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ORIGINAL COMMUNICATION

Long-term effect of a plant-based diet on magnesium status during pregnancy

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Objective: To compare dietary magnesium intake and magnesium concentrations in serum, red blood cells (RBC) and urine during pregnancy of women habitually following a long-term plant-based diet and of women following an average Western (control) diet.
Design: Prospective study during pregnancy.
Setting: Giessen, Germany.
Subjects: Healthy pregnant women ($n=108$) in their 9–12th, 20–22nd and 36–38th gestational week habitually following a plant-based diet for more than 3 y or an average Western diet. The vegetarians were subdivided into ovo-lacto vegetarians ($n=27$) and low-meat eaters ($n=43$).
Results: Significant higher dietary magnesium intakes were observed in pregnant women consuming a plant-based diet (508 ± 14 mg/day for ovo–lacto vegetarians, $P<0.001$ and 504 ± 11 mg/day for low-meat eaters, $P<0.001$) than in pregnant women consuming a control diet (412 ± 9 mg/day). Serum magnesium concentrations were similar in all diet groups whereas RBC magnesium was slightly higher in low-meat eaters than in controls ($P=0.058$). Urinary magnesium excretion was higher in ovo-lacto vegetarians ($P=0.023$), followed by low-meat eaters ($P=0.017$) when compared to the control group. During the third trimester of pregnancy, the frequency and the occurrence of calf cramps was lower in the plant-based diet group than in the control group ($P=0.004$ and 0.008).
Conclusions: Owing to a higher dietary magnesium intake confirmed by higher urinary magnesium excretion, habitual plant-based diets result in a slightly improved magnesium status during pregnancy and reduce the frequency of calf cramps during the third trimester of pregnancy compared to an average Western diet. Therefore, plant-based diets during pregnancy can be recommended with regard to magnesium supply.
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Introduction

An adequate magnesium status during pregnancy is essential for foetal development. A magnesium deficiency during pregnancy has been reported to be associated with preterm birth, eclampsia and pre-eclampsia (Almonte *et al*, 1999). Serum magnesium levels physiologically decrease during pregnancy since renal magnesium excretion increases (Spätling *et al*, 1985). The occurrence of calf muscle cramps during pregnancy has also been associated with low magnesium status (Riss *et al*, 1983).

There is substantial evidence from several dietary surveys that in some populations dietary magnesium intake during pregnancy is below the recommended dietary allowances

(Rogers & Emmett, 1998; Giddens *et al*, 2000; Pathak *et al*, 2003; Turner *et al*, 2003). Therefore, an appropriate dietary intake is necessary for maintaining an adequate magnesium status. Plant-based diets with a higher intake of fruits, vegetables, and whole grain products have been shown to be effective in the prevention of nutrition-related diseases and are widely recommended in dietary guidelines (Hoffmann *et al*, 2001; Koebnick *et al*, 2001; American Dietetic Association & Dietitians of Canada, 2003; Koerber *et al*, 2004). Among the beneficial effects of plant-based diets are the higher intake of magnesium and folate, both of which are needed in higher amounts during pregnancy. Except for folate intake, studies on the nutritional adequacy of vegetarian diets mainly exist for nonpregnant women. Moreover, studies on the adequacy of specific key nutrients during pregnancy in women consuming a plant-based diet are rarely found.

Therefore, we compared the magnesium status of pregnant women following a plant-based diet to the one of women consuming an average Western diet. Additionally, the occurrence of calf muscle cramps during pregnancy was assessed prospectively.

Methods

Study design

A prospective study with pregnant volunteers enrolled at any stage of pregnancy was conducted in Giessen, Germany. Dietary information and blood samples were collected at gestational weeks 9–12, 20–22 and 36–38. Ethical approval from the Ethics Committee of the Division of Human Medicine, University of Giessen, Germany and written informed consent from the participants were obtained.

Selection of participants

The recruitment and selection of participants has been described in detail (Koebnick *et al*, 2001). A total of 119 women were included in the study. Exclusion criteria were metabolic diseases, a twin-pregnancy or any known pregnancy complication or more than three previous deliveries. For the present study, 11 additional subjects had to be excluded who have taken medical drugs that interact with magnesium absorption (eg diuretics) or have used magnesium supplements before starting the study—resulting in a study population of $n=108$. The participants were assigned to different diet groups according to their dietary pattern of defined key foods. A plant-based diet was defined as a consumption of raw vegetables >100 g/day, a ratio of refined grain products to whole grain products <0.95 and a consumption <105 g/week of meat products and <300 g/week of meat. Women following a plant-based diet for at least 3 y were subdivided into ovo–lacto vegetarians (who did not consume any meat or meat products) and low-meat eaters. An average Western diet was defined in correspondence with the average German population as defined in the results of the German National Consumption Study (Projektträgergemeinschaft Forschung im Dienste der Gesundheit, 1991): a consumption of raw vegetables was <100 g/day, a ratio refined grain products to whole grain products >0.95 and a consumption >105 g/week of meat products or >300 g/week of meat.

The study population consisted of 70 women following a plant-based diet (27 ovo–lacto vegetarians and 43 low-meat eaters) and 38 consumed an average Western diet (control group). Because subjects were enrolled at different stages of pregnancy, in the 1st trimester all values are given for 16 ovo–lacto vegetarians, 29 low-meat eaters and 31 controls. In the 2nd trimester, values are given for 25 ovo–lacto vegetarians, 40 low-meat eaters and 37 controls, in the 3rd trimester for 20 ovo–lacto vegetarians, 34 low-meat eaters and 35 controls.

Blood and urine sampling and analyses

Fasting blood samples were collected in EDTA-containing vacutainers. Within 2 h after blood collection, plasma was separated from red blood cells by centrifugation. First morning urine was collected. The whole blood samples and urine were stored at 4–7°C and within 2 h magnesium concentrations were determined using an atomic absorption spectrophotometric method (AAS). The samples were diluted 500-fold in 0.2 M sulphuric acid and then measured in the flame atomic absorption at 286 nm. Magnesium in RBC was calculated according to the following formula: Mg in RBC = (Mg in whole blood − Mg in serum × (1 − Ht))/Ht (Paschen, 1978). Creatinine in urine was determined by the Jaffe-Method using a Hitachi 717 analyzer (Boehringer, Mannheim, Germany) (Bartels & Cikes, 1969). Serum potassium was determined using an indirect ion-sensitive electrode (Kulpmann, 1992; Burnett *et al*, 2000). Serum calcium was measured by direct colorimetric determination with *o*-cresolphthalein complexone in alkaline solution as colorimetric reagent (Walmsley & Fowler, 1981).

Dietary assessment

Information on dietary intake was assessed by an estimated 4-day food record. Except for minor changes the self-administered 4-day food record for dietary assessment was the same instrument as the validated semiquantitative food record used in comparable studies on plant-based diets (Hoffmann *et al*, 1994; Koebnick *et al*, 2001). The instrument included 152 food items with given portion sizes estimated by typical household measures. The nutrient and magnesium dietary intake was calculated based on the German Food Code and Nutrition Data Base BLS II.3 (Federal Institute for Health Protection of Consumers and Veterinary Medicine, 1999). Dietary magnesium intake does not include magnesium intake from supplements. Magnesium intake from supplements was calculated separately based on producer's data.

Diary for recording complaints during pregnancy

Information on complaints during pregnancy was recorded by using a complaint diary which was developed for the present study. The diary included daily information on typical complaints during pregnancy like calf cramps, changes in appetite and use of medicaments and supplements. This information was recorded retrospectively on a weekly basis for the first 8 weeks of pregnancy, and recorded directly on a daily basis from the 9th gestational week until delivery. Recording of complaints—not of supplement intake—was optional for participants. Therefore, these data are only available for compliant women participating during the entire pregnancy ($n=82$).

Statistical methods

Dietary intake; magnesium in serum, RBC and urine; calcium and potassium in serum are presented as arithmetic mean±s.e. Dietary intake was compared using the Mann–Whitney U-test. The magnesium status of the dietary groups was compared by using generalized estimating equations (GEE). GEE models allow an appropriate analysis of longitudinal data with repeated measurement and missing values; for all models a dependent working correlation matrix was chosen with simultaneous adjustment for BMI and use of magnesium supplements. All two-way interactions were tested, but no interactions with $P<0.15$ were found. For all analyses SAS 8.1 (SAS Institute Inc., Cary, NC, USA) was used.

Results

Population characteristics

A total of 108 women were included in the present study. Of those 70 women followed a plant-based diet (27 ovo–lacto vegetarians and 43 low-meat eaters) and 38 consumed an average Western diet (control group). The BMI at the beginning of pregnancy was higher in the control group (23.2±5.3) than the plant-based diet group (21.1±2.5, $P=0.017$). The low-meat eaters had also a slightly higher BMI (21.4±0.3) than the ovo–lacto vegetarians (20.6±0.4, $P=0.085$). The mean age was 30.8±0.9 y for ovo–lacto vegetarians, 30.6±0.6 y for low-meat eaters and 29.1±0.6 y for the control. The mean parity was 1.7±0.2 for ovo–lacto vegetarians, 1.7±0.1 for low-meat eaters and 2.0±0.2 for the control diet. No difference was observed between women following a plant-based diet and the control group with regard to age and parity. The plant-based diet group reported an average of 8.1±0.75 y of adherence to their diet.

Dietary intake

The dietary magnesium intake calculated as total magnesium/day and related to energy density and body weight is shown in Table 1. Magnesium intake was higher in the ovo–lacto vegetarians and low-meat eaters than in the control group. The contribution of single food groups as sources of magnesium intake is shown in Table 2. As a result of the selection criteria, on average during pregnancy, ovo–lacto vegetarians and low-meat eaters consumed higher percentages of whole grain products, raw food and bread than the control group.

The mean dietary protein intake was lower in the ovo–lacto vegetarians and low-meat eaters compared to the control group (Table 1). However, the mean protein intake in relation to total body weight did not markedly differed between diet groups and was above the RDA for protein (data not shown). A mean protein intake <0.8 g/kg body weight/day was observed in five (16 %) ovo–lacto vegetarians, in one (2%) low-meat eaters and in three (7 %) controls. Dietary fibre, calcium and phosphorous intake were higher in the ovo–lacto vegetarians and low-meat eaters than in the control group.

Serum, red blood cell and urinary magnesium

In the total course of pregnancy, serum magnesium concentrations were similar between diet groups (Table 3) after

Table 1 Average nutrient intake of pregnant women consuming a plant-based diet (subdivided into ovo–lacto vegetarians, OLV, and low-meat eaters, LME) or an average Western diet (control)

	Plant-based diet		*Control diet*	P[a]	
Dietary intake[b]	*OLV* n = *27*	*LME* n = *43*	n = *38*	*OLV vs control*	*LME vs control*
Magnesium (mg/day)	527±23	521±16	404±13	<0.001	<0.001
(mg/MJ)	63.9±2.1	59.7±1.5	44.2±0.8	<0.001	<0.001
(mg/kg body weight)	8.5±0.4	7.9±0.3	5.8±0.3	<0.001	<0.001
Protein (g/day)	67±3	76±2	89±3	<0.001	0.001
Fibre (g/day)					
Total	43±2	39±2	27±1	<0.001	<0.001
Soluble	13±1	12±1	8±1	<0.001	<0.001
Insoluble	30±2	27±1	19±1	<0.001	<0.001
Calcium (mg/day)	1193±63	1311±44	1093±44	0.180	0.001
Phosphorous (mg/day)	1625±74	1703±48	1483±46	0.090	0.001

[a]Significance of Student's independent samples *t*-test.
[b]Mean±s.e.

Table 2 Sources of dietary magnesium intake calculated as average percentages of consumed food groups as during pregnancy of ovo–lacto vegetarians (OLV), low-meat eaters (LME) and pregnant women on an average Western diet (control)

	Plant-based diet		*Control diet*	P[a]	
Food group[b]	*OLV* n = *27*	*LME* n = *43*	n = *38*	*OLV* vs *control*	*LME* vs *control*
Bread and cereals	19±2	19±2	19±1	0.997	0.996
Whole grain products	14±1	13±1	4±1	<0.001	<0.001
Potatoes	5±1	6±1	6±1	0.163	0.469
Milk and dairy products	20±2	22±1	23±1	0.201	0.881
Vegetables	19±1	19±1	13±1	<0.001	<0.001
Fruits	23±1	20±1	16±1	<0.001	0.001
Nuts	1±1	0±0	0±0	<0.001	0.003
Soy products	2±1	1±1	0±0	0.001	0.006

[a]Significance of Studentś independent samples *t*-test.
[b]Calculated as average percentage of total dietary magnesium intake during pregnancy.

Table 3 Serum, RBC and urinary magnesium concentrations of pregnant women consuming a plant-based diet (subdivided into ovo–lacto vegetarians, OLV, and low-meat eaters, LME) or an average Western diet (control)

	Plant-based diet			P[a]	
	OLV	*LME*	*Control diet*	*OLV* vs *control*	*LME* vs *control*
Serum magnesium (mmol/l)[b]				0.325	0.389
1 trimester	0.73±0.02	0.73±0.01	0.73±0.01		
2 trimester	0.69±0.01	0.69±0.01	0.69±0.01		
3 trimester	0.68±0.01	0.69±0.01	0.71±0.01		
RBC magnesium (mmol/l)[b]				0.957	0.058
1 trimester	2.08±0.09	2.34±0.09	2.19±0.05		
2 trimester	2.04±0.05	2.14±0.04	2.05±0.04		
3 trimester	2.04±0.06	2.09±0.06	2.03±0.04		
Urinary magnesium (mmol/mmol creatinine)				0.023	0.017
1 trimester	0.28±0.04	0.28±0.02	0.23±0.02		
2 trimester	0.28±0.03	0.31±0.02	0.21±0.02		
3 trimester	0.37±0.06	0.25±0.03	0.25±0.02		
Serum potassium (mmol/l)				0.659	0.123
1 trimester	4.39±0.14	4.46±0.09	4.66±0.19		
2 trimester	4.54±0.17	4.34±0.07	4.40±0.06		
3 trimester	4.58±0.17	4.50±0.11	4.74±0.21		
Serum calcium (mmol/l)				0.264	0.131
1 trimester	2.31±0.03	2.29±0.02	2.31±0.02		
2 trimester	2.22±0.02	2.23±0.02	2.26±0.02		
3 trimester	2.23±0.03	2.21±0.02	2.26±0.02		

[a]Significance of GEE model after simultaneous adjustment for BMI; for serum and RBC magnesium also adjusted for use of magnesium supplements, serum potassium and calcium concentrations.
[b]Mean±s.e. No significant differences were found between OLV and LME *vs* control after adjustment for BMI and use of magnesium supplements.

simultaneous adjustment for BMI, intake of magnesium from supplements, serum calcium and serum potassium concentrations. Magnesium concentrations in RBC were slightly higher in low-meat eaters but not in ovo–lacto vegetarians after simultaneous adjustment for BMI and intake of magnesium from supplements when controls were used as reference.

Calculated as an average during pregnancy, urinary magnesium excretion was highest in ovo–lacto vegetarians ($P = 0.023$), followed by low-meat eaters ($P = 0.017$) compared to the control group (Table 3).

Dietary magnesium intake per body weight was positively related to the magnesium concentrations in serum ($r = 0.158$; $P = 0.010$), RBC ($r = 0.163$, $P = 0.008$) and in urine ($r = 0.219$; $P < 0.001$).

Calf muscle cramps

In the course of pregnancy, a steady increase in the occurrence of calf muscle cramps was observed (Figure 1). In the 1st trimester, calf cramps were reported by about 4% of all subjects. The occurrence of calf cramps was similar

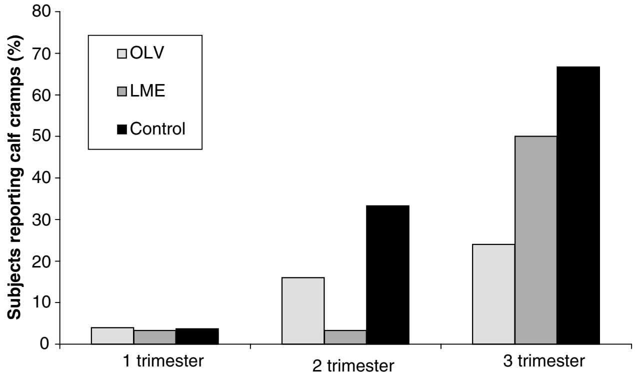

Figure 1 Subjects reporting calf cramp in the 1st, 2nd and 3rd trimester of pregnancy among ovo–lacto vegetarians (OLV, $n=25$), low-meat eaters (LME, $n=30$) and pregnant women consuming an average Western diet (control, $n=27$). The occurrence of calf cramps was higher in controls than in OLV and LME in the 2nd and 3rd trimester ($P=0.011$ and 0.008).

between diet groups. In the 2nd trimester calf cramps were reported by 17% of subjects, and the 3rd trimester by 48% of all subjects. The occurrence of calf cramps was significantly lower in ovo–lactoc vegetarians and low-meat eaters than in the control group during the 2nd ($P=0.011$) and the 3rd trimester ($P=0.008$). Additionally, the frequency of reported calf cramps assessed as number of reported calf cramps was higher in the control group (58%) than in the plant-based diet group (24%, $P=0.004$).

Serum magnesium concentrations were slightly lower in subjects reporting calf cramps than in subjects not reporting calf-cramps (0.67 ± 0.06 *vs* 0.70 ± 0.05 mmol/l, $P=0.054$). No significant differences were found for magnesium concentrations in RBC or urine.

Discussion

There is substantial evidence from many studies that plant-based or vegetarian diets can reduce the risk for many nutrition-related diseases. A high consumption of fruits and vegetables can contribute considerably to a well-balanced, healthy diet (White & Frank, 1994; Barr & Broughton, 2000). During pregnancy requirements for many nutrients are increased but little is known about beneficial effects of plant-based diets during pregnancy.

The present study shows that dietary magnesium intake is markedly higher in women following a habitual plant-based diet on a long-term consumption. Also the frequency of calf muscle cramps in the last trimester of pregnancy is significantly reduced in these women. Nevertheless, serum magnesium concentrations were not different between women following a plant-based diet and the control group despite a higher magnesium excretion and only slightly higher RBC magnesium in women following a habitual plant-based diet.

Magnesium depletion during pregnancy is due to increased demand (Black, 2001), higher renal excretion (Spätling *et al*, 1989) and haemodilution (Mende *et al*, 1977). A supplementation with magnesium during pregnancy has been associated with a lower incidence of eclampsia, pre-eclampsia and preterm labour (Kovacs *et al*, 1988; Spätling *et al*, 1989). However, controversy on the benefits of magnesium supplementation during pregnancy still exists due to inconsistency on the experimental designs of these studies (Mattar & Sibai, 1999; Makrides & Crowther, 2001). Moreover, an association between low magnesium status and low birth weight and small size for gestational age has been reported in animals but is still under discussion for humans (Wynn & Wynn, 1988). Nevertheless, a well-balanced diet including foods rich in magnesium (eg whole grain products, vegetables) should be of benefit during pregnancy.

The present study showed that during the course of pregnancy, women following a plant-based diet for an average period of more than 8 y had a higher dietary intake of magnesium and also a higher nutrient density for magnesium than the women on the control diet. The higher magnesium intake can be explained by the higher consumption of magnesium-rich food in women following a plant-based diet compared to the control group. However, the higher dietary magnesium intake was not associated with a concurrent higher magnesium concentration in serum but serum magnesium concentrations are strictly regulated by the kidney (Elin, 1994). RBC magnesium was only slightly higher in low-meat eaters when compared to controls. It has been shown recently, that RBC magnesium in humans are susceptible to dietary magnesium intake (Bohn *et al*, 2004b). Nevertheless, the observed magnesium concentrations for all participants were in the lower reference range as reported in several other studies (Baltzer & Daume, 1976; Spätling *et al*, 1985; Rogers & Emmett, 1998; Pathak *et al*, 2003).

A possible explanation for low serum magnesium concentrations even after a high magnesium intake in pregnant women adhering to a plant-based diet may be the high phytic acid intake from whole grain products. In animal studies, phytates have been shown to diminish the absorption of dietary magnesium (Miyazawa & Yoshida, 1991; Rimbach & Pallauf, 1999). In humans this effect is discussed controversial, but recent studies suggest similar effects of phytic acid as observed in animals (Coudray *et al*, 1997, 2003a; Hurrell, 2003; Bohn *et al*, 2004a). *In vitro* studies showed that phytic acid has a less pronounced negative effect on the bioavailability of magnesium than on the bioavailability of other minerals (eg calcium) (Wolters *et al*, 1993). In our study, no negative association between the consumption of whole grain products and serum or RBC magnesium concentrations was observed. This may be explained by the concurrent high intake of dietary fibre in women following a plant-based diet. A high dietary fibre intake, especially soluble fibre has been associated with an improved magnesium absorption when fermentable oligo- or polysaccharides were also part of the diet (Greger, 1999; Coudray *et al*, 2003b).

Moreover, urinary magnesium excretion was markedly increased in the plant-based diet group when compared to the control group suggesting a higher bioavailability of magnesium in the ovo–lacto vegetarians and low-meat eaters. After absorption, magnesium binds to proteins or nucleotides to become available for cell functions. Free magnesium is excreted in the urine (Golf, 1997). Pharmacological studies have shown that urinary magnesium excretion is increased when the body magnesium pool becomes saturated (Feillet-Coudray *et al*, 2002). It has been shown that measurement of total serum or plasma magnesium may not adequately reflect physiologic magnesium stores because plasma contains $<1\%$ of total body magnesium which is stored predominantly intracellular and in bone (Bardicef *et al*, 1995).

A high protein intake has also been associated with an increased magnesium absorption in young women (Lakshmanan *et al*, 1984). In our study, the total protein intake was higher among the control group whereas protein intake related to body weight was similar between diet groups. In our study, no association between protein intake and magnesium concentrations in serum or RBC was observed.

The most important finding of the present study was the reduced occurrence of calf muscle cramps in women following a plant-based diet compared to the controls. Pregnancy-induced calf muscle cramps are common complaints usually treated by oral supplementation with magnesium (Riss *et al*, 1983). Calf cramps result from an altered calcium metabolism in skeletal muscle cells. During pregnancy, calcium and potassium are kept under homeostatic regulation, while magnesium is more likely to be influenced by dietary intake (Pitkin, 1983). In addition, magnesium will be preferentially used for cellular functions which partially explain a higher availability in skeletal muscle cells rather than RBC (Golf, 1994). Our observations suggest that the higher magnesium intake of women following a plant-based diet is associated with a lower occurrence of calf muscle cramps. Additionally, comparable serum concentrations of calcium and potassium in all studied groups strengthens the role of dietary magnesium on the observed effect on calf muscle cramps.

In conclusion, the present study suggest that a habitual plant-based diet result in a slightly improved magnesium status during pregnancy compared to an average Western diet. Furthermore, a plant-based diet may contribute to alleviate pregnancy induced calf muscle cramps. Therefore, plant-based diets during pregnancy can be recommended with regard to magnesium supply.

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