Effect of Daily Consumption of Probiotic Yogurt on Oxidative Stress in Pregnant Women: A Randomized Controlled Clinical Trial

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8-oxo-7,8-dihydroguanine levels (–74.3 ng/ml, p = 0.04), no significant differences were found between the two yogurts in terms of their effects on the mentioned parameters.

Conclusion: Consumption of probiotic yogurt among pregnant women resulted in increased levels of erythrocyte GR as compared to the conventional yogurt, but could not affect other indices of oxidative stress.

Key Words
Probiotic yogurt • Oxidative stress • Pregnancy

Abstract
Background: Due to the enhanced oxygen requirement of the mitochondria-rich placenta primarily during the third trimester, pregnancy is associated with elevated levels of oxidative stress. This study was designed to determine the effects of daily consumption of probiotic yogurt on oxidative stress among Iranian pregnant women. Methods: This randomized single-blind controlled clinical trial was performed among 70 pregnant women, singleton primigravida, aged 18–30 in their third trimester. Subjects were randomly assigned to two groups to consume 200 g/day of either conventional yogurt (n = 33) or probiotic yogurt (n = 37) for 9 weeks. Fasting blood samples were taken at baseline and after a 9-week intervention to measure oxidative stress parameters. Results: Consumption of probiotic yogurt resulted in increased erythrocyte glutathione reductase (GR) levels as compared to the conventional yogurt (p = 0.01). Despite the significant effect of probiotic yogurt consumption on plasma glutathione (67.9 μmol/l, p = 0.01), erythrocyte glutathione peroxidase (163 mmol/min/ml, p = 0.04) and serum 8-oxo-7,8-dihydroguanine levels (–74.3 ng/ml, p = 0.04), no significant differences were found between the two yogurts in terms of their effects on the mentioned parameters. Conclusion: Consumption of probiotic yogurt among pregnant women resulted in increased levels of erythrocyte GR as compared to the conventional yogurt, but could not affect other indices of oxidative stress.

Introduction
Oxidative stress has been defined as an imbalance between oxidant and antioxidant forces in favor of oxidation [1]. Due to the increased oxygen requirement of the mitochondria-rich placenta [2] and the reduced scavenging power of antioxidants [3, 4], pregnancy is associated with increased susceptibility to oxidative stress.

Oxidative stress and generalized endothelial dysfunction appear to be the cause of the maternal syndrome of preeclampsia [5], the reduction of neonatal birth weight [6], premature delivery, fetal growth restriction, eclampsia, maternal infections and maternal malnutrition [2, 7–9]. It has been widely recognized as an important un-
underlying mechanism for diabetes mellitus, cancer, and renal failure [10]. Preeclampsia is estimated to occur in 2–7% of all pregnancies and is a leading cause of maternal and perinatal mortality and morbidity in the western world [11–13], as well as being responsible for about 60,000 deaths worldwide [14].

Various strategies for the management of oxidative stress during pregnancy have been suggested including, but not limited to, the use of antioxidants and vitamins E and A [2, 15]. Recently, clinical trials in nonpregnant women have shown that the consumption of probiotics can decrease oxidative stress [16–18]. However, these studies are limited and report conflicting findings [19–22]. Probiotics are live bacteria that could provide beneficial health effects upon consumption [23, 24]. Improvement of oxidative stress by probiotics might result from their effects on increasing glutathione (GSH) levels [25], reducing intestinal oxidative stress [25–27], scavenging superoxide and hydroxyl radicals [28], removing metals (prooxidants) from the environment [29], lowering levels of oxidized low-density lipoprotein [17, 30] and lowering the glutathione redox ratio in the blood, gut mucosa and skin [31].

We are unaware of any existing study indicating the effects of probiotic yogurt consumption on oxidative stress among pregnant women. Therefore, the aim of the current study was to investigate the effects of daily consumption of probiotic yogurt on indices of oxidative stress [including plasma total antioxidant capacity (TAC), plasma glutathione (GSH), erythrocyte glutathione peroxidase (GPx), glutathione reductase (GR) and serum 8-oxo-7,8-dihydroguanine (8-oxo-G)] in Iranian pregnant women.

Subjects and Methods

Participants

This randomized single-blind controlled clinical trial was carried out in Kashan, Iran, from October 2010 to March 2011. Pregnant women, singleton primigravida, aged 18–30 in their third trimester were recruited. The gestational age was assessed from the date of the last menstrual period and concurrent clinical assessment [26]. Individuals with the above-mentioned inclusion criteria were called for participation in the study from among those that attended maternity clinics affiliated to Kashan University of Medical Sciences, Kashan, Iran. Women with multiparity, maternal hypertension, liver or renal disease and gestational diabetes mellitus were not included in the study. A total of 82 pregnant women were recruited in the study and were randomly assigned to probiotic (n = 42) or conventional yogurt (n = 40) groups for 9 weeks. Among the individuals in the probiotic yogurt group, 5 women [gestational diabetes (n = 2), preeclampsia (n = 2) and bed rest (n = 1)] were excluded. The exclusions in the conventional yogurt group were 7 women [gestational diabetes (n = 3), pre-eclampsia (n = 2) and bed rest (n = 2)]. This gave a final total of 70 participants [probiotic yogurt (n = 37) and conventional yogurt (n = 33)] that completed the trial. The study was conducted according to the guidelines laid down in the Declaration of Helsinki. The ethical committee of Tehran University of Medical Sciences approved the study (No. 20402-89-7-18) and informed written consent was obtained from all participants.

Study Design

To obtain detailed information about the dietary intakes of study participants, all women were included in a 2-week run-in period during which they had to refrain from taking probiotic yogurt or any other probiotic food. At the end of the run-in period subjects were instructed to consume 200 g/day of either the conventional or probiotic yogurt for 9 weeks, depending on which group they were randomly assigned to. Participants were asked not to alter their routine physical activity or usual diets and not to consume any yogurt other than that provided to them by the investigators. They were also asked to avoid consuming any other probiotic or fermented products. The conventional or probiotic yogurts were provided for the participants every week. Compliance with the yogurt consumption was monitored once a week through phone interviews and was also double-checked by the use of 3-day dietary records completed throughout the study. To obtain the nutrient intakes of participants based on these 3-day food diaries, we used Nutritionist IV software (First Databank, San Bruno, Calif., USA) modified for Iranian foods.

Assessment of Variables

Anthropometric measurements were assessed at baseline and after 9 weeks of intervention. Body weight was measured in an overnight fasting status, without shoes and in minimal clothing by the use of a digital scale (Seca, Hamburg, Germany) to the nearest 0.1 kg. Height was measured using a nonstretched tape measure (Seca, Hamburg, Germany) to the nearest 0.1 cm. BMI was calculated as weight in kilograms divided by height in meters squared. Fasting blood samples (10 ml) were taken at baseline and after a 9-week intervention at Kashan reference laboratory in the early morning after an overnight fast. Plasma TAC was assessed by the use of the ferric reducing ability of plasma method developed by Benzie and Strain [detailed in 32]. The test was performed at 37°C and the 0- to 4-min reaction time window was used. The final results are expressed as mmol Trolox equivalent/l [32]. The plasma GSH was measured by the method of Beutler et al. [detailed in 33]. Erythrocyte GPx and GR activities were determined spectrophotometrically from a lysate of the washed and packed erythrocytes fraction using a test reagent kit (Cayman Chemical Co.; GPx with Ref. No. 703102 and GR with Ref. No. 703202). Absorbance was measured at 340 nm and results are expressed as nmol/min/ml protein. Serum 8-oxo-G was assayed by ELISA (Cusabio Biotech Co.; Ref No.: CSB-E10140h).

Characteristics of the Yogurts

The probiotic yogurt was a commercially available product prepared with the starter cultures of Streptococcus thermophilus and Lactobacillus bulgaricus, enriched with probiotic culture of two strains of lactobacilli (Lactobacillus acidophilus LAS) and bifidobacteria (Bifidobacterium lactis BB12) with a total of a minimum of 1 × 10^10 colony-forming units. The conventional yogurt contained the starter cultures of S. thermophilus and L. bulgari-
Both yogurts’ pH was in the range of 4.3–4.5 and their fat content was 1.5%.

**Statistical Analysis**

To ensure the normal distribution of variables, histogram and Kolmogrov-Smirnov tests were applied. We used paired-sample t tests to identify within-group differences (before and after intervention). Student’s t test was used to detect differences between groups. p < 0.05 was considered to be statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 17 (SPSS Inc., Chicago, Ill., USA).

**Results**

No serious adverse reactions were reported following the consumption of probiotic yogurt in the pregnant women throughout the study.

The mean age of the study participants was slightly higher in the conventional yogurt group than that in the probiotic yogurt group (25.7 ± 3.1 vs. 24.2 ± 3.3 years, p = 0.05). The mean baseline weights (71.6 ± 10.7 vs. 68 ± 12 kg) and BMI (27.5 ± 4.1 vs. 27 ± 4.1), and the mean end-of-trial weights (75.9 ± 9.8 vs. 72.2 ± 11.7 kg) and BMI (29.4 ± 3.8 vs. 28.7 ± 4) were not significantly different between the two groups. Individuals in the conventional yogurt group were taller than those in the probiotic yogurt group (160.81 ± 4.52 vs. 158.37 ± 5.54 cm; p = 0.049).

We found no statistically significant difference between the two groups in terms of dietary intakes of energy, vitamin E, β-carotene, vitamin C, selenium, magnesium and iron (table 1). Within-group differences in dietary intakes were also not significant.

The consumption of probiotic yogurt resulted in increased erythrocyte GR levels as compared to the conventional yogurt (p = 0.01). Despite the significant effect of probiotic yogurt consumption on plasma GSH (67.9 μmol/l, p = 0.01), erythrocyte GPx (163 mmol/min/ml,
p = 0.04) and serum 8-oxo-G levels (~74.3 ng/ml, p = 0.04), no significant differences were found between the two yogurts in terms of their effects on the mentioned parameters related to oxidative stress. Within-group differences in the conventional yogurt group revealed a significant reduction of plasma TAC levels (~63.8 mmol/l, p = 0.04; table 2).

**Discussion**

Our study revealed that the consumption of probiotic yogurt containing *L. acidophilus LA5* and *B. lactis* BB12 for 9 weeks among pregnant women in the third trimester resulted in a significant increase in erythrocyte GR levels. We did not find any significant effect of probiotic yogurt consumption on plasma GSH, TAC, erythrocyte GPx and serum 8-oxo-G levels as compared to conventional yogurt consumption.

Pregnant women are very susceptible to increased oxidative stress. Elevated oxidative stress during pregnancy can result in the development of preeclampsia [5], the reduction of neonatal birth weight [6], premature delivery, fetal growth restriction, eclampsia, maternal infections and maternal malnutrition [2, 7–9]. Our data demonstrated that among pregnant women probiotic and conventional yogurts did not differ in terms of their effect on several indices of oxidative stress, except for erythrocyte GR levels. Although the effect of probiotics on oxidative stress has previously been assessed among healthy adults [18, 19] and animal models [22, 34, 35], to the best of our knowledge, this study is the first study examining the effect of probiotics on oxidative stress among pregnant women.

Our data showed that probiotic yogurt consumption for 9 weeks did not affect plasma TAC levels; however, significant within-group differences were seen in the conventional yogurt group. In a study by Fabian and Elmadfa [19] a significant reduction of TAC levels was seen with daily consumption of conventional and probiotic yogurt containing a strain of *L. casei* after 4 weeks in young healthy women. A significant increase of TAC levels was also seen with a daily consumption of fermented goat’s milk and capsules containing *Lactobacillus fermentum* ME-3 [daily dosage 9.2 colony-forming units (CFU)] after 3 weeks in healthy volunteers [18]. Consumption of two strains of *lactobacilli* and *L. fermentum* ME-3 for 3 weeks has also resulted in increased TAC levels in healthy subjects [17]. The same findings have also been reached by the consumption of multistrain compositions (*Lactobacillus delbrueckii*, *L. bulgaricus* LAT, *L. acidophilus* LAT, *Lactobacillus helveticus* LAT, *L. delbrueckii* ssp, *lactis* LAT, *S. thermophilus* LAT and *Enterococcus faecium*) in broiler chickens [20]. The antioxidative effect of *L. fermentum* ME-3 has been confirmed by other investigations [30, 36, 37].

Throughout pregnancy the production of reactive oxygen species (free radicals and their nonradical intermediates) increases, especially from the placenta [38, 39], while TAC levels decrease [40]. Enzymatic and nonenzymatic compounds including vitamin E, glutathione, superoxide dismutase (SOD) and GPx that participated in the TAC structure attack oxidants and in doing so can cause a reduction of TAC compounds [38]. It can be assumed that in the current study probiotic yogurt consumption might have increased TAC levels, but due to an increased production of reactive oxygen species and their scavenging by antioxidants, a change in TAC levels could not be detected.

We did not find any significant effect of probiotic yogurt consumption on plasma GSH levels as compared to conventional yogurt. In contrast to our findings, previous studies performed among nonpregnant women have revealed a significant effect of probiotics on this biomarker [25, 41]. Two studies by Lutgendorff et al. [42, 43] in animal models have also reported a significant increase in mucosal GSH levels with the daily consumption of multispecies probiotics. In rats with colitis, the oral administration of *L. fermentum* (5 × 10⁸ CFU for 3 weeks) was counteracted by colonic GSH depletion induced by the inflammatory process [25]. GSH is an important regulator of intracellular redox homeostasis and it appears that probiotics can increase plasma GSH levels in several ways. It has been reported that certain probiotics including *L. fermentum, Lactobacillus reuteri, Bifidobacterium* and *Enterococcus* genera can release GSH from other tissues [25, 26, 44]. Probiotics can also cause GSH synthesis through enhanced glutamate-cysteine ligase (GCL) activity, increased mRNA expression of both of the GCL subunits, increased local synthesis and systemic GSH levels [42, 43].

Despite the significant reduction of serum 8-oxo-G levels by probiotic yogurt, we failed to find any significant difference between conventional and probiotic yogurts. To our knowledge, these data represent the first report of the effect of probiotics on 8-oxo-G, which is a biomarker of oxidative DNA damage [45]. The reduced scavenging power of antioxidants [3, 4] and increased oxygen requirement [2] during pregnancy is probably the most important factor in the production of serum 8-oxo-
G. It seems that the significant increase in the production of GSH and GPx by probiotic yogurt consumption in the present study might have reduced free radicals and reactive oxygen species, and therefore have indirectly resulted in a decline in serum 8-oxo-G levels.

The current study showed that probiotic yogurt consumption for 9 weeks resulted in significantly increased erythrocyte GPx and GR activity. However, after comparing the two groups, the difference was only significant for GR activity. In a similar study, feeding aging mice with L. acidophilus (La-Dahi) or combined L. acidophilus and Bifidobacterium bifidum (LaBb-Dahi) for 4 months increased GPx activity in RBCs and hepatic tissue [46]. GPx activity in fish fed a diet containing Lactobacillus plantarum at $10^8$ and $10^{10}$ CFU kg$^{-1}$ for 4 weeks was significantly increased as compared to those fed a $10^6$ CFU kg$^{-1}$ L. plantarum diet or a control diet [47]. Administration of Bifidobacteria in rats that were on a diet containing thermally oxidized soybean oil resulted in increased SOD and GR activities in the liver, kidneys, testes and brain [22]. Unlike our study, in a study by Paik et al. [21] administration of Bacillus polyfermenticus SCD ($3.1 \times 10^6$ CFU/day) for 6 weeks in hypercholesterolemic rats led to a significant reduction of erythrocyte GPx activity as compared to the control group. In the present study, a possible mechanism of increased erythrocyte GPx and GR activities by probiotics might be an increasing expression of GPx and GR. Upregulated SOD and GPx with probiotics supplementation have been reported in formula-fed neonatal rats [16]. Another explanation might be the increased production of cytokines (including leptin, IL-6 and TNF-α) by adipokines during pregnancy due to weight gain [48]. These cytokines are potent stimulators for the production of reactive oxygen, meaning a rise in the concentration of cytokines could be responsible for increased oxidative stress [49] and for decreasing the activity of antioxidant enzymes such as SOD and catalase [48, 50]. A significant decrease in the activity of SOD and GPx in individuals with obesity compared with healthy individuals has been reported [51]. It seems that probiotics can cause a decreased production of inflammatory factors including IL-6 and CRP which would indirectly affect the activity of antioxidant enzymes. In a study by Hegazy and El-Bedewy [52] probiotic use for 8 weeks significantly ameliorated the inflammation. We have reported in our previous study [53] that probiotic yogurt consumption for 9 weeks among pregnant women could decrease serum high-sensitivity C-reactive protein levels, a key inflammatory biomarker, as compared to conventional yogurt intake.

Several limitations must be considered in the interpretation of our findings. These include the limited 9-week duration of the trial. As the study participants were women in the 28th week of their pregnancy it was impossible to prolong the study. Furthermore, due to budget limitations, we were unable to use yogurts with higher concentrations of probiotics. In addition, we could not assess the effects of probiotic-containing yogurts on the biochemical indicators of newborn infants.

In conclusion, consumption of probiotic yogurt among pregnant women resulted in increased levels of erythrocyte GR as compared to the conventional yogurt, but could not affect other indices of oxidative stress.

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**Disclosure Statement**

None of the authors had any personal or financial conflict of interest.

**References**

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