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Magnesium intake, insulin resistance and markers of endothelial function among women

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Abstract

Objective: We investigated the association of dietary Mg intake with insulin resistance and markers of endothelial function among Iranian women.

Design: A cross-sectional study.

Setting: Usual dietary intakes were assessed using a validated FFQ. Dietary Mg intake was calculated by summing up the amount of Mg in all foods. A fasting blood sample was taken to measure serum concentrations of glycemic indices (fasting plasma glucose and insulin) and endothelial function markers (E-selectin, soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1). Insulin resistance and sensitivity were estimated using the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR), Homeostasis Model Assessment β-cell function (HOMA-β) and quantitative insulin sensitivity check index (QUICKI).

Participants: Iranian female nurses (n 345) selected by a multistage cluster random sampling method.

Results: The Mg intake across energy-adjusted quartiles was 205 (SE 7), 221·4 (SE 8), 254·3 (SE 7) and 355·2 (SE 9) mg/d, respectively. After adjustments for potential confounders, QUICKI level was significantly different across quartiles of Mg intake (Q_1: 0·34 (SE 0·02), Q_2: 0·36 (SE 0·01), Q_3: 0·40 (SE 0·01), and Q_4: 0·39 (SE 0·02), $P = 0·02$); however, this association disappeared after considering markers of endothelial function, indicating that this relation might be mediated through endothelial dysfunction. After controlling for all potential confounders, Mg intake was inversely, but not significantly, associated with serum concentrations of sICAM (Q_1: 239 (SE 17), Q_2: 214 (SE 12), Q_3: 196 (SE 12), and Q_4: 195 (SE 17), $P = 0·29$). There was no other significant association between dietary Mg intake and other indicators of glucose homoeostasis or endothelial markers.

Conclusions: Higher dietary Mg intake was associated with better insulin sensitivity in Iranian females. This linkage was mediated through reduced endothelial dysfunction.

Keywords
Diet
Endothelial function
Insulin resistance
Mg

Endothelial dysfunction is involved in the aetiology of insulin resistance, atherosclerosis, hypertension and some cancers[1–6]. Insulin resistance is fundamental to the aetiology of diabetes and is linked to a wide range of chronic diseases[5,7–10]. These non-communicable diseases (NCD) are the main causes of morbidity and mortality and impose enormous financial and social burden on Iranian population[11,12]. In Iran, 287 000 deaths in the year 2016 were

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from NCD and the number of NCD-related deaths has drastically increased during the past decade. The absolute number of years of life loss and disability-adjusted life years of NCD in Iran have accordingly grown by 98 % and 48 % from 1990 to 2017[12]. Improving the nutrition and diet would be an applicable strategy towards reducing the growing NCD burden[13]. Dietary intakes of whole grains, fish and *n*-3 rich foods, fruits and green leafy vegetables could improve insulin sensitivity and endothelial function, while red meat intake was linked to abnormal glucose homoeostasis and endothelial dysfunction[13–19]. The favourable association of these foods and chronic conditions might be mediated through their high content of fibre and antioxidants; however, they are also good sources of Mg. Nuts, legumes, seeds, whole grains, banana, avocado, leafy greens and some fatty fish such as halibut are rich sources of Mg in the diet. Earlier studies have indicated that Mg intake is associated with a lower risk of insulin resistance, hypertension, atherosclerosis and CVD[20–23]. It is unknown if the beneficial effect of Mg intake on these conditions could be mediated through influencing endothelial function[22].

Limited data are available linking Mg intake and endothelial function. We are aware of only two observational studies that examined Mg intake in relation to biomarkers of endothelial function[22,23]. Chacko *et al.* found that high Mg intake was associated with lower concentrations of soluble vascular cell adhesion molecule-1 (sVCAM-1) and E-selectin in 3713 post-menopausal women in the Women's Health Initiative Observational Study[22]. Song *et al.* reported the same association between Mg with E-selectin and soluble intercellular adhesion molecule (sICAM) in the Nurses' Health Study[23]. Both studies have been done in Western countries and it is unknown if these associations could be extrapolated to developing countries, in particular to the Middle Eastern population. Finding the association between Mg intake and endothelial function is particularly important for Middle Eastern countries, where dietary intake is mostly on the basis of low-Mg foods. In addition, other dietary behaviours of these populations are different from developed nations. Middle Eastern populations consume large amount of refined starches (white rice and bread), saturated fats and hydrogenated fats along with low amount of fruits, vegetables, legumes and nuts, as the main dietary sources of Mg[24]. Given the lack of any evidence in this part of the world, this study was done to investigate the association of Mg intake with insulin resistance and markers of endothelial function in Iranian women.

Methods and materials

Study procedure and subjects

This cross-sectional study was carried out among a representative sample of Iranian female nurses with the age range of 23–54 years, who were selected by a multistage cluster random sampling method. Seven hospitals were randomly selected based on the number of public and private hospitals. The required sample size for the present study was calculated using serum insulin level (SD: 6·54), as the key dependent variable, in the standard formula suggested for observational studies. Given the 80 % power, type I error of 5 % and the size of effect of 0·71 (5 % insulin level), a sample size of 326 participants was needed. Considering the high dropouts in epidemiologic investigations, we invited 510 female nurses working in the hospitals to participate in this study; 480 women agreed to do so. We did not include women with a prior history of CVD, diabetes, cancer, stroke (*n* 26) and current antibiotic use (*n* 7). In addition, those who had left ≥70 items blank on the FFQ (*n* 2), or reported total daily energy intake outside the range of 800–4200 kcal (*n* 9), or those who were taking medications affecting glucose homoeostasis (*n* 16) were excluded. After these exclusions (*n* 60) and also excluding individuals with incomplete data (*n* 75), the current analysis was done on 345 individuals. Written informed consent was obtained from each participant. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the University of Medical Sciences.

Assessment of dietary intakes

Usual dietary intakes were assessed using a self-administered validated 106-item dish-based semi-quantitative FFQ[25]. The FFQ contained information on frequency of consumption of foods or dishes over the last year, along with common portion sizes used in Iran. Daily intakes of Mg for each participant were calculated by summing up the amount of Mg in all kinds of foods in the FFQ using the Nutritionist IV software (First Databank, San Bruno), whose nutrient database was based on the US Department of Agriculture's (USDA) food composition table, modified for Iranian foods. Our earlier studies have indicated that data on foods and food groups' intake as well as nutrients intake from this FFQ provided reasonably valid data of long-term dietary intakes[16,26].

Assessment of biomarkers

To quantify serum concentrations of insulin, fasting plasma glucose (FPG), lipid profile and adhesion molecules (E-selectin, sICAM-1 and sVCAM-1), a 12-h fasting blood sample was taken from each participant. FPG concentration was measured on the day of blood collection with an enzymatic colorimetric method using glucose oxidase (Pars Azmoon commercial kits) and biochemical autoanalyser (Alpha Classic, Sanjesh Company). The blood samples were then centrifuged within 30–45 min of collection and serum was frozen at −70°C until analysis. Measurement of serum insulin was done using ELISA kits and an ELISA reader (Diagnostic Biochem Canada Inc.). Measurement of serum adhesion molecules was done

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using available commercial ELISA kits and standards (BioSource International) and (Bender MedSystems Diagnostica GmbH) and an ELISA reader (Diagnostic Biochem Canada Inc.). The sensitivity of the assays for sICAM-1, sVCAM-1 and E-selectin was 0·6, 2·3 and 0·3 mg/l, respectively. Inter- and intra-assay CV for all biomarkers were <10 %. Serum TAG concentrations were assayed with the use of TAG kits by enzymatic colorimetric tests with glycerol phosphate oxidase (Pars Azmoon commercial kits) and a biochemical autoanalyser (Alpha Classic, Sanjesh Company). Serum levels of HDL-C were measured with phosphotungstic acid, after precipitation of the apo B-containing lipoproteins (Pars Azmoon commercial kits) and a biochemical autoanalyser (Alpha Classic, Sanjesh Company). Insulin resistance and sensitivity were estimated using the homoeostasis model assessment (Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) and Homeostasis Model Assessment β-cell function (HOMA-β) and the quantitative insulin sensitivity check index (QUICKI), respectively. HOMA indexes were measured according to these formulas: HOMA-IR = (fasting insulin (mU/l) × fasting glucose (mmol/l)/22·5) and HOMA-β = 20 × fasting insulin (mU/l)/fasting glucose (mmol/ml) − 3·5.

Assessment of other variables

Weight was measured using digital scales while participants were wearing light clothes, without shoes and recorded to the nearest 0·1 kg. Height measurement was made while subjects were standing in a normal position without shoes by means of a tape measure. BMI was calculated as weight (kg) divided by height squared (m^2). Daily physical activity was assessed using the short form of a validated International Physical Activity Questionnaire[27] and was expressed as metabolic equivalent tasks-hours per week (MET-h/wk). Blood pressure of participants was measured by the standard method in a seated position through a standard mercury sphygmomanometer by a trained nurse. Measurements were repeated after 5-min interval, and the average of the two readings was considered as the individual's blood pressure. Socio-economic status was defined through a validated questionnaire[28] which included educational level, income, family size, being owner of a house or renting a house, house area, being owner of a car, number and type of cars, and number of bedrooms in house. Additional covariate information regarding age, smoking habits, marital status, education levels, menopausal situation, medical history, and current use of medications and supplements was obtained using self-administered questionnaires.

Statistical methods

First, energy-adjusted intakes of Mg based on residual method were calculated[29]. Energy-adjusted Mg intakes were used to categorise participants into quartiles. One-way ANOVA and chi-square tests were used for comparing continuous and categorical data, across quartiles of Mg intake, respectively. To assess food groups and nutrient intakes across quartiles of Mg intake, we used ANCOVA. Logarithmically transformed values of adhesion molecules in all statistical analyses were used due to the skewness of the distribution of these variables. Furthermore, we applied ANCOVA with Bonferroni correction to examine means of glycemic and endothelial markers across categories of Mg intake in different models. The covariates were chosen based on earlier publications[22,23]. Adjustments for age and total energy intake (kcal/d) were done in Model I. Further adjustment was made for physical activity (MET-h/wk), current corticosteroid use (yes or no), oral contraceptive pill use (yes or no), marital status (single, married, divorce and widow), menopausal status (yes or no), systolic blood pressure, diastolic blood pressure and socio-economic status (low, medium and high socio-economic status) in Model II. Further control was done for dietary intakes of fibre, total fat, carbohydrate, refined grains and Na in Model III. Additional adjustments were performed for BMI in Model IV. In case of variables related to glucose metabolism, we further controlled for markers of endothelial dysfunction to test the hypothesis that the effect of Mg intake on insulin resistance is mediated through endothelial dysfunction. Finally, for serum concentrations of adhesion molecules, we added FPG, serum TAG, serum total cholesterol, HDL and LDL cholesterol concentrations (all as continuous) in Model V. All statistical analyses were done using SPSS (SPSS, version 18). *P*-values less than 0·05 were considered to be statistically significant.

Results

General characteristics of study participants across quartiles of energy-adjusted dietary Mg intake are presented in Table 1. There was no significant difference in terms of weight, BMI, waist circumference, systolic and diastolic blood pressure and physical activity across different categories of Mg intake. Furthermore, distribution of participants according to marital status, menopausal status, socio-economic status, overweight and obesity, use of oral contraceptive pill and corticosteroids was not significantly different across quartiles of dietary Mg intake.

Dietary intakes of study participants across quartiles of Mg intake are illustrated in Table 2. Subjects with high dietary Mg intake had greater intakes of vegetables, fruits, low-fat dairy, nuts and legumes, K and folic acid compared with those with the lowest dietary Mg intake. Individuals in the top quartile of dietary Mg intake had lower intake of refined grains and whole grains compared with those in the bottom quartile. Furthermore, dietary intakes of white meat, high-fat dairy, energy, carbohydrates, Ca, Zn and total dietary fibre were significantly different across quartiles of Mg intake. No significant difference was found in

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Table 1 General characteristics of study participants across quartiles of dietary magnesium intake*

	Quartiles of energy-adjusted Mg intake								
	Q1 (205 (SE 67) mg/d) (*n* 86)		Q2 (221 (SE 71) mg/d) (*n* 86)		Q3 (254 (SE 66) mg/d) (*n* 87)		Q4 (355 (SE 86) mg/d) (*n* 86)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*†
Age (years)	35·1	7·8	35·4	7·0	35·0	7·0	36·5	7·2	0·56
Weight (kg)	62·0	10·3	62·0	10·3	60·8	8·4	62·7	9·7	0·24
BMI (kg/m^2)	23·5	3·4	24·6	3·5	23·4	3·4	24·0	3·7	0·12
Waist circumference (cm)	81·5	10·5	81·5	11·1	79·6	9·4	80·0	10·1	0·53
Systolic blood pressure (mm Hg)	110	14	109	9	109	12	107	10	0·43
Diastolic blood pressure (mm Hg)	68	10	71	8	70	10	70	9	0·45
Physical activity (MET-h/wk)	85	85	86	107	70	70	66	80	0·57
Current use of oral contraceptive pill									0·55
n	3		6		4		7		
%	3·7		7·1		4·7		8·3		
Current corticosteroids use									0·90
n	1		1		2		1		
%	1·2		1·2		2·3		1·2		
Menopausal									0·36
n	8		4		3		6		
%	9·5		4·7		3·5		7·0		
High-socio-economic status‡									0·98
n	25		25		25		26		
%	29·1		28·6		28·8		30·8		
Married									0·28
n	57		66		65		57		
%	66·7		76·7		74·7		65·9		
Overweight or obese§									0·09
n	27		39		25		35		
%	31·3		45·7		28·9		40·2		

*Q, quartile; MET-h/wk, metabolic equivalent-hour per week.
†Values obtained from ANOVA for continuous variable and chi-square for categorical variables.
‡High-socio-economic status was defined based on educational level, income, family size, being owner of the house or renting the house, house area, being owner of the car and number and kind of the car(s), number of bedrooms, and determination of who was in charge of the family.
§Values defined as BMI ≥ 25 kg/m^2.

terms of other dietary variables across different levels of dietary Mg intake.

Multivariable-adjusted means (±SE) for biomarkers of glucose metabolism (FPG, serum insulin, HOMA-IR, HOMA-β and QUICKI indexes) across quartiles of dietary Mg intake are presented in Table 3. After adjustments for potential confounders including dietary intakes in Model III, QUICKI level was significantly different across quartiles of Mg intake (Q_1: 0·34 (SE 0·01), Q_2: 0·36 (SE 0·01), Q_3: 0·39 (SE 0·01) and Q_4: 0·39 (SE 0·02), $P = 0·03$). This difference remained significant even after further controlling for BMI in Model IV (Q_1: 0·34 (SE 0·02), Q_2: 0·36 (SE 0·01), Q_3: 0·40 (SE 0·01) and Q_4: 0·39 (SE 0·02), $P = 0·02$); however, it became non-significant after further adjustment for markers of endothelial dysfunction in Model V, indicating that the effect of Mg intake on insulin function might be mediated through endothelial dysfunction. In addition, high dietary Mg intake was associated with lower serum insulin levels after adjustment for confounders in Model II (Q_1: 11·0 (SE 1·37), Q_2: 10·3 (SE 1·24), Q_3: 7·0 (SE 1·23) and Q_4: 6·8 (SE 1·35) mU/l, $P = 0·04$). This relationship did not quite reach statistical significance after further adjustments for dietary intakes and BMI in Model IV (Q_1: 11·8 (SE 1·73), Q_2: 10·1 (SE 1·29), Q_3: 6·4 (SE 1·28) and Q_4: 6·7 (SE 1·76) mU/l, $P = 0·08$). This association disappeared when other potential confounders including markers of endothelial dysfunction were taken into account in Model V. No significant association was found between dietary Mg intake and other biomarkers of glucose metabolism.

Multivariable-adjusted means (±SE) for markers of endothelial function (E-selectin, sICAM-1 and sVCAM-1**)** across quartiles of dietary Mg intake are indicated in Table 4. Concentrations of sICAM-1 were significantly different across categories of Mg intake in the crude model (Q_1: 206 (SE 8), Q_2: 196 (SE 8), Q_3: 200 (SE 8) and Q_4: 227 (SE 8), $P = 0·04$). After taking all potential confounders into account, this association reversed, such that more dietary Mg intake was associated with lower sICAM levels (Q_1: 239 (SE 17), Q_2: 214 (SE 12), Q_3: 196 (SE 12) and Q_4: 195 (SE 17), $P = 0·29$), although this difference was not significant in fully adjusted model. No other significant difference was seen in other markers of endothelial function across quartiles of Mg intake.

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Table 2 Dietary intakes (food group and nutrient) of study participants across quartiles of dietary magnesium intake*

	Quartiles of energy-adjusted Mg intake								
	Q1 (205 (SE 7) mg/d) (*n* 86)		Q2 (221 (SE 8) mg/d) (*n* 86)		Q3 (254 (SE 7) mg/d) (*n* 87)		Q4 (355 (SE 9) mg/d) (*n* 86)		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*P*†
Food groups									
Vegetables (g/d)	231[a]	12	260[b]	17	309[c]	14	534[abc]	34	<0·001
Fruits (g/d)	263[a]	25	309[b]	31	355[c]	27	569[abc]	39	<0·001
White meat (g/d)	82[a]	7	74[b]	5	102[ab]	9	90	7	0·02
Red meat (g/d)	128	8	115	8	128	10	126	8	0·63
Low-fat dairy (g/d)	253[a]	27	284[b]	23	339[c]	25	596[abc]	41	<0·001
High-fat dairy (g/d)	54[a]	4	53[b]	5	54[c]	4	79[abc]	10	0·007
Refined grains (g/d)	460[abc]	22	384[a]	23	353[b]	23	323[c]	20	<0·001
Whole grains (g/d)	73	12	55	8	47	12	46	6	0·18
Nuts and legumes (g/d)	40[a]	3	44[b]	3	61[c]	4	77[abc]	5	<0·001
Oils (g/d)	72	4	69	4	63	3	64	4	0·27
Nutrients									
Total energy	2801[ab]	89	2437[a]	86	2460[b]	78	2801[ab]	75	<0·001
Protein (g/d)	136	17	124	13	104	10	135	12	0·27
Carbohydrate (g/d)	347[ab]	14	286[a]	13	278[b]	10	341[ab]	12	<0·001
Fat (g/d)	114	13	97	4	98	3	106	3	0·27
SFA (g/d)	26	2	25	1	23	1	28	1	0·18
MUFA (g/d)	32	1	30	1	31	1	33	1	0·33
PUFA (g/d)	46	6	39	2	38	1	40	1	0·26
Cholesterol (mg/d)	224	13	216	11	239	12	253	13	0·13
Mg (mg/d)	205[a]	7	221[b]	8	254[c]	7	355[abc]	9	<0·001
Na (mg/d)	3816	132	3530	132	3916	224	3948	135	0·24
K (mg/d)	2722[a]	113	2914[b]	114	3320[c]	95	4795·3[abc]	152	<0·001
Ca (mg/d)	802[a]	40	797[b]	31	852[c]	30	1253[abc]	54	<0·001
Thiamin (mg/d)	7	3	3	0	2	0	3	0	0·30
Fe (mg/d)	28	7	19	1	18	1	22	0	0·15
Zn (mg/d)	9[a]	0	9[b]	1	8[c]	0	11[abc]	1	0·007
Vitamin B_6 (mg/d)	3	1	2	0	2	0	2	0	0·46
Folic acid (mg/d)	226[a]	8	248[b]	10	296[c]	9	436[abc]	17	<0·001
Total dietary fibre (g/d)	19[a]	3	17[b]	1	19[c]	1	27[abc]	1	<0·001

[a]$P < 0.05$ for pairwise comparison; [b]$P < 0.05$ for pairwise comparison; [c]$P < 0.05$ for pairwise comparison. In other words, values that shared a superscript letter are significantly different.
*Q, quartile.
†Values obtained from ANOVA.

Discussion

In the present study, individuals with more Mg intake had higher QUICKI compared with those with low intake; this relation might be mediated through endothelial dysfunction. Furthermore, Mg intake was associated with higher serum concentrations of sICAM. When all potential confounders were taken into account, this association reversed, such that higher Mg intake was related to lower sICAM concentrations, although this relation was not statistically significant. None of the other glucose homoeostasis indices (FPG, HOMA-IR, HOMA-B and serum insulin) or endothelial function biomarkers (E-selectin and sVCAM) was associated with dietary Mg intake. To the best of our knowledge, the current study is the first investigation that examined the association of dietary Mg intake with insulin resistance and markers of endothelial function in the Middle East.

The mean intake of Mg in our study population was 259 mg/d (SD: 93), which seems to be lower than general Iranian population. Another cross-sectional study in Iran (the third phase of Tehran Lipid and Glucose Study (2006–2008)) has reported an average Mg intake of 336 mg/d (SD: 107) in Iranian females[30]. Also, Mg intake in the current study was less than the estimated average requirement for Mg in women (265 mg/d), indicating that more than half of the participants might be Mg-deficient.

Endothelial dysfunction and insulin resistance are among the most important factors involved in aetiology of diabetes and CVD. These diseases are the leading causes of death in many developing and developed countries[1,3]. Diet, as a modifiable risk factor, has an important role in developing endothelial dysfunction and insulin resistance. Prior studies have assessed the association of different foods or nutrients intakes with endothelial dysfunction and insulin resistance. Previous research emphasised that a diet rich in Mg could affect glucose homoeostasis and endothelial function[31,32].

Based on our findings, after considering potential confounders, those with higher dietary Mg intake had lower

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Table 3 Multivariable-adjusted means of glycemic variables across quartiles of dietary magnesium intake*

	Quartiles of energy-adjusted Mg intake								
	Q1 (205 (SE 7) mg/d) (*n* 86)		Q2 (221 (SE 8) mg/d) (*n* 86)		Q3 (254 (SE 7) mg/d) (*n* 87)		Q4 (355 (SE 9) mg/d) (*n* 86)		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*P*†
FPG (mg/dl)									
Crude	82·0	1·3	81·7	1·3	82·9	1·3	82·3	1·3	0·93
Model ‡	82·0	1·4	81·4	1·4	83·0	1·3	82·4	1·4	0·87
Model II§	82·1	2·9	81·8	2·6	81·2	2·6	82·9	2·8	0·97
Model III‖	80·8	3·5	82·7	2·7	81·4	2·6	82·9	3·4	0·96
Model IV¶	80·3	3·6	82·7	2·7	81·2	2·7	83·2	3·5	0·93
Model V**	80·4	4·1	82·3	2·9	80·6	2·9	82·7	4·0	0·95
Insulin (mU/l)									
Crude	9·2	1·01	9·5	0·99	7·2	1·01	7·9	1·02	0·33
Model	9·6	1·03	9·5	1·02	7·1	1·01	7·6	1·03	0·19
Model II	11·0	1·37[a]	10·3	1·24	7·0	1·23	6·8	1·35[a]	0·04
Model III	11·6	1·72	10·1	1·28	6·7	1·25	6·9	1·76	0·12
Model IV	11·8	1·73	10·1	1·29	6·4	1·28	6·7	1·76	0·08
Model V	11·5	1·79	9·7	1·31	6·9	1·29	7·0	1·75	0·23
HOMA-IR									
Crude	1·9	0·2	1·9	0·2	1·5	0·2	1·6	0·2	0·40
Model	2·0	0·2	1·9	0·2	1·5	0·2	1·5	0·2	0·24
Model II	2·3	0·3	2·1	0·2	1·4	0·2	1·4	0·2	0·05
Model III	2·3	0·3	2·0	0·2	1·3	0·2	1·4	0·3	0·17
Model IV	2·4	0·3	2·0	0·2	1·3	0·2	1·4	0·3	0·11
Model V	2·3	0·3	2·0	0·2	1·4	0·2	1·4	0·3	0·26
HOMA-β									
Crude	183	54	135	53	224	54	118	54	0·50
Model	186	56	136	56	220	56	106	56	0·47
Model II	269	132	207	119	309	121	122	131	0·75
Model III	198	167	148	125	305	124	272	170	0·84
Model IV	193	169	144	126	316	127	273	172	0·81
Model V	143	180	147	132	347	131	285	176	0·73
QUICKI									
Crude	0·37	0·01	0·36	0·01	0·38	0·01	0·37	0·01	0·06
Model	0·37	0·01	0·36	0·01	0·38	0·01	0·37	0·01	0·07
Model II	0·35	0·01	0·36	0·01	0·39	0·01	0·38	0·01	0·05
Model III	0·34	0·01[a]	0·36	0·01	0·39	0·01[a]	0·39	0·02	0·03
Model IV	0·34	0·02[a]	0·36	0·01	0·40	0·01[a]	0·39	0·02	0·02
Model V	0·35	0·02	0·36	0·01	0·39	0·01	0·39	0·02	0·09

Q, quartile; FPG, fasting plasma glucose; HOMA-IR, homoeostatic model assessment of insulin resistance; HOMA-β, homoeostatic model assessment of beta-cell function; QUICKI, quantitative insulin sensitivity check index; MET-h/wk, metabolic equivalent tasks-hours per week.

[a]$P < 0.05$ for pairwise comparison. In other words, values in a row that shared a superscript letter of 'a' are significantly different.

*All values are means ± SE.

†Values obtained from ANCOVA.

‡Model I: adjusted for age and energy intake.

§Model II: further adjusted for physical activity (MET-h/wk), current corticoid steroids use (yes or no), current use of oral contraceptive pill (yes or no), marital status (categorical), menopausal status (yes or no), systolic blood pressure, diastolic blood pressure and socio-economic status (categorical).

‖Model III: further adjusted for intakes of fibre, total fat, carbohydrate, refined grains and Na.

¶Model IV: further adjusted for BMI.

**Model V: additionally adjusted for markers of endothelial function (E-selectin, sICAM-1 and sVCAM-1).

sICAM concentration, in a non-significant manner. It had been shown that adherence to a diet poor in Mg was positively associated with sICAM concentrations[31]. In contrast, no significant association was reported between dietary Mg intake and sICAM concentrations in a prospective study[22]. A cross-sectional study has also reported no significant association between a Mg-rich diet and sICAM concentrations[31]. Conflicting findings on the association between dietary Mg intake and sICAM concentrations might be explained by different study design and populations, adjusting for energy intake and other covariates and diversity in methods used to assess dietary intakes and blood markers. Therefore, finding the independent effects of dietary Mg can be a challenge.

Although independent effects of Mg intake on endothelial function are biologically plausible according to experimental evidence[32,33], any causal effects of Mg intake on inflammation and endothelial function warrant further investigation. The biological mechanism underlying the inverse association between dietary Mg intake and sICAM concentrations is unknown. An experimental study had shown that Mg deficiency may promote an inflammatory response[33]. Inflammation can adversely affect endothelial function and increase the concentrations of sICAM.

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Table 4 Multivariable-adjusted means for endothelial function indexes across quartile of dietary magnesium intake

	Quartiles of energy-adjusted Mg intake								
	Q1 (205 (SE 7) mg/d) (*n* 86)		Q2 (221 (SE 8) mg/d) (*n* 86)		Q3 (254 (SE 7) mg/d) (*n* 87)		Q4 (355 (SE 9) mg/d) (*n* 86)		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*P**
E-selectin (ng/l)									
Crude	84·5	6·1	96·7	6·1	78·5	6·1	85·6	6·2	0·20
Model I†	84·1	5·9	93·4	5·9	77·6	5·8	86·7	5·9	0·29
Model II‡	72·5	7·1	91·6	6·3	79·4	6·3	84·4	7·1	0·25
Model III§	79·8	9·0	91·3	6·6	77·2	6·4	80·1	9·2	0·45
Model IV‖	80·3	9·0	91·6	6·6	75·3	6·5	79·8	9·1	0·36
Model V¶	79·2	9·0	91·6	6·6	75·8	6·5	80·2	9·1	0·37
sICAM-1 (mg/l)									
Crude	206[a]	8	196[a]	8	200	8	227	8	0·04
Model	205	8	198	8	201	8	226	8	0·06
Model II	224	13	208	12	201	12	214	13	0·63
Model III	238	16	213	12	199	12	196	17	0·32
Model IV	239	17	213	12	197	12	195	17	0·30
Model V	239	17	214	12	196	12	195	17	0·29
sVCAM-1 (mg/l)									
Crude	504	17	496	16	506	16	484	17	0·79
Model	500	17	499	17	508	17	483	17	0·78
Model II	472	36	522	32	529	32	448	36	0·29
Model III	437	46	521	34	532	32	479	46	0·30
Model IV	441	46	521	34	524	33	476	46	0·38
Model V	442	47	523	34	520	33	476	47	0·41

E-selectin, endothelial selectin; Q, quartile; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; MET-h/wk, metabolic equivalent tasks-hours per week.
[a]$P < 0.05$ for pairwise comparison. In other words, values in a row that shared a superscript letter of 'a' are significantly different.
*Values obtained from ANCOVA.
†Model I: adjusted for age and energy intake.
‡Model II: further adjusted for physical activity (MET-h/wk), current corticoid steroids use (yes or no), current use of oral contraceptive pill (yes or no), marital status (categorical), menopausal status (yes or no), systolic blood pressure, diastolic blood pressure and socio-economic status (categorical).
§Model III: further adjusted for intakes of fibre, total fat, carbohydrate, refined grains and Na.
‖Model IV: further adjusted for BMI.
¶Model V: additionally adjusted for blood lipids (serum TAG, serum total cholesterol, HDL and LDL-cholesterol) and glucose.

Moreover, Mg increases glutathione synthesis which might limit or begin to resolve the inflammatory response and might result in lower circulating sICAM and inflammatory biomarkers concentrations. High level of sICAM and other inflammatory biomarkers could cause insulin resistance[32–34].

In the current study, we found that higher Mg intake was associated with higher QUICKI values. This relationship was mediated by endothelial dysfunction. Also, it should be considered that QUICKI, HOMA-IR and HOMA-β were calculated from the same fasting values of insulin and glucose. HOMA-B is a measure of pancreatic β-cell function, whereas the two other measures are indices of insulin sensitivity via slightly different calculations. A previous validation study[35] has proposed that QUICKI, as a logarithmic transformed index, could produce a normal distribution of data, could be preferable to the other measures and was more generalisable to the full range of metabolic conditions (including the relationship between Mg intake and insulin resistance). Our results might also suggest a support for the superiority of QUICKI over the two other indices of insulin resistance in Iranian women, although we could not evaluate correlations of these measures with hyperinsulinemic-euglycemic clamp, as the gold standard of insulin resistance. In line with our findings, several studies have shown that adherence to diets rich in Mg can increase insulin sensitivity[36,37]. It has been shown that a diet rich in Mg, particularly whole grain, is associated with a substantially lower risk of insulin resistance and type II diabetes[37]. Another study has shown that adherence to a diet rich in Mg and whole grains is associated with improved insulin sensitivity[38]. High serum Mg was also associated with a reduced risk of diabetes in a prospective study[39]. Inverse associations between serum Mg levels and serum glucose and insulin levels were also reported in a cross-sectional study[40]. Furthermore, intakes of other types of Mg-rich foods including dairy products, legumes and nuts have been linked to decreased risk of diabetes in prospective studies[37].

Mg is required for glucose utilisation and insulin signalling. Lower intake of foods rich in Mg might lead to metabolic alterations in cellular Mg and affect the development of insulin resistance by altering the glucose entry into the cell[41,42] Phosphorylation of the tyrosine kinase enzyme of the insulin receptor, required for post-receptor insulin sensitivity and subsequent insulin-mediated glucose

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uptake, is dependent on adequate intracellular concentrations of Mg[43]. A Mg-deficient diet caused a significant impairment of insulin-mediated glucose uptake[44]. In addition, dietary Mg is highly correlated with other micronutrients and dietary components believed to affect insulin sensitivity, such as K, Ca, vegetables, fruits and fibre. Thus, it is very difficult to separate their independent effects[45,46]. Furthermore, the reliability of serum Mg levels, as the marker of Mg deficiency, is unclear. Although intracellular Mg concentrations are believed to provide a more accurate estimation of Mg status, the technique of cell isolation and Mg measurement is not easily available[47].

Our study has several limitations. The major one is its cross-sectional nature, which would not allow conferring causality. However, the appropriate analysis of cross-sectional data would represent a valuable initial step in identifying diet–disease relations. Other limitations included self-reported diet associated with large measurement errors that could distort or attenuate the investigated associations. Potentially health conscious study population might also limit the range of Mg intake as well as the range of the outcome variables. In addition, our findings cannot be extrapolated to the general population, especially to men, due to its restriction to female nurses. Although we controlled for several lifestyle factors associated with dietary Mg intake, residual confounding due to unknown confounding factors cannot be excluded.

In conclusion, we found that higher dietary Mg intake was associated with better insulin sensitivity in Iranian females. This linkage was mediated through reduced endothelial dysfunction. Further investigations, particularly with prospective design, are required to confirm these findings.

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