	2	3	2
HbA1c (%)	9.1 ± 2.1	10.2 ± 2.7	11.5 ± 3.2	9.4 ± 1.6
Systolic blood pressure (mmHg)	127 ± 16	122 ± 15	125 ± 14	131 ± 19
Diastolic blood pressure (mmHg)	82 ± 9	78 ± 12	80 ± 9	94 ± 11
Vitamin C intake (mg/day)	83.5 ± 69.8	83.6 ± 87.3	77.8 ± 45.7	109.2 ± 114
Vitamin E intake (mg/day)	12.0 ± 15.0	9.3 ± 10.4	8.4 ± 9.1	13.5 ± 11.7
Magnesium intake (mg/day)	225 ± 93	213 ± 97	207 ± 66	248 ± 122
Zinc intake (mg/day)	7.9 ± 2.8	7.8 ± 2.5	7.2 ± 2.1	9.2 ± 4.5

Data are means ± SD, unless otherwise indicated. There were no significant differences between groups by ANOVA or  $\chi^2$ .

other medications during the study period. At baseline, 55.5% of patients had low concentration of serum magnesium (<1.9 mg/dl), and 3% of patients had low concentration of plasma vitamin C (<0.5 mg/dl). None of the subjects exhibited serum zinc levels below the deficient values of 70  $\mu$ g/dl and serum vitamin E levels <5  $\mu$ g/ml.

At the beginning of the study, ANOVA showed that the groups were similar based upon plasma vitamin C; serum levels of vitamin E, magnesium, and zinc; and urinary levels of minerals (Table 2). After 3 months of supplementation, levels of plasma vitamin C, serum vitamin E, and lipid-standardized  $\alpha$ -tocopherol increased significantly in groups V and MV compared with baseline and groups P and M. Serum and urinary levels of zinc increased significantly in groups M and MV. In spite of this increment in serum and urine magnesium in the M and MV groups, the changes were not statistically significant.

Table 3 shows the glycemic and neuropathy indexes in diabetic patients. At baseline by ANOVA, there were no significant differences among groups. Following 3 months of supplementation, fasting serum glucose decreased significantly in group MV using general linear modeling. Also, urinary albumin excretion decreased in groups V and MV (Fig. 1). There were no significant changes in other groups. The concentrations of fructosamine, HbA1c, urinary NAG, and protein were not altered after 3 months of supplementation in all four groups.

Diabetic patients were stratified by

low and high serum levels of magnesium (<1.9 mg/dl and  $\geq$ 1.9 mg/dl, respectively). Among patients with serum mag-

nesium <1.9 mg/dl, urinary albumin excretion significantly decreased in group MV using general linear modeling. There were no significant changes in patients with serum magnesium levels >1.9 mg/dl in all four groups (Table 4).

Table 5 shows the levels of lipid and lipoprotein profiles and MDA in all four groups. At baseline, there were no significant differences among groups. Following 3 months of supplementation, serum levels of HDL cholesterol and apoA1 increased significantly in group MV. There were no significant changes in the other three groups. There were significant changes in groups M and MV for MDA and lipid-standardized MDA. Total cholesterol, LDL cholesterol, triglyceride, and apoB were not altered after supplementation in all four groups.

Table 6 shows the means ± SD of systolic, diastolic, and mean blood pressure. At the beginning, ANOVA showed no statistically significant difference(s) between groups. After 3 months of supplementa-

**Table 2—Levels of vitamins and minerals in type 2 diabetic patients before and after 3 months of supplementation**

	Group P	Group M	Group V	Group MV
n	18	16	18	17
Plasma vitamin C (mg/dl)				
Before	1.04 ± 0.30	1.10 ± 0.33	1.13 ± 0.27 <sup>a</sup>	1.12 ± 0.40 <sup>a</sup>
After*	1.10 ± 0.33	1.02 ± 0.32	1.35 ± 0.24	1.45 ± 0.20
Serum vitamin E ( $\mu$ g/ml)				
Before	20.8 ± 6.1	22.6 ± 5.7 <sup>a</sup>	25.2 ± 6.9 <sup>b</sup>	24.0 ± 4.7 <sup>b</sup>
After†	20.4 ± 4.6	24.2 ± 5.7	36.9 ± 10.7	38.1 ± 10.7
Lipid-standardized $\alpha$ -tocopherol ( $\mu$ g/mg)				
Before	6.38 ± 1.43	6.79 ± 1.91	6.50 ± 1.37 <sup>b</sup>	6.06 ± 0.76 <sup>b</sup>
After†	6.15 ± 0.67	6.88 ± 2.17	9.98 ± 2.04	9.66 ± 2.01
Serum magnesium (mg/dl)				
Before	1.77 ± 0.32	1.82 ± 0.24	1.87 ± 0.24	1.85 ± 0.25
After	1.79 ± 0.34	1.87 ± 0.27	1.86 ± 0.40	1.96 ± 0.21
Serum zinc ( $\mu$ g/dl)				
Before	102.4 ± 24.8	99.3 ± 24.1 <sup>a</sup>	98.1 ± 12.7	95.8 ± 9.0 <sup>c</sup>
After‡	96.8 ± 13.1	114.3 ± 23.5	96.4 ± 13.4	114.9 ± 19.1
Urine magnesium (mg/g creatinine)				
Before	60.2 ± 36.2	57.1 ± 15.3	57.0 ± 18.6	58.6 ± 20.4
After	59.3 ± 16.1	66.0 ± 24.5	55.5 ± 24.6	66.4 ± 29.5
Urine zinc ( $\mu$ g/g creatinine)				
Before	942 ± 374	908 ± 452 <sup>c</sup>	1,066 ± 418	906 ± 279 <sup>c</sup>
After‡	824 ± 253	1,584 ± 889	1,073 ± 401	1,305 ± 456

Data are means ± SD. There were no significant baseline differences between groups by ANOVA. Lipid-standardized  $\alpha$ -tocopherol is calculated as micrograms serum  $\alpha$ -tocopherol concentration expressed per milligram (cholesterol + triglyceride). Statistically significant differences between before and after using general linear models: <sup>a</sup> $P < 0.01$ , <sup>b</sup> $P < 0.0001$ , <sup>c</sup> $P < 0.001$ . After supplementation: \*groups V and MV have significantly higher levels than groups P and M ( $P < 0.05$ ); †groups V and MV have significantly higher levels than groups P and M ( $P < 0.0001$ ); ‡groups M and MV have significantly higher levels than groups P and V ( $P < 0.05$ ).

Table 3—Levels of glycemic and nephropathy indices before and after 3 months of vitamin and mineral supplementation in type 2 diabetic patients

	Group P	Group M	Group V	Group MV
n	18	16	18	17
Fasting serum glucose (mmol/l)				
Before	9.02 ± 2.81	9.51 ± 2.81	10.89 ± 2.64	9.76 ± 2.25*
After	9.62 ± 2.69	9.68 ± 2.53	9.95 ± 2.31	9.07 ± 2.53
Fructosamine (μmol/l)				
Before	436 ± 136	451 ± 140	497 ± 123	413 ± 71
After	446 ± 121	481 ± 125	472 ± 96	413 ± 79
HbA1c (%)				
Before	9.21 ± 2.05	10.38 ± 2.70	11.23 ± 3.36	9.29 ± 1.58
After	10.04 ± 1.98	10.64 ± 2.25	11.01 ± 2.19	9.39 ± 2.18
Microalbumin (mg/g creatinine) (95% CI)				
Before	30.7 (8.7–52.7)	30.4 (12.3–48.4)	35.6 (6.2–64.9)†	29.3 (–3.2 to 61.9)‡
After	42.8 (11.8–73.8)	34.0 (13.9–54.1)	22.1 (5.2–39.0)	10.8 (4.2–17.3)
NAG (units/g Cr) (95% CI)				
Before	13.7 (8.1–19.3)	18.3 (12.8–23.8)	16.9 (13.1–20.7)	16.0 (11.1–20.9)
After	15.6 (10.3–21.0)	25.3 (13.4–37.3)	19 (11.0–26.9)	17.2 (10.2–24.2)
Urine protein (g/g creatinine)				
Before	1.38 ± 1.04	2.37 ± 0.96	2.06 ± 1.20	2.61 ± 1.05
After	2.25 ± 1.03	2.09 ± 0.74	2.11 ± 0.79	2.21 ± 0.72

Data are means ± SD, unless otherwise indicated. Statistically significant differences between before and after using general linear models: \* $P = 0.035$ , † $P = 0.034$ , ‡ $P = 0.005$ .

tion, significant decreases in the MV group for systolic and diastolic blood pressure and mean arterial pressure were observed from baseline and also when compared with group P. There were no significant changes in the other three groups. General linear modeling has been done to statistically control the effects of changes in blood pressure and glucose on microalbuminuria. The results showed that effects of these micronutrients on decreasing urine albumin excretion were in-

dependent of changes in blood pressure or glucose (ANCOVA,  $P = 0.009$ ).

There were significant positive correlations between the increase in serum vitamin E and the increase in HDL cholesterol ( $r = 0.359$ ,  $P = 0.002$ ) and the increase in serum magnesium and the increase in HDL cholesterol ( $r = 0.252$ ,  $P = 0.033$ ) and significant negative correlations between the increase in serum zinc and the reduction in HbA1c ( $r = -0.349$ ,  $P = 0.003$ ) and MDA ( $r = -0.247$ ,

$P = 0.038$ ) and the increase in plasma vitamin C and the reduction in fasting serum glucose ( $r = -0.502$ ,  $P < 0.0001$ ) and MDA ( $r = -0.267$ ,  $P = 0.024$ ).

**CONCLUSIONS**— The present study shows that 3 months of treatment with vitamins C and E and also combination of magnesium, zinc, and vitamins C and E supplementations significantly lowered urinary albumin excretion, which serves as a marker for glomerular renal function. In contrast to urinary albumin, urinary NAG, a lysosomal enzyme friction of proximal tubular renal cells, and urinary total protein have not been changed in any treatment groups.

These findings are consistent with recent studies that concluded that receiving 1,250 mg vitamin C and 680 IU vitamin E for 4 weeks (14), 1,800 IU vitamin E for 4 weeks (15), or receiving 1,000 mg vitamin C for 9 months (16) decreased the urinary albumin excretion ratio in diabetic patients.

Microalbuminuria predicts the onset of overt renal disease in diabetic patients. Albuminuria reflects glomerular dysfunction, whereas urinary excretion of NAG indicates proximal tubular dysfunction (30). The enzyme NAG is secreted into tubular filtrate through damaged epithelial tissue (31). However, some studies show that the increased excretion of NAG

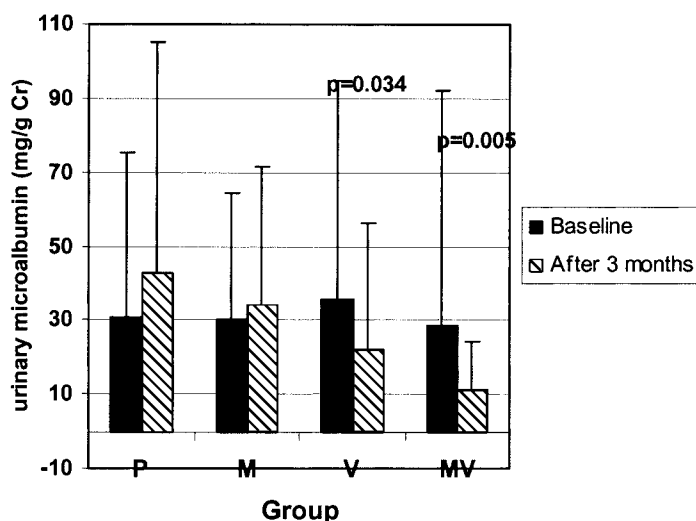


Figure 1—Levels of urine albumin excretion before and after 3 months of vitamin and/or mineral supplementation in type 2 diabetic patients.

**Table 4—Levels of urinary microalbumin before and after 3 months of vitamin and mineral supplementation in type 2 diabetic patients with low and high serum levels of magnesium (<1.9 mg/dl or ≥1.9 mg/dl)**

	Serum magnesium levels <1.9 mg/dl			Serum magnesium levels ≥1.9 mg/dl		
	n	Before	After	n	Before	After
Group P	11	40.5 (5.3–75.6)	58.1 (8.3–107.9)	7	15.5 (–5.7 to 36.8)	18.2 (–7.6 to 44)
Group M	8	42.5 (10.2–74.8)	50.5 (11.6–89.3)	8	18.1 (–2.8 to 39.2)	17.5 (4.1–30.9)
Group V	10	56.4 (3.4–109.3)	33.9 (3.3–64.5)	8	9.6 (5.4–13.7)	7.3 (4.1–10.5)
Group MV	12	30.1 (22.4–61.0)	7.6 (3.9–11.3)*	5	27.5 (–19 to 74.1)	18.4 (–8 to 44.9)

Data are means (95% CI). Statistically significant differences between before and after using general linear models: \**P* = 0.024.

seems to be a very sensitive marker for renal damage and the first renal marker measurable before microalbuminuria (32). O'Brien et al. (30) has shown tubular dysfunction occurring at a later stage of diabetic nephropathy than glomerular dysfunction. Using these micronutrients only has an effect on improvement of glomerular but not tubular renal function.

Over the last decade, there has been an intense interest in oxidative stress and its role in the development of nephropathy in diabetic patients (4,5). Chronic hyperglycemia increases oxidative stress and modifies the structure and function of

proteins and lipids considerably due to glycooxidation and peroxidation (6). These modified products could contribute to the morphological and functional abnormalities seen in the kidney of patients with diabetes (4–6,13). Our study provides support for the effect of these micronutrients in reducing MDA, a product of lipid peroxidation, as a known confounder of nephropathy in type 2 diabetes. Thus, the attenuation of microalbuminuria by these micronutrients may be linked to their antioxidant activity and inhibition of lipid peroxidation.

However, the mean levels of fasting

serum glucose decreased significantly in group MV. There were no significant changes in fructosamine or HbA1c in treatment groups. The role of intensified glycemic control in diabetic patients and constant microalbuminuria remains controversial. However, some studies revealed that poor glycemic control was significantly associated with the development of overt proteinuria in microalbuminuric patients with type 2 diabetes (33). In the Diabetes Control and Complications Trial (34), no advantage of intensified glycemic control was found in diabetic patients who had microalbuminuria at the beginning of the study. These results indicate that the renoprotective effects of vitamins C and E, and also combination of magnesium, zinc, and vitamins C and E, are not adequately explained by its hypoglycemic action. Therefore, the action of supplementary micronutrients reducing microalbuminuria may be related to their antioxidant functions.

Hypertension is well recognized as a risk factor for renal vascular and functional abnormalities (35,36), and antihypertensive therapies can halt the progression of renovascular disease and contribute to the preservation of renal function (37). Hypertension causes albuminuria, independent of diabetes, by a hemodynamic mechanism of increased glomerular capillary hydrostatic pressure (38). In the present study, although the combination of these micronutrients reduced blood pressure in these patients, it seems that reduction of urinary albumin excretion is statistically independent of changes in blood pressure. Since none of the patients took any antihypertensive agents including ACE inhibitors, whether patients benefit from combined treatment with micronutrients and ACE inhibitors agents remains to be elucidated.

Animal studies (39,40) support the evidence that hyperlipidemia is a risk factor for diabetic nephropathy. In type 2

**Table 5—Levels of serum lipids, lipoproteins, and MDA before and after 3 months of vitamin and mineral supplementation in type 2 diabetic patients**

	Group P	Group M	Group V	Group MV
n	18	16	18	17
Cholesterol (mmol/l)				
Before	4.56 ± 0.98	4.69 ± 0.85	4.87 ± 0.88	5.26 ± 0.80
After	4.66 ± 0.71	4.74 ± 0.80	4.87 ± 0.93	5.26 ± 0.96
Triglycerides (mmol/l)				
Before	1.83 ± 1.24	1.89 ± 0.95	2.44 ± 1.52	2.21 ± 0.93
After	1.74 ± 0.75	2.17 ± 1.20	2.15 ± 0.98	2.26 ± 1.19
HDL cholesterol (mmol/l)				
Before	1.01 ± 0.31	1.04 ± 0.41	0.93 ± 0.18	1.05 ± 0.28*
After	0.91 ± 0.19	1.00 ± 0.29	1.08 ± 0.40	1.31 ± 0.50
LDL cholesterol (mmol/l)				
Before	2.80 ± 0.83	2.77 ± 0.60	3.16 ± 0.88	3.31 ± 0.88
After	2.95 ± 0.83	2.72 ± 0.67	3.13 ± 1.11	3.21 ± 0.80
ApoA1 (mg/dl)				
Before	145 ± 22	142 ± 27	145 ± 25	156 ± 24†
After	143 ± 24	146 ± 21	146 ± 20	170 ± 34
ApoB (mg/dl)				
Before	128 ± 29	137 ± 36	135 ± 30	155 ± 24
After	140 ± 40	143 ± 38	135 ± 26	157 ± 22
MDA (μmol/ml)				
Before	1.32 ± 0.37	1.34 ± 0.44‡	1.41 ± 0.60	1.43 ± 0.61§
After	1.58 ± 0.51	1.31 ± 0.39	1.44 ± 0.68	1.31 ± 0.48
Lipid-standardized MDA (μmol/mg lipid)				
Before	0.43 ± 0.17	0.42 ± 0.20¶	0.41 ± 0.24	0.39 ± 0.21
After	0.50 ± 0.19	0.38 ± 0.16	0.41 ± 0.27	0.35 ± 0.15

Statistically significant differences between before and after using general linear models: \**P* = 0.018, †*P* = 0.019, ‡*P* = 0.049, §*P* = 0.004, ¶*P* = 0.026, ||*P* = 0.014.

**Table 6—Levels of systolic, diastolic, and mean blood pressure before and after 3 months of vitamin and mineral supplementation in type 2 diabetic patients**

	Group P	Group M	Group V	Group MV
n	18	16	18	17
Systolic blood pressure (mmHg)				
Before	127 ± 16	122 ± 15	125 ± 15	130 ± 19*
After	128 ± 18	119 ± 12	122 ± 16	122 ± 16
Diastolic blood pressure (mmHg)				
Before	82 ± 9	78 ± 12	81 ± 9	83 ± 11†
After	84 ± 11	78 ± 10	79 ± 12	77 ± 9
Mean arterial pressure (mmHg)				
Before	97 ± 9	93 ± 13	95 ± 10	99 ± 13‡
After	98 ± 12	92 ± 10	93 ± 12	92 ± 9

Data are means ± SD. Statistically significant differences between before and after using general linear models: \* $P = 0.008$ , † $P = 0.017$ , ‡ $P = 0.009$ .

diabetes, the relationship between lipid and lipoprotein concentrations and nephropathy have been reported (41). In type 2 diabetic patients, total triglycerides (41), LDL cholesterol (42), total cholesterol (43), and apoB (44) are risk factors for the progression of nephropathy, and clinical trials have suggested a beneficial effect of hyperlipidemic treatment for microalbuminuria in these patients. In the present study, there were no decreases in serum lipid and lipoprotein levels in type 2 diabetic patients who were given vitamins C and E and combination of magnesium, zinc, and vitamins C and E, suggesting that these micronutrients do not act through reducing lipid profiles in protecting renal cells and decreasing microalbuminuria.

The mechanisms of vitamin C and E action in treatment and prevention of diabetic nephropathy are based on prior animal studies. Administration of vitamin C or E can reduce glomerular hypertrophy and albuminuria in streptozotocin-induced diabetic animals (45,46). These vitamins may exert their beneficial effects through antioxidant action, too. Vitamin C also acts as an aldose reductase inhibitor reducing sorbitol conversion and decreasing cellular damage in the kidney (47).

Concerning the effect of antioxidant vitamin and mineral supplementation on the indicator of peroxidation, we did find significant reduction on plasma MDA in groups M and MV. The measurement of MDA is susceptible to artifacts caused by variations in sample lipid content (48). MDA were expressed per lipid (cholesterol and triglyceride), but there was no change in results. It seems that these micronutrients act synergistically, and hence a combination of these micronutrients provides a better effect. The ability of

ascorbic acid to reduce  $\alpha$ -tocopheroxyl radical to generate  $\alpha$ -tocopherol and possibly to inhibit oxidation induced by  $\alpha$ -tocopheroxyl radical has been demonstrated in many in vitro and in vivo studies (18,49). Also, synergistic action between vitamin E and magnesium has previously been shown (21,22). Increased oxygen free-radical production lowers the intracellular magnesium concentration (50), and in light of such evidence, vitamin E administration might also regulate the intracellular magnesium concentration (22). A synergistic effect of vitamins C and E and zinc could be expected based on the different environments in which they act. Vitamin C acts in the hydrophilic milieu, scavenging reactive oxygen species (49); Zn, located in the interphase of the bilayer, will prevent iron or copper binding to the membrane and  $\alpha$ -tocopherol and in the hydrophobic domains of the bilayer, will inhibit the lipid oxidation free-radical chain reaction (19). Effects of magnesium and zinc on lipid peroxidation have previously been reported. Magnesium inhibits MDA formation in endothelial cells (51) and low [Mg(2+)](o)-induced lipid peroxidation (52). MgSO<sub>4</sub> administration to pregnant women induced significant changes in lipid peroxidation (53). This study does have limitations. Our estimate of urine excretion of albumin and activities of NAG were based on gram creatinine excretion, and collection of 24-h urine would be required for their exact excretion determinations.

In summary, our study provides evidence for the effects of vitamins C and E and also combination of vitamins C and E, magnesium, and zinc supplementations on improvement of glomerular but not

tubular renal function in type 2 diabetic patients.

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