Medicines Adverse Reactions Committee

Meeting date	**9 March 2023**	Agenda item	3.2.2
Title	**Probiotics and pre-eclampsia**		
Submitted by	Medsafe Pharmacovigilance Team	Paper type	For advice
Active constituent **Medicines** **Sponsors** Comprehensive list of probiotic supplements not available - see section 2.1.			
Funding	Not funded.		
Previous MARC meetings	No safety issues relating to probiotics have been discussed by the MARC previously.		
International action	N/a		
Prescriber Update	N/a		
Schedule	Dietary supplement (not medicines)		
Usage data	Not available for unfunded dietary supplements.		
Advice sought	**The Committee is asked to advise whether:** – Whether the currently available evidence supports a plausible causal relationship between probiotic supplementation and increased risk of pre-eclampsia, noting that the available information is for overweight or obese pregnant people only. – Whether any regulatory action or communication is required, other than MARC's remarks.		

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1.0 PURPOSE

Probiotics are widely available in New Zealand as dietary supplements to support gut health. There are a number of products that are marketed specifically for use in pregnancy and breastfeeding.

A recent Cochrane review analysed the use of probiotic supplements for the prevention of gestational diabetes mellitus (GDM) and related outcomes (see Annex 1). The review did not find evidence of a reduced risk of gestational diabetes with probiotics compared to placebo. However, the review did find evidence of an increased risk of pre-eclampsia with the use of probiotics.

This report reviews the available scientific information on a possible association between probiotic supplementation and pre-eclampsia.

2.0 BACKGROUND

2.1 Pre-eclampsia

Hypertensive disorders of pregnancy include pre-existing hypertension, gestational hypertension, pre-eclampsia, eclampsia and HELLP syndrome. Hypertension is defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg on at least two separate occasions [1].

Pre-eclampsia is defined as hypertension occurring after 20 weeks' gestation, either new in onset or superimposed on existing hypertension with the development of at least one additional clinical feature. Other clinical features of pre-eclampsia include proteinuria, renal insufficiency, elevated liver transaminases, neurological complications, haematological complications and uteroplacental complications. Severe pre-eclampsia may involve severe treatment-resistant hypertension (systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg), impaired liver function, progressive renal insufficiency, worsening thrombocytopenia, pulmonary oedema, HELLP syndrome (haemolysis, elevated liver enzymes, low platelet count), eclampsia (seizures) and worsening fetal growth restriction [1].

Signs and symptoms of severe pre-eclampsia include:

- Severe headache
- Visual disturbances
- Severe epigastric pain
- Shortness of breath
- Retrosternal pressure/pain
- Nausea, vomiting
- Sudden swelling of face, hands, or feet
- Hyperreflexia [1].

Pathogenesis

The clinical manifestations of pre-eclampsia arise from microangiopathy of target organs such as the brain, liver, kidney and placenta. Reduced blood flow to the placenta results in the release of antiangiogenic factors that cause maternal endothelial dysfunction with resulting hypertension, proteinuria and other manifestations. [2].

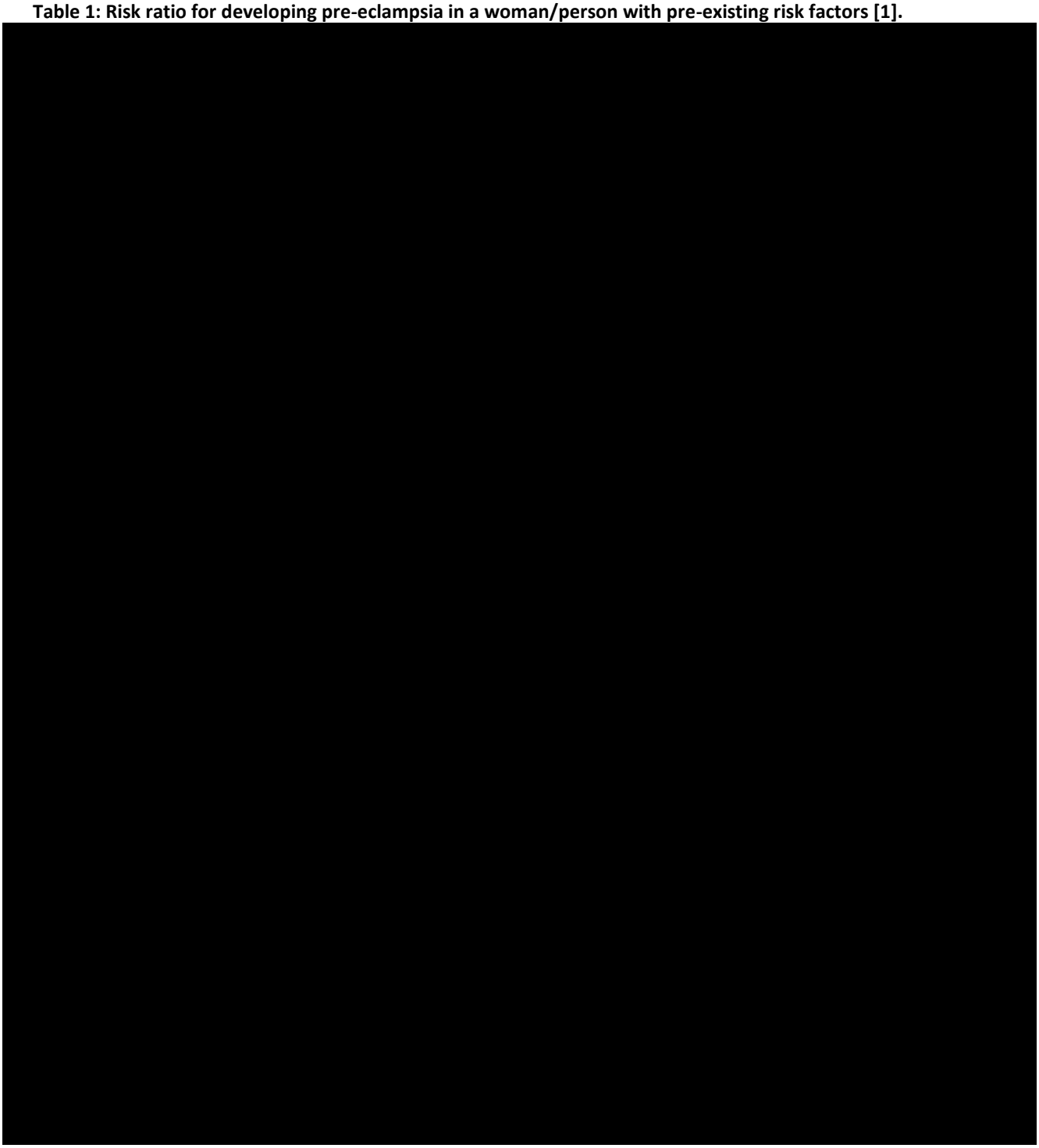
Abnormal placental development involves abnormal remodelling of spinal arteries and defective trophoblast differentiation. Placental hypoperfusion is both a cause and consequence of abnormal placental development. Antiangiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1) and endoglin are released that bind vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), which results in widespread maternal vascular inflammation, endothelial dysfunction,

and vascular injury. Immunological and genetic factors are thought to play a role in susceptibility to pre-eclampsia [2].

Risk factors

Risk factors, and relative risk/odds ratios for development of pre-eclampsia in the presence of these risk factors, are shown in Table 1.

Table 1: Risk ratio for developing pre-eclampsia in a woman/person with pre-existing risk factors [1].



ART = assisted reproductive technology; BMI = body mass index; CI = confidence interval; SLE = systemic lupus erythematosus.

Comments
Note that references 3 to 13 are referred to in the above table only.

Prognosis

Growth restriction, preterm birth, abruption and stillbirth can result from pre-eclampsia. Recurrence of pre-eclampsia and other hypertensive disorders in future pregnancies is common. Patients with hypertensive disorders of pregnancy are at increased risk of hypertension, cardiovascular disease and renal disease later in life [14].

Management

Patients should be educated about optimal weight gain and healthy eating. Low-dose aspirin is recommended starting from 12 to 16 weeks' gestation in people with a major risk factor for pre-eclampsia. Calcium supplementation is also recommended until birth for those with a major risk factor.

Antihypertensives such as labetalol, nifedipine or methyldopa should be considered for patients with gestational hypertension. Severe hypertension is treated urgently with labetalol, nifedipine or hydrazaline.

Antenatal monitoring consists of blood pressure monitoring, full blood count, electrolytes, creatinine, liver function tests, coagulation studies and monitoring of fetal growth.

Induction of labour may be needed if there is deterioration of the maternal or fetal condition [1].

2.2 Probiotics and pregnancy

Probiotics are defined as live micro-organisms which when administered in adequate quantities confer a health benefit on the host [15].

There are a wide variety of strains of bacteria included in commercially available probiotics. Most commonly, members of the genera *Lactobacillus*, *Bifidobacterium* and *Enterococcus* are included. Some examples of micro-organisms commonly included in probiotic products are listed in Table 2.

Table 2: Micro-organisms considered as probiotics [16]

The bacteria used as probiotics are non-pathogenic. However, theoretical risks associated with the use of probiotics include bacteraemia in immunocompromised patients, malabsorption due to disruption of bile salts, lactate production resulting in lactic acidosis, modification of immune responses and degradation of intestinal mucus [17].

Probiotics products are dietary supplements and as such there is no registration process in New Zealand. A complete list of probiotics marketed for use in pregnancy in New Zealand is not available. However, it is known that there are a number of probiotic products in New Zealand marketed specifically to people who are pregnant or breastfeeding. As dietary supplements, the labels state health benefits such as:

- 'Support baby's immune system development'
- 'Soothe baby's dry skin'
- 'Support baby's health and wellbeing'
- 'Supports immune system & gut health'
- 'Supports intestinal flora growth'
- 'Supports nutrient uptake'
- 'Supports pregnancy and breastfeeding'.

Relationship between gut microbiota and health

There is a complex relationship between gut microbiota and overall health. It has been demonstrated that diet and health conditions can influence the gut microbiome [18]. Conversely, the gut microbiota may influence metabolism and contribute to the development of chronic health conditions such as obesity and diabetes [19]. Women with gestational diabetes have been found to have different gut microbiota composition compared to normoglycaemic pregnant women [20]. It has been theorised that use of probiotics may alter the balance of gut bacteria and consequently be used for the prevention of conditions such as GDM.

Some studies have reported differences in the gut microbiome of women with pre-eclampsia. One study examining faecal samples from the SPRING trial reported lower abundance of butyrate-producing *Coprococcus* species was associated with late-onset pre-eclampsia. The authors previously reported an association between presence of butyrate-producing species and reduced systolic blood pressure [21].

A study found depletion of *Lactobacillus* in women with pre-eclampsia relative to healthy pregnant patients [22]. Another analysis found decreased abundance of bacteria of the family Bifidobacteriaceae, of the genus *Bifidobacterium,* of the phylum Actinobacteria and increased abundance of bacteria of the genus *Blautia* and *Ruminococcus* [23].

The precise relationship between the composition of the gut microbiome and the development of conditions such as GDM or pre-eclampsia has not been established.

Cochrane reviews on the use of probiotics to improve maternal and infant outcomes in women with gestational diabetes, and to prevent preterm birth, did not find any difference to placebo due to limited evidence [24, 25]. Benefits of probiotic supplementation on pregnancy outcomes do not appear to be supported currently by high-quality controlled trials.

3.0 SCIENTIFIC INFORMATION

3.1 Literature

3.1.1 Davidson et al (2021) – Probiotics for preventing gestational diabetes (Cochrane review) [26]

The full Cochrane systematic review is attached as Annex 1. This is an update of a previous version of the review (Barrett, 2014) [27].

Aim

This review aimed to systematically assess the effects of probiotic supplements used either alone or in combination with pharmacological and non-pharmacological interventions on the prevention of GDM.

Methods

Inclusion criteria: Randomised and cluster-randomised trials were eligible for inclusion. Quasi-randomised and crossover design studies were not included.

Studies were eligible for inclusion if pregnant participants had not previously been diagnosed with diabetes mellitus. Studies where participants had GDM in a previous pregnancy but no evidence of diabetes mellitus or GDM in the current pregnancy were also eligible.

Interventions included probiotic supplementation with or without pharmacological treatment or diet and lifestyle measures for prevention of GDM.

The Cochrane Core Outcome Set for GDM prevention was used for eligible outcomes. The primary maternal outcomes were diagnosis of GDM, hypertensive disorders of pregnancy and caesarean section. A large number of other short-term and long-term maternal outcomes, infant outcomes and health service use outcomes were also eligible for inclusion.

Search methods: A search of the Cochrane Pregnancy and Childbirth Trial Register was undertaken according to standard protocols. The World Health Organization International Clinical Trials Registry Platform and ClinicalTrials.Gov were searched for unpublished, planned and ongoing trials. The reference lists of retrieved trials were searched.

Selection and analysis of studies: The retrieved studies were assessed by two independent reviewers. Disagreement was resolved through discussion or adjudication by a third reviewer.

The studies were evaluated against standard, predefined criteria for scientific integrity and trustworthiness. Risk of bias assessment for the included studies was conducted against the criteria in the Cochrane Handbook for Systematic Reviews of Interventions. The risk of bias criteria relate to the methods and level of detail regarding:

- Random sequence generation
- Allocation concealment
- Blinding of participants and personnel
- Blinding of outcome assessment
- Incomplete outcome data
- Selective reporting
- Other bias.

Levels of attrition were noted and the impact was examined with sensitivity analysis. Analysis was on an intention-to-treat basis where possible. Heterogeneity was assessed and considered substantial if

the I^2 statistic was greater than 30% and either the Tau^2 was greater than zero, or there was a low P value (less than 0.10) in the Chi^2 test for heterogeneity.

The review used fixed-effect meta-analysis for combining data where the studies were estimating the same treatment effect (ie, population, intervention and methods were similar). For studies with significant clinical or statistical heterogeneity, random-effects meta-analysis was used to produce a summary which was treated as the mean range of possible treatment effects. A discussion of the clinical implications of treatment effects that were differing between trials was included.

Subgroup analysis was conducted for the primary outcomes. Data on family history of GDM or diabetes mellitus was not available. Sub-group analysis was conducted for probiotic dose, bacterial species and treatment duration.

The findings of the review were assigned a level of certainty of the evidence according to the GRADE Handbook.

Results

Description of studies: There was one study (Laitinen, 2009) included in the 2014 version of this review by Barrett et al. There were 43 new trials assessed for eligibility, of which two were excluded, three await further classification and eight are ongoing (see comments). Six new trials were added. A total of seven trials were included in the meta-analysis. These trials were conducted between 2002 and 2017. Two studies were excluded because the intervention was started during the third trimester of pregnancy, after GDM would be diagnosed.

All studies were parallel randomised controlled trials. The sample sizes ranged from 60 to 438 and the total number of participants included in the analysis was 1,647. The settings for the studies were Iran, Australia, Finland, Ireland and New Zealand. The participants were pregnant women with singleton pregnancies without pre-existing diabetes or other health conditions. However, two studies included women with a history of atopic disease. On review of the main publications, it appears that most of the trials did not exclude women with GDM in a previous pregnancy. However, the trial reported by Lindsay et al did exclude women with previous GDM, and it is unclear whether these women were excluded from the Jamilian et al and Laitinen et al studies.

Two studies were conducted in overweight and obese pregnant women, two in obese women only and three studies did not exclude women based on body mass index (BMI). All studies compared probiotics to placebo. Two studies also included a dietary intervention and one included a fish oil capsule. Six trials started the intervention prior to 20 weeks' gestation and one trial started at 20 weeks’ gestation or later.

A variety of bacterial species were used in the probiotics across the studies: *Lactobacillus rhamnosus* GG, *Lactobacillus rhamnosus* HN001, *Lactobacillus acidophilus* LA5, *Lactobacillus casei, Lactobacillus salivarius* UCC118, *Bifidobacterium animalis* subspecies lactis BB12, *Bifidobacterium animalis* subspecies lactis 420 and *Bifidobacterium bifidum*. All probiotic interventions were administered as a once daily capsule. For the purpose of analysis of the effect of dose, studies were categorised as using doses greater than five billion CFUs or fewer than five billion CFUs. Three studies used a dose of fewer than five billion CFUs per species and four used a dose of greater than five billion CFUs per species.

Six studies reported the incidence of GDM and one study reported laboratory measurements of glucose metabolism. A variety of diagnostic criteria were applied (Table 4). Other outcomes included hypertensive disorders of pregnancy, caesarean sections, large-for-gestational-age (LGA) infants, perinatal mortality, and neonatal mortality or morbidity composite measures.

The characteristics of the studies are described in Table 3.

Table 3: Characteristics of included studies (adapted from Davidson, 2021) [26]

Author, year	Design	Setting	Participants	BMI (kg/m^2)	Sample size	Interventions	Probiotic formulation	Intervention started	Primary outcomes	Diagnostic criteria**	Risk of bias
Callaway, 2019*	Parallel randomised controlled trial, double blind	Australia	< 16 weeks' gestation (changed to < 20 weeks' gestation during the study) Aged > 18 years	>25	433	Probiotic (n = 219) Placebo (n = 214) Enrolment until birth	*Lactobacillus rhamnosus* GG 10^9 CFU *Bifidobacterium lactis* BB12 10^9 CFU	< 20 weeks' gestation*	Diagnosis of GDM	IADPSG	Low
Jamilian, 2016	Parallel randomised controlled trial, double blind	Iran	< 20 weeks' gestation Aged 18-37 years	Any	60	Probiotic (n = 30) Placebo (n = 30) Taken for 12 weeks	*Lactobacillus acidophilus* 2 x 10^9 CFU *Lactobacillus casei* 2 x 10^9 CFU *Bifidobacterium bifidum* 2 x 10^9 CFU	< 20 weeks' gestation	Insulin levels	N/a	Method of allocation conceal-ment unclear
Laitinen, 2009	Parallel randomised controlled trial, double blind for probiotics/placebo, single blind for dietary intervention	Finland	< 17 weeks' gestation	Any	256	Probiotic + dietary intervention (n = 85) Placebo + dietary intervention (n = 86) Placebo + routine diet (n = 85) Early pregnancy to end of exclusive breastfeeding	*Lactobacillus rhamnosus* GG, ATCC 53 103 10^{10} CFU *Bifidobacterium lactis* BB12 10^{10} CFU	< 20 weeks' gestation	Maternal glucose metabolism	Modified Fourth International Workshop-Conference on GDM	Low
Lindsay, 2014*	Parallel randomised controlled trial, double blind	Ireland	< 20 weeks' gestation Aged >18 years	30-39.9	175	Probiotic (n = 83) Placebo (n = 92) From 24 to 28 weeks' gestation	*Lactobacillus salivarius* UCC118 10^9 CFU	> 20 weeks' gestation	Change in fasting maternal glucose*	Carpenter and Coustan	Low
Okesene-Gafa, 2019*	Parallel 2×2 factorial randomised controlled trial, double blind for probiotic, no blinding for dietary intervention	New Zealand	12–17.6 weeks' gestation	≥ 30	230	First randomisation: Dietary intervention (n = 116) Routine diet (n = 114) Second randomisation: Probiotic (n = 115) Placebo (n = 115) From enrolment to delivery	*Lactobacillus rhamnosus* GG 7 x 10^9 *Bifidobacterium lactis* BB12 7 x 10^9	< 20 weeks' gestation	Proportion of women with excessive gestational weight gain and infant birthweight*	IADPSG	Low

Author, year	Design	Setting	Participants	BMI (kg/m^2)	Sample size	Interventions	Probiotic formulation	Intervention started	Primary outcomes	Diagnostic criteria**	Risk of bias
Pellonpera, 2019*	Parallel 4-arm randomised controlled trial of 2 interventions, double blind	Finland	< 18 weeks' gestation	≥ 25	438	Probiotics + fish oil (n = 109) Probiotics + placebo (n = 110) Placebo + fish oil (n = 109) Placebo + placebo (n = 110) From enrolment to six months post-partum	*Lactobacillus rhamnosus* HN001 10^{10} CFU *Bifidobacterium animalis* ssp lactis 420 10^{10} CFU	< 20 weeks' gestation	Prevalence of GDM and fasting glucose levels*	IADPSG Finnish criteria	Low
Wickens, 2017	Randomised controlled trial, double blind	New Zealand	14-16 weeks' gestation Aged ≥ 16 years	Any	423	Probiotic (n = 212) Placebo (n = 211)	*Lactobacillus rhamnosus* HN001 6×10^9 CFU	< 20 weeks' gestation	Infant eczema and atopic sensitisation at age 12 months	IADPSG Australasian Diabetes in Pregnancy Society	Low

*****Studies reporting secondary outcomes of hypertensive disorders of pregnancy, pre-eclampsia

**See Table 4 for a comparison of the diagnostic criteria used.

Table 4: Diagnostic criteria for GDM used in the included studies [26]

GDM: Analysis of the six studies reporting GDM (including 1,440 participants in total) showed a non-significant reduction in the risk of GDM with probiotics (Table 5). The risk ratio was 0.80 (95% CI: 0.54 – 1.20). There was substantial heterogeneity between the studies.

Subgroup analysis by dose gave a similar result for doses greater than 5 billion CFU per species (RR 0.67; 95% CI: 0.46 – 0.98). There was a non-significant increase in the risk of GDM for doses less than 5 billion CFU (RR 1.47; 95% CI: 0.94 – 2.30). A non-significant risk reduction was seen for probiotics containing *Lactobacillus rhamnosus* and *Bifidobacterium animalis* (RR 0.83; 95% CI: 0.5 – 1.37). The risk ratio for GDM associated with starting probiotics in early pregnancy was 0.78 (95% CI: 0.51 – 1.20) and the risk ratio when started after 20 weeks' gestation was 1.19 (95% CI: 0.35 to 5.70).

Table 5: Analysis of probiotics versus placebo for the outcome of gestational diabetes mellitus [26]

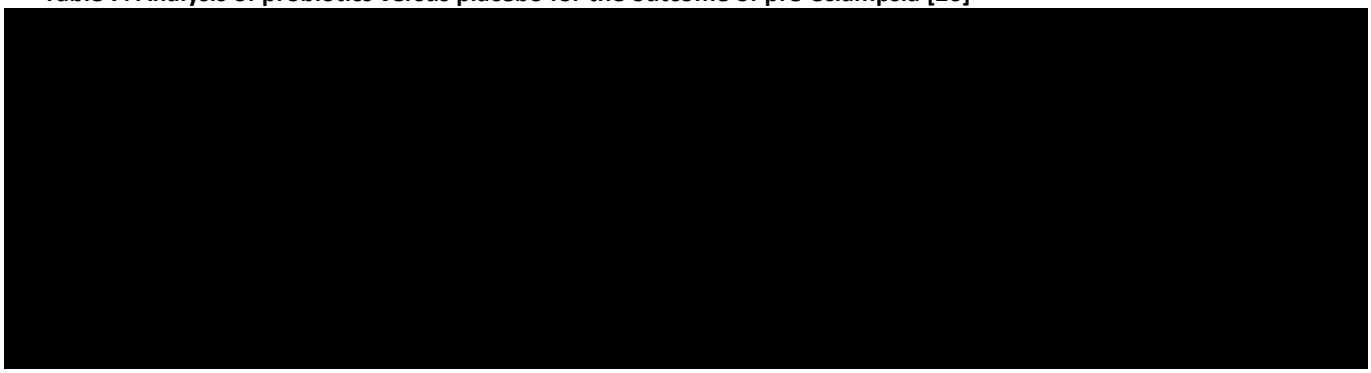
Hypertensive disorders of pregnancy: Four studies with a total of 955 participants reported the outcome of hypertensive disorders of pregnancy (Table 6). The risk was elevated with probiotics compared to placebo but the difference was not statistically significant. The risk ratio was 1.39 (95% CI: 0.96 – 2.01).

Subgroup analyses by dose, bacterial species and duration of treatment showed non-significant advantages for placebo over probiotics. Doses less than 5 billion CFU per bacterial species had a risk ratio of 1.35 (95% CI: 0.87 – 2.12) and doses greater than 5 billion CFU per species had a risk ratio of 1.47 (95% CI: 0.77 – 2.81). Subgroup analysis for probiotics containing *Lactobacillus rhamnosus* and *Bifidobacterium animalis* had a risk ratio of 1.35 (95% CI: 0.92 – 1.98). When probiotics were started in early pregnancy, the risk ratio was 1.35 (95% CI: 0.92 - 1.98) and when started after 20 weeks' gestation the risk ratio was 1.99 (95% CI: 0.49 – 7.99).

Table 6: Analysis of probiotics versus placebo for the outcome of hypertensive disorders of pregnancy [26]

Pre-eclampsia: Four studies with a total of 955 participants reported the outcome of pre-eclampsia (Table 7). The risk of pre-eclampsia was higher in those treated with probiotics compared to placebo and the difference was statistically significant. The risk ratio was 1.85 (95% CI: 1.04 – 3.29).

Table 7: Analysis of probiotics versus placebo for the outcome of pre-eclampsia [26]



Other outcomes: There were few differences for the outcomes of caesarean section, maternal weight gain, LGA infants or neonatal adiposity. The effects on perinatal mortality, composite neonatal morbidity and neonatal hypoglycaemia are unknown due to substantial heterogeneity and wide confidence intervals.

Discussion

There were a total of seven trials included in the Cochrane review with 1,647 participants. The findings of the review are summarised in Table 8 . Each finding was assigned a level of certainty based on the GRADE Working Group grades of evidence.

The risk ratio for GDM had wide confidence intervals and there was substantial heterogeneity between the studies. The review identified eight ongoing studies that will add to the body of evidence.

Table 8: Summary of findings - probiotics compared to placebo for preventing gestational diabetes (maternal outcomes) [26]

Authors' conclusions

'Probiotics may increase, decrease or make little to no difference in the risk of gestational diabetes mellitus (GDM), although the current evidence is of low certainty due to concerns regarding imprecision and inconsistency. While analysis revealed a small reduction in insulin levels with probiotics, this is unlikely to be clinically meaningful. Given the substantial heterogeneity observed between studies in the risk of GDM, there may be certain populations in which probiotics are effective, but there is currently insufficient evidence to identify these populations.

High-certainty evidence suggests that probiotics probably increase the risk of pre-eclampsia and could increase hypertensive disorders of pregnancy but the 95% confidence intervals for hypertensive disorders of pregnancy includes the possibility of no effect. While further research is needed to explore the underlying potential physiology of this relationship, given the potential risk of harm and little observed benefit, we urge caution in using probiotics during pregnancy at this time [26].'

Comments

It is noted that pre-eclampsia was a secondary outcome for the studies in the analysis and therefore some risk factors for pre-eclampsia may not be balanced across the study arms. However, the risk factors for GDM and pre-eclampsia are similar. Some common risk factors for pre-eclampsia (eg, BMI

> 25 kg/m^2, older age, pre-existing diabetes, chronic hypertension, nulliparity, multifetal pregnancy) were either excluded from the studies or reported and balanced across the arms. However, the possibility that some unreported risk factors for pre-eclampsia were unbalanced cannot be ruled out.

Each of the included studies used different bacterial species in the probiotic formulations. Three of the four studies reporting hypertensive disorders of pregnancy, included *Lactobacillus rhamnosus* and *Bifidobacterium animalis* in the probiotic formulation. It is unknown how the probiotic formulation may influence the apparent harmful effect on risk of pre-eclampsia and whether this effect might be generalisable to all probiotics. The wide variety of bacterial species and doses in probiotics makes analysis of specific bacterial species difficult.

The potential risk of harm is especially concerning as the studies do not indicate a benefit for probiotics in preventing GDM. The health benefits of probiotics in relation to pregnancy outcomes are not currently supported by high quality randomised controlled trials.

Of the eight ongoing studies, two have been completed with journal articles published since publication of the Cochrane review.

Godfrey et al (2021) reported on a randomised controlled trial to investigate whether a nutritional formulation containing *myo*-inositol, probiotics and multiple micronutrients could improve gestational glycaemia when taken pre-conception and throughout pregnancy. The study did not show an improvement in glycaemia in the treatment group (n = 870) compared with placebo and did not include any hypertensive disorders of pregnancy as secondary outcomes [28].

Halkjaer et al (2020) reported on a randomised controlled trial to investigate the effect of a daily probiotic on GWG, GDM and HbA1c in obese pregnant women. The probiotic contained *Streptococcus thermophilus* DSM 24,731, bifidobacteria (*Bifidobacterium breve* DSM 24,732, *Bifidobacterium longum* DSM 24,736, *Bifidobacterium infantis* DSM 24,737) and lactobacilli (*Lactobacillus acidophilus* DSM 24,735, *Lactobacillus plantarum* DSM 24,730, *Lactobacillus paracasei* DSM 24,733*, Lactobacillus delbrueckii subsp. bulgaricus* DSM 24,734*).* The study included 50 women, allocated 1:1 to probiotic or placebo. The trial did not find a significant difference between the groups with regard to GWG, GDM or HbA1C. There were six diagnoses of pre-eclampsia, of which three were in the probiotic group and three were in the placebo group. There were 11 cases of hypertension, of which six were in the probiotic group and five were in the control group [29].

There were three studies noted as awaiting classification.

Asgharian et al (2020) reported on a randomised controlled trial of a probiotic yoghurt for the improvement of gestational glycaemia. There were 128 women in the trial. The authors reported significantly lower plasma glucose (fasting and 2-h OGTT) in the probiotic yoghurt group compared to conventional yoghurt. There was no statistical difference between the groups for GDM or pre-eclampsia. There was one event of pre-eclampsia in the probiotic yoghurt group and no events in the conventional yoghurt group [30].

Si et al (2019) reported on the effectiveness of black garlic, prepared with or without *Lactobacillus bulgaricus*, in the prevention of GDM. There were 226 participants allocated to the groups in a 1:1 ratio. The authors reported that the probiotic intervention reduced blood glucose measurements (FBG, 1hBG, 2hBG). There was no difference in the outcome of pre-eclampsia, with 2 events in the control group and no events in the probiotic group [31].

The review also makes reference to a conference abstract from Charles et al (2018). A secondary analysis of the PrePro pilot study assessed the effects of probiotic supplementation (*Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-1) on the risk of GDM in pregnant people (n = 304). The study did not find a reduction in the incidence of GDM with probiotic supplementation and did not report on pre-eclampsia as an outcome [32].

The numbers of events of pre-eclampsia in the individual studies noted as ongoing or awaiting classification are too small to allow for meaningful comparison.

3.1.2 Callaway et al (2019) - Probiotics for the prevention of gestational diabetes mellitus in overweight and obese women: findings from the SPRING double-blind randomized controlled trial [33]

This study was included in the Cochrane meta-analysis and is attached as Annex 2.

Aim

This study aimed to determine whether probiotics (*Lactobacillus rhamnosus* and *Bifidobacterium animalis* subspecies *lactis*) administered from the second trimester in overweight and obese women prevent GDM as assessed by an oral glucose tolerance test (OGTT) at 28 weeks' gestation.

Methods

Setting: The study was conducted at the Royal Brisbane and Women's Hospital (RBWH), Redcliffe Hospital, and the Mater Mothers' Hospital in Brisbane, Australia. Recruitment started in November 2012.

Participants: The participants were people aged over 18 years with singleton pregnancies at less than 20 week's gestation and with a BMI greater than 25 kg/m^2. The participants underwent a random venous plasma glucose (RVPG) test prior to enrolment. Those with RVPG greater than 8.0 mmol/L proceeded to an oral glucose tolerance test (OGTT) and were excluded if any values met or exceeded criteria for GDM. People with pre-existing diabetes, impaired glucose tolerance, concomitant medicines that affect glucose metabolism, known major fetal abnormality or prior ingestion of probiotics were also excluded. Patients with prior GDM were not excluded.

Intervention: Participants were randomly assigned to take probiotics or placebo once daily, beginning prior to 20 weeks' gestation and continuing until birth. The composition of the probiotic was 10^9 CFU each of *Lactobacillus rhamnosus* (LGG) and *Bifidobacterium animalis* subspecies *lactis* (BB-12).

Compliance with the treatment regimen was monitored through patient interviews and checked in a subset of patients by testing faecal samples for BB-12 DNA.

Outcomes:

The primary outcome of the study was the frequency of GDM at 28 weeks' gestation by a 75-g OGTT using IADPSG criteria. The secondary maternal outcomes were gestational weight gain, preeclampsia, hypertensive disorders of pregnancy, caesarean delivery, and gestational age at delivery. The secondary neonatal outcomes were prematurity, neonatal special care admission, jaundice, hypoglycemia, birth weight, SGA, large for gestational age, stillbirth, birth injury, congenital anomaly, fat-free mass, and percentage fat.

Analysis: Intervention group comparisons were analysed using binary logistic regression or general linear models as appropriate, with adjustment for centre and BMI category. Adjusted odds ratios and difference of means with 95% CIs were reported. Where a model did not converge due to small cell counts, the effect estimate and P value were not reported.

Results

There were 204 participants in the placebo group and 207 participants in the probiotic group. The characteristics of the patients were similar between the two groups. The probiotic group had a slightly higher proportion of participants with a BMI of 30-39 (55.1% vs 49.5%) and a lower

proportion of participants with a BMI of 25-29 (31.9% vs 37.3%). However, the median BMI and interquartile range were similar between the groups.

For the outcome of GDM, there were 25 occurrences in the placebo group and 38 occurrences in the probiotic (Table 9). The odds ratio (OR) was 1.62 (95% CI: 0.91 to 2.89). The association was not statistically significant. There was a small, statistically significant increase in fasting glucose in the probiotic group that is unlikely to be clinically significant.

For the outcome of pre-eclampsia, there were 10 occurrences in the placebo group and 19 occurrences in the probiotics group (Table 10). The OR was 2.00 (95% CI: 0.91 to 2.89). The association was not statistically significant.

Table 9: GDM at 28 weeks' gestation (primary outcome) [33]

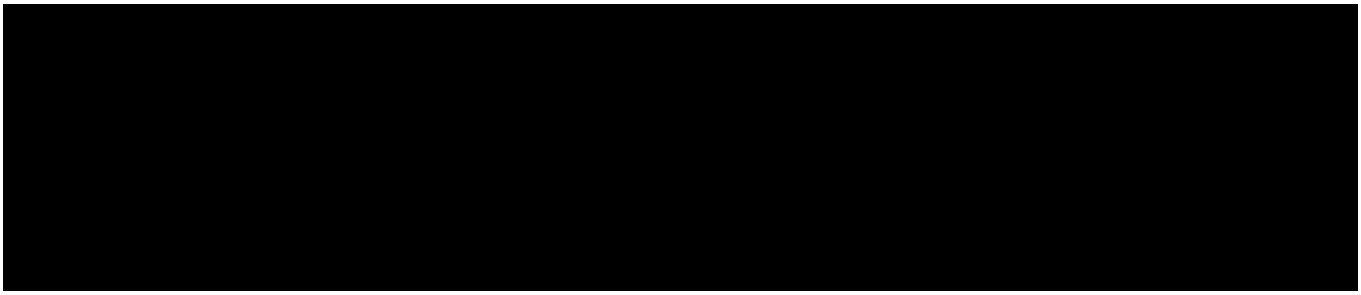


Table 10: Secondary maternal outcomes [33]

Conclusions

The administration of probiotics prior to 20 weeks' gestation did not reduce the frequency of GDM or any of the secondary outcomes at 28 weeks' gestation.

3.1.3 Lindsay et al (2014) - Probiotics in obese pregnancy do not reduce maternal fasting glucose: a double-blind, placebo-controlled, randomized trial (Probiotics in Pregnancy Study) [34]

This study was included in the Cochrane meta-analysis and is attached as Annex 3.

Aim

This study aimed to investigate the effect of a probiotic capsule on maternal fasting glucose in obese pregnant women.

Methods

Setting: The study recruited from antenatal clinics at the National Maternity Hospital, Dublin, Ireland.

Participants: Pregnant people with singleton pregnancies at fewer than 20 weeks' gestation, with a BMI between 20 and 39.9. People with prior GDM or diabetes mellitus or fetal anomalies were excluded.

Intervention: The participants were randomly assigned to take a probiotic or placebo capsule once daily from 24 to 28 weeks' gestation. The composition of the probiotic was *Lactobacillus salivarius* UCC118 10^9 CFU. All participants were provided with information on healthy eating and asked to avoid consuming other probiotic-containing supplements or foods for the duration of the study. Compliance was assessed by counting the capsules remaining in the bottle after two weeks and four weeks of treatment.

Outcomes: The primary outcome was change in maternal fasting glucose. Secondary maternal outcomes were GDM or impaired glucose tolerance (IGT) (according to Carpenter and Coustan criteria), metabolic variables, gestational weight gain, pre-eclampsia and delivery complications. Secondary fetal/neonatal outcomes were cord blood metabolic variables, fetal growth, Apgar score and NICU admission.

Analysis: BMI differed significantly between the groups. Therefore, an additional analysis for continuous variables was conducted by using the general linear model with BMI as the covariate and intervention group as the fixed factor. For categorical variables, binary logistic regression was used with placebo as the referent group. Statistical significance was set at $P < 0.05$ for the primary outcome. A Bonferroni correction was applied to analysis of all other outcomes to control for multiple comparisons. Significance was set at $P < 0.0017$ for the secondary outcomes.

Results

Of the 175 participants assigned to a treatment group, there were 63 participants in the probiotic group and 75 participants in the placebo group that completed the study and were included in the intention-to-treat analysis. There were 11 participants in the probiotic group and 16 participants in the placebo group that were excluded from the secondary analysis due to poor compliance or antibiotic usage. The characteristics of the participants were similar between the groups, with the exception of BMI which was significantly higher in the placebo group. There was a higher proportion of smokers in the placebo group (18.3% vs 6.8%) but this felt just short of statistical significance.

The effect size for diagnosis of IGT/GDM was 0.04 (-0.09 to 0.16). The effect size for diagnosis of pre-eclampsia was 0.03 (95% CI: -0.04 to 0.10) (Table 11).

Table 11: Maternal, fetal, and neonatal outcomes associated with a probiotic intervention in obese pregnant women (n = 138) [34]

Continuous variables are reported as means 6 SDs with the corresponding coefficient for the effect size; P values were calculated by using a general linear model with adjustment for maternal BMI. Categorical variables are reported as n (%) with the corresponding OR for the effect size; P values were calculated by using binary logistic regression with adjustment for maternal BMI. Significance was set at P , 0.0017 after Bonferroni correction for multiple comparisons. AAW, anterior abdominal wall; ACOG, American College of Obstetricians and Gynecologists; GDM, gestational diabetes mellitus; GWG,

gestational weight gain; IGT, impaired glucose tolerance; NICU, neonatal intensive care unit; PIH, pregnancy-induced hypertension.

Conclusions

There were no differences detected between the groups for the primary or secondary outcomes. This study did not show any effect of a four-week antibiotic intervention on maternal glycaemia, diagnosis of GDM/IGT or any other maternal outcome, including pre-eclampsia.

3.1.4 Okesene-Gafa et al (2019) - Effect of antenatal dietary interventions in maternal obesity on pregnancy weight-gain and birthweight: Healthy Mums and Babies (HUMBA) randomized trial [35]

This study was included in the Cochrane meta-analysis and is attached as Annex 4.

Aim

This trial aimed to determine whether a culturally tailored dietary intervention and/or daily probiotic capsules in obese pregnant woman reduce the co-primary outcomes of (1) excessive gestational weight gain (mean >0.27 kg/week) and (2) birthweight.

Methods

Design and setting: This was a single-centre, 2x 2 factorial randomized controlled demonstration trial in the Counties Manukau Health Region of South Auckland, NZ.

Participants: People with singleton pregnancies and BMI > 30 mg/m^2, at 12 to 17 weeks' gestation. The exclusion criteria were pre-existing diabetes or HbA1c ≥ 50 mmol/mol, known congenital abnormality, concomitant probiotic intake, previous bariatric surgery, severe hyperemesis, and medications or medical conditions that alter glucose metabolism.

Interventions: The dietary intervention consisted of an educational handbook, four home-based educational sessions on behaviour change techniques, positive reinforcement and motivational text messages. Routine dietary advice consisted of Ministry of Health pamphlets with no personalised input. Women allocated to probiotics received capsules containing *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB12 (minimum dose 6.5 x 10^9 CFU) which was taken once daily until birth. Compliance with capsules was assessed by verbal feedback.

Outcomes: The primary maternal outcome was the proportion of women with excessive gestational weight gain (GWG). The primary infant outcome was birthweight (adjusted for demographic factors). Secondary maternal outcomes included total GWG adjusted for gestation, OGTT and HbA1c results, GDM diagnosis by NZ criteria, pregnancy-induced hypertension, depression, anxiety and mode of delivery. Secondary infant outcomes included neonatal anthropometry, body composition, gestation at birth, LGA, SGA, NICU admission and composite neonatal morbidity.

Analysis: A 2-sided alpha level of <0.025 was specified for the co-primary outcomes (Bonferroni adjustment) and <0.05 for the secondary outcomes. Binary endpoints were analysed using modified Poisson regression models to estimate relative risks for each of the interventions. Continuous outcomes were calculated using generalised linear models to estimate any changes in outcomes with the interventions compared to controls. Primary analyses reported marginal effects for each randomized exposure, with adjustment for cointervention and prespecified covariates, as defined previously. Interactions between the main effects were tested for primary outcomes, although the trial was only powered for the main effects. Sensitivity analysis for the primary outcomes was performed in women who were compliant with the trial interventions.

Results

There were 115 people allocated to probiotic capsules and 115 people allocated to placebo capsules. In the probiotics group, seven people were not included in the intention-to-treat (ITT) analysis as they withdrew from the study or had missing outcome data. In the placebo group, seven people were not included in the ITT analysis due to missing outcome data. The demographic characteristics of those who declined to participate were similar to those who consented. The mean age of the participants was 28.8 years and the mean BMI was 38.8 kg/m^2. The characteristics of the participants were similar across the intervention groups.

The analysis of the primary maternal outcome of excessive GWG showed no difference between probiotics and placebo (RR 1.14; 95% CI: 0.99 to 1.31). There was no difference between probiotic and placebo for the diagnosis of GDM according to IADPSG criteria (RR 0.94; 95% CI: 0.59 to 1.49).

There were 11 cases of pregnancy-induced hypertension in the probiotic group and 7 cases in the placebo group, with a non-significant RR of 1.61 (95% CI: 0.64 to 4.09). The outcome of pre-eclampsia, which was included in the Cochrane analysis, was not reported in this publication.

Table 12: Maternal primary and secondary outcomes [35]

Conclusions

Neither the dietary intervention nor probiotic intervention had a significant effect on excessive GWG, GDM or pregnancy-induced hypertension.

3.1.5 Pellonpera et al (2019) - Efficacy of fish oil and/or probiotic intervention on the incidence of gestational diabetes mellitus in an at-risk group of overweight and obese women: a randomized, placebo-controlled, double-blind clinical trial [36]

This study was included in the Cochrane meta-analysis and is attached as Annex 5.

Aim

This study aimed to assess whether the risk of gestational diabetes mellitus (GDM) may be lowered and glucose metabolism improved by daily administration of fish oil and/or probiotic supplements in overweight and obese pregnant women.

Methods

Setting: Turku University Hospital and University of Turku in Finland with recruitment between October 2013 and July 2017.

Participants: Participants had a BMI greater than 25 kg/m^2, singleton pregnancy at fewer than 18 weeks' gestation, and no chronic diseases other than asthma or allergies. People with pre-existing diabetes or concomitant use of probiotics, fish oil or anticoagulants were excluded.

Interventions: The probiotic intervention consisted of a once daily capsule containing 10^{10} CFU each of *Lactobacillus rhamnosus* HN001 and *Bifidobacterium animalis* ssp. *lactis* 420. The intervention groups were probiotic + placebo, fish oil + placebo, probiotic + fish oil, and placebo + placebo. The interventions were taken from the first study visit until six months postpartum.

Outcomes: The primary outcome was the incidence of GDM (Finnish and IADPSG criteria) and the change in fasting plasma glucose between the early and late pregnancy study visits. Secondary outcomes included change in insulin and HOMA2-IR values, use of medicines for GDM, gestational hypertensive disorders, mode of delivery, postpartum haemorrhage, birth weight, and neonatal macrosomia.

Analysis: Postpartum haemorrhage was not normally distributed and was reported as median with interquartile range, and a Kruskal-Wallis test was applied when comparing the intervention groups. The comparisons of baseline characteristics, OGTT test result, GDM diagnosis, and maternal/neonatal outcomes among the intervention groups were conducted by one-way ANOVA for continuous variables and x2 test or Fisher exact test for categorical variables, when applicable. Differences in the change of glucose, insulin, and HOMA2-IR were also compared with one-way ANOVA. General linear models with binomial distribution and log link function were used to compare the relative risk of GDM in each intervention group with the placebo + placebo group. The effect of possible confounders for GDM diagnosis was analysed using the generalised linear model. Two-way ANOVA was used to analyse the effect of possible confounders for change in fasting plasma glucose, insulin, and HOMA2-IR.

Results

There were 439 women randomised to the intervention groups. The characteristics of the participants were similar across the groups, except there was a higher proportion of women with a family history of diabetes in the fish oil + placebo group.

There was no difference in the incidence of GDM, use of insulin or metformin, or OGTT results across the groups. The potential confounders were not found to have an effect on the results.

The number of diagnoses of pregnancy-induced hypertension was not significantly different across the groups (P = 0.8) (Table 13). The outcome of pre-eclampsia was also not different across the groups, with 4 diagnoses in the probiotic + placebo group, 4 in the fish oil + placebo group, 3 in the probiotic + fish oil group, and 2 in the placebo + placebo group.

Table 13: Pregnancy outcomes in the intervention groups [36]

Conclusions

The fish oil supplement, probiotic supplement, or a combination of the two did not lower the incidence of GDM. Fasting glucose or insulin resistance in overweight and obese women. The frequencies of pregnancy complications, including pre-eclampsia were similar across the group.

3.1.6 Nordqvist et al (2017). Timing of probiotic milk consumption during pregnancy and effects on the incidence of preeclampsia and preterm delivery: a prospective observational cohort study in Norway [37]

This study is attached as Annex 6. This study was not part of the Cochrane review as it is a cohort study.

Aim

This study aimed to investigate whether the timing of probiotic milk intake before, during early or late pregnancy influences associations with preeclampsia and preterm delivery.

Methods

Setting and design: This was a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health. Participants were recruited in Norway between 1999 and 2008. The cohort includes 114,500 children, 95,200 mothers and 75,200 fathers. The women completed questionnaires at gestational weeks 15, 22 and 30. The questionnaires covered diet, health, exposures, lifestyle and other background factors. The records were linked to the Medical Birth Registry of Norway (MBRN).

Exposures: The first and third questionnaires contained questions about intake of two different milk products containing probiotic lactobacilli before and during pregnancy. Exposure was defined in terms of 'cups/glasses per day' (0.5 L = 4 cups) and a participant was considered exposed if they consumed any amount of milk. The probiotic milk products were product A, containing *Lactobacillus acidophilus* (LA-5), *Bifidobacterium lactis* (Bb12), and *Lactobacillus rhamnosus* GG (LGG), and product B, containing *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium lactis* (Bb12). These were the only probiotic food items commonly available in Norwegian stores at the time of the study. The content of probiotic bacteria in these beverages is 10^8 probiotic bacteria/mL according to the manufacturer. Exposure was categorised as before pregnancy, during early pregnancy (prior to the first questionnaire at 15 weeks) or during late pregnancy (between the first questionnaire and third questionnaire at 30 weeks). Intake of probiotic supplements was not factored into the analysis as the proportion of women who took these was very low (< 0.5%).

Outcomes: The main outcomes were pre-eclampsia and preterm birth delivery as registered in the MBRN by doctors or midwives. Multiparous women were excluded from the pre-eclampsia analysis. Preterm delivery was defined as delivery between 22+0 and 36+6 weeks of gestation. Early (22+0 – 33+6) and late (34+0 – 36+6) spontaneous preterm delivery subgroups were analysed separately.

Analysis: The logistic regression models were adjusted for known risk factors for preeclampsia and preterm delivery. The maternal characteristics and lifestyle variables initially examined as potential confounding variables were maternal age, height and educational level, parity, history of late miscarriage, history of preterm delivery, BMI, marital status, smoking and alcohol intake during current pregnancy, household income, fetal sex, IVF, intake of non-probiotic milk, and use of dietary supplements as a marker of health-conscious behaviour.

Intake of probiotic milk in relation to maternal characteristics was examined using Pearson´s $\chi 2$, while mean intake of probiotic milk according to maternal characteristics was examined using the Kruskal Wallis test. Adjusted odds ratios were estimated for the association between intake of probiotic milk (as categorised variables) and preeclampsia and preterm delivery using multiple logistic regression models with exposure at all three periods and covariates in the model. In categorical variables, missing data were given a category of their own. In a sensitivity analysis, missing values regarding food/beverage frequencies were classified as non-consumers.

Results

There were 98,725 participants with singleton births of whom 91,038 completed the questionnaires. After exclusions for chronic health conditions and adverse pregnancy outcomes such as diabetes, GDM, hypertension and malformations, there were 70,149 pregnancies included in the study.

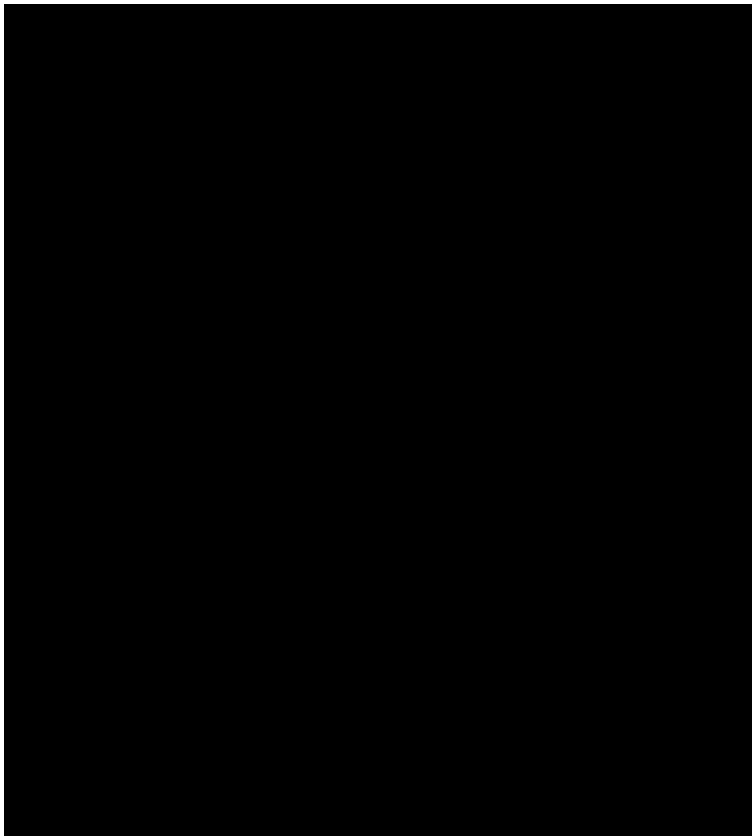
Consumption of probiotic milk was reported before pregnancy by 6,502 participants (mean 1.56 cups per day), in early pregnancy by 11,221 participants (mean 1.6 cups/day) and in late pregnancy by 12,784 participants (mean 1.51 cups/day). Intake of probiotic milk was more common in women who were older, primiparous, had BMI <25, did not smoke, used dietary supplements, consumed non-probiotic yoghurt, and had higher educational levels and family income. Alcohol intake during pregnancy was more common among probiotic consumers. Most participants reported exposure during more than one period (Figure 1).

Figure 1: Venn-diagram illustrating the probiotic consumption pattern among the study population during the three time periods [37].

n= 49,300.

There were 37,050 nulliparous women included in the pre-eclampsia analysis of whom 1,851 were diagnosed with pre-eclampsia. Intake of probiotic milk during late pregnancy was significantly associated with a lower risk of pre-eclampsia (aOR 0.80; 95% CI: 0.64 to 0.94), while there was no significant association with pre-pregnancy probiotic milk consumption and consumption during early pregnancy (Table 14). When the subtypes mild and severe preeclampsia were examined separately, a statistically significant association was only found for probiotic milk consumption during late pregnancy and severe preeclampsia (aOR 0.68; 95% CI 0.50 to 0.92). There was no significant dose response effect when consumption was divided into zero cups/day, one cup/day and more than two cups/day.

Table 14: Intake of probiotics before and during pregnancy and risk of preeclampsia in nulliparous women, n=37 050 [37].



In the 34,458 women included in the preterm delivery analysis, preterm delivery occurred in 2,858 cases, of which 1,795 were spontaneous preterm deliveries and 1,063 were iatrogenic preterm deliveries. There was a significant association between consumption of probiotic milk during early pregnancy and reduced risk of preterm delivery (aOR 0.79; 95% CI 0.64 to 0.97). There was no significant association with pre-pregnancy probiotic milk consumption and consumption during late pregnancy.

Conclusions

Probiotic milk intake may reduce the risk of preeclampsia and preterm birth and the timing of consumption may be relevant. Strengths of the study were a large sample size and linking to the national birth registry. A weakness was that exposures were self-reported retrospectively via two surveys and may not have been recalled accurately. Dietary habits may vary throughout pregnancy. The authors note that the analysis adjusted for a number of confounding variables, including education and income, there may have been unmeasured confounding.

Comments

A key limitation is that exposure was self-reported via surveys at two points during pregnancy (15 and 30 weeks' gestation) and may not have been recalled accurately. Exposure was categorised as being during early pregnancy (prior to the first questionnaire) and late pregnancy (between the first questionnaire and the third questionnaire). It appears that women answered the questionnaires at slightly different points in pregnancy, as the methods state that the first questionnaire at 15 weeks' gestation was answered on average at 17 weeks' gestation. Therefore, there may be misclassification of exposure. It appears that the questionnaire asked for consumption of probiotic milk as the average number of cups/glasses per day/week/month. This may vary greatly over the course of pregnancy and this question is unlikely to provide a precise measure of exposure. It does not appear that there was analysis by duration of exposure.

As there were substantial differences between consumers and non-consumers, the possibility of unmeasured confounding cannot be ruled out.

Data on probiotic milk may have limited relevance to probiotic supplements, especially given the role of calcium supplementation in the prevention of pre-eclampsia.

3.2 CARM data

There have been no relevant suspected adverse reaction reports for pregnant people taking probiotics.

4.0 DISCUSSION AND CONCLUSIONS

A recent Cochrane review analysed the available literature examining the impact of probiotic supplementation on the risk of GDM. There were six studies included in the review. The meta-analysis did not find a difference in the incidence of GDM with probiotics compared to placebo (RR 0.8; 95% CI: 0.54 to 1.20), with the evidence considered to be of low certainty according to GRADE criteria due to unexplained heterogeneity between the studies and wide confidence intervals.

The meta-analysis did not find any difference for any of the other maternal or neonatal outcomes, with the exception of pre-eclampsia. Four of the included studies (Callaway et al, Lindsay et al, Okesene-Gafa et al and Pellonpera et al) reported outcomes of hypertensive disorders of pregnancy, including pre-eclampsia, in overweight and obese women. There was a significantly higher risk of pre-eclampsia with probiotics compared to placebo (RR 1.85; 95% CI: 1.04 to 3.29) and the evidence

was considered to be of high certainty. A similar trend was seen for hypertensive disorders of pregnancy, although the effect was not statistically significant.

A key limitation of the analysis was the limited number of studies and small numbers of participants in the studies. As pre-eclampsia was a secondary outcome, it is possible that risk factors could be imbalanced across the arms, although many risk factors are shared with GDM. The probiotics studied differed across the studies and included a range of bacterial species and strains, making it difficult to ascertain whether any adverse treatment effects can be attributed to the presence of certain constituents in the formulation. There are eight ongoing studies awaiting classification for the Cochrane analysis which, if eligible for inclusion, may add information about the association.

The individual studies included in the analysis did not find any significant effect on GDM/markers of glucose metabolism or on the outcome of pre-eclampsia, both in the Cochrane analysis and in the original publications. It is noted that the numbers of events of pre-eclampsia in each study were small, resulting in wide confidence intervals. However, in each case the results trended towards favouring placebo.

A cohort study found a protective effect for pre-eclampsia when probiotic milk was consumed in late pregnancy, but not early pregnancy. The study was limited by imprecise quantification of exposure, and the potential for misclassification of exposure and inaccurate recall of exposure. As there were significant differences between the exposed and unexposed participants, there may have been residual confounding. Data on probiotic milk may have limited relevance to probiotic supplements, especially given the role of calcium supplementation in the prevention of pre-eclampsia.

The relationship between the composition of the gut microbiota and the outcome of pre-eclampsia is complex and requires further elucidation.

The possible increased risk of pre-eclampsia requires careful consideration due to the availability of probiotic products specifically marketed for use in pregnancy and the lack of high-quality evidence that probiotics improve pregnancy outcomes.

5.0 ADVICE SOUGHT

The Committee is asked to advise whether:

- Whether the currently available evidence supports a plausible causal relationship between probiotic supplementation and increased risk of pre-eclampsia, noting that the available information is for overweight or obese pregnant people only.
- Whether any regulatory action or communication is required, other than MARC's remarks.

6.0 ANNEXES

1. Davidson et al (2021) – Probiotics for preventing gestational diabetes (Cochrane review)
2. Callaway et al (2019) - Probiotics for the prevention of gestational diabetes mellitus in overweight and obese women: findings from the SPRING double-blind randomized controlled trial
3. Lindsay et al (2014) - Probiotics in obese pregnancy do not reduce maternal fasting glucose: a double-blind, placebo-controlled, randomized trial (Probiotics in Pregnancy Study)
4. Okesene-Gafa et al (2019) - Effect of antenatal dietary interventions in maternal obesity on pregnancy weight-gain and birthweight: Healthy Mums and Babies (HUMBA) randomized trial

5. Pellonpera et al (2019) - Efficacy of fish oil and/or probiotic intervention on the incidence of gestational diabetes mellitus in an at-risk group of overweight and obese women: a randomized, placebo-controlled, double-blind clinical trial

6. Nordqvist et al (2017) - Timing of probiotic milk consumption during pregnancy and effects on the incidence of preeclampsia and preterm delivery: a prospective observational cohort study in Norway

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