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Randomized Study of the Effects of Vitamin D and Magnesium Co-Supplementation on Muscle Strength and Function, Body Composition, and Inflammation in Vitamin D-Deficient Middle-Aged Women

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Abstract

This study aimed to investigate the effects of vitamin D and magnesium co-supplementation on muscle strength and function, body composition, and inflammation in vitamin D-deficient middle-aged women. In this study, 83 healthy middle-aged women (40–55 years) with vitamin D deficiency were randomly assigned into two groups: (1) intervention: receiving a 50,000-IU vitamin D soft gel (weekly) plus a 250-mg magnesium tablet (daily); (2) control: receiving a vitamin D placebo (weekly) plus a magnesium placebo (daily), for 8 weeks. Before and after the intervention, anthropometric indices, muscle strength, muscle function, and some inflammatory markers were measured. After 8 weeks of supplementation, significant difference was observed in handgrip strength and time for Time Get Up and Go (TGUG) test between the intervention and placebo groups ($P < 0.05$). Regarding percentage of fat mass (FM%) and fat free mass (FFM%), and knee extension strength, there was no significant difference between the two groups at the end of intervention ($P > .05$). Serum 25(OH)-D levels increased significantly ($P < 0.001$) and its change was significantly different between the two groups, at the end of the intervention ($P < 0.001$). Serum level of hs-CRP decreased significantly in the intervention group compared to baseline ($P < 0.001$), and the change in hs-CRP was significant between the two groups at the end of the intervention ($P < 0.01$). Furthermore, serum level of TNF-α declined significantly in the intervention group compared to baseline ($P < 0.001$) but, no significant differences were seen between the two groups in regard of serum levels of TNF-α and IL-6 after the intervention ($P > 0.05$). Our findings show that vitamin D and magnesium co-supplementation, for 8 weeks, in healthy middle-aged women with vitamin D deficiency have beneficial impacts on muscle strength, muscle function, and probably inflammation.

Keywords Vitamin D · Magnesium · Muscle strength · Muscle function · Body composition · Inflammatory markers · Middle-aged women

Introduction

Aging is associated with sarcopenia, the age-related decline of muscle mass, muscle strength, and function [1]. This decline is related to dependence, poor quality of life, hospitalization, and premature death [1]. The cause of sarcopenia is multifactorial and includes poor nutritional intake [1].

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Moreover, in both genders, aging is associated with a significant rise in serum levels of inflammatory biomarkers, independently of comorbidities and cardiovascular risk factors [2]. This state of chronic low-grade inflammation, known as inflammaging [2], has outstanding clinical implications. The oxidative stress-induced redox imbalance and the sustained upregulation of pro-inflammatory markers are shown to act as the pathophysiological basis causing inflammatory disorders such as sarcopenia [3]. A growing body of evidence has reported that inflammatory cytokines activate many of the molecular pathways involved in skeletal muscle wasting resulting to an imbalance between protein synthesis and catabolism [3, 4]. High levels of inflammatory cytokines have been indicated to be negatively associated with muscle strength and mass [5].

The role of nutrition in these processes is of great importance. Nutritional intervention can probably help to inhibit "anabolic resistance." Molecular studies have shown that many nutrients including vitamin D and magnesium might be able to modulate systemic inflammation [2] and to exert beneficial effects on muscle metabolism and muscle function [1, 6].

With the recognition of vitamin D receptor in muscle cells and neuronal cells, studies in cell culture and experimental animals suggested that vitamin D affects muscle growth, development, and contraction [6]. Some observational investigations have reported a relationship between serum 25-hydroxyvitamin D (25(OH)-D) levels and measures of muscle performance [7–9] and appendicular muscle mass [7] in community-dwelling older adults. In addition, vitamin D decreases cytokine secretion via its impact on the NF-kB pathway in lymphocytes and adipocytes, resulting to immunomodulation and reduction of chronic inflammation [2]. Older people are susceptible to develop vitamin D deficiency because of reduced dietary intake, diminished sun exposure, decreased skin thickness, reduced intestinal absorption, and impaired hydroxylation in the liver and kidneys [10].

Magnesium, the second most abundant intracellular cation, plays central roles in different physiological processes involve in muscle function such as energy production, transmembrane transport, electrolyte balance, and muscle contraction and relaxation [11]. In addition, a number of cross-sectional studies have demonstrated inverse associations between magnesium intake and some inflammatory parameters, such as high sensitive CRP (hs-CRP) and intrerluekin-6 (IL-6) [12–15]. In spite of the several dietary sources of magnesium, subclinical or marginal magnesium deficiency is prevalent worldwide, especially in aging [16, 17].

Furthermore, the adequate balance of magnesium and vitamin D is important for maintaining the physiologic functions of various organs. It was shown that magnesium is required to activate vitamin D. Optimal health advantageous of exogenous and endogenous vitamin D might not be achieved without the adequate presence of magnesium, as the bioactivity of vitamin D is a magnesium-dependent process [18]. Abnormal levels in either of these nutrients can result to serious organ dysfunctions [18].

Given the importance of the investigation the effects of vitamin D and magnesium on muscle strength and function, muscle mass, and inflammation, and due to the impact of co-supplementation of them on muscle metabolism and inflammatory state in vitamin D-deficient middle-aged women is not clear, and also, the high prevalence rate of deficiency/insufficiency in these two nutrients worldwide, specifically in aging, the aim of this study was to evaluate the effects of vitamin D and magnesium co-supplementation on muscle strength and function, body composition, and inflammation in vitamin D-deficient middle-aged women.

Methods and Materials

The present study was a randomized, double blind, placebo-controlled clinical trial. Participants were enrolled from the staff at Iran University of Medical Sciences (IUMS), Tehran, Iran, between March 2018 and April 2019. The protocol of this study was approved by the Medical Ethics Committee of Iran University of Medical Sciences, is in conformity with the declaration of Helsinki (approval number IR.IUMS.FMD.REC 1396.9413468001), and was registered at the Iranian Registry of Clinical Trials (IRCT registration number IRCT20090822002365N200) which is available at http://irct.ir/user/trial/20288/view. Signed informed consent was obtained from all participants. Volunteers could participate in the study if they were healthy female, aged 40–55 years, had a body mass index (BMI) ≥ 25 kg/m^2, were not suffering from any chronic disease such as liver and gastrointestinal disorders, diabetes or other endocrine disorders, and kidney disease, were not smoker, and were not taking vitamin D and/or magnesium and other vitamin-mineral supplements, laxative, or hormone medications. We excluded the women who had less than 80% compliance with the treatment, and those who were involving in weight loss programs or taking nutritional supplements.

Sample Size

The number of participants calculated for each group was 40 at 98% power and α of 0.05 to detect a difference of 1.5 kg in handgrip strength between groups with an S of 52.8, as described previously [11]. To allow for attrition, 45 participants were enrolled for each group. Collectively, the sample of 90 middle-aged women was enrolled.

Intervention and Randomization

The 90 participants, who met the inclusion criteria, were randomly allocated into two groups: intervention group (n = 45) received 50000-IU vitamin D soft gel (weekly) plus a 250-mg magnesium tablet (in the form of magnesium oxide) (daily), and a placebo group (n = 45) received a vitamin D placebo (weekly) plus a magnesium placebo (daily). The vitamin D soft gels and magnesium tablets were provided by Zahravi, Iran, and Jalinous, Iran, respectively. The placeboes for vitamin D and magnesium contained oral paraffin and maltodextrin, respectively, and were supplied by Zahravi, Iran. These were matched in appearance, smell, and taste of the corresponding soft gels and tablets. Participants received verbal and written counseling on how to consume the supplements. Compliance with the intervention was evaluated by tablet count every 2 weeks and through the face-to-face visits.

The participants were allocated randomly using a random number table; for this, a person who was not involved in the protocol made the randomization list assigning the participants to the intervention or placebo group. Vitamin D soft gels and magnesium tablets were placed in to unlabeled identical containers. The study leader labeled these containers with participant numbers using the randomization list. All investigators and participants were blinded to the random assignments.

Outcome Measurement

A questionnaire on subjects' demographic state, diseases, and probable use of supplements and/or medications was recorded before the beginning of the intervention.

Anthropometric indices, dietary intakes, and physical activity level were evaluated twice, before and after the intervention. Height and body weight were assessed using standard protocols, while the participants were minimally clothed and without shoes, to the nearest 0.5 cm and 0.1 kg, respectively. BMI was computed by dividing body weight (kg) to height squared (m^2). Body composition including percentage of body fat mass (FM%) and fat free mass (FFM%) were evaluated using bioelectrical impedance method by Omron BF-511 apparatus, Japan.

Dietary intakes were assessed with a 24-h food recall for 3 days (2 week day and 1 weekend day), and nutrient intakes were estimated by nutritionist 4 software. Physical activity level was measured by the Persian and short form of the International Physical Activity Questionnaire (IPAQ) and demonstrated in MET-min/week [10].

Muscle Strength and Function

In this study, we evaluated upper and lower body strength by handgrip strength test and knee extension strength test, respectively. Muscle function was measured quantitatively using Time Get Up and Go (TGUG) test, twice, before and after the intervention.

Handgrip strength was assessed in kilogram in dominant hand by using a hand-held dynamometer (digital hand dynamometer "DIGI-II" Korea). The participants were seated on a standard armchair with shoulder adducted and neutrally rotated, elbow bent to 90^0, and the forearm and wrist were in a neutral situation [11].

Isometric knee extension strength was evaluated with a calibrated hand held dynamometer (Nicholas Manual Muscle Tester; Lafayette Inc) in kilogram in dominant leg. The participants were seated on straight-back standard chair, their hips and knees bent 90^0. The dynamometer was put proximal to the ankle joint of the subjects, and they were asked to raise their leg throughout the testing; the subjects were encouraged to raise the force to the greatest gradually while the tester was opposing [10].

For TGUG test, the women were seated on the standard chair. They were asked to stand up and walk at normal step 3 m, turn to the chair, and sit down. Value in seconds (s) was reported [19, 20].

Biochemical Parameters

Blood samples were collected after 10 h of night fasting, at baseline and after the intervention, and serum was obtained by centrifugation at 800–1000 RPM for 10 min; the serum samples were frozen and stored at -80^0. Serum level of 25(OH)-D was measured by ELISA and Euro Immune kit. Serum level of magnesium was detected using the spectrophotometric method by an autoanalyzer (Hitachi 912; Roche Diagnostics). Serum hs-CRP was assessed using the ZellBio kit (ZellBio, Germany). Serum levels of TNF-α and IL-6 were evaluated by ELISA and Crystalday kit (Crystalday, China).

Statistical Analysis

Statistical analysis was performed using SPSS (Version 22.0; SPSS Inc. Chicago, IL). Normal distribution of the variables was assessed by Kolmogorov-Smirnov test. All results were present as mean ± SD or median (interquartile). Categorical variables were reported as frequencies and percentages. Paired-samples t test was used to compare the baseline and postintervention values in each group. Differences among the two groups, at baseline and postintervention, were assessed by independent-samples t test. For non-normally distributed data, within- and between-group comparison was performed using Wicoxon-signed ranks and Mann-Whitney U test, respectively. Chi-square test was used to evaluate the differences between categorical variables. Analysis of covariance (ANCOVA) was used to determine differences between the two groups at the end of the intervention, adjusting for baseline values. $P < 0.05$ was considered statistically significant.

Results

In the present study, 90 participants were enrolled. Seven subjects (3 in the intervention group and 4 in the placebo group) were excluded from the study because of withdraw, noncompliance with the treatment, and private reasons. Finally, statistical analysis was conducted on 83 participants (Fig. 1). General characteristics of the participants are shown in Table 1. There were no significant differences between the two groups in age, menstrual status, and physical activity level, at baseline of the study ($P > 0.05$) (Table 1). In addition, dietary intake of energy, protein, total fat, carbohydrate, fiber, vitamin D, magnesium, and calcium was not significantly different between the two groups at baseline and postintervention ($P > 0.05$) (Table 1). Also, the dietary intake of these components did not change significantly within each group during the study ($P > 0.05$). Compliance with the intervention was more than 90% for each group. No side effects were reported after supplementation with magnesium and vitamin D in participants through the study.

Anthropometric indices including weight, BMI, and body composition variables were not different significantly, at baseline and after the intervention ($P > 0.05$) (Table 2). At the end of the study, FM% decreased and FFM% increased in the intervention group compared to baseline. However, the alterations were not significant ($P > 0.05$) and also, did not reach significant level in compared to placebo group, even after adjusted for baseline values ($P > 0.05$).

There were no statistically significant differences between the two groups in terms of handgrip strength, knee extension strength, and the time for TGUG test, at baseline ($P > 0.05$) (Table 3). Handgrip strength increased significantly from 23.57 ± 5.98 to 26.67 ± 5.96 kg ($P < 0.001$) in the intervention group and non-significantly from 22.82 ± 7.18 to 23.38 ± 7.19 kg ($P = 0.12$) in the placebo group, at the end of the intervention in compared with baseline. Moreover, the change was significantly different between the two groups, even after adjustment for baseline values ($P < 0.05$) (Table 3). Knee extension strength elevated from 8.99 (7.39–9.93) to 9.49 (7.44–10.11) kg ($P < 0.001$) in the intervention group and from 8.40 (7.14–10.31) to 8.70 (7.60–10.41) kg ($P < 0.001$) in the placebo group, at the end of the intervention compared to the baseline. But, the changes did not reach significant between the two groups ($P = 0.41$), even after controlling for the baseline values ($P = 0.25$) (Table 3). The median time for TGUG test reduced from 8.19 (7.91–9.23) to 7.72 (6.94–8.34) s in the intervention group ($P < 0.001$) but, no significant alteration was seen in the placebo group ($P = 0.18$). However, significant difference was observed in time for TGUG test between the two groups, at the end of the intervention ($P = 0.01$), even after for adjustment for baseline values ($P = 0.02$) (Table 3).

Table 4 demonstrates the effects of vitamin D and magnesium co-supplementation on serum levels of 25(OH)-D, magnesium, and some inflammatory biomarkers. There were no statistically significant differences between the two groups in terms of serum levels of 25(OH)-D, magnesium, and inflammatory parameters, at the beginning of the study ($P > 0.05$). At the end of the intervention, serum 25(OH)-D levels increased significantly ($P < 0.001$) in the intervention group and declined non-significantly in the placebo group compared to baseline and also, its change was significantly different between the two groups, at postintervention ($P < 0.001$). The median level of serum magnesium increased from 1.85 to 1.90 mg/dl in the intervention group and decreased from 1.90 to 1.85 mg/dl in the placebo group. However, the changes were

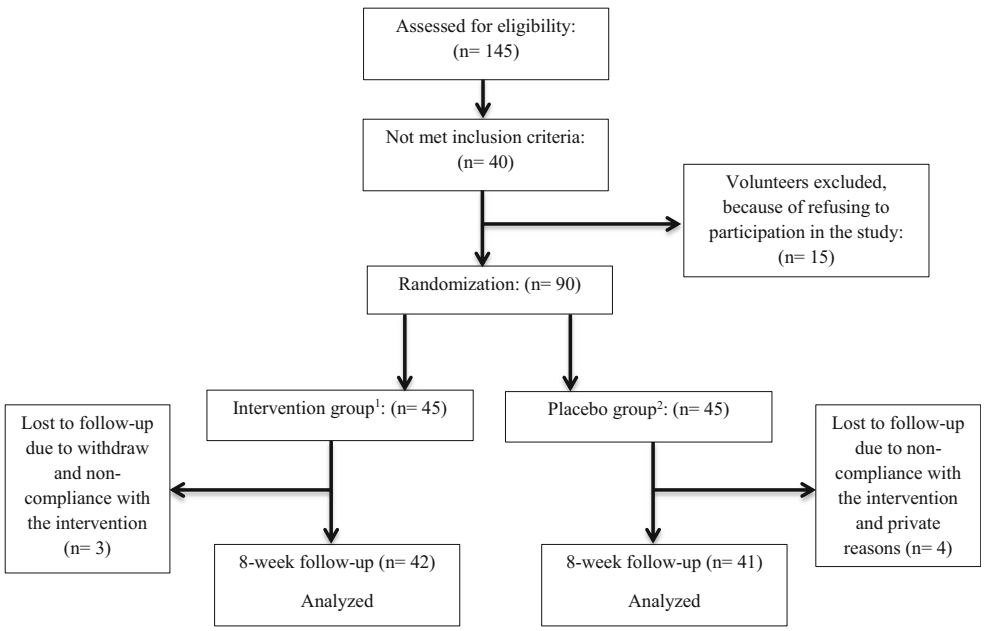

Fig. 1 Flow diagram of the study. [1] Participants received a 50000-IU vitamin soft gel (weekly) plus a 250-mg magnesium tablet (daily); [2] Participants received a vitamin D placebo (weekly) plus a magnesium placebo (daily)

Table 1 General characteristics, food intake, and physical activity level of the participants in the intervention and placebo groups at baseline and after 8 weeks

Variable	Intervention group (n = 42)	Placebo group (n = 41)	P
Age, years[a]	45 (41.00–49.00)	47.00 (42.00–51.00)	0.36*
Pre-menopause, n(%)	25 (59)	27 (66)	0.61**
Height, cm[b]	157.72 ± 5.56	158.29 ± 5.49	0.64***
Physical activity, MET-min/week[a]			
Baseline	396.00 (198.00–742.12)	462.00 (297.00–826.50)	0.42*
Week 8	429.00 (243.00–1004.25)	462.00 (297.00–767.25)	0.98*
Energy intake, kcal/day[a]			
Baseline	1258.75 (1031.20–1634.00)	1264.50 (1047.92–1494.50)	0.88*
Week 8	1261.25 (1044.80–1503.00)	1214.50 (976.37–1407.75)	0.18*
Total fat intake, g/day[a]			
Baseline	54.09 (38.92–65.10)	47.14 (32.32–57.00)	0.13*
Week 8	48.32 (36.52–4.75)	43.97 (31.88–52.48)	0.15*
Carbohydrate intake, g/day[b]			
Baseline	57.43 ± 50.17	67.43 ± 57.17	0.43***
Week 8	162.17 ± 138.08	166.80 ± 125.20	0.50***
Protein intake, g/day[b]			
Baseline	6.94 ± 15.47	54.74 ± 12.64	0.69***
Week 8	70.91 ± 12.45	21.35 ± 12.44	0.56***
Fiber intake, g/day[a]			
Baseline	9.75 (7.44–12.94)	9.28 (7.74–13.03)	0.65*
Week 8	8.98 (6.67–11.01)	9.62 (6.62–11.39)	0.95*
Vitamin D intake, μg/day[a]			
Baseline	0.15 (0.00–1.24)	0.00 (0.00–1.20)	0.31*
Week 8	0.00 (0.00–0.83)	0.00 (0.00–0.17)	0.72*
Magnesium intake, mg/day[a]			
Baseline	140.35 (99.90–175.67)	120.00 (96.59–149.69)	0.24*
Week 8	115.68 (95.22–158.11)	110.00 (86.40–145.60)	0.38*
Calcium intake, mg/day[a]			
Baseline	468.50 (320.04–593.36)	409.55 (305.64–591.35)	0.60*
Week 8	396.02 (299.04–526.65)	379.70 (273.52–465.52)	0.46*

[a] Values are shown as median (interquartile range)

[b] Values are shown as mean ± standard deviation (SD)

*P value is based on Mann Whitney U test

**P value is based on chi-square test

***P value is based on independent samples t test

not statistically significant ($P > 0.05$) and also, there were no significant differences between the two groups at the end of the intervention ($P > 0.05$). The median level of serum hs-CRP decreased significantly from 2.49 to 1.28 mg/L in the intervention group compared to baseline ($P < 0.001$), whereas no significant alteration was observed in the placebo group ($P = 0.61$). In addition, the change in hs-CRP was significant between the two groups at the end of the intervention ($P < 0.01$). No significant differences were seen between the two groups in regard of serum levels of TNF-α and IL-6 at baseline and after the intervention ($P > 0.05$). The median serum level of TNF-α declined significantly from 7.07 to 6.88 pg/ml in the intervention group compared to baseline ($P < 0.001$); however, either the TNF-α or IL-6 concentrations at the end of the intervention or the alterations during the study were not significantly different between the two groups ($P > 0.05$).

Discussion

To the best of our knowledge, the present study is the first randomized, double-blind, placebo-controlled clinical trial

Table 2 Anthropometric indices of participants at baseline and after 8 weeks

Variable	Intervention group (n = 42)				Placebo group (n = 41)				P_2	P_3	P_4
	Baseline	Week 8	Change	P_1	Baseline	Week 8	change	P_1			
Weight, kg[a]	81.80 (74.42–86.32)	81.25 (73.40–86.10)	− 0.32 ± 1.46	0.19	75.90 (70.40–84.05)	76.10 (70.50–84.05)	0.07 ± 1.04	0.52	0.18	0.24	0.59
BMI, kg/m^2 [a]	32.35 (29.20–35.05)	32.15 (28.85–35.20)	0.20 (− 0.20–0.60)	0.05	31.00 (27.60–33.60)	30.80 (27.65–33.90)	− 0.10 (–0.30–0.35)	0.68	0.18	0.27	0.24
FFM, %[a]	24.30 (23.22–24.95)	24.25 (23.62–24.90)	0.17 ± 0.74	0.21	24.00 (22.60–24.90)	23.20 (22.65–25.00)	− 0.06 ± 0.66	0.35	0.31	0.09	0.33
FM, %[b]	44.75 ± 4.34	44.60 ± 4.55	− 0.35 (− 0.97–0.62)	0.52	44.80 ± 4.68	44.98 ± 4.82	0.10 (− 0.25–0.60)	0.12	0.95	0.71	0.49

BMI, body mass index; *FFM*, fat free mass; *FM*, fat mass

[a] Values are shown as median (interquartile range)

[b] Values are shown as mean ± standard deviation (SD)

P_1: Within-group differences; P value is based on paired-samples t test or Wilcoxon Signed ranks test

P_2: Difference between groups at baseline; P value is based on independent-samples t test or Mann-Whitney U test

P_3: Difference between groups at the end of the intervention; P value is based on independent-samples t test or Mann-Whitney U test

P_4: Difference between groups at the end of the intervention; P value is based on parametric or non-parametric analysis of covariance (ANCOVA) and adjusted for baseline values

that evaluate the effects of vitamin D and magnesium co-supplementation, during an 8-week period, on muscle strength and function, body composition, and inflammation in vitamin D-deficient middle-aged women. We found that vitamin D (50000 IU, weekly) plus magnesium (250 mg, daily) supplementation for 8 weeks in vitamin D-deficient middle-aged women had beneficial effects on muscle strength and function, and some inflammatory markers but, the intervention showed no significant changes in FM% and FFM% at the end of the study.

In our previous trials, we evaluated the effects of vitamin D [10] and magnesium supplementation [11], individually, on muscle strength, muscle function, and body composition in middle-aged women. In one of these studies [10], with the aim of the investigation of the effects of physiologic dose of vitamin D supplementation (1000 IU, daily, for 12 weeks) on muscle strength, muscle function, and body composition in healthy vitamin D-deficient middle-aged women (40–55 years), results of the intervention revealed amelioration in muscle function (a decreased in the time needed for doing the TGUG test) in vitamin D group compared to the placebo but, the intervention showed no significant differences in FM% and FFM% between the two groups. In addition, muscle strength (handgrip strength and knee extension strength) did not differ significantly after a 12-week supplementation. This study suggested that the physiologic dose of vitamin D might have been insufficient to exert any significant amelioration in body composition and muscle strength in vitamin D-deficient middle-aged women. So, in the present trial, we used therapeutic dose (50000 IU) of vitamin D in the vitamin D-deficient participants. In our another study, with the aim of the investigation of the effects of magnesium supplementation on muscle strength, muscle function, body composition, and inflammation in healthy middle-aged women, we found that

Table 3 Comparison of the baseline and postintervention values of muscle strength and muscle function in the intervention and placebo groups

Variable	Intervention group (n = 42)				Placebo group (n = 41)				P_2	P_3	P_4
	Baseline	Week 8	Change	P_1	Baseline	Week 8	change	P_1			
HS, kg[a]	23.57 ± 5.98	26.67 ± 5.96	3.00 ± 1.87	< 0.001	22.82 ± 7.18	23.38 ± 7.19	0.00 ± 0.05	0.12	0.60	0.02	0.04
KES, kg [b]	8.99 (7.39–9.93)	9.49 (7.44–10.11)	0.28 (0.15–0.47)	< 0.001	8.40 (7.14–10.31)	8.70 (7.60–10.41)	0.25 (0.01–0.46)	< 0.001	0.31	0.41	0.25
TGUG, s[b]	8.19 (7.91–9.23)	7.72 (6.94–8.34)	− 0.67 (− 1.02–0.33)	< 0.001	8.84 (7.44–9.45)	8.29 (7.16–9.03)	− 0.03 (− 0.51–0.11)	0.18	0.92	0.01	0.02

HS, handgrip strength; *KES*, knee extension strength; *TGUG*, time get up and go test

[a] Values are shown as mean ± standard deviation (SD)

[b] Values are shown as median (interquartile range)

P_1: Within-group differences; P value is based on paired-samples t test or Wilcoxon signed ranks test

P_2: Difference between groups at baseline; P value is based on independent-samples t test or Mann-Whitney U test

P_3: Difference between groups at the end of the intervention; P value is based on independent-samples t test or Mann-Whitney U test

P_4: Difference between groups at the end of the intervention; P value is based on parametric or non-parametric analysis of covariance (ANCOVA) and adjusted for baseline values

Table 4 Serum levels of biochemical factors of the participants at baseline and after 8 weeks

Variable	Intervention group (n = 42)			P_1	Placebo group (n = 41)			P_1	P_2	P_3	P_4
	Baseline	Week 8	Change		Baseline	Week 8	change				
25(OH)-D, ng/ml[a]	13.93 (12.16–17.40)	38.00 (35.85–40.59)	24.26 (20.48–25.51)	< 0.001	14.35 (11.34–17.05)	15.24 (12.00–18.01)	0.56 (− 0.43–1.24)	0.07	0.70	< 0.001	< 0.001
Serum mg, mg/dl [a]	1.85 (1.80–2.10)	1.90 (1.80–2.10)	0.00 (− 0.10–0.10)	0.94	1.90 (1.80–2.10)	1.85 (1.80–2.00)	0.00 (− 0.20–0.05)	0.18	0.86	0.35	0.13
hs-CRP, mg/l[a]	2.49 (1.78–3.14)	1.28 (1.08–1.79)	− 1.03 (− 1.50–0.64)	< 0.001	1.94 (1.24–2.78)	2.12 (1.62–2.90)	0.03 (− 0.23–0.28)	0.61	0.06	< 0.01	0.03
TNF-α, pg/ml[a]	7.07 (6.09–10.38)	6.88 (5.99–9.91)	− 0.18 (− 0.33–0.03)	< 0.001	8.06 (5.92–9.89)	7.98 (6.19–10.17)	− 0.04 (− 0.15–0.31)	0.41	0.76	0.34	0.63
IL-6, pg/ml[b]	2.30 ± 0.69	2.23 ± 0.79	− 0.03 ± 0.1	0.19	2.23 ± 0.68	2.21 ± 0.71	− 0.02 ± 0.02	0.36	0.66	0.61	0.45

mg, magnesium; *hs-CRP*, high sensitive C-reactive protein; *TNF-α*, tumor necrosis factor alpha; *IL-6*, interluekin-6

[a] Values are shown as median (interquartile range)

[b] Values are shown as mean ± standard deviation (SD)

P_1: Within-group differences; P value is based on paired-samples t test or Wilcoxon signed ranks test

P_2: Difference between groups at baseline; P value is based on independent-samples t test or Mann-Whitney U test

P_3: Difference between groups at the end of the intervention; P value is based on independent-samples t test or Mann-Whitney U test

P_4: Difference between groups at the end of the intervention; P value is based on parametric or non-parametric analysis of covariance (ANCOVA) and adjusted for baseline values

daily intake of 250 mg magnesium for 8 weeks did not result to improvement in handgrip strength and knee extension strength in the magnesium group compared to the placebo group. Moreover, no significant difference was seen in time for TGUG test between the two groups at the end of the intervention; however, the mean time for TGUG test reduced significantly in the magnesium group compared to the baseline. In regard to body composition, the changes at the end of the trial did not reach significant compared to the placebo group. Moreover, the supplementation did not reduce inflammatory markers (hs-CRP and IL-6) in the magnesium group compared to the placebo [11, 17].

In the present study, magnesium (250 mg, daily) plus vitamin D (50000 IU, weekly) supplementation for 8 weeks in vitamin D-deficient middle-aged women resulted to improvement in muscle function and grater gains in handgrip strength in the intervention group compared to the placebo, but the intervention failed to exert a significant difference in knee extension strength and body composition in the intervention group compared to the placebo.

Magnesium intake was related to the prevalence of sarcopenia in some observational studies [21, 22]. In addition, the results of the SarcoPhAge study [23] showed a significantly decreased in consumption of some micronutrients including magnesium in sarcopenic individuals even after adjusting for covariates. In a randomized controlled trial, magnesium supplementation (300 mg/day) in combination with mild fitness program in healthy women (mean ± SD age 71.5 ± 5.2 years) ameliorated physical performance [24]. These findings are approved by studies conducted by Scott et al. [25] and Dominguez et al. [26]. Magnesium intake was significantly and positively related to appendicular lean mass, according to a cohort study [25]. Serum magnesium level associated independently with muscle strength [26]. Mechanistically, magnesium has an important effect on muscle function and muscle metabolism. Furthermore, it is involved in protein and ATP synthesis and so, is responsible for muscle relaxation [27, 28]. Magnesium is also contributed to the exchange of calcium and potassium ions across cell membranes, which is essential for neuronal activity and muscle contractions [29]. Evidence indicates that magnesium is involved in modification of the secretion of anabolic hormones [30] and inflammation [31], the established leading causes of decreasing muscle strength [32]. Furthermore, magnesium deficiency leads to nausea, fatigue, and muscle weakness [29]. Hence, it is probable that magnesium may have long-term influences on muscle strength and function through its impact on inflammation and anabolic hormone. In contrary, serum magnesium did not differ between sarcopenic and nonsarcopenic subjects in the study by Ter Borg et al. [21]. This might be justified by the strict regulation of serum levels of magnesium by bone stores, gastrointestinal tract, and urinary excretion [33]. This finding has been established by a randomized clinical trial that a significant elevate in serum magnesium levels was reported after magnesium supplementation (300 mg/day) for 12 weeks; in this study, the impact of magnesium on physical performance was more obvious in individuals with a dietary magnesium intake below the recommended dietary allowance (RDA) at baseline [24]. This finding is in line with the previous studies [28, 34]. Unlike the results of the present research, in a study of young men, with the aim of the evaluation the effect of magnesium supplementation on muscle strength in healthy participants, magnesium supplementation (in the form of magnesium oxide) parallel to strength training for 7 weeks resulted to grater knee extension torque in the magnesium group compared to the placebo [35]. It is probable that to observe any significant amelioration in muscle strength, magnesium in muscle should be reached to an optimal level, which remains

to be clarified in our present study. The duration of the magnesium supplementation has been too short to elevate muscle strength. In addition, bioavailability of magnesium from magnesium oxide could also influence the responses to the subjects to magnesium supplementation. Both elemental magnesium content in a magnesium supplement and its bioavailability can affect the response to the supplementation. Lower bioavailability of magnesium in the form of magnesium oxide has been reported [36] despite of the fact that this form of the magnesium supplement has higher elemental magnesium content in compared with other forms of supplements [37]. It was also suggested that baseline magnesium level or baseline muscle strength might have impacts on the response to the magnesium supplementation. Those with lower baseline serum magnesium or baseline muscle strength respond better to magnesium supplementation. In our present study, serum magnesium levels of the subjects were within normal range (1.8–2.6 mg/dl) at the baseline. Collectively, magnesium may be an important nutrient to prevent and treat sarcopenia in older adults with lower baseline serum magnesium.

On the other hand, a prospective study in older population aged 55–85 years has demonstrated that individuals with 25(OH)-D level < 25 nmol/L were 2.51 times more possible to experience decline of grip strength and 2.38 times more possible to experience decline appendicular skeletal muscle mass, compared to those with a high 25(OH)-D concentration [38]. A population based-study in elderly men and women has reported that total body fat content is inversely associated with 25(OH)-D levels [39]. In contrary, another research [40] evaluated the relationship between serum 25(OH)-D level with skeletal muscle mass and strength (handgrip and isometric knee extension strength) and showed no constant relationship between 25(OH)-D with muscle mass and strength in either men or women. The findings of this study are in agreement to some cross-sectional studies [41, 42], but not to others [7–9]. In a randomized controlled trial [43], 242 healthy women and men (aged ≥ 70 years) with serum 25(OH)-D levels less than 78 nmol/L were enrolled and administered 1000 mg calcium plus 400 IU vitamin D or 1000 mg calcium alone for 12 months. Results of this study demonstrated a reduce in the number of subjects with first fall, amelioration in quadriceps strength of 8%, a decrease in body wobble of 28%, and a decreased in the time for TUG (Timed Up and Go) test of 11%. However, methodologies differed widely between these studies; for example, one of the major causes has led to divergent results when trying to demonstrate the association between vitamin D and muscle strength is testing methods. Handgrip strength is one of the most common indicators for measuring muscle strength but different equipment was used on different muscle groups to conduct these measurements and possibly lead to different outcomes. The discrepant results may be justified by several reasons. Low levels of vitamin D associated with muscle weakness and atrophy of type II fibers [44]; type II fibers are fast twitch and involve in high intensity but short duration activities [45]. Moreover, there are several polymorphisms in vitamin D receptors, which resulted from variations in DNA sequence of vitamin D receptor genes that are related to different biological characteristics including muscle strength [45]. Furthermore, in these studies, latitude or other environmental factors that led to these differences are not clear. In addition, cross-sectional design could not clarify the temporality of vitamin D and sarcopenia. It is probable that sarcopenia leads to low vitamin D condition. Firstly, sarcopenia has established to be a major predictor of disability in elderly population [46]. Declined physical activity and less time spent outdoors leads to serum vitamin D decreasing [10]. Secondly, sarcopenia is mainly demonstrated in obese older adults with significant muscle loss and elevated fat mass [6]. Elevation in body fat mass results to vitamin D trapping in the adipose tissue and reduces the serum level of vitamin D [46]. Third, sarcopenia is associated with aging [6]. Old age is an important risk factor for vitamin D deficiency/insufficiency [6]. Several possible mechanisms have demonstrated that vitamin D may influence muscle function. The genomic influence of vitamin D on muscle includes alterations in mRNA that will lead to de novo protein synthesis and control cell proliferation and induction of terminal differentiation [6]. Moreover, the non-genomic influence of vitamin D on muscle includes the activation of protein kinase C and releasing calcium in to the cytosol [6]. Hence, this impact results to the active transportation of calcium in to the sarcoplasmic reticulum by Ca-ATPase enhancing the calcium pool which is important for muscle contraction. Furthermore, the activation of protein kinase C has an influence on protein synthesis in muscle cells [6]. Because inflammation is a potential risk factor for sarcopenia, the anti-inflammatory influences of vitamin D could have beneficial effects on skeletal muscle metabolism [47]. However, environmental factors and polymorphisms in the genes encoding vitamin D receptors may impact the metabolism of this vitamin.

In the present study, supplementation with magnesium plus vitamin D led to significant elevate in serum 25(OH)-D level in the intervention group compared to the placebo, while, it had no impact on serum levels of magnesium in the intervention group. Furthermore, the intervention had beneficial effects on some inflammatory markers. However, all previous trials do not report magnesium supplementation to lead to constant elevation in levels of serum magnesium; in some trials, it did elevate, following magnesium supplementation [48–50] while in others not [17, 51, 52] or even demonstrated a reduction [53]. It is established that serum magnesium cannot be a sensitive marker for magnesium intake in spite of frequent usage. In response to magnesium supplementation, the magnesium level of the subjects at baseline of the study may be also important. In some cases, in whom magnesium supplementation elevated serum magnesium, the subjects

were hypomagnesemic at baseline [49, 50]. In our present study, serum magnesium levels of the subjects were within normal range (1.8–2.6 mg/dl) at the baseline.

Kim et al. investigated the relationship between serum magnesium and some inflammatory parameters including hs-CRP, IL-6, and fibrinogen in 4497 Americans aged 18–30 years, and they found an inverse relationship between serum magnesium level and only hs-CRP [54]. In a clinical trial among the adults older than 51 years with poor sleep quality, supplementation with 320 mg magnesium (in the form of magnesium citrate), daily, for 7 weeks reduced levels of CRP in subjects with baseline values of CRP > 3 mg/L [55]. Individuals with high inflammatory stress or low magnesium level might respond better and faster to magnesium supplementation. However, high dose of magnesium (600 mg) as pidolate for 12 weeks could elevate serum level of magnesium significantly in the subjects with normal serum magnesium [56], proposing that differences in form of magnesium salt and doses administered might be also probable sources of differences in serum magnesium response after magnesium supplementation between trials. The mechanisms by which magnesium influences inflammation are not fully understood; elevated intracellular calcium due to magnesium deficiency is one of the suggested underlying mechanisms [16]. In the present study, the dose and duration of magnesium supplementation may not be enough to achieve the desirable outcomes; also, serum magnesium levels of the subjects were within normal range at the baseline; due to unavailability of similar studies on the anti-inflammatory impact of magnesium, we cannot compare and justify precisely why we observed no desirable impacts on all inflammatory factors for magnesium in compared with the placebo.

Moreover, in elderly subjects, 25(OH)-D concentrations decrease with age [57]. A systematic review has indicated that the prevalence of vitamin D deficiency/insufficiency is high across all age groups and geographical areas, but the prevalence in older subjects is higher, approaching 90% [58]. The causes of vitamin D deficiency/insufficiency in the elderly have not been fully recognized and mostly remain suppositional. They may include inadequate sunlight exposure, poor nutritional intake, chronic diseases, changes in body composition with elevate in fat mass content, cognitive and physical disability, and polypharmacy [10]. In healthy adult individuals, the association between 25(OH)-D levels and inflammation is controversial. Data from the National Health and Nutrition Examination Survey (NHANES) study, involving 15167 adults (mean age 46 years), demonstrated an inverse association between 25(OH)-D and CRP only for 25(OH)-D concentrations below 21 ng/ml, and a positive association above 21 ng/ml [59]. The effect of vitamin D in modifying inflammation has been investigated also in a large number of clinical trials. However, most of them were conducted on adult participants with specific diseases such as diabetes, obesity, asthma, COPD, chronic kidney disease, and sepsis [60]. Vitamin D administration with different doses in a large cohort of healthy adult was not related to variations in inflammatory biomarkers, although an inverse association between 25(OH)-D and CRP was observed at baseline [61]. So far, the only clinical trial reporting an anti-inflammatory impact of vitamin D supplementation in elderly is a small randomized clinical trial conducted on 40 female community dwellers. According to this trial, the administration of a single dose of 200000 IU vitamin D led to a significant reduce of hs-CRP levels after 4 weeks. The reduction was more evident in those with the BsmI polymorphism of vitamin D receptor [62]. The molecular anti-inflammatory properties of vitamin D are well known [2]. Vitamin D receptor is expressed by cells having an effect on inflammation and immunity, such as macrophages, that also have the ability of converting 25(OH)-D in to its active metabolite by expressing 1α-hydroxylase. The activation of vitamin D receptor in macrophages leads to the inhibition of NF-kB and expression of TLR2 and TLR4, leading to reduced generation of TNF-α and induced hyporesponsiveness to antigenic stimulation [2]. Vitamin D decreases cytokine via its impacts on the NF-kB pathway in lymphocytes and adipocytes, resulting to immunomodulation and reduction of chronic inflammation [2]. In addition, vitamin D causes the production of IGF-1, the key agent in the cross road relating nutrition and inflammatory pathways [2]. In spite of large body of molecular evidence proposing a key role for vitamin D in modification of inflammatory markers, observational studies in adults show conflicting results and clinical trials are focused only on disease-specific groups. So, the level of clinical evidence connecting vitamin D with inflammaging in older population is even more discrepant. In a systematic review, the authors concluded that 25(OH)-D may act as an acute phase reactant, emphasizing that the association between vitamin D and inflammation may be bidirectional [63]. A meta-analysis suggested that sarcopenic subjects experienced significantly higher levels of CRP, while no significant differences observed for IL-6 and TNF-α in compared with controls [3]. These findings are in line with the research regarding the association between frailty and inflammation [64] demonstrating that CRP is more strictly associated with frailty than IL-6 and TNF-α. While the exact causes for this difference are not clear, it might be hypothesized that CRP could be a potential biomarker for detecting sarcopenia. In addition, some researchers noted that sarcopenia may result other mechanisms including age-associated decrease in hormones, neurodegenerative processes, and disability, but not certainly from inflammation [3]. Our findings may propose that the plasma titer of some inflammatory biomarkers (hs-CRP and probably TNF-α) could be associated with the aspects of muscle decline and function disturbance; whether this relationship could be clinically applicable is conflicting because the phenotypical and pathophysiological complexity

of sarcopenia could not be conquered by single biological markers but possibly require a multidimensional approach [3].

The essential micronutrients magnesium and vitamin D are each related to chronic diseases of global concern, such as metabolic syndrome, systemic inflammation, and musculoskeletal disorders. The adequate balance of magnesium and vitamin D is important for maintaining the physiologic functions of various organs, and abnormal levels in either of these nutrients can result to serious organ dysfunctions [18]. Magnesium, the second most intracellular cation, plays a crucial role in the synthesis and metabolism of parathyroid hormone (PTH) and vitamin D [65]. Evidence demonstrates that the activities of 3 major enzymes that affect 25(OH)-D levels, including 25-hydroxylase, 1α-hydroxylase, and 24-hydroxylase, and also vitamin D binding protein (VDBP), are magnesium dependent. Magnesium deficiency results to declined $1,25(OH)_2D$ and impairment in PTH response [65]. In 2 case studies of vitamin D-resistant rickets, magnesium supplementation considerably reversed the resistance to vitamin D therapy [66], while intramuscular vitamin D (600000 IU) alone did not result to any amelioration in biochemical values of advanced vitamin D deficiency [66]. Collectively, these findings propose that a probable interaction between magnesium and vitamin D affects vitamin D condition [65]. On the basis of this biological plausibility, the results of NHANES reported a high intake of total, dietary, or supplemental magnesium was significantly related to decreased risks of both vitamin D deficiency and insufficiency in the general population [67]. Moreover, an inverse relationship between total magnesium intake and vitamin D insufficiency was observed between populations at high risk of vitamin D insufficiency, including overweight/obese subjects and African Americans. In addition, this study showed inverse relationships of serum 25(OH)-D with mortality that were modulated by high magnesium intake [65].

The grater improvements in muscle strength, muscle function, and inflammation in the present research compared to our previous trials with vitamin D [10] and magnesium supplementation [11, 17], individually, may be due to the synergistic effects of vitamin D and magnesium and/or vitamin D administration in therapeutic dose in vitamin D-deficient middle-aged women.

Altogether, vitamin D and magnesium co-supplementation, for 8 weeks in vitamin D-deficient middle-aged women, have a strong synergistic impact on inflammation and musculoskeletal system. Given the high prevalence rate of deficiency/insufficiency in these two nutrients worldwide, specifically in aging, and because the elderly population has had an increasing trend during the last century, and also based on the importance of vitamin D and magnesium for the normal function of different organs, aforementioned nutritional intervention can be a successful strategy for improving systemic inflammation, muscle strength and performance, quality of life in elderly, and consequently public health status.

To our knowledge, this is the first clinical trial that has investigated the impacts of vitamin D plus magnesium supplementation on muscle strength and function, body composition, and some indicators of inflammation in healthy middle-aged women. By excluding the participants with known conditions influencing inflammation, it was tried to evaluate the impact of vitamin D plus magnesium on chronic subclinical inflammation, an important risk factor for age-associated diseases. However, there were some limitations in the current study. First, the period of the study was short and the dose of magnesium administered was rather modest. Second, we did not evaluate the impact of the supplementation on urinary magnesium concentration. Third, the study only included the women who had low levels of serum 25(OH)-D and it is not clear if the same effect would be obtained in women with normal serum vitamin D levels.

Conclusion

Vitamin D (50,000 IU/week) plus magnesium (250 mg/day) supplementation, for 8 weeks in healthy middle-aged women with vitamin D deficiency, have beneficial impacts on inflammation, muscle strength, and muscle function.

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Author Contributions FK and MV designed this study. FK and JS participated in the conduction of the study. AFH analyzed the data. BA drafted the manuscript. FK, JS, AFH, and MV critically revised the manuscript. All authors read and approved the final manuscript.

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Data Availability All data generated or analyzed during this study are included in this article.

Compliance with Ethical Standards

Ethics Approval and Consent to Participate Written informed consent was obtained from all participants on recruitment. The protocol of this study was approved by the Medical Ethics Committee of Iran University of Medical Sciences, is in conformity with the Declaration of Helsinki (approval number: IR.IUMS.FMD.REC 1396.9413468001), and was registered at the Iranian Registry of Clinical Trials (IRCT registration number IRCT20090822002365N200) which is available at http://irct.ir/user/trial/20288/view.

Consent for Publication All authors have given consent for the paper to be published by the corresponding author.

Competing Interests The authors declare that they have no conflict of interest.

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