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Do Probiotics Prevent Gestational Diabetes in Obese/Overweight patients? A Systematic Review

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ABSTRACT

Introduction: While some studies suggest probiotic supplements may prevent Gestational Diabetes Mellitus (GDM), it is unclear if probiotics effectively prevent GDM among overweight and obese patients. This systematic review synthesizes recommendations for clinical practice and future research by evaluating the quality of evidence regarding *Lactobacillus* and *Bifidobacterium* containing probiotics to prevent GDM among obese and overweight patients.

Methods: PubMed, Embase, CINAHL, and Web of Science were searched using appropriate MeSH terms. Results were limited to randomized controlled trials published between 2011-2021. Titles and abstracts were screened for relevance after duplicates were removed. Included studies diagnosed GDM according to by International Association of Diabetes and Pregnancy Study Group criteria, suspended probiotic use prior to intervention, excluded participants with altered glucose metabolism, included participants with a BMI $\geq 25 kg/m^2$, and provided a specified dose of probiotic supplements. Articles without statistical analysis were excluded. Resulting articles were critically appraised using Version 2 of the Cochrane Risk of Bias tool.

Results: This search strategy resulted in 24 articles after duplicates were removed. Five double-blind randomized controlled trials found the incidence of GDM during the third trimester was not significantly different between probiotic and control groups. There was wide variation in the bacterial species, dose and duration of probiotic treatments used. All studies have a high risk of bias due to non-adherence to the treatment.

Discussion: This review used a highly sensitive criteria for GDM diagnosis that may mask a preventative effect of probiotics. Non-compliance may bias results towards the null given insufficient analysis of the effect of adhering to the intervention. No patterns between length of probiotic intervention or probiotic species and improved glucose tolerance were noted.

Conclusions: Current evidence is not sufficient to recommend probiotic supplements to prevent GDM in overweight and obese patients. Future evidence should address the effect of adhering to probiotic interventions and develop consistent probiotic intervention protocols.

INTRODUCTION

The rising prevalence of Gestational Diabetes Mellitus (GDM) represents a public health concern. During a healthy pregnancy, increased Human placental lactogen and decreased insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation decreases maternal insulin sensitivity to improve glucose availability for the fetus.[1,2] GDM refers to pathological insulin resistance that occurs when pancreatic β-cells cannot produce sufficient insulin to compensate for this increased demand.[3] GDM is associated with reduced tyrosine phosphorylation of IRS-1 and the insulin receptor's intracellular domain that attenuates insulin response and persists after delivery.[2] GDM affected 6% of pregnancies in the US from 2012-2016,[4] which increased from 0.3% of pregnancies from 1979-1980.[4,5] Despite the development of more sensitive diagnostic criteria, the rise in GDM has been attributed to the increasing prevalence of obesity as GDM is more common among patients whose body mass index (BMI) classifies as being overweight (BMI 25-29.9 kg/m^2) or obese (BMI ≥30 kg/m^2).[4]

The impact of GDM on healthcare spending and patient outcomes demands novel preventative measures. The total economic burden of GDM and complications was approximately $1.6billion in the USA during 2017.[6] Neonatal complications from GDM includes macrosomia, respiratory distress syndrome, and hyperbilirubinemia.[7–9] Neonates may also develop seizures, obesity and metabolic syndrome later in life.[7,8] Maternal long-term complications include type-2 diabetes mellitus and metabolic syndrome.[8] While diet and exercise can prevent GDM,[10] multiple reviews found no significant changes in the incidence of GDM when patients of diverse BMI categories were assigned to lifestyle interventions.[11,12,13] These results were attributed to poor self-efficacy and low adherence to recommendations, especially for overweight and obese patient populations with a higher prevalence of GDM.[4]

Host-microbiome interactions that influence glucose metabolism suggest modulating the gut microbiome using probiotics could be a novel target to prevent GDM. Hasain and colleagues (2020) have previously reviewed the molecular pathway by which gram-negative bacteria and lipopolysaccharides (LPS) in the colon can attenuate insulin signalling.[14] The mechanism by which *Bifidobacterium*[14] and *Lactobacillus*[15] modulate this pathway and improve glucose metabolism has also been described. This basic science research aligns with results from a randomized controlled trial (RCT) that found a statistically significant decreased risk of GDM among women who received *Lactobacillus rhamnosus* and *Bifidobacterium lactis* containing probiotics compared to those who did not, with a risk ratio of 0.37 (95% CI 0.15-0.89).[15] The majority of participants in this study had a healthy BMI, which limits the generalizability of this trial due to the high prevalence of obesity in the US.[16] This discrepancy is especially important, as obesity is associated with changes in the gut microbiome and a greater risk of GDM due to increased insulin resistance and low-grade inflammation.[17]

It is unclear if probiotics effectively prevent GDM among overweight and obese patients. A meta-analysis pooled results found probiotics did not significantly prevent GDM among obese and overweight patients, as diagnosed by International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria.[18] However, the substantial methodological diversity across RCTs limits the utility of this meta-analysis. This systematic review aims to synthesize recommendations for clinical practice and future research by evaluating the quality of evidence regarding *Lactobacillus* and *Bifidobacterium* containing probiotics to effectively prevent GDM, as defined by the IADPSG criteria, among obese and overweight patients.

METHODS

PubMed, Embase, CINAHL, and Web of Science were searched for articles investigating the incidence of GDM among overweight or obese women receiving probiotics. PubMed was searched using the following MeSH terms: probiotic, *Lactobacillus*, *Bifidobacterium*, overweight, obesity, gestational diabetes, gestational, and gestational weight gain. CINAHL, EMBASE, and Web of Science were searched using the same terms. Results were limited to RCTs published within the last 10 years. Literature searches resulted in 24 articles after duplicates were removed, as illustrated in **Figure 1**. Titles and abstracts were screened for relevance to the research question. Full texts of relevant articles were reviewed for inclusion and exclusion criteria by the first and single author of this review. Studies were included if:

1. GDM was diagnosed according to IADPSG criteria.[19] It is important to use IADPSG criteria as it provides a consistent criterion to compare research produced in different countries. This is the most sensitive definition of GDM, which will lead to more conservative results for prevention studies.
2. Participants received a specified dose of probiotics with a noted species of *Lactobacillus* or *Bifidobacterium*, or a placebo/vehicle control.
3. All participants had a BMI $\geq 25 kg/m^2$.
4. Probiotic use prior to the intervention was suspended.
5. Participants on medications or diagnosed medical conditions that alter glucose metabolism before the intervention period were excluded from the trial.

Articles were excluded if they did not provide results using statistical tests appropriate to the study design. Inclusion and exclusion criteria resulted in five selected articles, as shown in **Figure 1**.

The first author assessed the quality of evidence using Version 2 of the Cochrane Risk of Bias tool for randomized controlled trials (RoB2) after studying the RoB2 guidance document.[20] A narrative synthesis focused on critical appraisal and methodological heterogeneity of probiotic interventions was completed. An institutional review board was not needed given the nature of a systematic review.

Study Tabulations and Outcomes Measured

The incidence of GDM during the third trimester of pregnancy was the primary outcome in this review. Differences between intervention and control groups were determined by appropriate statistical tests for each study design, specifically odds ratios,[21,22] relative risks,[23] unpaired t-test[24] and ANOVAs.[25] The included primary studies completed statistical analysis using SPSS Version 21[21] SPSS Statistics 23[22], SAS 9.4[23,25], and R 3.2.3[24] Statistical significance was defined by $p<0.05$. The odds ration of developing GM in the probiotic group compared to placebo/vehicle controls along with 95% confidence intervals is listed in **Table 1**.

RESULTS

Probiotic Supplements on GDM

This literature search resulted in five RCTs summarized in **Table 1**. Sample sizes ranged from 49- 460 participants. Three studies included overweight and obese patients.[21,22,25] Two studies included obese patients only.[23,24] All studies included patients in their second trimester with inclusion criteria ranging from 12-22 weeks of gestation. Three studies compared the incidence of GDM among patients who received probiotic supplements and placebo/vehicle controls.[21,22,24] One study compared the incidence of GDM among those treated with a combination of probiotics and fish oil, probiotics only, fish oil only, or a double placebo control.[25] Another study compared the incidence of GDM among patients treated with probiotic

capsules, placebo capsules, dietary interventions, and routine dietary advice.[23] Three studies reported a decrease in the incidence of GDM among the intervention compared to the control group,[21,23,25] while two studies found an increase in GDM. Of the two studies that included obese women only: one study found decreased rates of GDM among the probiotic group compared to placebo controls,[23] while the other found an increase.[24] The incidence of GDM among those treated with probiotic supplements compared to placebo controls was not statistically significant in all five studies, as outlined in **Table 1**.

Probiotic Supplements on Maternal Outcomes

Although GDM results were not significant for all articles included in this review, there were several significant secondary outcomes. One study found fasting plasma glucose (FPG) was 4mg/dl lower (95% CI −6.9, −1.1) at 28 weeks gestation among the probiotic yogurt group compared to placebo controls when adjusted for baseline FPG and BMI, which was significant according to Analysis of Covariance (ANCOVA) (p value = 0.008).[21] The mean 2-hour oral glucose tolerance of 103.9 ± 21.0mg/dL in the probiotic group was significantly lower than the mean 2-hour oral glucose tolerance of 115.5 ± 26.3g/dL in the vehicle control group at 28 weeks of gestation according to ANCOVA tests (p value = 0.002).[21] Oral glucose tolerance at 1 hour was not significantly different between the two groups in this study.[21] Another study found the mean FPG of 77.5 ± 8.1mg/dL among women who received probiotic supplements was significantly higher than the mean FPG of 79.3 ±9.0 mg/dL among the placebo group at 28 weeks gestation according to a general linear model adjusted for clinical center and BMI category (25-29 kg/m^2, 30-39 kg/m, ≥40 kg/m^2) (p value = 0.049).[22] However, these changes are not clinically significant as changes in FPG do not cross the threshold for GDM diagnostic criteria. In addition, oral glucose tolerance at 1- and 2-hour time points were not statistically

significant between groups in this study.[22] A significant interaction between previous GDM and the probiotic group with respect to change in FPG was found in another study.[25] The change in blood glucose concentration was significantly different among the probiotic and fish-oil groups depending on the duration of the intervention or pre-pregnancy BMI after excluding women with GDM in early pregnancy.[25] There were no significant differences between FPG and oral glucose tolerance found in the two other studies.[23,24]

There were inconsistent results regarding the effect of probiotic supplements on gestational weight gain (GWG) and preeclampsia. One study found 33% of participants in the probiotic group experienced excessive GWG, which was significantly lower than 85% of participants receiving placebos at 28 weeks of gestation according to a binary logistic regression adjusted for clinical center and BMI category.[22] Mean GWG was not significantly different between the two groups. No study found a significant change in GWG between the probiotic and placebo-control groups. One study reported 10% of participants with preeclampsia in the probiotic treated group was greater than the 5% of controls according to a binary logistic regression adjusted for clinical center and BMI category (p value = 0.09).[22] However, no other study found a significant difference between preeclampsia or gestational hypertension.[21,23–25]

Methodological Heterogeneity Between Study Designs

Methodological diversity between study designs included in this review are summarized in **Table 2.** *Lactobacillus rhamnosus* and *Bifidobacterium lactis* were the most common strains of bacteria contained in probiotic supplements. Two studies that used these species observed a non-significant decrease in the incidence of GDM among the probiotic group compared to placebo controls,[23,25] while one study observed an increased rate of GDM among the intervention group.[22] One study provided the intervention group with probiotics containing *Lactobacillu*s

acidophilus and *Bifidobacterium lactis*, and observed a non-significant decrease in GDM rates compared to placebo controls.[21] This study provided probiotics via yoghurt compared to vehicle control created with the same starting bacteria. The other four studies provided probiotics using capsules. One study provided the intervention group with a multi-strain probiotic supplement containing strains of *Streptococcus*, *Bifidobacterium*, and *Lactobacillus.* This study found a non-significant increase in the incidence among GDM between groups.[24]

There were differences in regards to the daily dose of probiotics and duration of the intervention. The daily dose of probiotic supplements ranged from $5x10^8$ to $4.5x10^{11}$ colony forming units (CFUs). There did not appear to be a pattern with decreased GDM rates in the probiotic group compared to placebo controls reported by studies with higher doses of probiotics. The duration of probiotic supplementation ranged from 4 weeks total to 13 weeks of gestation to delivery. A decreased incidence of GDM in the intervention compared to the control group was not found with longer durations of treatment.

Each study defined adherence to probiotic interventions differently. One study did not define a threshold for compliance but reported a mean yogurt intake of 27.8/28days and 27.6/28days among the probiotic and conventional yogurt groups respectively.[21] Four studies provided different thresholds to define adherence to the intervention. For example, Hajlker *et., al* (2020) defined compliance at ≥80% of capsule intake, which was met by 21/25 participants in the intervention and 17/24 participants in the placebo group.[24] Another study defined compliance as ≥75% of capsule intake, which was met by 76% of participants assigned to either probiotic or placebo capsules.[23] In comparison, 81% of participants complied with dietary intervention in the same study. Pellonpera et., al (2019) considered probiotic intake ≥ 5 days per week as a threshold for compliance, which was met by 89% of participants among all four study groups.[25]

These figures were derived from self-reported data. Interestingly, one studied defined compliance at ≥ 75% self-reported capsule use, which was verified by faecal sample analysis.[22] While over 90% of participants in the probiotic group were compliant according to self-reported data, only 76% compliance was found on faecal sample analysis.

Quality Assessment

Four included studies had a low risk of bias arising from the randomization process in the studies, as indicated in **Table 3**. All studies randomly allocated participants to study groups and performed blocked randomization stratified by pre-pregnancy BMI categories to ensure each study group had an equal number of overweight and obese participants. Intervention and control groups were not statistically significant for all baseline characteristics included in two studies.[21,24] Despite adequate randomization, a significantly higher prevalence of family history for diabetes among the fish-oil/placebo group raised some concerns for bias in one study.[25] Two studies did not statistically identify differences among study groups at baseline,[22,23] which was considered a low risk of bias according to the RoB2.

All studies had a low risk of bias due to deviations from the intended interventions with respect to the assignment to the intervention. These double-blind placebo-controlled trials used appropriate measures to conceal the intervention assignment from participants, investigators and those involved in data analysis. All studies analyzed participants according to the intervention they have been assigned to as part of an intention to treat (ITT) analysis.

However, all studies had a high risk of bias due to deviations from the intended intervention with respect to adherence to the treatment. Despite adequate concealment of the intervention from participants and researchers, non-adherence to daily probiotic use in all studies was sufficient to raise concerns. Three studies did not complete an analysis to estimate the effect

of adhering to the intervention.[21,22,25] Two studies performed a per-protocol sensitivity analysis, which excluded participants who did not adhere to the probiotic or placebo capsule directions from further analysis.[23,24] A second study performed a per-protocol analysis for GWG outcome only.[23] The RoB2 does not consider per-protocol analysis an acceptable method for studying the effect of adherence.

Results from the included studies may be biased due to missing outcome data. Three studies included in this review have a low risk of bias due to missing data, as data was reported for 95% of randomized participants.[21,22,24] Two studies did not perform a sensitivity analysis to demonstrate outcomes were not biased by missing data.[23,25] However, it is unlikely that missing outcome data depended on the true value of measured outcomes, because the proportions of missing data were approximately equal between the study groups and reasons for missing data were consistent across study groups. For these reasons, the two studies raised some concern for bias due to missing data.

There was a low risk of bias due to the outcome measurements for all included studies. Four studies did not describe how blood glucose concentrations during the oral glucose tolerance test (OGTT) were measured.[21–24] Inappropriate methods may have been used, as portable blood glucose monitors have poor validity.[26] However, the non-differential bias on outcome measures and blinding of outcome assessors suggests a low risk of bias. One study specified that they measured blood glucose concentrations using an enzymatic hexokinase assay,[25] which is a valid method.[27] In addition, measuring blood glucose concentration to diagnose GDM is an automated test that requires no judgement from outcome assessors, which eliminates observer bias.

All studies had a low risk of bias due to the selection of the reported results. All studies analyzed results according to the pre-specified plan outlined in the methods section. There is

only one way to report GDM according to IADPSG guidelines, which eliminates bias due to selecting outcomes from multiple measures. All of these analyses eligible for consideration, as statistical tests between probiotic and placebo control groups at comparable time points were provided and appropriate for the study designs.

DISCUSSION

Although probiotic supplements did not significantly affect the development of GDM among overweight or obese patients, the high sensitivity of IAPDSG may not capture the preventative effect of probiotic supplements. A study from New Zealand that included of participants in all weight categories found probiotics contain *Lactobacillus rhamnosus* or placebo capsules resulted in comparable rates of GDM according to IAPDSG guidelines, but a significant decrease in the incidence of GDM among the probiotic group when using New Zealand's diagnostic criteria.[28] While IAPDSG enables research to compare the incidence of GDM across countries, the highly sensitive criteria for diagnosing GDM provides a high number of false positives may mask the potential preventative effect in probiotics.[29] It would have been helpful if primary studies reported the incidence of GDM according to population specific criteria in addition to IAPDSG, as primary studies included in this review included populations from Iran,[21] Australia,[22] Denmark,[24] New Zealand[23] and Finland.[25] However, the incidence of GDM diagnosed by Carpenter and Coustan criteria was not significantly different among overweight or obese participants at 28 weeks gestation who received *Lactobacillus* and *Bifidobacterium* containing probiotics or placebo control according to a generalized linear model adjusted for pre-pregnancy BMI (p value = 0.561).[30] Therefore, probiotics may not effectively prevent GDM among overweight or obese women who enter pregnancy in a state of increased insulin resistance regardless of the sensitivity of the diagnostic criteria.

One study reported significant differences in glucose tolerance. After excluding patients with a history of GDM, one study found a significantly decreased FPG and 2-hour glucose tolerance test in the probiotic group compared to placebo controls.[21] Although this difference in FPG may not be clinically significant, this improvement in glucose metabolism aligns with a study of patients from diverse weight groups that found 6 weeks of L. *casei, L. acidophilus* and *B. bifidum* containing probiotics significantly decreased FPG and insulin resistance compared to placebos.[31] However, an included study that did not exclude patients with a history of GDM or perform statistical tests to identify differences in sample characteristics at baseline found a significantly greater FPG among patients who received probiotics compared to placebos.[22] History of GDM may influence the effect of probiotics on glycemic control in obese and overweight patients, as another included article reported a significant interaction between GDM history and the intervention group with respect to changes in FPG.[25] This interaction was attributed to the association of previous GDM with decreased glucose values in the probiotic group when compared with the increase in fish oil and placebo group.[25] Patients with a history of GDM may have persistently altered cell signaling pathways, such as decreased IRS-1 tyrosine phosphorylation, attenuating a potentially preventative effect of probiotic supplements for subsequent pregnancies.[2] For this reason, it is recommended that future research excludes patients with a previous history of GDM among the study sample.

Outcomes related to GWG or preeclampsia may also be affected by the prevalence of previous GDM. One study reported excessive GWG was significantly lower among those receiving probiotics compared to placebos, while the mean GWG was comparable between both groups.[22] The higher proportion of participants with a history of GDM in the probiotic group may contribute to the significantly decreased excessive GWG and higher prevalence of

preeclampsia found in this group.[22] The four other studies reported no significant differences in GWG among probiotic and placebo-treated groups.[21,23–25] These results align with a study that found GWG was not significantly different among pregnant participants of diverse weight categories who received conventional or probiotic yoghurt enriched in *L. acidophilus* and *B. animalis*.[32] Another study found women of diverse weight categories who received L. *rhamnosus* and *B. lactis* containing probiotics were at a significantly decreased risk of central adiposity than placebo controls at 6 months post-partum.[33] Future studies should investigate the effect of probiotics at longer post-partum time points using multiple body composition outcomes. Although central adiposity is defined as a waist circumference >80 cm, which is not a suitable measure of body composition during pregnancy. A decreased hydration constant may underestimate fat mass in BMI calculations during pregnancy, although this would result in a non-differential bias.

The systematic quality appraisal is a strength of this review, which suggests the results may be biased due to the effect of adhering to the intervention. All RCTs completed an ITT analysis. However, high rates of non-compliance in an ITT analysis may increase type 2 error, as low rates of compliance can substantially impact on the power of an equivalence trial.[34] The threshold of capsule uptake to define compliance was inconsistent across all included studies. In addition, self-reported data likely over-estimated the proportion of compliant participants. Although two studies completed a per-protocol analysis,[23,24] such results may be biased by factors that influence participants' willingness or ability to comply with protocol guidelines. Future studies should measure compliance using fecal sample analysis and complete inverse probability weighting (IPW) to estimate causal effects, as suggested by the RoB2. As part of IPW, outcomes of each study arm are weighted by the inverse of the probability for receiving the

treatment they were assigned, which creates an average potential outcome based on a pseudo-population where every participant received the treatment value. Currently, there are no relevant studies that complete such an analysis.

The heterogeneity among included interventions is a limitation of this review. Differences between study designs, as summarized in **Table 2**, make it difficult to draw meaningful conclusions from pooled data from individuals. Future studies should apply a more consistent probiotic supplement and dosing schedule before the efficacy of probiotic supplements for preventing GDM among high-risk patients can be concluded.

While one might presume increased exposure to probiotics would have beneficial effects on glucose metabolism, this may not be the case for overweight and obese patients. An included study that provided four weeks of probiotic supplements starting at 24 weeks gestation found a significant decrease in FPG but no change in the incidence of GDM among study groups.[21] Similarly, a study of patients from diverse weight categories who received probiotic supplements for six weeks starting at 22 weeks gestations found a significant decrease in FPG.[31] However, GDM diagnosis was not a measured outcome in this study. This study design is comparable to the study design by Wickens *et., al* (2017), which found a significant decrease in FPG and GDM according to New Zealand guidelines compared to placebo controls.[28] Other studies included in this review provided probiotic supplements for a longer duration and began treatment earlier in gestation but found no significant differences in FPG or GDM.[22,24,25] While the optimal time to provide probiotic interventions during gestation is unclear, this comparison suggests that high risk patients may benefit from probiotic interventions later in gestation. Furthermore, the study that provided the lowest dose of probiotic supplements was the only included study to report an improvement in glycemic control among obese and overweight patients.[21] This dose was also

less than that provided in studies that improved glycemic control among normal-weight patients.[28,31] Potential differences in doses and treatment time for probiotic interventions to prevent GDM may relate to the rise in insulin resistance starting at 19-22 weeks of gestation and peak at 33-36 weeks of gestation among normal-weight patients compared to overweight or obese individuals who begin pregnancy with a higher insulin requirement. However, this argument conflicts with evidence from a meta-analysis reporting a dose-dependent improvement in glucose metabolism among GDM patients of diverse BMI categories.[35]

Current literature contains variation in bacterial species contained in probiotic supplements without any trends regarding the most effective combination for improving glycemic control. *L. acidophilus* and *B. lactis* containing probiotics were found to significantly decrease FPG among overweight and obese women.[21] However, probiotics containing *B. lactis* combined with *L. rhamnosus* did not produce such results among this patient population.[25] Conflicting results are also found among studies with healthy weight participants.[32,35] These inconsistencies suggest the effectiveness of probiotic species at improving glycemic control depends on the host gut microbiome at baseline. This explanation is likely, as *Lactobacillu*s and *Bifidobacterium* improve host metabolism by increasing the availability of substrates for enteric butyrate-producing bacteria.[37]

CONCLUSIONS

Current evidence is not sufficient to recommend probiotic supplements to prevent GDM in overweight and obese patients, as the incidence of GDM among intervention and control groups were not statistically significant in all included studies. The majority of articles included in this review did not report any adverse effects of probiotic supplements. Therefore, there is insufficient evidence to suggest probiotics should be avoided due to adverse effects or

contraindications. Clinical recommendations for preventing GDM among this high-risk patient population using probiotics may become more conclusive as future evidence addresses the effect of adhering to probiotic interventions using fecal sample analysis and IPW. In addition, a more consistent regime, possibly consistent Ing of lower dose probiotics later in gestation, should be established. Future studies may benefit from excluding participants with a history of GDM in addition to using multiple GDM diagnostic criteria and body composition measurements.

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