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Original Contribution

Intake of Probiotic Food and Risk of Preeclampsia in Primiparous Women

The Norwegian Mother and Child Cohort Study

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Probiotics have been suggested to modify placental trophoblast inflammation, systemic inflammation, and blood pressure, all potentially interesting aspects of preeclampsia. The authors examined the association between consumption of milk-based probiotic products in pregnancy and development of preeclampsia and its subtypes. The study was performed in the Norwegian Mother and Child Cohort Study by using a prospective design in 33,399 primiparous women in the years 2002–2008. The intake of milk-based products containing probiotic lactobacilli was estimated from a self-reported food frequency questionnaire. Preeclampsia diagnoses were obtained from the Norwegian Medical Birth Registry. Intake of probiotic milk products was associated with reduced risk of preeclampsia. The association was most prominent in severe preeclampsia (adjusted odds ratio (OR) = 0.79, 95% confidence interval (CI): 0.66, 0.96). With probiotic intakes divided into categories representing no, monthly, weekly, or daily intake, a lower risk for preeclampsia (all subtypes) was observed for daily probiotic intake (OR = 0.80, 95% CI: 0.66, 0.96). Lower risks for severe preeclampsia were observed for weekly (OR = 0.75, 95% CI: 0.57, 0.98) and daily (OR = 0.61, 95% CI: 0.43, 0.89) intakes. These results suggest that regular consumption of milk-based probiotics could be associated with lower risk of preeclampsia in primiparous women.

cohort studies; pre-eclampsia; pregnancy; primiparity; probiotics

Abbreviations: CI, confidence interval; MBRN, Medical Birth Registry of Norway; MoBa, Norwegian Mother and Child Cohort Study; OR, odds ratio.

Preeclampsia is a serious pregnancy condition associated with raised blood pressure (hypertension) and proteinuria. The condition may affect multiple organs and is associated with a poor pregnancy outcome. Preeclampsia is one of the leading causes of maternal death worldwide and estimated to influence between 2% and 8% of all pregnancies (1, 2).

Endothelial dysfunction with concomitant activation of the clotting cascade is assumed to be an important part of the pathogenesis of preeclampsia (3) and, to this end, a causal link has been suggested between abnormal hemostasis and inflammation (4). Modifications of the maternal immune system and the underlying inflammatory level may therefore be important in the pathogenesis of preeclampsia, a notion further implicated through association of maternal infections with an increased risk of preeclampsia (5, 6). There is, in addition, a growing consensus for differences in the underlying pathogenesis of subtypes of preeclampsia, with an increased inflammatory response seeming to play a more prominent role in early onset (7, 8) and severe (9–11) preeclampsia.

The numerous metabolic markers of systemic inflammation increased in preeclampsia are closely related to oxidative stress (12), but intervention trials have failed to show beneficial influence of antioxidant vitamin supplementation on preeclampsia incidence (13). The gastrointestinal tract represents the largest immune interface with the environment, but controlled intervention trials with diverse diets are not feasible in pregnancy. However, several epidemiologic studies have

indicated that consumption of fruits, vegetables, and dietary fiber is associated with lower preeclampsia risk (14–16). In addition, plant foods may influence preeclampsia through intestinal antiinflammatory mechanisms (17).

Probiotics are known to modulate gastrointestinal health through suppression of pathogenic bacteria and, more recently, to affect human health by pathophysiological processes involved in hypertension, inflammation, renal function, and diabetes (18).

Intake of food with probiotics might therefore influence and reduce pregnancy complications associated with hypertension and inflammation (19, 20). The suggested rationale for this is a reduction in the systemic inflammatory state through probiotic modification of the inflammatory response. Probiotics have been shown to modulate human gene expression in the gut lining similar to that of drugs for conditions including inflammation and high blood pressure (21). Probiotic strains from the species *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus rhamnosus* each induced differential gene-regulatory networks and pathways in the human mucosa similar to response pathways associated with the regulation of immune responses (21).

In a recent study by Yeganegi et al. (22), it was shown that the supernatant of the probiotic bacteria *L. rhamnosus* GR-1 modified the lipopolysaccharide inflammatory response in placental trophoblast cells, potentially a key cell type in preeclampsia.

We hypothesized that intake of food with probiotics might delay and reduce incidences of preeclampsia in general and particularly of early onset or severe preeclampsia through the reduction of inflammatory responses. The aim of the present study was to investigate the association between consumption of probiotics in milk-based products and development of preeclampsia in a large prospective cohort of nulliparous Norwegian women.

MATERIALS AND METHODS

Population and study design

The data set is part of the Norwegian Mother and Child Cohort Study (MoBa), initiated by and maintained at the Norwegian Institute of Public Health (23). In brief, MoBa is a nation-wide pregnancy cohort that, in the years from 1999 to 2009, included 108,000 pregnancies. Women were recruited to the study through a postal invitation in connection with their first routine ultrasound examination (23). By 2006, the participation rate among primiparous women was 43.2% (24). Evaluation of a potential bias due to self-selection in MoBa showed that, despite differences in prevalence estimates between the cohort participants and the total population of pregnant women, no statistically relative differences in association measures were found regarding the 8 exposure-outcome associations evaluated (24). The women were asked to provide biologic samples and to answer 3 questionnaires during pregnancy. The data included in this study are from 2 questionnaires answered in gestational weeks 15 (questionnaire 1) and 17–22 (questionnaire 2), respectively. Questionnaire 2 is a food frequency questionnaire, while questionnaire 1 is a general questionnaire covering health, exposures, lifestyles, and background factors. Pregnancy and birth records from the Medical Birth Registry of Norway (MBRN) are linked to the MoBa database (25). Informed consent was obtained from each participant before the study. The study was approved by the Regional Committee for Ethics in Medical Research and the Norwegian Data Inspectorate.

This study uses the quality-ensured data files released for research in 2009 (version 4). At the time of this analysis, 39,199 primiparous women had answered the first MoBa questionnaire and were recorded in the MBRN with singleton births. Of these, 34,160 (87%) had also answered version 2 of the food frequency questionnaire (26), and 33,549 had registered a valid food intake (total energy: $>$4,500 kJ and $<$20,000 kJ per day). The range of acceptable energy intake in MoBa has been evaluated elsewhere (26). We excluded women with chronic hypertension, resulting in a final study sample of 33,399 (85%). Women in the study were slightly older, included more nonsmokers, and were more highly educated women than those excluded. The incidence of preeclampsia was lower in the study sample (5.3%) than in the excluded women (7.8%).

Dietary information

The MoBa food frequency questionnaire (downloadable at http://www.fhi.no/dokumenter/011fbd699d.pdf) was completed in weeks 17–22 of gestation. The dietary data used in this study were collected from February 2002 to November 2008. The food frequency questionnaire is a semiquantitative questionnaire designed to capture dietary habits and intake of dietary supplements during the first 4–5 months of pregnancy (26). The food frequency questionnaire asked how often the women had consumed subtypes of milk and yogurt, clearly distinguishing probiotic milk or yogurt from other milk items. The probiotic items were Biola milk (Tine SA, Oslo, Norway), Biola yogurt (Tine SA), and Cultura milk (Tine SA). Intake was reported by marking 1 of 11 alternative intake frequencies ranging from ''never'' to ''8 or more times per day.'' The lowest possible intake for each item was 6.6 mL/day (1 glass monthly), and the highest possible intake was 1,600 mL/day (maximum of 8 glasses daily).

FoodCalc (27) and the Norwegian Food Composition Table (28) were used to calculate food and nutrient intakes. A validation study showed that, relative to a dietary reference method and several biologic markers, the MoBa food frequency questionnaire produces a realistic estimate of habitual intake and is a valid tool for ranking pregnant women according to high and low intakes of energy, nutrients, and food (29). The relative validity of reported milk and dairy intakes was evaluated separately. The correlation coefficients for milk and yogurt intakes ranged from 0.51 to 0.70, indicating good agreement between the MoBa food frequency questionnaire and the reference method for estimating milk and yogurt intakes (30).

Intake of probiotic milk products was first treated as a dichotomous variable (intake yes or no), and then the estimated intake was categorized into 4 groups: none, low, moderate, and high intakes. These groups were based on intake quartiles among consumers. The 2 middle quartiles were combined, and the groups reflected low, moderate, and high

Table 1. Reported Intake of Probiotic and Nonprobiotic Milk Products and Selected Nutrient Intakes for Women With and Without Preeclampsia, the Norwegian Mother and Child Cohort Study, 2002–2008

	Preeclampsia Yes (n = 1,755)		Preeclampsia No (n = 31,644)	
	Mean	95% CI	Mean	95% CI
Milk and yogurt				
Probiotic milk and yogurt, mL/day	29	25, 33	35	34, 36
Nonprobiotic yogurt, mL/day	72	66, 77	72	71, 73
Nonprobiotic milk, mL/day	352	335, 368	340	336, 344
Nutrients				
Total energy intake, MJ/day	9.64	9.52, 9.76	9.59	9.56, 9.62
Total fat intake, g/10 MJ	81.6	81.0, 82.2	81.4	81.3, 81.6
Saturated fat intake, g/10 MJ	31.5	31.2, 31.7	31.4	31.3, 31.5
Total protein intake, g/10 MJ	90.9	90.3, 91.5	91.6	91.4, 91.7
Calcium intake, mg/10 MJ[a]	1,081	1,065, 1,096	1,095	1,092, 1,099
Vitamin D intake, μg/10 MJ[a]	3.51	3.41, 3.61	3.48	3.46, 3.51
Dietary fiber intake, g/10 MJ	31.1	30.7, 31.4	31.9	31.8, 32.0

Abbreviation: CI, confidence interval.
[a] From food only, not supplements.

intakes corresponding to less than 1 portion weekly, 1–6 portions weekly, and 1 or more portions daily.

The products classified as containing probiotics in this study are milk-based beverages containing probiotic lactobacilli. Biola probiotic milk and yogurt contain *L. acidophilus* LA-5, *B. lactis* Bb12, and *L. rhamnosus* GG, while Cultura probiotic milk contains *L. acidophilus* LA-5 and *B. lactis* Bb12. These were the only widely used probiotic food items available in the market at the time of the study. The content of probiotic bacteria in these beverages is 10^8 probiotic bacteria/mL, which ranges from $\approx 1.4 \times 10^{10}$ to 1.6×10^{11} probiotic bacteria per day in the high intake group.

Information regarding the use of probiotic supplements was not included in the current study. However, in a more recent subsample of the cohort, less than 0.5% of the women had reported use of supplements containing probiotic substances.

The outcome variable

The main outcome was preeclampsia in the present pregnancy as registered in the MBRN (25). Information provided to the registry is based on forms completed by the midwives after birth. The form has 5 check-off boxes relevant to preeclampsia: hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome); eclampsia; early onset preeclampsia (diagnosed before 34 weeks); mild preeclampsia; and severe preeclampsia. For the present study, the diagnosis of preeclampsia was given if any of the above-mentioned diagnoses were present. In Norway, all pregnant women receive free antenatal care. Blood pressure measurement and urinalysis for protein are carried out at each antenatal visit. According to guidelines issued by the Norwegian Society of Obstetrics and Gynecology, the diagnostic criterion for preeclampsia is blood pressure $>140/90$ after 20 weeks' gestation, combined with proteinuria $>+1$ dipstick on at least 2 occasions. Preeclampsia is diagnosed as severe preeclampsia if blood pressure is $\geq 160/110$ (31).

Other variables

Adjusting variables in the logistic regression model were chosen according to known risk factors for preeclampsia development: maternal prepregnant body mass index, height, educational attainment, smoking status, dietary supplement use, and total energy intake. Self-reported prepregnancy weight and height were used to calculate body mass index (weight (kg)/height $(m)^2$). Body mass index was divided into World Health Organization categories (<18.5, 18.5–24.9, 25–29.9, 30–34.9, and ≥ 35 kg/m^2), maternal height into quartiles, length of education into 3 categories (≤ 12 years, 13–16 years, ≥ 17 years), and first trimester smoking into 3 categories (nonsmokers, occasional smokers, and daily smokers). Dietary supplement use and dietary fiber intake were included among the confounding variables on the basis of previous studies of preeclampsia in MoBa (15, 32). Dietary supplement use was divided into 3 categories (no supplement use, supplements without vitamin D, and supplements including vitamin D). Maternal age at delivery was retrieved from the MBRN and used as a continuous variable except in Table 2, where it was divided into 4 categories (<20, 20–29, 30–39, and ≥ 40 years). Total energy and fiber intakes were divided into quartiles for Table 2 but used as continuous variables in the regression models. We examined sex of the fetus among potential confounding variables on the basis of the results of a significant sex difference in inflammatory response to probiotics as reported by Yeganegi et al. (22).

Statistical methods

We used independent sample t tests for differences between groups and Pearson's chi square for nominal categories. We estimated odds ratios for developing preeclampsia with and without adjustment for potential confounders using multivariate logistic regression models. Preeclampsia (all types)

Table 2. Intake of Probiotic Milk Products During Pregnancy for 33,399 Primiparous Women, According to Maternal Characteristics, the Norwegian Mother and Child Cohort Study, 2002–2008

	Total Consumers		Probiotic Consumers		Mean Daily Intake of Probiotics, mL/day
	No.	%	No.	%	
All	33,399	100	13,295	39.8	34 (91)[a]
Maternal age at delivery, years					
<20	609	1.8	138	22.7	16 (63)
20–29	19,838	59.3	7,487	37.7	31 (86)
30–39	12,628	37.9	5,526	43.8	40 (97)
≥40	324	1.0	144	44.4	51 (124)
Maternal height, m					
<1.65	9,007	27.0	3,350	37.2	30 (83)
1.65–1.68	8,415	25.2	3,366	40.0	34 (95)
1.69–1.72	7,856	23.5	3,204	40.8	36 (91)
≥1.73	7,814	23.4	3,272	41.9	38 (92)
Missing	307	0.9	103	33.6	37 (114)
Prepregnancy body mass index, kg/m^2					
<18.5	1,102	3.3	390	35.4	33 (92)
18.5–24.9	22,169	66.4	9,408	42.4	37 (85)
25–29.9	6,610	19.8	2,408	36.4	32 (91)
30–34.9	2,039	6.1	648	31.8	25 (82)
≥35	736	2.2	194	26.4	18 (53)
Missing	743	2.2	247	33.2	31 (114)
Maternal education, years					
≤12	9,493	28.4	2,885	30.4	27 (86)
13–16	14,289	42.8	5,799	40.6	34 (87)
≥17	8,888	26.6	4,358	49.0	43 (99)
Missing	729	2.2	253	34.7	34 (106)

Table continues

and subtypes of preeclampsia were examined as separate outcome variables. The maternal characteristics and lifestyle variables examined as potential confounding variables were as follows: maternal age at delivery, maternal height, prepregnant body mass index, education, marital status, pregnant smoking status, dietary supplement use, sex of the fetus, total energy intake, intake of nonprobiotic milk and nonprobiotic yogurt, fiber intake, and intakes of protein, calcium, and fat. Variables were included in the final model if their inclusion influenced the association between probiotics and preeclampsia or if there was a strong theoretical reason for keeping them in the model. The variables included in the final models were as follows: prepregnancy body mass index, height, education, smoking, and intakes of total energy, fiber, and nonprobiotic milk and yogurt.

A total of 1,444 (5.0%) women had missing values on maternal weight, height, or educational attainment. Participants with missing data on a variable were categorized in a ''missing'' category. This may, however, introduce bias. A new data set with imputed missing data values was created by using the multiple imputations option in SPSS statistical software (SPSS, Inc., Chicago, Illinois). Running the adjusted logistic regression models in the data set with imputed values rather than with missing strata did not change the results.

The significance level was set at 5% (2 tailed), and all analyses were performed by using PASW statistics 17 (SPSS, Inc.).

RESULTS

Among the 33,399 nulliparous women, 1,755 (5.3%) developed preeclampsia. Women with preeclampsia reported lower consumption of probiotic milk products than women without preeclampsia, while the intakes of other nonprobiotic milk products did not differ between the 2 groups (Table 1). The energy-adjusted intakes of protein and dietary fiber were lower in women who developed than in those who did not develop preeclampsia (Table 1). The reported intake of probiotic milk products increased with increasing maternal age, height, length of education, dietary fiber intake, and total energy intake and decreased with increasing body mass index. The intake was lower in smokers, in single women, and in women who did not use dietary supplements (Table 2).

Table 2. Continued

	Total Consumers		Probiotic Consumers		Mean Daily Intake of Probiotics, mL/day
	No.	%	No.	%	
Marital status					
Married/cohabitant	31,887	95.4	12,722	40.1	35 (91)
Single	1,522	4.6	508	33.4	30 (79)
Smoking in pregnancy					
Nonsmokers	30,859	92.4	12,651	41.0	36 (92)
Occasional smokers	963	2.9	288	29.9	25 (73)
Daily smokers	1,577	4.7	356	22.6	19 (79)
Dietary supplement use					
None	3,915	11.7	958	24.5	18 (67)
Supplement without vitamin D	2,066	6.2	662	32.0	27 (84)
Supplement with vitamin D	27,418	82.1	11,675	42.6	37 (94)
Sex of the fetus					
Male	17,062	51.1	6,837	40.1	35 (90)
Female	16,320	48.9	6,456	39.6	34 (92)
Total energy intake, MJ/day					
Quartile 1 (4.5–7.8)	8,349	25.0	2,897	34.7	19 (51)
Quartile 2 (>7.8–9.2)	8,350	25.0	3,282	39.3	29 (70)
Quartile 3 (>9.2–11.0)	8,350	25.0	3,511	42.0	36 (84)
Quartile 4 (>11.0)	8,350	25.0	3,605	43.2	54 (134)
Dietary fiber intake, g/10 MJ[b]					
Quartile 1 (13–27)	8,349	25.0	2,611	31.3	30 (101)
Quartile 2 (>27–31)	8,350	25.0	3,316	39.7	34 (93)
Quartile 3 (>31–36)	8,350	25.0	3,618	43.3	36 (88)
Quartile 4 (>36)	8,350	25.0	3,750	44.9	38 (81)

[a] Numbers in parentheses, standard deviation.
[b] From food only, not supplements.

We examined the intake of any probiotic milk products compared with no intake in all preeclampsia cases and in the registered subtypes of preeclampsia (Table 3). In crude analyses, any probiotic use was associated with reduced risk of preeclampsia (all types), as well as with late-onset, mild, and severe preeclampsia. After adjustment for confounders, probiotic use was associated only with reduced risk of the subtype severe preeclampsia (odds ratio (OR) = 0.79, 95% confidence interval (CI): 0.66, 0.96).

When probiotic intake was grouped into no, low, moderate, and high intakes, high intake was associated with reduced risk of all preeclampsia. The incidence of preeclampsia was 5.6% among nonconsumers and 4.1% among high consumers. In the adjusted model, high consumers had 20% lower risk of preeclampsia than did nonconsumers (OR = 0.80, 95% CI: 0.66, 0.96) (Table 4). The incidence of severe preeclampsia was 1.8% among nonconsumers of probiotics and 1.0% in the high intake group. Compared with no intake, moderate and high intakes were associated with reduced risk of severe preeclampsia, with the lowest risk in the high intake group (OR = 0.61, 95% CI: 0.43, 0.89) (Table 4). Adjustment for gestational or preexisting diabetes mellitus did not change the association between probiotics and severe preeclampsia (data not shown).

Because severe preeclampsia often leads to medically indicated preterm delivery (2), we examined the association between any probiotic intake and severe preeclampsia in preterm (<37 weeks) and term (≥37 weeks) pregnancies separately. The prevalence of severe preeclampsia was 12.8% in preterm and 0.9% in term pregnancies. However, the protective effect of any probiotic intake was similar in both preterm (crude OR = 0.78, 95% CI: 0.58, 1.05) and term (crude OR = 0.79, 95% CI: 0.62, 1.01) pregnancy groups. We also examined the association in normal weight (body mass index, <25) and overweight (body mass index, ≥25) women separately, because obesity is a known risk factor for preeclampsia (2). The prevalence of severe preeclampsia was 2.5% in overweight and 1.2% in normal weight women. The protective effect of any probiotic intake was evident in both groups but was stronger in normal weight women (crude OR = 0.74, 95% CI: 0.58, 0.95) than in overweight women (OR = 0.83, 95% CI: 0.62, 1.10).

Table 3. Association Between Probiotic Milk Consumption (Yes or No) During Pregnancy and Preeclampsia (All Subtypes) and Subtypes, the Norwegian Mother and Child Cohort Study, 2002–2008

	No.	%	Probiotic Consumers, %	Crude Model		Adjusted Model[a]	
				OR	95% CI	OR	95% CI
Preeclampsia all types							
Yes	1,755	5.3	35.7	0.83	0.75, 0.92	0.91	0.82, 1.01
No	31,644	94.7	40.0				
Subtypes by time of onset							
Early onset preeclampsia[b]							
Yes	169	0.5	37.9	0.91	0.67, 1.25	1.05	0.76, 1.45
No	31,644	94.7	40.0				
Late-onset preeclampsia							
Yes	1,586	4.7	35.4	0.82	0.74, 0.91	0.90	0.81, 1.00
No	31,644	94.7	40.0				
Subtypes by clinical severity							
Mild preeclampsia							
Yes	997	3.0	36.4	0.86	0.75, 0.98	0.95	0.83, 1.08
No	31,644	94.7	40.0				
Severe preeclampsia[c]							
Yes	514	1.5	32.7	0.73	0.60, 0.88	0.79	0.66, 0.96
No	31,644	94.7	40.0				
Unspecified preeclampsia							
Yes	244	0.7	38.9	0.95	0.75, 1.19	1.02	0.81, 1.31
No	31,644	94.7	40.0				

Abbreviations: CI, confidence interval; HELLP, hemolysis, elevated liver enzymes, and low platelet count; OR, odds ratio.

[a] Adjusted for prepregnancy body mass index, height, education, smoking in pregnancy, intake of fiber, energy, nonprobiotic milk, and nonprobiotic yogurt.

[b] Diagnosed before 34 weeks.

[c] Severe preeclampsia including eclampsia and HELLP syndrome.

DISCUSSION

In this study, we investigated the relation between intake of probiotic milk products during the first half of pregnancy and the risk of developing preeclampsia. High intake of probiotics (daily intake of at least 140 mL) was associated with reduced risk of all preeclampsia (OR = 0.80, 95% CI: 0.66, 0.96), although the association was most prominent for severe preeclampsia (OR = 0.61, 95% CI: 0.43, 0.89). The results indicated a weak dose-dependent protection with increasing intake compared with no intake.

Preeclampsia is one of the leading causes of maternal and perinatal morbidity and mortality also in developed countries (2). The etiology and pathogenesis of preeclampsia are not fully understood, but increasing evidence suggests an excessive maternal systemic inflammatory response to pregnancy (4, 5, 33, 34). Previous studies in MoBa reported reduced risk of preeclampsia with use of vitamin D-containing supplements (32) and adherence to a healthy dietary pattern (15). The underlying biologic explanation for the association of these dietary components with preeclampsia remains unclear but may be related to modification of immunologic, oxidative, and inflammatory responses to pregnancy. Dietary fiber is an essential constituent of a healthy diet, reflecting intake of vegetables, fruits, and unrefined grains, and has been shown to reduce systemic inflammation (17). A previous study in MoBa reported a protective effect of probiotic milk products on preterm delivery (20). Compared with women reporting no intake, those reporting daily intake of probiotics had significantly lower risk of spontaneous preterm delivery. Women who developed preeclampsia were not included in the previous study. Both spontaneous preterm delivery and preeclampsia have been associated with increased levels of systemic inflammatory markers (5, 35).

Our biologic working hypothesis was dual with both a probiotic-mediated local modifying effect on placental trophoblasts and a modifying effect on overall systemic inflammation levels. We observed a protective effect of probiotic intake and an association between moderate or high intake of probiotic dairy products and severe preeclampsia (Table 4). The results are especially interesting in light of a study by Yeganegi et al. (22), in which supernatant fluid of *L. rhamnosus* GR-1 was found to influence the lipopolysaccharide response in placental trophoblast cells, connecting an observable modification of inflammatory response directly to cell types highly interesting in the development of

Table 4. Association Between Probiotic Milk Consumption (in mL) During Pregnancy and Preeclampsia, the Norwegian Mother and Child Cohort Study, 2002–2008

Probiotic Consumption Categories[a]	Total (n = 33,399)		All Preeclampsia (n = 1,755)[b]		Crude		Adjusted[c]	
	No.	%	No.	%	OR	95% CI	OR	95% CI
No intake	20,104	60.2	1,129	5.6	1	Referent	1	Referent
Low	4,630	13.9	235	5.1	0.90	0.78, 1.04	0.97	0.84, 1.12
Moderate	5,357	16.0	255	4.8	0.84	0.73, 0.97	0.94	0.81, 1.08
High	3,308	9.9	136	4.1	0.72	0.60, 0.86	0.80	0.66, 0.96
	Total (n = 32,158)		Severe Preeclampsia[d] (n = 514)					
	No.	%	No.	%				
No intake	19,321	60.1	346	1.8	1	Referent	1	Referent
Low	4,467	13.9	72	1.6	0.90	0.70, 1.16	0.97	0.75, 1.26
Moderate	5,165	16.1	63	1.2	0.67	0.52, 0.89	0.75	0.57, 0.98
High	3,205	10.0	33	1.0	0.57	0.40, 0.82	0.61	0.43, 0.89

Abbreviations: CI, confidence interval; HELLP, hemolysis, elevated liver enzymes, and low platelet count; OR, odds ratio.

[a] Low—median: 13.2 mL/day, minimum: 6.6 mL/day, maximum: 13.2 mL/day (less than weekly); moderate—median: 28.5 mL/day, minimum: 19.7 mL/day, maximum: 114 mL/day (1–6 times weekly); high—median: 200 mL/day, minimum: 142 mL/day, maximum: 1,600 mL/day (greater than or equal to daily intake).

[b] All preeclampsia represents 5.3% of the total.

[c] Adjusted for prepregnancy body mass index, height, education, smoking, intake of fiber, energy, nonprobiotic milk, and nonprobiotic yogurt.

[d] Severe preeclampsia, including eclampsia and HELLP syndrome, represents 1.6% of the total.

preeclampsia. Preeclampsia is a hypertensive disorder involving abnormal angiogenic factors (4, 36). Clinical intervention trials of milk-based probiotic products have reported reduced blood pressure in nonpregnant individuals with probiotic intake (37, 38). It could be speculated that probiotics may influence the risk of preeclampsia through an indirect modification of blood pressure. A daily intake of probiotic milk products in the present study is comparable to the amounts sufficient to show effect on blood pressure in the prior mentioned intervention studies (37, 38).

The main strengths of this study include the large sample of nulliparous women and the link to the Medical Birth Registry of Norway. MoBa is a large pregnancy cohort, and participants were recruited from both urban and rural regions and represented all age and socioeconomic groups (23). The study has a prospective design, and information about dietary intake and potential confounders was collected prior to onset of preeclampsia. The food frequency questionnaire asks about dietary intake during the first 4–5 months of pregnancy and was developed and validated for use in this cohort (26, 29). However, dietary intake of probiotic milk products was assessed only once during pregnancy, and there may have been changes in consumption that have not been registered. Food frequency questionnaires are considered rather imprecise instruments and are especially challenging to answer for the first period of pregnancy when many women experience nausea and changes in appetite and eating patterns. Nevertheless, the validation study showed that, relative to a dietary reference method and a number of biomarkers, including iodine as a marker of milk and dairy intake, the MoBa food frequency questionnaire produces realistic estimates of habitual intakes and is a valid tool for ranking pregnant women according to high or low intakes of foods and nutrients (29, 30).

We reduced the possibility of confounding by adjusting for relevant factors, but in spite of this we cannot rule out the possibility that residual or unmeasured confounding may still exist. The intake of probiotic milk products was associated with characteristics indicative of a health-conscious lifestyle, with higher probiotic intake in nonsmoking, lean, and well-educated women (Table 2). We included dietary fiber among confounding variables in order to adjust for an overall healthy dietary behavior. The association between probiotic intake and severe preeclampsia was stronger in women with a body mass index of less than 25 than in the group having a body mass index of 25 or greater. Recent studies have established a link between increasing body mass index and low-grade systemic inflammation (39, 40). As maternal obesity is an established risk factor for all major pregnancy complications including gestational diabetes, preeclampsia, large-for-gestational-age babies, preterm births, and cesarean delivery (41–44), the increased possibility of other adverse pregnancy outcomes may explain why the influence of probiotic intake was not as pronounced in the high body mass index group.

The prevalence of preeclampsia in the present study is similar to that seen in the overall Norwegian population (MBRN). The validity of the preeclampsia diagnosis in

MBRN is presently being assessed. Preliminary results indicate a positive predictive value of the preeclampsia diagnosis of more than 80% (unpublished data). In this study, the information about diet and potential confounding factors was collected prior to the onset of preeclampsia. Thus, systematic (differential) misclassification of preeclampsia according to consumption of probiotic milk products seems unlikely. However, nondifferential misclassification may exist, but any misclassification of preeclampsia is likely to be randomly distributed across consumers and nonconsumers of probiotic milk products. Such nondifferential misclassification will result in underestimation of the true association between probiotic intake and preeclampsia.

In conclusion, this large observational study indicates an independent protective association between intake of probiotic milk products and preeclampsia, especially severe preeclampsia, suggesting that probiotics might specifically "target" and modify the type of inflammation underlying severe preeclampsia. Further strain/species-specific investigation is warranted with the use of randomized controlled trials for further evaluation of the effect of probiotics on preeclampsia.

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REFERENCES

1. Geographic variation in the incidence of hypertension in pregnancy. World Health Organization International Collaborative Study of Hypertensive Disorders of Pregnancy. *Am J Obstet Gynecol*. 1988;158(1):80–83.
2. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet*. 2005;365(9461):785–799.
3. Roberts JM. Preeclampsia: what we know and what we do not know. *Semin Perinatol*. 2000;24(1):24–28.
4. Dusse LM, Rios DR, Pinheiro MB, et al. Pre-eclampsia: relationship between coagulation, fibrinolysis and inflammation. *Clin Chim Acta*. 2011;412(1-2):17–21.
5. James JL, Whitley GS, Cartwright JE. Pre-eclampsia: fitting together the placental, immune and cardiovascular pieces. *J Pathol*. 2010;221(4):363–378.
6. Le J, Briggs GG, McKeown A, et al. Urinary tract infections during pregnancy. *Ann Pharmacother*. 2004;38(10):1692–1701.
7. Goswami D, Tannetta DS, Magee LA, et al. Excess syncytiotrophoblast microparticle shedding is a feature of early-onset pre-eclampsia, but not normotensive intrauterine growth restriction. *Placenta*. 2006;27(1):56–61.
8. von Dadelszen P, Magee LA, Roberts JM. Subclassification of preeclampsia. *Hypertens Pregnancy*. 2003;22(2):143–148.
9. Hu W, Wang H, Wang Z, et al. Elevated serum levels of interleukin-15 and interleukin-16 in preeclampsia. *J Reprod Immunol*. 2007;73(2):166–171.
10. Mellembakken JR, Aukrust P, Hestdal K, et al. Chemokines and leukocyte activation in the fetal circulation during preeclampsia. *Hypertension*. 2001;38(3):394–398.
11. Cudihy D, Lee RV. The pathophysiology of pre-eclampsia: current clinical concepts. *J Obstet Gynaecol*. 2009;29(7):576–582.
12. Borzychowski AM, Sargent IL, Redman CW. Inflammation and pre-eclampsia. *Semin Fetal Neonatal Med*. 2006;11(5):309–316.
13. Rumbold A, Duley L, Crowther CA, et al. Antioxidants for preventing pre-eclampsia. *Cochrane Database Syst Rev*. 2008;(1):CD004227.
14. Qiu C, Coughlin KB, Frederick IO, et al. Dietary fiber intake in early pregnancy and risk of subsequent preeclampsia. *Am J Hypertens*. 2008;21(8):903–909.
15. Brantsæter AL, Haugen M, Samuelsen SO, et al. A dietary pattern characterized by high intake of vegetables, fruits, and vegetable oils is associated with reduced risk of preeclampsia in nulliparous pregnant Norwegian women. *J Nutr*. 2009;139(6):1162–1168.
16. Longo-Mbenza B, Tshimanga KB, Buassa-bu-Tsumbu B, et al. Diets rich in vegetables and physical activity are associated with a decreased risk of pregnancy induced hypertension among rural women from Kimpese, DR Congo. *Niger J Med*. 2008;17(3):265–269.
17. North CJ, Venter CS, Jerling JC. The effects of dietary fibre on C-reactive protein, an inflammation marker predicting cardiovascular disease. *Eur J Clin Nutr*. 2009;63(8):921–933.
18. Lye HS, Kuan CY, Ewe JA, et al. The improvement of hypertension by probiotics: effects on cholesterol, diabetes, renin, and phytoestrogens. *Int J Mol Sci*. 2009;10(9):3755–3775.
19. Reid G, Dols J, Miller W. Targeting the vaginal microbiota with probiotics as a means to counteract infections. *Curr Opin Clin Nutr Metab Care*. 2009;12(6):583–587.
20. Myhre R, Brantsæter AL, Myking S, et al. Intake of probiotic food and risk of spontaneous preterm delivery. *Am J Clin Nutr*. 2011;93(1):151–157.
21. van Baarlen P, Troost F, van der Meer C, et al. Human mucosal in vivo transcriptome responses to three lactobacilli indicate how probiotics may modulate human cellular pathways. *Proc Natl Acad Sci U S A*. 2011;108(suppl 1):S4562–S4569.
22. Yeganegi M, Watson CS, Martins A, et al. Effect of *Lactobacillus rhamnosus* GR-1 supernatant and fetal sex on lipopolysaccharide-induced cytokine and prostaglandin-regulating enzymes in human placental trophoblast cells: implications for treatment of bacterial vaginosis and prevention of preterm labor. *Am J Obstet Gynecol*. 2009;200(5):532.e1–532.e8.
23. Magnus P, Irgens LM, Haug K, et al. Cohort profile: the Norwegian Mother and Child Cohort Study (MoBa). *Int J Epidemiol*. 2006;35(5):1146–1150.

24. Nilsen RM, Vollset SE, Gjessing HK, et al. Self-selection and bias in a large prospective pregnancy cohort in Norway. *Paediatr Perinat Epidemiol*. 2009;23(6):597–608.
25. Irgens LM. The Medical Birth Registry of Norway. Epidemiological research and surveillance throughout 30 years. *Acta Obstet Gynecol Scand*. 2000;79(6):435–439.
26. Meltzer HM, Brantsæter AL, Ydersbond TA, et al. Methodological challenges when monitoring the diet of pregnant women in a large study: experiences from the Norwegian Mother and Child Cohort Study (MoBa). *Matern Child Nutr.* 2008;4(1):14–27.
27. Lauritsen J. FoodCalc. Copenhagen, Denmark: Danish Cancer Society; 2006. (http://www.ibt.ku.dk/jesper/foodcalc). (Accessed February 2006).
28. Norwegian Food Safety Authority, Norwegian Directorate of Health, and Department of Nutrition, University of Oslo. The Norwegian Food Composition Table 2006. Oslo, Norway: Matportalen; 2006. (http://www.matportalen.no/Matvaretabellen).
29. Brantsæter AL, Haugen M, Alexander J, et al. Validity of a new food frequency questionnaire for pregnant women in the Norwegian Mother and Child Cohort Study (MoBa). *Matern Child Nutr.* 2008;4(1):28–43.
30. Brantsæter AL, Haugen M, Julshamn K, et al. Evaluation of urinary iodine excretion as a biomarker for intake of milk and dairy products in pregnant women in the Norwegian Mother and Child Cohort Study (MoBa). *Eur J Clin Nutr.* 2009;63(3):347–354.
31. Norwegian Medical Association, the Norwegian Society of Obstetrics and Gynecology. Clinical guidelines in obestetrics. (In Norwegian). Oslo, Norway: Norwegian Medical Association; 2005. (http://www.legeforeningen.no/id/78144.0). (Accessed October 2008).
32. Haugen M, Brantsæter AL, Trogstad L, et al. Vitamin D supplementation and reduced risk of preeclampsia in nulliparous women. *Epidemiology.* 2009;20(5):720–726.
33. Saito S, Shiozaki A, Nakashima A, et al. The role of the immune system in preeclampsia. *Mol Aspects Med*. 2007;28(2):192–209.
34. Szarka A, Rigó J Jr, Lázár L, et al. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array [electronic article]. *BMC Immunol*. 2010;11:59. (doi: 10.1186/1471-2172-11-59).
35. Romero R, Espinoza J, Kusanovic JP, et al. The preterm parturition syndrome. *BJOG*. 2006;113(suppl 3):17–42.
36. Shah DM. Preeclampsia: new insights. *Curr Opin Nephrol Hypertens*. 2007;16(3):213–220.
37. Agerholm-Larsen L, Raben A, Haulrik N, et al. Effect of 8 week intake of probiotic milk products on risk factors for cardiovascular diseases. *Eur J Clin Nutr.* 2000;54(4):288–297.
38. Aihara K, Kajimoto O, Hirata H, et al. Effect of powdered fermented milk with *Lactobacillus helveticus* on subjects with high-normal blood pressure or mild hypertension. *J Am Coll Nutr.* 2005;24(4):257–265.
39. Schmatz M, Madan J, Marino T, et al. Maternal obesity: the interplay between inflammation, mother and fetus. *J Perinatol*. 2010;30(7):441–446.
40. Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. *Am J Clin Nutr.* 2006;83(2):S461–S465.
41. Siega-Riz AM, Viswanathan M, Moos MK, et al. A systematic review of outcomes of maternal weight gain according to the Institute of Medicine recommendations: birthweight, fetal growth, and postpartum weight retention. *Am J Obstet Gynecol*. 2009;201(4):339.e1–339.e14.
42. Bhattacharya S, Campbell DM, Liston WA, et al. Effect of body mass index on pregnancy outcomes in nulliparous women delivering singleton babies [electronic article]. *BMC Public Health*. 2007;7:168. (doi: 10.1186/1471-2458-7-168).
43. Jansson N, Nilsfelt A, Gellerstedt M, et al. Maternal hormones linking maternal body mass index and dietary intake to birth weight. *Am J Clin Nutr.* 2008;87(6):1743–1749.
44. Kramer MS, Morin I, Yang H, et al. Why are babies getting bigger? Temporal trends in fetal growth and its determinants. *J Pediatr.* 2002;141(4):538–542.