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Effect of Daily Consumption of Probiotic Yoghurt on Serum Levels of Calcium, Iron and Liver Enzymes in Pregnant Women

Zatollah Asemi[1], Ahmad Esmaillzadeh[2,3]

[1]Department of Nutrition and Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, I.R. Iran, [2]Food Security Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [3]Department of Community Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran

Correspondence to:
Dr. Ahmad Esmaillzadeh, Department of Community Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, PO Box 81745-151, Iran.
E-mail: esmaillzadeh@hlth.mui.ac.ir

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ABSTRACT

Background: To reach fetal appropriate growth during the third trimester, the requirements for dietary calcium and iron intakes during the pregnancy increases. This study was carried out to determine the effects of daily consumption of probiotic yoghurt on serum calcium and iron levels and liver enzymes among Iranian healthy pregnant women.

Methods: In this controlled clinical trial, 70 primigravida pregnant women carrying singleton pregnancy at their third trimester were participated. Participants were randomly divided into two groups of consuming 200 g/d of conventional (n = 33) or probiotic yogurts (n = 37) for 9 weeks. The probiotic yogurt contained *Lactobacillus acidophilus* and *Bifidobacterium lactis* with a total of min 1×10^7 CFU. To measure serum calcium, iron, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, blood samples were drawn in a fasting state at baseline and after 9 weeks intervention.

Results: Consumption of probiotic yogurt resulted in maintaining serum calcium levels compared with the conventional yogurt ($P = 0.01$). Within-group differences in the conventional yogurt group revealed a significant reduction of serum calcium levels (−1.7 mg/dL, $P < 0.0001$).No significant differences were found between the two yogurts in terms of their effects on serum iron, AST and ALT levels.

Conclusions: Consumption of probiotic yogurt among pregnant women resulted in maintaining serum calcium levels compared with the conventional yogurt; however, it could not affect serum iron, ALT and AST levels.

Keywords: Calcium, iron, liver enzymes, pregnant women, probiotic yoghurt

Original Article

INTRODUCTION

Pregnancy is associated with increased requirements of both calcium and iron to maintain growth and development to the fetus.[1-3] Calcium deficiency during pregnancy is associated with abnormal fetal programming,[4] hypocalcaemia in newborns,[5]

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neonatal rickets and tetany, infantile rickets[6] and pre-eclampsia.[7] Iron deficiency during pregnancy can cause anemia, small-for-gestational-age infants, preterm birth and decreased behavioral, emotional and cognitive development, particularly during the first half of pregnancy.[2,8-10] Iron-deficiency anemia during pregnancy is a global health problem affecting nearly half of all pregnant women world-wide.[1] Besides iron and calcium, it has also been reported that liver enzymes are slightly elevated during the third trimester of pregnancy compared with the non-pregnant women.[11,12] Weight gain during the pregnancy might explain the increased levels of liver enzymes.[11,12]

Numerous strategies for the prevention of calcium and iron deficiency during pregnancy have been suggested including, but not limited to, the use of fortified food supplements,[13] calcium and iron supplementation,[14,15] dietary calcium and iron supply.[16,17] Recently, limited number of experimental studies has shown that consumption of probiotics in animal models has been resulted in maintaining serum calcium and iron levels in the normal range.[18,19] On the other hand, the beneficial effects of probiotics on reducing serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) levels have also been documented.[20,21] Some investigators believe that probiotic consumption might inhibit the increments in serum ALT and AST levels.[22] Findings from other studies in this regard are conflicting.[20,23-26] Probiotics might affect serum calcium and iron levels through their beneficial effects on increased intestinal absorption of these minerals.[24] Attenuated liver injury and reduced tissue tumor necrosis factor-alpha level by probiotics might explain their effects of liver enzymes.[27,28]

Previous studies on the effect of probiotics on serum calcium and iron levels as well as liver enzymes have been mostly performed in animal models. Limited data are available in this regard in humans, particularly in pregnant women. We are aware of no study indicating the effects of probiotic yoghurt consumption on serum calcium, iron, AST and ALT levels among pregnant women. Therefore, the aim of this study was to investigate the effects of daily consumption of probiotic yoghurt on serum calcium, iron, AST and ALT levels in Iranian pregnant women.

METHODS

Subjects

We conducted a randomized single-blinded controlled clinical trial in Kashan, Iran, during October 2010 to March 2011. On the basis of that sample size formula suggested for randomized clinical trials, we considered the type I error of 5% ($\alpha = 0.05$) and type II error of 20% ($\beta = 0.20$; power = 80%) and serum ALT as a key variable,[29] we reached the sample size of 35 persons for each group. In this study, women who were carrying singleton pregnancy at their third trimester were recruited.

Individuals were selected from among those attended maternity clinics affiliated to Kashan University of Medical Sciences, Kashan, Iran. A total of 100 pregnant women aged 18-30 years were screened, of them 82 pregnant women met the inclusion criteria (12 women due to multiparous and 6 women because of not living in Kashan were excluded). We did not include those with severe and mild pre-eclampsia, hypertension, gestational diabetes mellitus (GDM), intrauterine fetal death as well as those with a history of rheumatoid arthritis, thyroid, parathyroid or adrenal diseases, hepatic or renal failure. Participants were randomly assigned to consume probiotic ($n = 42$) or conventional yogurt ($n = 40$) for 9 weeks. Among individuals in the group of probiotic yogurt, five women (GDM [$n = 2$], pre-eclampsia [$n = 2$] and bed rest [$n = 1$]) were excluded. The exclusions in conventional yogurt group was seven persons (GDM [$n = 3$], pre-eclampsia [$n = 2$] and bed rest [$n = 2$]). Finally, 70 participants (probiotic yogurt [$n = 37$] and conventional yogurt [$n = 33$]) completed the trial. The study was ethically approved by Tehran University of Medical Sciences (No: 20402-89-7-18). All participants provided informed written. The trial was registered in the Iranian website (www.irct.ir) for registration of clinical trials (IRCT code: 138811282394N3).

Methods

At first, participants were entered into a 2-weekrun-inperiod. The logic behind this period was obtaining detailed information about the dietary intakes of study participants. Women were asked not to consume probiotic yoghurt or any other probiotic food in this period. At the end of run-in period, subjects were randomly assigned to consume 200 g/d of conventional or the probiotic group for 9 weeks. To reduce confounding effect of dietary intakes and physical activity, participants were asked not to change their routine physical activity or usual diets throughout the study. They were also asked not to consume any yogurt other than the one provided to them by the investigators, any other probiotic and fermented products. Conventional or probiotic yogurts were provided for participants every week. The probiotic yoghurt was a commercially available product prepared with the starter cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, enriched with probiotic culture of two strains *Lactobacillus acidophilus* (LA5) and *Bifidobacterium lactis* (BB12) with a total of min 1×10^7 colony-formingunits (a total of 200×10^7 CFU/day). The conventional yoghurt contained the starter cultures of *S. Thermophilus* and *L. Bulgaricus* (the same substance without probiotic bacteria) was packed in identical packages and coded by the producer to guarantee blinding. Quality control of probiotic yogurt was carried out in the laboratory of Food and Drug Administration in Kashan, Iran with microbiological culture method. Both yogurts' pH was in the range of 4.3-4.5 and their fat content was 1.5%. The probiotic and conventional yogurts were provided by Pegah Company, Tehran, Iran. Compliance with the yoghurt consumption was monitored once a week through phone interviews. The compliance was also double-checked by the use of 3 day dietary records completed throughout the study. To obtain nutrient intakes of participants based on these 3-day food diaries, we used nutritionist IV software (First Databank, SanBruno, CA) modified for Iranian foods.

All measurements, including anthropometric and biochemical assessments, were carried out at baseline and after 9 weeks of intervention. Maternal weight was assessed by trained midwifes at maternity clinic in an overnight fasting status, without shoes and in a minimal clothing state using a digital scale (Seca, Hamburg, Germany) to the nearest 0.1 kg. Height was measured using a non-stretched tape measure (Seca, Hamburg, Germany) to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight in kg divided by height in meters squared. Fasting blood samples (5 mL) were taken at baseline and after 9-week intervention at Kashan reference laboratory in a nearly morning after an overnight fast. Blood samples were immediately centrifuged (HettichD-78532, Tuttlingen, Germany) at 3500 rpm for 10 min to separate serum. Then, the samples were stored at −70°C before analysis at the KUMS reference laboratory. Serum levels of calcium, iron, ALT and AST were quantified. All biochemical assessments were performed using commercially available kits (Pars Azmoon, Tehran, Iran) by automatic biochemistry analyzer (BT 1500, Monsano, Italy).

Statistical analysis

We used Histogram and Kolmogrov-Smirnov test to ensure the normal distribution of variables. To identify within group differences, paired-samples *t*-tests were applied. Student's *t*-test or Mann-Whitney U test was used to detect differences between groups, where appropriate. $P < 0.05$ was considered as statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 17 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Study participants were young, with the average age of 25 y. Women in the conventional yoghurt group had a mean age of 25.7 y while those in the probiotic yogurt group had a mean age of 24.2 ($P = 0.05$). We did not find any significant difference in baseline weight and BMI comparing the two groups (mean weight for conventional yogurt group: 71.6 kg and for probiotic yogurt group 68.0 kg; mean BMI for conventional yogurt group: 27.5 kg/m^2 and for probiotic yogurt group 27.0 kg/m^2). Lack of significant difference in weight and BMI was also reached after intervention. Consumption of probiotic yogurt in the pregnant women did not result in any serious adverse reactions.

Comparing conventional and probiotic yogurts groups, participants' energy intake were not significantly different at run-in period (2376 ± 291 vs. 2395 ± 266 kcal/d) as well as throughout the study (2394 ± 203 vs. 2448 ± 190 kcal/d). We found no statistically significant difference between the two groups in terms of dietary intakes of calcium, phosphorus, vitamin D and iron [Table 1]. Comparing the dietary intake at run-in period with those throughout the study, we found that dietary intakes were not altered.

The effect of probiotic yogurt consumption of serum biochemical indicators are provided in [Table 2]. Consumption of the probiotic yogurt resulted in maintaining serum calcium levels compared with the conventional yogurt ($P = 0.01$). Within-group differences in the conventional yogurt group revealed a significant reduction of serum calcium levels (−1.7 mg/dl, $P < 0.0001$). We did not find any significant differences between the two yogurts in terms of their effects on serum iron ($P = 0.45$), AST ($P = 0.32$) and ALT levels ($P = 0.61$). Within-group changes in these biochemical indicators were also non-significant.

DISCUSSION

Our findings indicated that consumption of probiotic yogurt containing LA5 and BB12 for 9 weeks among pregnant women in the third trimester maintained serum calcium levels compared with the conventional yogurt. We did not find any significant effect of probiotic yogurt consumption on serum iron, AST and ALT levels compared with conventional yogurt.

Due to increased requirements of both calcium and iron during the third trimester, pregnant women are very susceptible to decreased levels of serum calcium and iron. Insufficient calcium and iron intake during pregnancy would result in several complications in maternal and their infants.[2,8-10] The current study showed that probiotic yogurt consumption during the third trimester prevented the reduction in serum calcium levels; however, consumption of conventional yogurt resulted in decreased levels of serum calcium. Due to increased blood volume and increased calcium levels, the reduction in serum calcium levels particularly during the third trimester of pregnancy is an expected finding. Therefore, what has happened to serum calcium levels in the conventional yogurt group is expected and the interesting finding of the current study is that consumption of probiotic yogurt prohibited the reduction in serum calcium levels. This finding highlights the importance of probiotic yogurt intake during pregnancy. Our finding on the effect of probiotics on serum calcium levels are in line with previous studies.[18,19,24,30] However, the majority of previous studies have been conducted in animal models. A probiotic preparation with multi-strains composition caused a significant increase of serum calcium levels in broiler chickens.[18] This finding was also confirmed when just two probiotic strains, namely *Lactobacillus fermentum* and *Enterococcus faecium* M74, were given to broiler chickens.[19] In another study, use of *Enterococcus faecium* EK13 with a dosage of 10^9 CFU/mL in piglets for 14 days resulted in increased serum calcium levels.[30]

Table 1: Dietary intakes of study participants at run-in period and throughout the study[a]

Micronutrients	Run-in period			Throughout the study		
	Probiotic yogurt (*n*=37)	Conventional yogurt (*n*=33)	*P* value†	Probiotic yogurt (*n*=37)	Conventional yogurt (*n*=33)	*P* value†
Calcium (mg/d)	1136.8±205.1	1134.9±173	0.96	1180.5±164.7	1104.9±158.1	0.06
Phosphorus (mg/d)	1175±190.8	1159.8±195.6	0.74	1221.8±137	1192.5±248.8	0.53
Vitamin D (μg/d)	2.71±0.78	2.86±0.86	0.81*	2.88±0.82	2.95±0.79	0.16*
Iron (mg/d)	15.4±3.7	14.7±3.2	0.35	15.8±3.2	15±2.8	0.27

[a]Data are means±standard deviation, †Obtained from independent *t*-test, *Obtained from Mann-Whitney U test

Table 2: Means (±standard deviation) of serum calcium, iron, AST and ALT levels at baseline and after the intervention

Variables	Conventional yogurt (*n*=33)				Probiotic yogurt (*n*=37)				*P* value††
	Baseline	Week 9	Change	*P* value†	Baseline	Week 9	Change	*P* value†	
Calcium (mg/dL)	10.4±1.8	8.7±1.1	−1.7±2	<0.0001	9.8±1.3	9.3±1.1	−0.5±1.8	0.07	0.01
Iron (mg/dL)	124±88.3	112.9±47.9	−11.1±94.3	0.50	118±61.8	122.1±59	4.1±74.6	0.74	0.45
AST[a] (mg/dL)	22.4±10.2	20.2±5.4	−2.2±11	0.26	24.4±10.4	25.1±14.4	0.7±13.4	0.74	0.32
ALT[b] (mg/dL)	9.3±6.5	8.2±5.1	−1.1±7.3	0.38	10.1±7.8	9.9±8.3	−0.2±8.5	0.90	0.61

†For within-group differences (paired samples *t*-test), ††For the differences in change between the two groups (independent samples *t*-test), [a]AST=Serum aspartate aminotransferase, [b]ALT=Serum alanine aminotransferase

A significant increase of serum calcium levels was also seen with a daily consumption of probiotic supplementation at a rate of 100 or 200 mg/kg feed in White Leghorn layers.[31] In contrast to our findings, consumption of *lactobacillus*-containing soymilk did not cause increase calcium absorption among postmenopausal women;[24] however, this product enhanced calcium solubility.[32] The exact mechanisms by which probiotics might affect serum calcium levels are unknown. The beneficial effects of probiotics might be explained by their effects on enhanced calcium solubility.[24] Probiotics might also influence intestinal tract to increase the absorption of calcium.[33] Furthermore, the production of short-chain fatty acids by probiotics might improve protein digestibility and result in increased calcium release and absorption.[23]

We did not find any significant effect of probiotic yogurt consumption on serum iron levels. Addition of two probiotic strains *L. fermentum* (1×10^9 CFU/g) and *E. faecium* M74 (2×10^9 CFU/g) to the drinking water in broiler chickens caused a significant increase in serum iron levels after 42 days.[19] However, when a combination of multi-strains was used, no significant effect on serum iron levels were found.[18] In a study by Silva *et al*.,[23] consumption of iron fortified milk containing *L. Acidophilus* for 101 days in children aged 2-5 years resulted in the reduction of red blood cell count, hemoglobin and hematocrit compared with the control group. Unfortunately, no reports are available about the effects of probiotics on serum iron levels among pregnant women. The differences between our findings with those of previous reports might be explained by the type and dosage of probiotic used the intervention time or the study participants.

We did not find any significant effect of probiotic yogurt consumption on serum AST and ALT levels compared with conventional yoghurt. In contrast to our findings, previous studies have reached a significant decrease in serum levels of these liver enzymes with the consumption of *saccharomyces boulardii* in broilers[21] and *L. acidophilus, Lactobacillus rhamnosus* ATCC 53103, *L. rhamnosus* DSM 6594 + *L. plantarum* DSM 9843 in an acute liver injury model.[29] In alcoholic liver disease in mice, administration of heat-killed *L. brevis* at a dose of 100 or 500 mg/kg once a day for 35 days inhibited the increase in serum ALT and AST levels.[22] However, others have reported a rise in serum levels of these enzymes by *S. parauberis* (2.1×10^7 CFU/ml) in animal models.[26] It seems that the effect of probiotics on serum levels of ALT and AST is dependent on the species and strains of probiotic. The addition of *Lactobacillus plantarum* and *Bifidobacterium infantis* to the rat feed resulted in lowered levels of serum ALT,[34] but consumption of *Saccharomyces cerevisiae* caused a significant increase in serum ALT levels in rats.[35] Limited data are available in humans; therefore, we were unable to compare our findings with earlier studies.

Several limitations in the current study must be taken into account. It is well-known that serum ferritin levels and serum transferring receptors are better biomarkers for assessing body iron status. Furthermore, serum calcium levels are under tight control of hormones. However, due to budget limitations, we were unable to assess other biomarkers related to iron and calcium metabolism in the body. Some findings of the current study might be explained by the short duration of intervention. We were unable to continue the intervention for more than 9 weeks. In addition, we used yogurts with limited number of strains of probiotics. Future studies are recommended to assess the effects of probiotics consumption on inflammatory factors, biomarkers of oxidative stress, serum minerals and liver enzymes with higher dosage and combination of bacteria strains in pregnant women.

CONCLUSIONS

Consumption of probiotic yogurt, compared with conventional yogurt, among pregnant women resulted in maintenance of serum calcium levels without any significant effect on serum iron, ALT and AST levels.

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