The effects of combined magnesium and zinc supplementation on metabolic status in patients with type 2 diabetes mellitus and coronary heart disease

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Abstract

Background: The present research aimed to analyze the impacts of magnesium and zinc supplement on the metabolic level in the patients suffering from CHD (coronary heart disease) and T2DM (type 2 diabetes mellitus).

Methods: According to the research design, a randomized, double-blind, placebo-controlled trial has been implemented on 55 women suffering from CHD and T2DM. Therefore, the participants have been randomly divided into 2 groups for taking placebo (n = 28) or 250 mg magnesium oxide plus 220 mg zinc sulfate (n = 27) or for 12 weeks.

Results: Magnesium and zinc considerably declined the levels of fasting plasma glucose (FPG) (β -9.44 mg/dL, 95% CI, -18.30, -0.57; P = 0.03) and levels of insulin (β -1.37 μIU/mL, 95% CI, -2.57, -0.18; P = 0.02). Moreover, levels of HDL-cholesterol have been remarkably enhanced (β 2.09 mg/dL, 95% CI, 0.05, 4.13; P = 0.04) in comparison to the placebo. There have been an association between magnesium and zinc intake and a considerable decrease of C-reactive protein (CRP) (β -0.85 mg/L, 95% CI, -1.26, -0.45; P < 0.001), a considerable enhancement in the total nitrite (β 5.13 μmol/L, 95% CI, 1.85, 8.41; P = 0.003), and total anti-oxidant capacities (TAC) (β 43.44 mmol/L, 95% CI, 3.39, 83.50; P=0.03) in comparison to the placebo. Furthermore, magnesium and zinc remarkably reduced the Beck Depression Inventory index (BDI) (β -1.66; 95% CI, -3.32, -0.009; P = 0.04) and Beck Anxiety Inventory (BAI) (β -1.30; 95% CI, -2.43, -0.16; P=0.02) in comparison to the placebo.

Conclusions: In patients with T2DM and CVD the 12-week intake of magnesium and zinc affected FPG, HDL-cholesterol, CRP, insulin, NO, TAC levels, and BDI and BAI score usefully. Trial registration: Current Controlled Trials http://www.irct.ir: IRCT20130211012438N31 at 11 May 2019 of registration. This study retrospectively registered.

Background

As reported by the World Health Organization (WHO), CHD consistently is a primary cause of the men and women's mortality, which results with more than 7 million deaths annually. It is widely accepted that diabetes mellitus (DM) is also one of important risk parameters for CHD. Consequently, the risk of CHD in the patients with diabetes is double as high as in non-diabetic subjects [1]. Many studies have shown that there is a correlation between CHD and metabolic syndrome (MetS), T2DM, and elevated levels of inflammatory and oxidative stress biomarkers [2, 3]. Several researches indicated that levels of magnesium and zinc considerably decreased in patients suffering from CHD and T2DM [4, 5].

Researchers already reported valuable impacts of trace elements on the metabolic profiles in the patients suffering from metabolic disorders. For example, Asemi et al.'s[6] study showed that magnesium supplementary with a dose of 250 mg for each day in the form of magnesium oxide to the pregnant female suffering from gestational diabetes (GDM) considerably improved glycemic control and lipoproteins and oxidative stress and inflammation bio-markers. Taking zinc supplementation at a dose
of 30 mg for each day in the form of zinc sulfate by subjects with prediabetes for six months caused a considerable improvement in glycemic control [7]. There are some studies that used trace elements co-supplementation. It was reported that co-supplementing possibly is more effective compared to single element supplementations. A recent study on patients with diabetes indicated that joint zinc, magnesium, and vitamin C and E co-supplementations significantly reduced fasting glucose and malondialdehyde (MDA) levels and considerably enhanced the levels of HDL-cholesterol [8]. Results also showed that zinc and magnesium co-supplementation to the female suffering from polycystic ovary syndrome (PCOS) affected usefully the serum high sensitivity C-reactive protein (hs-CRP) as well as the TAC; however, it had no effect on the other markers of oxidative stress [9]. Researchers also examined co-supplementing magnesium-zinc-calcium-vitamin D in the patients who had PCOS, and showed that there is an association between them and a remarkable improvement in insulin levels, quantitative insulin sensitiveness check index (QUICKI), inflammatory markers, decline in triglycerides, total cholesterol and VLDL-cholesterol homeostatic model of insulin resistance (HOMA-IR), without any considerable modifications in the level of fasting glucose, HDL-cholesterol, and LDL-cholesterol [10].

Both zinc and magnesium contribute importantly to the glucose homeostasis and lipoprotein metabolisms. Magnesium participates as a cofactor in different adenosine triphosphate depended reactions which are important in carbohydrate and insulin metabolism [11]. Magnesium also contribute importantly to lipoprotein metabolism by modulating the 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase enzyme [12]. Some studies also demonstrated that zinc affected glucose homeostasis in terms of formation, storage, and secretion of insulin [13–15]. However, there is limited information of the impacts of combined magnesium and zinc supplementary on the metabolic parameters in subjects with CHD and T2DM. Hence, this research aimed to check the hypothesis that consuming magnesium plus zinc may improve metabolic condition of these patients. This study assessed the impacts of combination of magnesium and zinc supplementary on inflammation biomarker, metabolic profile, and oxidative stress but also some mental health parameters in the participants suffering from CHD and T2DM.

**Methods**

**Population of the study**

This is a double-blind, randomized placebo-controlled research, which has been registered in the Iranian registry of clinical trials at http://www.irct.ir: no, IRCT20130211012438N31. This research has been done at the cardiology clinic, which is affiliated to Kashan University of Medical Sciences (KAUMS), Kashan, Iran. It lasted from February 2019 to May 2019. The study has been performed with regard to the Declaration of Helsinki principles. Moreover, the research design has been confirmed by the Research Ethics Committee of KAUMS, Iran. Then, each patient has been given a written informed consent. The research also considered criteria for including in the study, which are patients suffering from T2DM in the age ranging between 40 and 85 years old with 2- and 3-vessel CHD. In addition, diagnosing T2DM and CHD has been accomplished on the basis of the criteria American Diabetes Association [16] as well as the American Heart Association criteria [17]. Moreover, some cases have been excluded based on the
following criteria: taking magnesium and zinc supplements 3 months before this study, consuming anti-
oxidant and or anti-inflammatory supplements and or omega3 fatty acids, renal or hepatic failure, acute
myocardial infarction, or cardiac surgery in the last three months.

**Research design** Patients have been randomly assigned into 2 treatment groups for taking 250 mg per
day magnesium (magnesium oxide) and 120 mg/day and zinc (zinc sulfate) or placebo (starch) (Barij
Essence; Kashan: Iran) (n = 30 in each group) for 12 weeks. Color, form, size, and package of the placebo
and magnesium plus zinc supplementations have been similar. Randomizing has been done via the
computer-generated random numbers. The investigators and patients have been blinded concerning the
randomization and supplements/placebo till the resulting analyses have been accomplished. Enrolling
the patients, randomizing, and assigning them to treatment or placebo have been performed by the
qualified personnel at the clinic. Agreement with taking placebo and supplement has been made by
examining the capsule containers. Then, each patient finished the three-day dietary intake records at 1, 6,
and 12 weeks of the trial. Moreover, Nutritionist IV software (First Databank; San Bruno; CA) adapted for
the Iranian food pattern has been employed to achieve the patients’ nutrient intakes in accordance with
three-day food records. At this stage, the anthropometric measures (Seca; Hamburg: Germany) have been
registered at base-line and 12 weeks after clinical interventions. It should be noted that a qualified
nutritionist performed each anthropometric measure.

**Outcomes**

According to our research design, FBC (10 mL) specimens have been drawn out at the base-line and after
passing a 12-week period of intervention at Kashan Reference Laboratory. Measurement of the levels of
serum insulin has been done via ELISA kit (DiaMetra; Milano: Italy) through inter- and intra-assay
coefficient variances (CVs) beneath 5%. Then, QUICKI and HOMA-IR have been evaluated on the basis of
the standard formulation [18]. Afterwards, in this stage, enzymatic kits (Pars Azmun; Tehran: Iran) have
been utilized to estimate magnesium, zinc, levels of the FPG, and serum lipoproteins that had inter-assay
and intra-assay CVs beneath 5%. Next, the levels of CRP have been checked via an ELISA kit (LDN;
Nordhorn: Germany) with inter-assay and intra-assay CVs beneath 7%. In the next stage, total nitrite has
been estimated by Griess assay [19], TAC by the technique published by Benzie and Strain [20], levels of
total glutathione (GSH) via the procedure described by Beutler et al. [21], and concentration of MDA have
been specified by a spectro-photometric experiment [22] with inter-assay and intra-assay CVs beneath 5%.

**Clinical evaluation**
According to the protocol of the study, the Beck Depression Inventory (BDI) has been evaluated by a modified questionnaire [23]. It should be noted that anxiety has been gauged by the Beck Anxiety Inventory (BAI) designed by Beck et al. [24].

Statistical procedures and size of the sample

According to the present research design, the sample size formula has been employed for the randomized clinical trial, in which type 1 (α) and type 2 errors (β) have been 0.05, and 0.20 (power = 80%). One of the studies conducted in the field [6] applied 0.80 as the SD and 0.64 as the changes in the mean (d) of HOMA-IR. Considering the power computation, we required 25 subjects in each group. Upon the 20% drop-outs in each group, the resulting dimension of the sample has been 30 subjects.

As it is known, Kolmogorov-Smirnov statistic has been employed to control data normality. Moreover, independent-sample $t$-test has been utilized for determining the difference in the anthropometric measure and dietary intake between the two groups. In this stage, multiple linear regression model has been employed to evaluate the treatment impacts on the research outputs after setting for base-line values of the bio-chemical parameters. In addition, the effect size has been provided as the mean difference with 95% confidence interval. Then, Pearson Chi-square test has been utilized to compare categorical variables. Moreover, P-values less than 0.05 have been viewed significant. Afterwards, SPSS18 (SPSS Inc.; Chicago, Illinois: USA) have been employed for statistical analysis of the present trial.

Results

55 females [magnesium plus zinc (n = 27) and placebo (n = 28)] participated in the trial (Fig. 1). It should be noted that the agreement rate has been high; both groups took > 90% of capsules during this trial. However, there has been not any side impacts in the T2DM patients suffering from CHD when taking magnesium plus zinc supplement.

Moreover, there has been not any considerable difference between the two groups regarding the height, the end of trial weight, mean age, base-line, and body mass index (Table 1).
### Table 1
General characteristics of study participants at baseline study

|                               | Placebo group (n = 28) | Magnesium plus zinc group (n = 27) | p₁     |
|-------------------------------|------------------------|-----------------------------------|--------|
| Age (y)                       | 62.6 ± 10.8            | 61.7 ± 9.4                        | 0.74   |
| Height (cm)                   | 159.5 ± 10.9           | 162.1 ± 7.8                       | 0.32   |
| Weight at study baseline (kg) | 76.9 ± 12.6            | 81.3 ± 11.7                       | 0.19   |
| Weight at end-of-trial (kg)   | 77.0 ± 12.5            | 80.9 ± 11.4                       | 0.23   |
| Weight change (kg)            | 0.1 ± 1.2              | -0.4 ± 1.0                        | 0.12   |
| BMI at study baseline (kg/m²) | 30.2 ± 3.7             | 30.9 ± 3.8                        | 0.47   |
| BMI at end-of-trial (kg/m²)   | 30.2 ± 3.8             | 30.8 ± 3.7                        | 0.59   |
| BMI change (kg/m²)            | 0.03 ± 0.5             | -0.1 ± 0.4                        | 0.10   |

Data are means ± SDs.

₁ Obtained from independent t-test.

Macronutrient and micronutrient supplementations as computed on the basis of the three-day food records did not differ considerably between magnesium plus zinc and the controls (Data not shown).

Magnesium and zinc co-supplementations remarkably reduced the FPG (β -9.44 mg/dL, 95% CI, -18.30, -0.57; P = 0.03) and insulin levels (β -1.37 µIU/mL, 95% CI, -2.57, -0.18; P = 0.02), and considerably augmented the levels of HDL-cholesterol (β 2.09 mg/dL, 95% CI, 0.05, 4.13; P = 0.04) in comparison to the placebo (Table 2). Magnesium plus zinc taking was associated with a remarkable decline in CRP (β -0.85 mg/L, 95% CI, -1.26, -0.45; P < 0.001), and a considerable enhancement in the total nitrite (β 5.13 µmol/L, 95% CI, 1.85, 8.41; P = 0.003) and the TAC (β 43.44 mmol/L, 95% CI, 3.39, 83.50; P = 0.03) when contrasted with the placebo. Magnesium and zinc co-supplementations also remarkably declined BDI (β -1.66; 95% CI, -3.32, -0.009; P = 0.04) and BAI scores (β -1.30, 95% CI, -2.43, -0.16; P = 0.02) when contrasted with the placebo. Magnesium and zinc co-supplementation did not have any considerable effects on the other metabolic parameters when contrasted with the placebo.
Table 2
The effect of combined magnesium and zinc supplementation on metabolic status in patients with type 2 diabetes and coronary heart disease

| Variables                    | Placebo group (n = 28) | Magnesium and zinc group (n = 27) | Difference in outcome measures between magnesium and zinc treatment groups¹ | β (95% CI) | p²  |
|------------------------------|------------------------|-----------------------------------|--------------------------------------------------------------------------------|------------|-----|
|                              | Baseline | Week 12 | Baseline | Week 12 |                                |          |     |
| Magnesium (mg/dL)           | 1.84 ± 0.22 | 1.83 ± 0.25 | 1.94 ± 0.21 | 2.08 ± 0.22 | 0.15 (0.08, 0.22) | < 0.001 |
| Zinc (µg/dL)                | 93.3 ± 24.8 | 95.8 ± 25.9 | 98.1 ± 22.5 | 116.5 ± 21.5 | 16.21 (11.98, 20.44) | < 0.001 |
| FPG (mg/dL)                 | 123.7 ± 31.4 | 128.4 ± 32.7 | 119.2 ± 38.0 | 115.6 ± 28.7 | -9.44 (-18.30, -0.57) | 0.03    |
| Insulin (µIU/mL)            | 13.8 ± 4.5 | 13.9 ± 4.4 | 12.9 ± 5.1 | 11.7 ± 4.9 | -1.37 (-2.57, -0.18) | 0.02    |
| HOMA-IR                     | 4.2 ± 1.8 | 4.2 ± 1.7 | 3.7 ± 1.7 | 3.4 ± 1.8 | -0.36 (-0.75, 0.02) | 0.06    |
| QUICKI                      | 0.31 ± 0.02 | 0.31 ± 0.02 | 0.32 ± 0.02 | 0.32 ± 0.02 | 0.006 (-0.002, 0.01) | 0.12    |
| Triglycerides (mg/dL)       | 123.7 ± 50.7 | 124.9 ± 46.5 | 128.2 ± 55.7 | 134.6 ± 53.9 | 5.66 (-5.40, 16.73) | 0.30    |
| VLDL-cholesterol (mg/dL)    | 24.7 ± 10.1 | 24.9 ± 9.3 | 25.6 ± 11.1 | 26.9 ± 10.8 | 1.10 (-1.08, 3.34) | 0.30    |
| Total cholesterol (mg/dL)   | 147.3 ± 35.3 | 145.6 ± 31.0 | 137.1 ± 28.3 | 140.7 ± 32.5 | 3.72 (-5.26, 12.71) | 0.41    |

Data are mean ± SDs.

¹"Outcome measures" refers to the change in values of measures of interest between baseline and week 12. β [difference in the mean outcome’s measures between treatment groups (magnesium and zinc group = 1 and placebo group = 0)].

² Obtained from multiple regression model (adjusted for baseline values of each biochemical variables).

BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; FPG, fasting plasma glucose; GSH, total glutathione; HOMA-IR, homeostasis model of assessment-estimated insulin resistance; CRP, C-reactive protein; MDA, malondialdehyde; QUICKI, quantitative insulin sensitivity check index; TAC, total antioxidant capacity.
| Variables                              | Placebo group (n = 28) | Magnesium and zinc group (n = 27) | Difference in outcome measures between magnesium and zinc treatment groups<sup>1</sup> |
|----------------------------------------|------------------------|----------------------------------|----------------------------------------------------------------------------------|
|                                        | Baseline | Week 12  | Baseline | Week 12 | β (95% CI) | P<sup>2</sup> |
| LDL-cholesterol (mg/dL)                | 77.2 ± 29.5 | 76.6 ± 26.5 | 70.8 ± 22.4 | 71.6 ± 25.7 | 0.13 (-8.32, 8.59) | 0.97 |
| HDL-cholesterol (mg/dL)               | 45.3 ± 7.7 | 43.9 ± 7.0 | 40.6 ± 8.4 | 42.1 ± 8.2 | 2.09 (0.05, 4.13) | 0.04 |
| Total-/HDL-cholesterol ratio          | 3.3 ± 0.8 | 3.3 ± 0.7 | 3.4 ± 0.7 | 3.4 ± 0.8 | -0.06 (-0.30, 0.17) | 0.59 |
| CRP (mg/L)                            | 3.0 ± 1.0 | 3.2 ± 1.2 | 2.7 ± 1.7 | 2.1 ± 1.3 | -0.85 (-1.26, -0.45) | <0.001 |
| Total nitrite (µmol/L)                | 49.8 ± 6.1 | 48.7 ± 6.1 | 43.7 ± 5.3 | 50.1 ± 6.5 | 5.13 (1.85, 8.41) | 0.003 |
| TAC (mmol/L)                          | 898.4 ± 168.4 | 894.9 ± 178.9 | 940.9 ± 107.2 | 976.8 ± 105.3 | 43.44 (3.39, 83.50) | 0.03 |
| GSH (µmol/L)                          | 508.3 ± 72.9 | 524.9 ± 95.3 | 556.8 ± 92.9 | 578.2 ± 56.3 | 29.49 (-8.83, 67.82) | 0.12 |
| MDA (µmol/L)                          | 2.2 ± 0.6 | 2.2 ± 0.5 | 1.9 ± 0.4 | 1.8 ± 0.4 | -0.15 (-0.32, 0.007) | 0.05 |
| BDI score                             | 19.7 ± 5.8 | 19.6 ± 5.7 | 21.7 ± 4.7 | 19.6 ± 4.9 | -1.66 (-3.32, -0.009) | 0.04 |
| BAI score                             | 15.7 ± 4.3 | 14.1 ± 4.8 | 17.0 ± 5.3 | 14.0 ± 5.1 | -1.30 (-2.43, -0.16) | 0.02 |

Data are mean ± SDs.

<sup>1</sup>“Outcome measures” refers to the change in values of measures of interest between baseline and week 12. β [difference in the mean outcome’s measures between treatment groups (magnesium and zinc group = 1 and placebo group = 0)].

<sup>2</sup> Obtained from multiple regression model (adjusted for baseline values of each biochemical variables).

BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; FPG, fasting plasma glucose; GSH, total glutathione; HOMA-IR, homeostasis model of assessment-estimated insulin resistance; CRP, C-reactive protein; MDA, malondialdehyde; QUICKI, quantitative insulin sensitivity check index; TAC, total antioxidant capacity.
Discussion

This is the first research that dealt with the evaluation of the impacts of magnesium and zinc combined supplementations on the metabolic profiles in the female suffering with T2DM and CHD. This RCT demonstrated that magnesium plus zinc combined supplementation to the female suffering from CHD and T2DM affected usefully the FPG, insulin, HDL-cholesterol, CRP, total nitrite, levels of TAC as well as BDI and BAI scores.

Impacts on the glycemic control and serum lipoproteins

Our findings showed that magnesium and zinc combined supplement in the female suffering from CHD and T2DM during 12 weeks caused a considerable decline in the FPG and insulin levels, and considerable enhancement in the level of HDL-cholesterol, but it had no effect on the HOMA-IR, QUICKI, total cholesterol, triglyceride, VLDL-cholesterol and LDL-cholesterol level. According to previous studies, chronic hyperglycemia and dyslipidemia increase the risk of diabetic and atherogenic complications [25, 26]. A meta-analysis indicated that magnesium intake is associated a significant improvement in FPG, triglycerides, HDL-cholesterol and LDL-cholesterol levels [27]. Our previous study showed that supplementing with magnesium oxide (250 mg/day) in the pregnant female suffering from GDM during 6 weeks caused a remarkable improvement in the FPG, insulin concentration, HOMA-IR, QUICKI, and triglycerides levels, but it caused no change other serum lipoproteins [6]. However, magnesium lactate with a dose of 360 mg per day during 12 weeks did not apply remarkable effects on HbA1c, FPG, levels of insulin, HOMA-IR and lipid profiles in normomagnesemic patients suffering from T2DM [28]. One of the meta-analyses conducted in the field showed that supplementing zinc to the patients suffering diabetes mellitus has been associated with a considerable decline in FPG, HbA1c, total cholesterol, and a remarkable augment in the levels of HDL-cholesterol, but there has been no significant association with triglycerides [29]. Another research performed by Islam et al. [7] revealed that zinc supplementations (30 mg per day of zinc sulphate) for 6 months improved FPG, insulin resistance, insulin sensitivity and decreased triglycerides levels without causing any considerable changes in the level of the LDL-cholesterol and HDL-cholesterol. A combination of magnesium-zinc-calcium-vitamin D during a 12 week period has been correlated with a remarkable decline in the levels of HOMA-IR, insulin, triglyceride, total cholesterol, and VLDL-cholesterol and a considerable increase in the QUICKI scores, but it has been not accompanied by changes in the level of LDL-cholesterol, FPG, and HDL-cholesterol in the PCOS patients [10]. Hyper-insulinemia and insulin resistance have been related to hyperglycemia which is the main symptom of diabetes mellitus. Hyperglycemia affects the glycation of lipoproteins but has a lot of other unfavorable effects which cause accelerated atherosclerosis [30, 31]. Moreover, researchers demonstrated that magnesium is one of the crucial cofactors in the enzymatic processes that require adenosine triphosphate and kinase, and therefore it plays an important role in glucose metabolic pathways [11]. Zinc is also important in physiological processes such as glucose metabolism. For
instance, zinc is involved in the phosphorylation of insulin and the regulation of signaling by tyrosine phosphatase [13, 32].

**Effects on oxidative stress and inflammation**

In this research, we showed a considerable decline of CRP and a remarkable enhancement in the levels of TAC and total nitrite via the combined magnesium and zinc supplementations to the patients suffering from CHD and T2DM over a 12 weeks period. Oxidative stress and inflammation are important risk factors for diabetes and diabetes-associated atherosclerosis [33]. Asemi et al.'s [6] research indicated that magnesium supplement (250 mg/day of magnesium oxide) in pregnant women with GDM for 6 weeks considerably decreased hs-CRP, but changes in TAC and GSH concentrations were not significant. Nevertheless, magnesium supplement (magnesium oxide) with a dose of 250 mg per day during eight weeks to overweight female did not associate with any changes in inflammatory markers [34]. In another study, zinc supplement (30 mg per day of zinc sulphate) in the women with prediabetes for 6 months did not associate with any considerable change in the levels of CRP [7]. However, combining magnesium-zinc-calcium-vitamin D supplement for 12 weeks in the female suffering from PCOS has been correlated to the considerable decline of CRP level [10]. Combined magnesium-zinc-calcium-vitamin D supplementation in another similar study had association with a remarkable decline decrease in the levels of hs-CRP and MDA, and a considerable enhancement in the level of TAC without any significant change in nitric oxide (NO) and GSH levels [35]. A recent study reported that combined magnesium and zinc supplementation (250 mg/day of magnesium oxide plus 220 mg/day zinc sulfate) to the female suffering from PCOS during 12 weeks affected usefully the levels of hs-CRP and TAC; yet, it no significant effects have been seen on NO, MDA and GSH levels [9].

Magnesium is assumed to possess anti-inflammatory properties caused by the respective antagonist effects to the calcium which contributes significantly to the inflammations, transmembrane ion transport, and protein synthesis [36]. Magnesium also increases production of NO and prostacyclins [37]. Zinc seems to have effects on hemostasis by influencing on coagulation and platelet accumulation [38]. Zinc deficiency influences calcium channels and calcium uptake defects and subsequent second-messenger performance most probably stems from an unusual sulfhydryl redox states in the membrane channel protein having an impact on CVD. [39].

**Effects on depression and anxiety**

Our findings indicated that combined magnesium and zinc supplementation to patients with CHD and T2DM during 12 weeks improved BDI and BAI scores. The prevalence of depression and anxiety in CHD subjects is high and it is responsible for an increased risk of mortality [40]. A study using food frequency questionnaire and general health questionnaire reported a reverse correlation between dietary magnesium
intake and depression and anxiety [41]. Data from a review suggested that zinc deficiencies are prevalent in mood disorders and that zinc supplement can have therapeutic impacts in these subjects [42]. Nonetheless, Fard et al.'s [43] research showed that supplementing with 27 mg per day zinc sulfate or 320 mg/day magnesium sulfate did not improve postpartum anxiety and depressive symptoms after 8 weeks. In another study by Nikseresht et al.[44], acute administration of combined 30 mg/kg zinc chloride, 30 mg/kg magnesium chloride and 50 mg/kg thiamine-HCl in mice with postpartum depression symptoms improved depression symptoms and anxiety-like behavior. Zinc and magnesium act as cofactors and contribute significantly to synthesis and release of neurotransmitters and thereby can have antidepressant and anxiolytic effects [45]. For example, zinc and magnesium prevent binding of N-methyl-D-aspartate receptors to glutamate and may be associated with an antidepressant and anxiolytic effects [46].

This study has some limitations. The most important one is relatively small number of patients despite the fact that the power analysis showed that the number of participants is sufficient.

**Conclusions**

The combined magnesium and zinc supplementation during 12 weeks beneficially affected the FPG, insulin, total nitrite, HDL-cholesterol, CRP, TAC levels, BDI and BAI score; however, it had no considerable impacts on the other metabolic variables in suffering from T2DM and CVD.

**Declarations**

**Ethics approval and consent to participate**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments.

The protocol of this study was approved by Research Ethics Committee, KAUMS, Iran and written informed consent was obtained from all patients.

**Consent for publication**

Not applicable.
Availability of data and material

The primary data for this study is available from the corresponding author (Mohsen Taghizadeh) on reasonable request.

Competing interests

The authors declare no conflict of interest.

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Author contributions

MT and ZA: Conception, design, and statistical analysis, drafting of the manuscript and supervised the study.

ZH, ZR, FB and MG: Data collection and manuscript drafting.

All authors have read and approved the manuscript.

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Clinical trial registration number

www.irct.ir: http://www.irct.ir: IRCT20130211012438N31.

References
1. Kautzky-Willer A, Kamyar MR, Gerhat D, Handisurya A, Stemer G, Hudson S, et al. Sex-specific differences in metabolic control, cardiovascular risk, and interventions in patients with type 2 diabetes mellitus. Gend Med. 2010;7:571-83.

2. Onat A, Can G, Cicek G, Ayhan E, Dogan Y, Kaya H. Fasting, non-fasting glucose and HDL dysfunction in risk of pre-diabetes, diabetes, and coronary disease in non-diabetic adults. Acta Diabetol. 2013;50:519-28.

3. Patel RS, Ghasemzadeh N, Eapen DJ, Sher S, Arshad S, Ko YA, et al. Novel Biomarker of Oxidative Stress Is Associated With Risk of Death in Patients With Coronary Artery Disease. Circulation. 2016;133:361-9.

4. Wu J, Xun P, Tang Q, Cai W, He K. Circulating magnesium levels and incidence of coronary heart diseases, hypertension, and type 2 diabetes mellitus: a meta-analysis of prospective cohort studies. Nutr J. 2017;16:60.

5. Cikim G, Canatan H, Gursu MF, Gulcu F, Baydas G, Kilicoglu AE. Levels of zinc and lipid peroxidation in acute coronary syndrome. Biol Trace Elem Res. 2003;96:61-9.

6. Asemi Z, Karamali M, Jamilian M, Foroozanfard F, Bahmani F, Heidarzadeh Z, et al. Magnesium supplementation affects metabolic status and pregnancy outcomes in gestational diabetes: a randomized, double-blind, placebo-controlled trial. The American journal of clinical nutrition. 2015;102:222-9.

7. Islam MR, Attia J, Ali L, McEvoy M, Selim S, Sibbritt D, et al. Zinc supplementation for improving glucose handling in pre-diabetes: A double blind randomized placebo controlled pilot study. Diabetes Res Clin Pract. 2016;115:39-46.

8. Farvid MS, Jalali M, Siassi F, Hosseini M. Comparison of the effects of vitamins and/or mineral supplementation on glomerular and tubular dysfunction in type 2 diabetes. Diabetes Care. 2005;28:2458-64.

9. Afshar Ebrahimi F, Foroozanfard F, Aghadavod E, Bahmani F, Asemi Z. The Effects of Magnesium and Zinc Co-Supplementation on Biomarkers of Inflammation and Oxidative Stress, and Gene Expression Related to Inflammation in Polycystic Ovary Syndrome: a Randomized Controlled Clinical Trial. Biol Trace Elem Res. 2018;184:300-7.

10. Jamilian M, Maktabi M, Asemi Z. A Trial on The Effects of Magnesium-Zinc-Calcium-Vitamin D Co-Supplementation on Glycemic Control and Markers of Cardio-Metabolic Risk in Women with Polycystic Ovary Syndrome. Archives of Iranian medicine. 2017;20:640-5.

11. Barbagallo M, Dominguez LJ. Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance. Arch Biochem Biophys. 2007;458:40-7.

12. Rasheed H, Elahi S, Ajaz H. Serum magnesium and atherogenic lipid fractions in type II diabetic patients of Lahore, Pakistan. Biol Trace Elem Res. 2012;148:165-9.

13. Shan Z, Bao W, Zhang Y, Rong Y, Wang X, Jin Y, et al. Interactions between zinc transporter-8 gene (SLC30A8) and plasma zinc concentrations for impaired glucose regulation and type 2 diabetes. Diabetes. 2014;63:1796-803.
14. Capdor J, Foster M, Petocz P, Samman S. Zinc and glycemic control: a meta-analysis of randomised placebo controlled supplementation trials in humans. J Trace Elem Med Biol. 2013;27:137-42.

15. Maruthur NM, Clark JM, Fu M, Linda Kao WH, Shuldiner AR. Effect of zinc supplementation on insulin secretion: interaction between zinc and SLC30A8 genotype in Old Order Amish. Diabetologia. 2015;58:295-303.

16. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014;37 Suppl 1:S81-90.

17. Luepker RV, Apple FS, Christenson RH, Crow RS, Fortmann SP, Goff D, et al. Case definitions for acute coronary heart disease in epidemiology and clinical research studies: a statement from the AHA Council on Epidemiology and Prevention; AHA Statistics Committee; World Heart Federation Council on Epidemiology and Prevention; the European Society of Cardiology Working Group on Epidemiology and Prevention; Centers for Disease Control and Prevention; and the National Heart, Lung, and Blood Institute. Circulation. 2003;108:2543-9.

18. Pisprasert V, Ingram KH, Lopez-Davila MF, Munoz AJ, Garvey WT. Limitations in the use of indices using glucose and insulin levels to predict insulin sensitivity: impact of race and gender and superiority of the indices derived from oral glucose tolerance test in African Americans. Diabetes Care. 2013;36:845-53.

19. Tatsch E, Bochi GV, Pereira Rda S, Kober H, Agertt VA, de Campos MM, et al. A simple and inexpensive automated technique for measurement of serum nitrite/nitrate. Clin Biochem. 2011;44:348-50.

20. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239:70-6.

21. Beutler E, Gelbart T. Plasma glutathione in health and in patients with malignant disease. J Lab Clin Med. 1985;105:581-4.

22. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med. 1990;9:515-40.

23. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry. 1961;4:561-71.

24. Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. J Consult Clin Psychol. 1988;56:893-7.

25. Goldberg IJ. Clinical review 124: Diabetic dyslipidemia: causes and consequences. The Journal of clinical endocrinology and metabolism. 2001;86:965-71.

26. Yan L, Xu MT, Yuan L, Chen B, Xu ZR, Guo QH, et al. Prevalence of dyslipidemia and its control in type 2 diabetes: A multicenter study in endocrinology clinics of China. J Clin Lipidol. 2016;10:150-60.

27. Verma H, Garg R. Effect of magnesium supplementation on type 2 diabetes associated cardiovascular risk factors: a systematic review and meta-analysis. Journal of human nutrition and dietetics: the official journal of the British Dietetic Association. 2017;30:621-33.

28. Navarrete-Cortes A, Ble-Castillo JL, Guerrero-Romero F, Cordova-Uscanga R, Juarez-Rojop IE, Aguilar-Mariscal H, et al. No effect of magnesium supplementation on metabolic control and insulin
sensitivity in type 2 diabetic patients with normomagnesemia. Magnes Res. 2014;27:48-56.

29. Jayawardena R, Ranasinghe P, Galappatthy P, Mankanthi R, Constantine G, Katulanda P. Effects of zinc supplementation on diabetes mellitus: a systematic review and meta-analysis. Diabetol Metab Syndr. 2012;4:13.

30. Keymel S, Heinen Y, Balzer J, Rassaf T, Kelm M, Lauer T, et al. Characterization of macro-and microvascular function and structure in patients with type 2 diabetes mellitus. Am J Cardiovasc Dis. 2011;1:68-75.

31. Georg P, Ludvik B. Lipids and diabetes. Journal of clinical and basic cardiology. 2000;3:159-62.

32. Wijesekara N, Chimienti F, Wheeler MB. Zinc, a regulator of islet function and glucose homeostasis. Diabetes Obes Metab. 2009;11 Suppl 4:202-14.

33. Pickering RJ, Rosado CJ, Sharma A, Buksh S, Tate M, de Haan JB. Recent novel approaches to limit oxidative stress and inflammation in diabetic complications. Clinical & translational immunology. 2018;7:e1016.

34. Moslehi N, Vafa M, Rahimi-Foroshani A, Golestan B. Effects of oral magnesium supplementation on inflammatory markers in middle-aged overweight women. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2012;17:607.

35. Maktabi M, Jamilian M, Asemi Z. Magnesium-Zinc-Calcium-Vitamin D Co-supplementation Improves Hormonal Profiles, Biomarkers of Inflammation and Oxidative Stress in Women with Polycystic Ovary Syndrome: a Randomized, Double-Blind, Placebo-Controlled Trial. Biol Trace Elem Res. 2018;182:21-8.

36. Almoznino-Saraan D, Berman S, Mor A, Shteinshnaider M, Gorelik O, Tzur I, et al. Magnesium and C-reactive protein in heart failure: an anti-inflammatory effect of magnesium administration? Eur J Nutr. 2007;46:230-7.

37. Sontia B, Touyz RM. Role of magnesium in hypertension. Archives of biochemistry and biophysics. 2007;458:33-9.

38. Marx G, Eldor A. The procoagulant effect of zinc on fibrin clot formation. American journal of hematology. 1985;19:151-9.

39. O’Dell BL. Role of zinc in plasma membrane function. The Journal of nutrition. 2000;130:1432s-6s.

40. Watkins LL, Koch GG, Sherwood A, Blumenthal JA, Davidson JR, O'Connor C, et al. Association of anxiety and depression with all-cause mortality in individuals with coronary heart disease. Journal of the American Heart Association. 2013;2:e000068.

41. Anjom-Shoae J, Sadeghi O, Hassanzadeh Keshteli A, Afshar H, Esmailzadeh A, Adibi P. The association between dietary intake of magnesium and psychiatric disorders among Iranian adults: a cross-sectional study. The British journal of nutrition. 2018;120:693-702.

42. Cope EC, Levenson CW. Role of zinc in the development and treatment of mood disorders. Current opinion in clinical nutrition and metabolic care. 2010;13:685-9.
43. Fard FE, Mirghafourvand M, Mohammad-Alizadeh Charandabi S, Farshbaf-Khalili A, Javadzadeh Y, Asgharian H. Effects of zinc and magnesium supplements on postpartum depression and anxiety: A randomized controlled clinical trial. Women & health. 2017;57:1115-28.

44. Nikseresht S, Etebary S, Karimian M, Nabavizadeh F, Zarrindast MR, Sadeghipour HR. Acute administration of Zn, Mg, and thiamine improves postpartum depression conditions in mice. Arch Iran Med. 2012;15:306-11.

45. Etebary S, Nikseresht S, Sadeghipour HR, Zarrindast MR. Postpartum depression and role of serum trace elements. Iranian journal of psychiatry. 2010;5:40-6.

46. Sowa-Kucma M, Szewczyk B, Sadlik K, Piekoszewski W, Trela F, Opoka W, et al. Zinc, magnesium and NMDA receptor alterations in the hippocampus of suicide victims. Journal of affective disorders. 2013;151:924-31.

Figures
Figure 1

Summary of patient flow diagram.

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