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The impact of vitamin and/or mineral supplementation on lipid profiles in type 2 diabetes

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Abstract

Objective: The purpose of the present study was to assess the impact of Mg + Zn, Vitamins C + E, and combination of these micronutrients on serum lipid and lipoprotein profiles in type 2 diabetic patients. *Materials 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: 200 mg Mg and 30 mg Zn ($n = 16$), group V: 200 mg Vitamin C and 150 mg Vitamin E ($n = 18$), group MV: minerals plus vitamins ($n = 17$), group P: placebo ($n = 18$). Fasting blood and urine samples were collected at the beginning and at the end of the trial. Serum triglyceride, total cholesterol, high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) were measured enzymatically. Apolipoproteins (apo) A1 and B were measured by immunoturbidimetric method. Adjustment for differences in baselines covariates and changes in variables during study were performed by analysis of covariance using general linear models. *Results*: Results indicate that after 3 months of supplementation mean serum levels of HDL-c and apo A1 increased significantly in the MV group by 24% (50.4 ± 19.3 mg/dl versus 40.6 ± 10.8 mg/dl) and 8.8% (169.8 ± 33.8 mg/dl versus 156.1 ± 23.9 mg/dl), respectively ($P < 0.01$). There were no significant changes in the levels of these parameters in the other three groups. Serum levels of total cholesterol, LDL-c, triglyceride, and apo B were not altered after supplementation in all four groups. *Conclusion*: It is concluded that since co-supplementation of Mg, Zn, Vitamins C and E significantly increases HDL-c and apo A1, supplementation of these micronutrients could be recommended for the type 2 diabetic patients based on their daily requirements.

Keywords: Diabetes; Lipid; Vitamin C; Vitamin E; Magnesium; Zinc

Abbreviations: Apo, apolipoprotein; BMI, body mass index; Cr, creatinine; CVD, cardiovascular disease; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; LS-α-TOH, lipid standardized α-tocopherol; M, mineral; Mg, magnesium; MV, mineral and vitamin; P, placebo; TG, triglyceride; V, vitamin; Zn, zinc

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1. Introduction

The major cause of disability and death associated with diabetes is complications involving large and small blood vessels [1]. Patients with diabetes have an increased incidence of vascular disease compared with non-diabetic control groups. Cardiovascular disease (CVD) is the major cause of death in patients with diabetes [2]. It is estimated that incidence of CVD is three to seven-fold higher for individuals with diabetes than in the non-diabetic population [3]. Lipoprotein abnormalities have been identified among the several risk factors that could account for this increase in CVD incidence in diabetes [4]. Patients with type 2 diabetes have low levels of high-density lipoprotein cholesterol (HDL-c), and elevated triglyceride (TG) levels [5].

Numerous epidemiological studies have investigated the association between dietary intake and plasma levels of some micronutrients and plasma lipid concentrations in a variety of populations. Serum levels of Vitamin C, Mg and Zn have been reported to be correlated inversely with serum levels of cholesterol and/or TG [6–9], and positively with serum levels of HDL-c [7,9,10]. Several studies have pointed out that dietary Vitamins E and C supplementation are useful in improving plasma lipid levels [11–13]. But the effects of these vitamins remain unclear with some other studies showing no changes [14,15]. While several studies have shown no benefit of Mg therapy [16–18], other studies have demonstrated beneficial effects of oral Mg supplementation on lipid metabolism in human subjects [19,20].

Because of the known synergistic action between Vitamins E and C [21], Vitamin E and Zn [22,23] and Vitamin E and Mg [24,25], a further important question is whether a combination of these micronutrients provides a better protection against CVD. Thus the present study was designed to assess the effects of long term supplementation with Mg and Zn, Vitamins E and C and a combination of these micronutrients on blood lipid levels in type 2 diabetic patients.

2. Materials and methods

A randomized double blind placebo controlled clinical trial was conducted on 76 type 2 diabetic patients, 30–69 years old and having diabetes for at least 1 year. The calculated sample size is 18 patients in each group, to have 80% power to detect the postulated differences in HDL-c with a α-error of 5%.

Data on dietary habits, dietary supplements, anthropometric indices including body mass index (BMI), medical history, smoking addiction, and taking medication were obtained from face to face interview. A patient had to meet the following criteria to be included in the study: not taking vitamin and/or mineral supplement, thyroid hormones, estrogens, progesterons, diuretics or β-blockers and having normal renal and hepatic function with no history of myocardial infarction and for the females, 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.

The mineral doses were used as tolerable upper intake levels that is 40 mg for zinc and 350 mg for magnesium. Since the mean intake of Zn and Mg in our diabetic patients were 8.3 and 227 mg per day, respectively, we increased the level of intakes near to tolerable upper intake levels by giving them 30 mg zinc and 200 mg magnesium supplementations.

Diabetic patients were stratified by sex and randomly assigned to one of the four treatment groups using block randomization procedure. Depending upon the treatment groups, each subject received two capsules per day for a period of 3 months. Each capsule contained one of the following preparations and hence determined the corresponding group: group M: Zn sulfate and Mg oxide (providing 15 mg Zn and 100 mg Mg); group V: Vitamin C (100 mg) and Vitamin E (75 mg); group MV: both of the above mineral and vitamin supplements; group P: lactose (placebo). The supplement and placebo capsules looked identical and were specially prepared for this study by Darou-Pakhsh Co.

After 12–14 h overnight fasting, between 8 and 10 a.m. and before taking any oral hypoglycemic agent(s), 20 ml blood and spot urine samples were collected from each subject at the beginning and at the end of 3 months trial. Blood and urine samples were collected in trace element free tubes. Aliquots of serum and urine were transferred to polystyrene

tubes which were immediately stored at −70 °C until analysis. Ascorbic acid was measured in the whole blood with colorimetric method [26], serum α-tocopherol was determined by high performance liquid chromatography [27] and lipid-standardized α-tocopherol (LS-α-TOH) was calculated as serum α-tocopherol concentration expressed per milligram TG plus cholesterol (μg/mg). Zn and Mg were measured in serum and urine sample by colorimetric methods [28,29] and urine creatinine (Cr) was determined using Jaffe reaction [30]. All urine results were expressed in relation to creatinine excretion.

Serum TG and total cholesterol were measured enzymatically. HDL-c was determined after precipitation with phosphotangestate/magnesium. Since some of the patients had serum TG concentrations more that 400 mg/dl and some studies have shown up to 50% misclassification of patients using Friedewald equation [31], low density lipoprotein cholesterol (LDL-c) was measured by Okada et al. method with intra assay CV 0.58% and inter assay CV 1.61% [32]. Apolipoproteins (apo) A1 and B were measured by immunoturbidimetric method.

Nutrients intakes were estimated using 24 h dietary recall questionnaire at the beginning and at the end of the 3 months trial for 2 days 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 assessed by counting number of the capsules had used and also by measuring changes in the serum and/or urine levels of Mg, Zn, Vitamins E and C.

3. Statistical analysis

All values are expressed as mean ± S.D. Differences between four groups were determined by one-way analysis of variance (ANOVA) for continuous data and the χ^2-test for group data. Post hoc comparisons were performed with Tukey test. Log transformation was used to normalize the distribution of TG. All other variables were normally distributed. Adjustment for differences in baselines covariates and changes in variables during study were performed by analysis of covariance using general linear models. A value of $P < 0.05$ was considered to be statistically significant. All data were analyzed using SPSS software.

4. Results

During the follow up five patients withdrew and two were excluded from statistical analysis because they interrupted trial treatment or changed their medication. Four patients were diet-controlled only and the others were treated with metformin and/or sulfonylurea. As shown in Table 1, at the beginning of the study, the groups were similar with respect to the sex, age, duration of diabetes, body mass index (BMI), smoking, daily intake of Vitamins C and E, Mg and Zn. There were no significant changes in BMI, physical activity, dietary intake or medication during the study period (data not shown).

At the beginning of study, the groups were similar base upon whole blood Vitamin C and serum levels of Vitamin E, Mg and Zn, and urinary levels of

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

	Group P	Group M	Group V	Group MV
No. of subjects	18	16	18	17
Male/female	9/9	7/9	8/10	9/8
Age (years)	49.6 ± 9.2	51.1 ± 7.5	49.9 ± 9.2	50.6 ± 9.7
Duration of diabetes (years)	8.4 ± 4.4	9.4 ± 6.2	9.2 ± 5.4	7.9 ± 4.7
BMI (kg/m^2)	27.6 ± 3.6	28.0 ± 4.7	27.5 ± 4.7	28.8 ± 3.9
Smoking	3	2	3	2
Vitamin C intake (mg per day)	83.5 ± 69.8	83.6 ± 87.3	77.8 ± 45.7	109.2 ± 114
Vitamin E intake (mg per day)	12.0 ± 15.0	9.3 ± 10.4	8.4 ± 9.1	13.5 ± 11.7
Mg intake (mg per day)	225.5 ± 92.6	212.9 ± 96.6	206.9 ± 66.5	248.1 ± 122.4
Zn intake (mg per day)	7.9 ± 2.8	7.8 ± 2.5	7.2 ± 2.1	9.2 ± 4.5

Mean ± S.D. There were no significant differences between groups by ANOVA or χ^2-test. BMI: body mass index.

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

	Group P ($n = 18$)	Group M ($n = 16$)	Group V ($n = 18$)	Group MV ($n = 17$)
Whole blood Vitamin C (mg/dl)				
Before	1.37 ± 0.44	1.45 ± 0.56	1.44 ± 0.48[a]	1.40 ± 0.66[a]
After[†]	1.36 ± 0.5	1.38 ± 0.56	1.75 ± 0.32	1.69 ± 0.3
Serum Vitamin E (μg/ml)				
Before	20.8 ± 6.1	22.6 ± 5.7[a]	25.2 ± 6.9[b]	24.0 ± 4.7[b]
After[‡]	20.4 ± 4.6	24.2 ± 5.7	36.9 ± 10.7	38.1 ± 10.7
LS-α-TOH (μg/mg)				
Before	6.38 ± 1.43	6.79 ± 1.91	6.50 ± 1.37[b]	6.06 ± 0.76[b]
After[‡]	6.15 ± 0.67	6.88 ± 2.17	9.98 ± 2.04	9.66 ± 2.01
Serum Mg (mg/dl)				
Before	1.77 ± 0.32	1.82 ± 0.24	1.87 ± 0.24	1.85 ± 0.25
After	1.79 ± 0.34	1.86 ± 0.27	1.86 ± 0.40	1.96 ± 0.21
Serum Zn (μg/dl)				
Before	102.4 ± 24.8	99.3 ± 24.1[c]	98.1 ± 12.7	95.8 ± 9.0[d]
After[§]	96.8 ± 13.1	114.3 ± 23.5	96.4 ± 13.4	114.9 ± 19.1
Urine Mg (mg/g Cr)				
Before	60.2 ± 36.2	57.1 ± 15.3	57.0 ± 18.6	58.6 ± 20.4
After	59.3 ± 16.0	66.0 ± 24.5	55.5 ± 24.6	66.4 ± 29.5
Urine Zn (μg/g Cr)				
Before	942 ± 374	908 ± 452[d]	1066 ± 418	906 ± 279[d]
After[§]	824 ± 253	1584 ± 889	1073 ± 401	1305 ± 456

Mean ± S.D. There were no significant baseline differences between groups by ANOVA. LS-α-TOH: lipid standardized α-tocopherol is calculated as μg serum α-tocopherol concentration expressed per mg (cholesterol + triglyceride).

[a] Statistically significant differences between before and after: $P < 0.05$.

[b] Statistically significant differences between before and after: $P < 0.0001$.

[c] Statistically significant differences between before and after: $P < 0.01$.

[d] Statistically significant differences between before and after: $P < 0.001$.

[†] Group V has significantly higher levels than group P ($P < 0.05$) (after supplementation).

[‡] Groups V and MV have significantly higher levels than groups P and M ($P < 0.0001$) (after supplementation).

[§] Groups M and MV have significantly higher levels than groups P and V ($P < 0.05$) (after supplementation).

minerals (Table 2). After 3 months of supplementation, whole blood of Vitamin C in group V and MV increased significantly as compared with baseline and group P. Serum levels of Vitamin E and LS-α-TOH increased significantly in group V and MV as compared with baseline and groups P and M. Serum and urinary levels of Zn increased significantly in group M (15.1 and 74.4%, respectively) and MV (19.9 and 44.5%, respectively). In spite of 2 and 5.4% increment in serum Mg in M and MV groups, respectively, and 15.6 and 13.3% increment in urine Mg in M and MV groups, respectively, the changes were not statistically significant.

Table 3 shows the serum lipid, lipoprotein and apolipoprotein concentrations before and after supplementation for subjects who completed the study. At baseline there were no significant differences between groups by ANOVA. Following 3 months of supplementation, serum levels of HDL-c and apo A1 increased significantly in the MV group by 24 and 8.8%, respectively, as compared with baseline and groups P, M and V ($P < 0.01$, Table 3). There were no significant changes in the other three groups. Total cholesterol, LDL-c, cholesterol/HDL-c and LDL-c/HDL-c ratios, TG and apo B were not altered after supplementation in all four groups.

Table 3
Levels of serum lipid and lipoproteins before and after 3 months vitamin and mineral supplementation in type 2 diabetic patients

	Group P ($n = 18$)	Group M ($n = 16$)	Group V ($n = 18$)	Group MV ($n = 17$)
Total cholesterol (mg/dl)				
Before	176.0 ± 38.0	181.1 ± 33.1	188.2 ± 34.1	203.3 ± 30.6
After	179.6 ± 27.7	182.5 ± 30.7	187.6 ± 36.3	202.6 ± 37.4
HDL-c (mg/dl)				
Before	39 ± 11.8	40.3 ± 15.5	35.8 ± 7.0	40.6 ± 10.8*
After†	35.3 ± 7.3	38.6 ± 11.1	41.8 ± 15.5	50.4 ± 19.3
LDL-c (mg/dl)				
Before	107.6 ± 31.9	107.4 ± 23.1	122.0 ± 34.0	127.8 ± 34.2
After	114.9 ± 23.4	105.5 ± 26.2	120.8 ± 42.6	123.6 ± 31.1
TC/HDL-c				
Before	4.68 ± 1.36	4.92 ± 1.57	5.40 ± 1.49	5.24 ± 1.08
After	5.18 ± 0.79	4.95 ± 1.02	4.85 ± 1.41	4.52 ± 1.47
LDL-c/HDL-c				
Before	2.91 ± 1.10	2.96 ± 1.17	3.53 ± 1.27	3.27 ± 1.00
After	3.31 ± 0.67	2.80 ± 0.46	3.09 ± 1.11	2.84 ± 1.27
Triglyceride (mg/dl)				
Before	162.4 ± 109.7	167.0 ± 84.5	215.9 ± 134.5	196.0 ± 81.6
After	153.8 ± 66.2	192.1 ± 105.3	189.7 ± 86.5	200.0 ± 104.8
Apo A1 (mg/dl)				
Before	144.8 ± 22.0	141.9 ± 24.5	144.9 ± 25.2	156.1 ± 23.9*
After†	142.9 ± 24.5	146.1 ± 21.0	146.2 ± 19.6	169.8 ± 33.8
Apo B (mg/dl)				
Before	127.6 ± 28.6	137.0 ± 36.0	135.0 ± 30.2	155.1 ± 24.2
After	140.4 ± 39.7	143.0 ± 37.8	135.5 ± 25.6	157.5 ± 22.3

Mean ± S.D. There were no significant baseline differences between groups by ANOVA.
* Statistically significant differences between before and after: $P < 0.01$.
† Group MV has significantly higher levels than groups P, M and V ($P < 0.01$).

5. Discussion

Over the last two decades a number of observational studies and epidemiological trials have provided data that establish HDL-c as the most potent lipid predictor for coronary artery disease [33]. A large base of epidemiological evidence suggests that a 1 mg/dl (0.02 mmol/l) increment in HDL-c would be associated with a significant 2–3% decrement in CVD risk [34]. Furthermore, The Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Clinical Trail supports the idea that increasing HDL-c can protect against clinical coronary artery disease [35].

Our data for the first time shows that supplementation of Vitamins C and E, Mg and Zn for at least 3 months can increase HDL-c by 24% and apo A1 by 8.8%. Separate supplementation of vitamins or minerals did not improve serum HDL-c or apo A1 levels. No significant changes in the other lipid parameters were observed for all the study groups.

The absence of such effects of Vitamins C and E or Mg and Zn supplementation separately should not be attributed to the interference of weight, physical activity, dietary intake or medication, because these variables did not change during the study period and the patients had a good compliance in taking their supplements.

Due to ability of ascorbic acid to reduce α-tocopheroxyl radical to generate α-tocopherol and possibly to inhibit oxidation induced by α-tocopheroxyl radical [36,37], and known synergistic action between Vitamin E and Zn [22,23] and Mg [24,25], combination of vitamins and minerals may theoretically have more beneficial effects on HDL-c and apo A1 than each one

alone. In the present study, the higher increase in fasting serum Mg and Zn concentrations, together with the lesser increase in Mg/Cr and Zn/Cr ratios excretion in the MV group compared to the M group may imply an in vivo interaction among these micronutrients.

Besides increases in HDL-c and apo A1 in MV group in the present study, no other significant changes were observed in the other serum lipid and lipoprotein profiles in any one of the studied groups. These findings confirm the results obtained by Miller et al. in a 2–4 month placebo-controlled supplementation trial with Vitamins E, C and β-carotene (400 IU, 500 and 6 mg, respectively) in 297 retired teachers [14]. Also, the levels of total cholesterol, HDL-c, LDL-c, apo A1 and apo B did not vary significantly after 2 months administration of 1200 mg per day Vitamin E to the diabetic patients [15]. In contrast, in patients with type 1 diabetes, elevated triglyceride levels were significantly decreased following Vitamin E supplementation (100 IU per day for 3 months), but plasma total cholesterol levels did not change [11]. Tofler et al. demonstrated that 2 mg Vitamin C supplementation resulted in lower levels of total cholesterol, LDL-c and HDL-c in healthy male with low normal Vitamin C levels [12]. Paolisso et al. showed that chronic Vitamin C administration versus placebo (0.5 g twice daily for 4 months) was associated with significant decline in total plasma cholesterol, LDL-c and triglyceride levels in type 2 diabetic patients [13].

Inconsistent effects of oral Mg supplementation on serum lipid levels have been reported. In several studies serum lipid levels did not improve in type 2 diabetic patients supplemented with Mg [16–18]. However, type 1 diabetic patients supplemented with 500 mg Mg twice daily for 24 weeks showed decreased serum levels of total cholesterol, LDL-c and apo B [19]. Healthy subjects receiving 411–548 mg Mg per day also showed decreased ratio of cholesterol to HDL-c and increased in HDL-c and apo A1 levels [20]. Nevertheless, type 2 diabetic patients receiving similar dose (30 mg per day) or higher dose (50 mg per day) of Zn supplementation as to the present study showed no adverse effect on lipid parameters [38,39].

In addition to the differences in the length of the studies, type of the participants, and different doses of Vitamins C, E, Mg and Zn supplementation, the serum levels of these micronutrients might explain part of the inconsistency. Several reports have suggested that oral supplementation with vitamins and minerals may be beneficial only in subjects with a low vitamin and mineral status and, therefore, may have little or no effects in subjects with a normal status of these micronutrients [12,40,41]. The type 2 diabetic patients in the present study did not have necessarily low plasma levels of Mg, Zn, Vitamin C or E.

The more increase In HDL-c (24%) than apo A1 (8.8%) in MV group suggest but do not prove that the benefits of this supplementation may extent to reversing cholesterol transport. Our data do not establish a mechanism by which such increase in HDL-c or apo A1 might take place.

Furthermore, Mg supplementation to their usual diets (200 mg per day) for a 3-month period did not significantly change their serum Mg levels. This is in agreement with the findings of other studies which showed that serum Mg levels are unaffected by Mg supplementation [17,42]. This could be related to shift of Mg into intracellular storage, thus causing Mg compartment redistribution on, however, because we did not measure intracellular Mg, we can not be sure that this is actually the case.

However, a significant increase in the urinary Mg excretion was observed during the Mg treatment in Eriksson and Kohvakka study [17]. In the present study, in spite of an increment in the urinary Mg/Cr ratio in M and MV groups after 3 months supplementation, the changes were not statistically significant. It seems that since Mg has a different diurnal excretion rates, collection of 24 h urine is required for its exact excretion determination [43]. Furthermore, the average dietary intake of Mg in these subjects was about 227 mg per day (258 mg for men and 189 mg for women), which is considerably lower than the recommended dietary allowance (RDA) for this age group (420 mg for men and 320 mg for no gravid women). Provision of 200 mg Mg supplementation per day, only increased their dietary intake a few more than RDA levels. It seems, for demonstrating clinical effects of this mineral higher levels of supplementation were needed. Whether Mg has a different diurnal excretion rates, amount of the supplementation is not enough, or the magnesium absorption is impaired, is open to question.

In summary, we have shown that a combination of relatively low dose of Mg, Zn, Vitamins C and E is an effective dietary supplementation in increasing serum

levels of HDL-c and apo A1 in type 2 diabetic patients. Understanding the mechanism by which a combination of these micronutrients increases serum levels of HDL-c and apo A1 may lead to the development of new approaches to the treatment of lipoprotein disorders and more effective prevention of CVD disease in diabetic patients.

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