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Research Article

# The Relationship between Dietary Fibre Intake, *Lacticaseibacillus Rhamnosus* HN001 Supplementation, and Incidence of Gestational Diabetes in the Probiotics in Pregnancy Study

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## ABSTRACT

**Background:** The New Zealand Probiotic in Pregnancy study randomised women to receive either HN001 or placebo daily from 14-16 weeks' gestation until 6 months postpartum and showed that probiotic *Lacticaseibacillus rhamnosus* HN001 supplementation reduced gestational diabetes mellitus incidence. We investigated whether high dietary fibre intake was critical for achieving these benefits.

**Aim:** This study was a secondary analysis from the Probiotic in Pregnancy study aiming to investigate participants' macronutrient intake, particularly dietary fibre in relation to gestational diabetes and HN001.

**Methods:** Dietary fibre and macronutrient intake was estimated in 348/423 Probiotic in Pregnancy study women who completed three-day food diaries at 26-28 weeks gestation, just prior to their gestational diabetes screening test.

**Results:** Women who developed gestational diabetes (n=42) reported lower dietary fibre ($p$=0.027), higher total protein ($p$=0.001) and lower carbohydrate intake ($p$=0.013) compared to women without diabetes. Incidence of gestational diabetes was not significantly different by HN001 supplementation (9.4% in probiotic vs 14.6% in placebo, p=0.143), but was reduced by 4.6% for each gram increase in fibre intake (odds ratio 0.954; 95% confidence interval 0.914, 0.996; $p$=0.030). The percentage of total energy intake from fat was significantly lower among women with gestational diabetes who were taking HN001 compared to all other groups ($p$<0.05).

**Conclusion:** The development of gestational diabetes was associated with low dietary fibre intake. The probiotic effect on gestational diabetes did not differ by fibre intake.

## INTRODUCTION

The incidence of Gestational Diabetes Mellitus (GDM) is increasing globally, spanning up to 45% in some populations, depending on ethnicity, environment and diagnostic

criteria used [1-4]. Uncontrolled or poorly controlled GDM can result in short-term and long-term health consequences for the mother and her offspring [5,6]. Significantly, metabolic dysregulation caused by GDM in utero perpetuates the "vicious diabetes cycle" whereby the offspring is placed at greater risk of developing metabolic syndrome later in life conferred through fetal programming [7,8]. This trans-generational effect of GDM places considerable emotional, physiological, and economic burdens on both the mother and her offspring [8]. Therefore, the development of effective and sustainable preventive measures for GDM is crucial.

With growing evidence linking microbial imbalance with glucose intolerance [9-11], probiotics are a potential option for GDM prevention as compliance is better than dietary change [12]. Probiotics are defined as "live microorganisms that, when administered in adequate doses, confer a benefit to the host" [13]. The New Zealand Probiotic in Pregnancy (PiP) study demonstrated that probiotic *Lacticaceibacillus* (formerly *Lactobacillus) rhamnosus* HN001 ($6x10^9$ CFU/d) supplementation from 14-16 weeks' gestation may reduce the incidence of GDM in New Zealand women, particularly among older women (aged 35 years or older) and those with a history of GDM during previous pregnancies [14]. The results from this study were consistent with an earlier Finnish study, which demonstrated that probiotic *L. rhamnosus* GG ($10^9$ CFU/d) and *Bifidobacterium animalis* subspecies *lactis* Bb12 ($10^9$ CFU/d) combined with dietary counselling was effective for the prevention of GDM [15]. In contrast, other probiotic supplementation studies [16-18] have demonstrated that probiotic supplementation with *L. rhamnosus* (LGG) and *B. animalis* subsp. *lactis* (BB-12) (>$1x10^9$CFU/d), *L. rhamnosus* GG and *B. animalis* subsp. *lactis* BB12 (≥$6.5x10^9$ CFU/d) and *Ligilactobacillus salivarius* UCC118 ($10^9$ CFU/d), respectively, were ineffective for the prevention of GDM. Heterogeneity in background dietary fibre intake may partially explain the variable effects of probiotics in preventing GDM. Studies showing a benefit included women with BMI measurements within relatively healthy ranges, while the negative studies were conducted in women with obesity/overweight. While dietary fibre intake was not reported in these studies, obesity may be considered a marker for lower dietary fibre intake, with which it has been frequently associated [19,20]. Both obesity and diet, including habitual dietary fibre intake, alter gut microbiota [21]. The baseline gut microbiota may influence host responsiveness to specific probiotics by their susceptibility to the antimicrobial substances produced by the probiotic, creating antagonism of potentially harmful bacteria or direct competition with the probiotic for nutrients or epithelial adhesion, as well as immunomodulatory effects of the probiotic on the host [21].

Dietary intake has a considerable effect on the gut microbiota. The so-called Western obesogenic diet (i.e., high in simple sugars and saturated fat and low in dietary fibre) is linked to gut microbiota taxonomic profiles that may contribute to metabolic syndrome [22-24]. In contrast, diets high in dietary fibre (non-digestible plant polysaccharides found in vegetables, fruit, wholegrains, nuts, and legumes) are observed to shift the composition of the gut microbiota towards a health-promoting taxonomic profile [21-24]. Prebiotics are non-digestible carbohydrates that act as food for probiotic bacteria. Thus, a diet high in dietary fibre is also likely high in prebiotics [25]. Prebiotics act as a fermentation substrate within the colon that target the proliferation of beneficial lactobacilli and *Bifidobacterium* species [26,27] and enhance the production of short-chain fatty acids (SCFA) [25]. The production of SCFA is thought to result in favourable changes to energy harvest (the ability to extract energy from food) and satiety [28,29], maintain the integrity of the intestinal epithelium, reduce systemic inflammation [27,29-31], and ultimately, enhance the metabolic regulation of glucose by insulin [28]. Emerging evidence also suggests that prebiotics can improve the functionality of probiotics, owing to a synergistic approach [32].

Therefore, the current study aimed to investigate macronutrient intake, focusing on dietary fibre, among the New Zealand PiP study participants, which showed a beneficial effect of probiotic *L. rhamnosus* HN001 supplementation in preventing GDM [14]. It was hypothesised that high dietary fibre intake (above minimum recommended 28g/day) is required for the efficacy of probiotic *L. rhamnosus* HN001 to prevent GDM. Thus, women who develop GDM despite probiotic supplementation were hypothesised to have low dietary fibre intakes.



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## MATERIALS AND METHODS

### Primary study

This study evaluated the dietary intake of women who participated in the PiP study [33]. The PiP study was a two-centre, randomised, double-blind, placebo-controlled trial investigating if probiotic HN001 improves maternal health during pregnancy by reducing GDM, bacterial vaginosis, and Group B Streptococcal vaginal colonisation before birth. The PiP study took place between December 2012 and November 2014. For this study, we focused on investigating the effects of the probiotic HN001 on the incidence of GDM (Australia NZ Clinical Trials Registry ACTRN12612000196842). Pregnant women with a personal or partner history of atopic disease and who intended to breastfeed their infant were eligible to be enrolled in the study between 14 and 16 weeks gestation. Other key exclusions were age under 16 years, not intending to stay in either of the study regions for the 18 months following enrolment, serious immunological disorder that suppresses immune function, or taking immune suppressant drugs, known cardiac valve disease for which antibiotic prophylaxis is required when undergoing dental procedures, has a history of a transplant or human immunodeficiency virus, were on long-term continuous antibiotic therapy, is already using or intending to use probiotic drinks or supplements themselves or in their child. Those with pre-existing type 1 or type 2 diabetes were excluded from the gestational diabetes outcomes. PiP study was granted ethical approval by the New Zealand Multi-region Ethics Committee (MEC/11/09/077).

All participants provided written informed consent before commencing the study. 423 pregnant women in Wellington and Auckland, New Zealand, were randomised to receive capsules containing either HN001 ($6\times10^9$ CFU/d) or placebo (maise-derived maltodextrin, identical in appearance and smell to the probiotic) daily from enrolment (14-16 weeks gestation) until 6 months postpartum. Study capsules containing *L. rhamnosus* HN001 ($6x10^9$ cfu) were manufactured by Fonterra Co-operative Group Ltd. Shelf life was managed to ensure minimum viable counts of $6\times10^9$cfu were maintained in the HN001 capsules. The placebo powder was corn-derived maltodextrin, manufactured by Grain Processing Corp. Oregon, USA and were supplied to Fonterra Co-operative Group Ltd by Salkat New Zealand Ltd, Auckland. Both probiotic and placebo powders were encapsulated by Alaron Products Ltd, Nelson, New Zealand and provided in opaque bottles. Quality and safety testing was performed to a pharmaceutical standard (Therapeutic Goods Act) by a registered external laboratory. Randomisation was managed by Fonterra Co-operative Group Ltd and was concealed from all study staff and participants. Randomisation was stratified by study centre and performed in blocks of random lengths according to a computer-generated list with an allocation ratio of 1:1. Research staff screened and enrolled participants, providing eligible participants with the next available sequentially numbered capsule container, without knowing whether these contained placebo or HN001. A total of 212 women were allocated to receive the *L. rhamnosus* HN001 probiotic, and 211 women were allocated to receive the placebo. Returned capsules were counted by staff not involved in the study assessments to calculate adherence rates (number taken divided by time). Median adherence rates were 94.9% (interquartile range (IQR) 75.7-98.8% (n=179) in the HN001 group and 94.05% (IQR 85.9-98.8% (n=183) in the placebo group (Wilcoxon rank-sum test, p=0.59).

GDM was assessed at 26-30 weeks gestation and was classified using the International Association of Diabetes and Pregnancy Study criteria (either fasting plasma glucose $\geq$5.1 mmol/L, 1 hour post 75 glucose level at $\geq$10 mmol/L, or a 2-hour level $\geq$8.5 mmol/L) [34] and the New Zealand criteria (either fasting plasma glucose $\geq$5.5 mmol/L, 1 hour post 75 glucose level at $\geq$10 mmol/L, or a 2-hour level $\geq$9 mmol/L [35]. For a detailed description of study methods and outcomes, refer to Barthow *et al.* [33]. For this substudy analysis, GDM was classified according to IADSPG criteria.

### Outcomes for secondary analysis

The primary outcome for this secondary analysis was dietary fibre at 26-28 weeks gestation. Secondary outcomes included total energy intake and macronutrient intake (protein, carbohydrate, fat). Participating women did not receive any specific nutrition counselling. Participants were required to complete a 3-day food diary between 26-28 weeks gestation before an Oral Glucose Tolerance Test (OGTT) for GDM was conducted. Participants were requested to record all food and drinks consumed in real-time using a provided paper form. Participants were asked to include specific details of the food

(e.g. breakfast cereal type, milk as whole or trim), portion size, method of cooking (e.g. fried, grilled, boiled, roasted), and any additions (e.g. sauces, dressings, spreads). Records of any dietary supplements taken during the three days, including the study capsule, were also requested.

352 women (83%) provided food diaries. Four were incomplete and thus excluded. Therefore, 348 dietary records (HN001 *n*=170, Placebo *n*=178) were entered into Food works 9 Professional (Xyris™ software) for dietary analysis by the first author. Food works 9 Professional uses nutritional data from New Zealand FOOD files™ 2016 Version 01 [35]. When unspecific descriptions of amounts taken or missing details of foods were encountered, the primary researcher replaced missing data with standard substitutions based on published nutritional guidelines or manufacturer recommendations [36-39]. Another researcher, who was blinded to the coding of the first author, entered 10% of the food diaries (*n* = 35) into Food works 9 Professional following the assumptions outlined by the first author. Both researchers were blinded to participant characteristics and intervention during food diary data entry.

Food works 9 Professional was used to obtain dietary intake of total energy, protein, carbohydrate, fibre, total fat, saturated fat, polyunsaturated fat, and monounsaturated fat. Percentage of energy intake from carbohydrate, fat, and protein was also obtained from Food works 9 Professional. Adequate dietary fibre (≥28g/d) was classified according to The Food and Nutrition Guidelines for Healthy Pregnant and Breastfeeding Women (19-50 years) [40,41].

**Statistical analysis**

For this secondary analysis, comparisons were made across the four groups generated by splitting each of the two treatment groups (HN001/placebo) according to GDM status (positive/negative). Data distributions were checked for normality and variance homogeneity by visual observation of histograms and quantile-quantile plots. For continuous data, pairwise comparisons were carried out using Student's *t*-test. Categorical data were examined using Fisher's exact test. For each macronutrient, group comparisons were carried out using two-factor analysis of variance (ANOVA) with GDM status and treatment group as fixed factors. Individual means were compared if a statistically significant interaction effect was detected. Logistic regression was undertaken to examine the impact of HN001 intervention and fibre intake on incidence of GDM. Statistical analysis was carried out using SAS software, Version 9.4 (SAS Institute, Cary, NC) [42]. Results are expressed as mean ± SD unless otherwise stated. The Bland & Altman approach [43] was adopted as a quality control measure for dietary fibre data using the 10% of food diaries (*n* = 35) which were double entered. This approach to compare two independent dietary fibre intake assessments entailed examining the mean difference for the paired data (bias) as an estimate of a systematic difference and the SD of the differences. Limits of agreement (i.e., mean difference 1.96 SD) then indicate the likely range of differences for most of the paired assessments.

## RESULTS

Table 1: Baseline characteristics of women participating in the Probiotics in Pregnancy study investigating the effect of HN001 probiotic supplementation on GDM development and who completed 3-day food diaries at 24-26 weeks gestation (n=348).

| | | GDM | | no GDM | |
|---|---|---|---|---|---|
| | | **HN001** | **Placebo** | **HN001** | **Placebo** |
| | | *n 16* | *n 26* | *n 154* | *n 152* |
| Age (years, mean ± SD) | | 33.0[a]± 3.7 | 37.2[b]± 4.4 | 33.4[a]± 4.2 | 33.7[a]± 3.9 |
| Weight (kg, mean ± SD) | | 82.9 ± 19.2 | 83.9 ± 20.6 | 71.7 ± 12.1 | 72.4 ± 13.5 |
| BMI (kg/m$^2$, mean ± SD) | | 30.4 ± 7.2 | 31.1 ± 7.6 | 26.0 ± 4.3 | 26.4 ± 4.7 |
| *Ethnicity* (*n*, %) | | | | | |
| | Māori | 3 (19%) | 4 (15%) | 14 (9%) | 20 (13%) |
| | Pacific Island | 1 (6%) | 1 (4%) | 3 (2%) | 1 (1%) |
| | Asian | 1 (6%) | 2 (8%) | 8 (5%) | 8 (5%) |
| | European | 11 (69%) | 19 (73%) | 129 (84%) | 122 (80%) |
| | Other | 0 (0%) | 0 (0%) | 0 (0%) | 1 (1%) |

[a b] Means in the same row with different superscripts were significantly different at p<0.005.

**Participant characteristics**

Baseline summary data for the participants at 14-16 weeks gestation, for whom dietary records were available (*n* = 348), are detailed in Table 1. The mean age of the GDM/HN001 group, no GDM/HN001 group, and no GDM/placebo group was 33 years. The GDM/placebo group was older than all other groups (*p*<0.005) with a mean age of 37.3 ± 4.4 years.

New Zealand European was the predominant ethnicity in this study (*n* = 281, 81%), followed by Māori (*n* = 41, 12%). Participants who did not develop GDM had a lower BMI, irrespective of the study intervention (26.2 ± 4.5 kg/m$^2$ vs 30.8 ± 7.5 kg/m$^2$, *p* <0.001). The incidence of GDM was 9.4% (16/170) in the HN001 group, vs 14.6% (26/178) in the placebo group, p=0.143, using Fishers test of proportions.

**Dietary fibre intake**

For the 10% (*n* = 35) of food diaries double-entered, Bland-Altman analysis indicated no relationship between the differences in fibre intake and the averages of fibre intake for each paired assessment (Pearson correlation coefficient = 0.23, p=0.18) with a constant bias of 1.1 g/d (95% confidence interval [CI] –0.3, 2.5 g/d) , suggesting minimal systematic error (Supplementary Figure 1). However, the limits of agreement spanned –7.3 (95% CI –9.6, –4.6) to 9.3 (95% CI 6.8, 11.7) g/d. Dietary fibre intakes at 26-28 weeks gestation of each of the four study groups were compared to evaluate the impact of dietary fibre on the efficacy of *L. rhamnosus* HN001 supplementation (Figure 1).

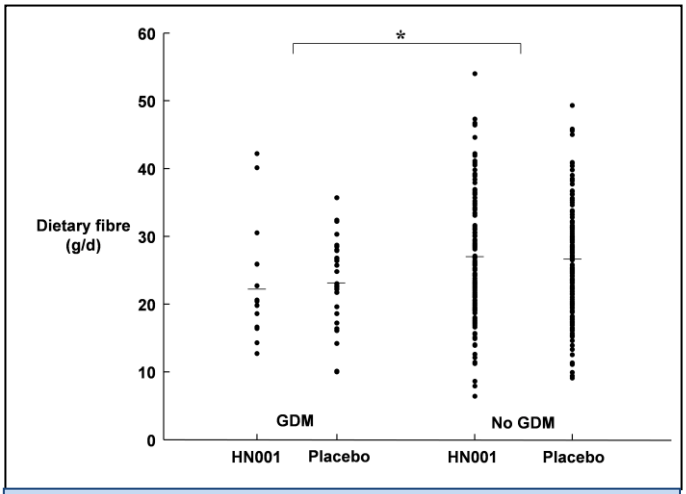


Figure 1: Self-reported dietary fibre intake (bars indicate mean values) among women in the Probiotic in Pregnancy study at 26-28 weeks gestation (*n*=348) was significantly greater among women who did not have GDM (*n*=306) than women with GDM (*n*=52). *p = 0.027.

Adequate dietary fibre intake during pregnancy (≥28 g/d) was reported by 37.4% of total participants. There was no statistically significant association between adequate dietary fibre intake and GDM status (p=0.062). However, women who developed GDM had significantly lower dietary fibre intake at 26 to 28 weeks gestation compared to women without GDM (GDM *n* = 42; 23.1 ± 7.3 g/d vs no GDM *n* = 306; 26.1 ± 8.5 g/d; *p* = 0.027), irrespective of study intervention (Table 2). Women supplemented with HN001 had a similar dietary fibre intake to those on placebo (*p* = 0.893) irrespective of GDM status (Table 2). Incidence of GDM was not related to treatment (p = 0.147) but was related to fibre intake with lower odds of developing GDM as fibre intake increased (odds ratio 0.954; 95% CI 0.914, 0.996; p=0.030). Participants with dietary fibre intake ≥28 g/d had a lower BMI (*n* = 130, mean 25.6 ± 4.2 kg/m$^2$) compared to participants with intakes of dietary fibre <28 g/day (*n* = 218, 27.5 ± 5.6 kg/m$^2$) (*p* <0.001).

**Other macronutrient intake**

Women with GDM reported a higher percentage of total energy intake derived from protein than women without GDM (*p* = 0.001, Table 2). The percentage of total energy derived from carbohydrate was similar across all four study groups. However, when evaluated as grams per day, women who subsequently developed GDM had statistically significant lower carbohydrate intake than those without GDM (GDM: 206.9 ± 64.60 g/d vs no GDM: 232.4 ± 56.94 g/d; *p* = 0.013), irrespective of probiotic supplementation.

Total and saturated fat intakes were not significantly different between women who did and did not develop GDM. However, women who developed GDM and were supplemented with HN001 had the lowest dietary fat intake at 26-28 weeks gestation compared to all other groups (*p*<0.05). Among women supplemented with placebo, women who developed GDM had a higher percentage of total energy intake derived from fat than all other groups (*p*<0.05). Saturated, monounsaturated, and polyunsaturated fat intake did not differ statistically according to GDM status or treatment (data not shown).

**Table 2:** Macronutrient intake reported by participants who completed a 3-day food diary at 26-28 weeks gestation grouped according to GDM status and probiotic/placebo supplementation.

| | | GDM | | No GDM | | Two-way ANOVA | | |
|---|---|---|---|---|---|---|---|---|
| | NRV | HN001 *n* 16 | Placebo *n* 26 | HN001 *n* 154 | Placebo *n* 152 | GDM vs No GDM | HN001 vs Placebo | Interaction |
| | | *Mean± standard deviation* | | | | *P* | *P* | *P* |
| Energy, kcal/d | 2297-2990 | 1756 ± 500 | 1809 ± 410 | 2010 ± 437 | 1969 ± 383 | 0.305 | 0.986 | 0.512 |
| Protein, g/d | - | 80.99 ±16.62 | 81.50 ± 23.48 | 81.32 ± 20.06 | 79.88 ± 18.87 | 0.846 | 0.890 | 0.769 |
| Protein, %TE | 15-25 | 19.28 ± 3.51 | 17.98 ± 4.18 | 16.99 ± 3.15 | 16.68 ± 3.20 | **0.001** | 0.148 | 0.376 |
| Carbohydrate, g/d | >175 | 207.5 ± 86.09 | 206.5 ± 48.96 | 234.2 ± 62.75 | 230.4 ± 56.43 | **0.013** | 0.814 | 0.893 |
| Carbohydrate, %TE | 45-65 | 46.19 ± 6.73 | 43.77 ±6.74 | 46.80 ± 5.94 | 46.77 ± 5.89 | 0.077 | 0.229 | 0.240 |
| Dietary Fibre, g/d | ≥28 | 22.54 ± 8.48 | 23.40 ± 6.68 | 26.33 ± 8.79 | 25.86 ± 8.10 | **0.027** | 0.893 | 0.637 |
| Fat, g/d | - | $60.89^{a}$± 18.56 | $76.19^{b}$± 24.39 | $72.42^{b}$± 22.35 | $73.40^{b}$± 20.58 | 0.232 | **0.026** | 0.051 |
| Fat, %TE | 20-35 | $30.84^{a}$± 6.46 | $35.12^{b}$± 5.70 | $32.36^{a}$± 5.58 | $32.78^{a}$± 5.39 | 0.665 | **0.013** | **0.040** |

**Abbreviations:** NRV: Nutrient Reference Value; g/d: Grams per day; kcal/d: Kilocalories per day; %TE: Percentage of total energy intake; $^{a\ b}$ Means in the same row with different superscripts were significantly different at $p<0.05$

## Discussion

In this food diary subgroup analysis of the PiP study, the incidence of gestational diabetes was not affected by treatment with *L. rhamnosus* HN001 but was reduced by a clinically and statistically significant 5% for each one gram increase in dietary fibre intake. It is most likely that there was insufficient power to detect the GDM lowering impact of HN001 that was observed in the main PiP study in the subgroup of women who returned food diaries in this study. The sample size reduced from 373 women, in which the HN001 protective result on GDM incidence was only marginally significant (p=0.08) in the main study [14], to 348 women analysed in this food diary substudy (p=0.15) although the effect size estimate for the protective effect of HN001 on GDM were similar in both analyses: 8.2% (15/184) in HN001 vs 13.8% (26/189) in placebo in the main study, compared to 9.4% (16/170) in HN001 vs 14.6% (26/178) in the food diary substudy. Nonetheless, a clinically and statistically significant difference in mean fibre intake of 3 g was seen between those who did and did not develop GDM. Although small, this amount equates to an additional daily serve of fruit or substitution of a serve of white bread with a wholegrain option. Our finding is concordant with the lower GDM risk that is associated with higher-fibre dietary patterns shown in a recent New Zealand GDM study [45].

Adequate levels of dietary fibre intake during pregnancy (≥28 g/d) were only reported by 37.4% of total participants.

The statistically significant interaction detected between treatment and GDM status for dietary fat intake was unexpected. A significantly lower dietary fat intake was reported by women who developed GDM while on HN001 supplementation compared to women who developed GDM on placebo. Among the placebo group, women who developed GDM reported much higher dietary fat intakes relative to total energy intake than those without GDM. This finding is consistent with previous studies showing high dietary fat consumption, particularly saturated fat, trans fat and cholesterol, increase GDM risk [44,46,47]. A plausible explanation is that HN001 supplementation affects fat intake, specifically among women with GDM, through a gut microbial mediated effect. Supplementation with *L. rhamnosus* LPR has been reported to reduce food cravings and food choice disinhibition among women in another double-blind, randomised, placebo-controlled study [48]. Gut microbiota in women with GDM is different from women without GDM [49], and changes to gut microbiota have been shown to influence food preferences [50].

The first study to report a successful effect of probiotic supplementation (*L. rhamnosus* GG ($10^{10}$ CFU) and *B. animalis* subsp. *lactis* Bb12 ($10^{10}$ CFU)) for the prevention of GDM was conducted in Finland [15]. Notably, all participants received basic dietary counselling based on the Nordic Nutrition Recommendations (aiming for a dietary fibre intake of 25-35

g/d [51]. Participants in the New Zealand PiP study were not provided with any dietary counselling. Only 37.4% of participants in the PiP study had adequate dietary fibre intake (≥28 g/d) during pregnancy, consistent with previous research showing that women in New Zealand consume approximately 24g of dietary fibre per day during pregnancy [52,53]. Moreover, low dietary fibre intake is associated with BMI, ethnicity (Māori and Pacific), age (≤30 years), lower education level (≤5 years high school and further education), low occupation, socioeconomic status, and welfare groups [20,52]. Therefore, ethnicity, BMI, and socioeconomic status are considered proxies for adequate dietary fibre intake.

In contrast to the Finnish probiotic study and the New Zealand PiP study, which both found a beneficial GDM lowering effect of probiotics, the SPRING [17] and HUMBA [18] studies reported no GDM lowering effect of the same probiotic used in the Finnish study. Notably, the participants in the latter negative studies were selected for overweight (SPRING) or obese (HUMBA) status, unlike women in the positive studies (PiP and Finnish). While dietary data were not reported for the other studies, we found no evidence from dietary records collected from PiP study participants to suggest that dietary fibre significantly influences the probiotic effect on lowering GDM. However, given that low dietary fibre was associated with GDM and higher BMI, we cannot exclude the possibility that sufficient dietary fibre is necessary for the beneficial effect of the HN001 probiotic on GDM development.

Prospective cohort studies have demonstrated that diets high in fat and protein might contribute to an increased risk of GDM [53-55]. A similar result was found in this study as a higher percentage of total energy from protein was reported among women with GDM. Previous literature suggests this is due to an association between animal protein and GDM development [46,56]. The negative effects of animal protein on GDM development can be attributed to dysregulation in brain-chain amino acid catabolism [57,58], as well as its high saturated fat content which can contribute to systemic inflammation [59].

A strength of this study included the collection of dietary data at 26-28 weeks gestation, prior to the GDM diagnostic test and any formal nutrition counselling. The diagnosis of GDM has been described as a 'teachable moment' [60], motivating health-related behaviour change, thus collecting dietary data prior to the diagnosis means that we were more likely to have captured habitual intake. This study has several limitations. The first is that this was a secondary analysis of a previously published randomised controlled trial testing whether probiotic HN001 had benefits for GDM. The study was not specifically designed for evaluating the hypothesis of whether sufficient dietary fibre is required for the beneficial effects of probiotics on GDM. As such, women were randomised only by probiotic and not by fibre intake. Secondly, our dietary assessment relied on 3-day food diaries. A longer period of food recording may have captured intake data more similar to usual dietary patterns; however, a period of three consecutive days was chosen as a compromise to maintain response and compliance. Missing or incomplete data were entered using standard substitutions based on nutritional guidelines and manufacturer recommendations which could be further affected by researchers' systematic errors when entering the food diary data [61]. Also, free or added sugar intake was not assessed, which may have affected GDM development. Although knowing that food intake must be recorded can alter dietary behaviours [18], participants provided dietary information as part of a double-blinded, placebo-controlled, randomised study, prior to their GDM screening test. Hence, our dietary data is unlikely to have been influenced by either the treatment arm or by knowledge of GDM status, minimising any systematic bias. Fibre intake estimated from food diaries was completed by two researchers rather than one. While there appeared to be no systematic bias introduced, the limits of agreement evaluated on a subset of diaries assessed by both researchers showed considerable inter-individual variability which may have compromised the results. Finally, the type of fibre was not accounted for, as specifically fermentable fibres consumed by probiotics may be more beneficial [21].

In summary, there was no significant association between HN001 supplementation and GDM development, or any difference in the probiotic effect on GDM by fibre intake. However, low dietary fibre intake was associated with both GDM and higher BMI. Therefore, achieving adequate fibre intake should continue to be recommended for the prevention of GDM.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. This content has not been published elsewhere.

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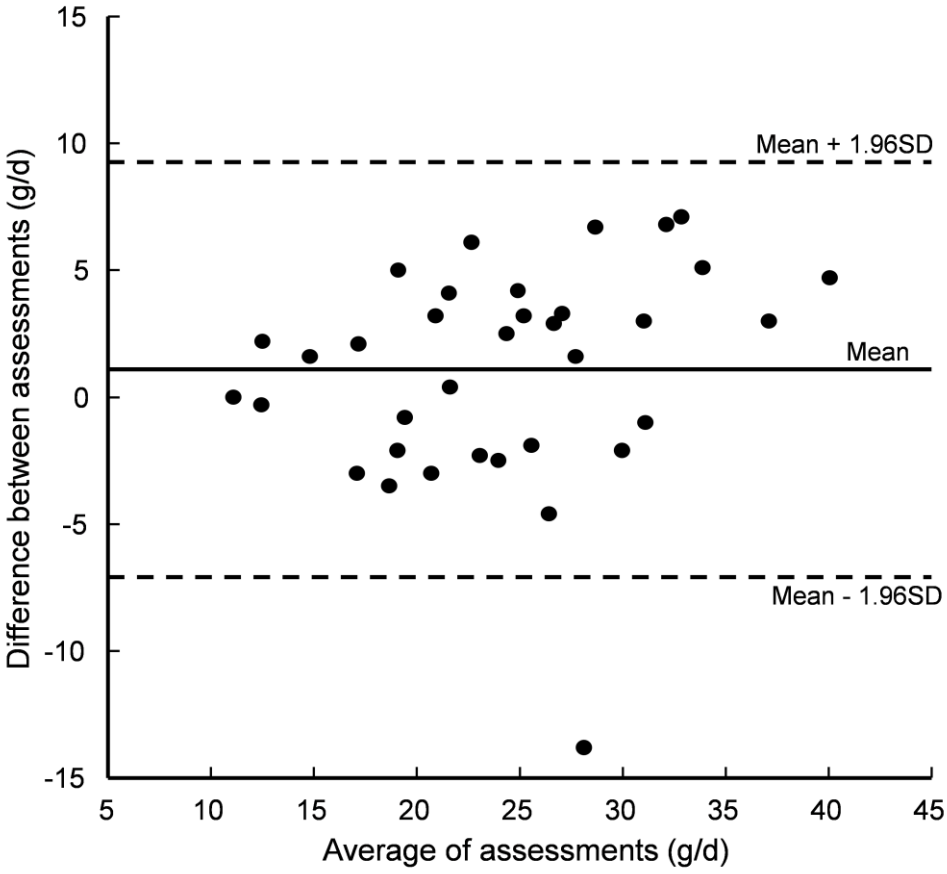
## Supplementary Figure




**Supplementary Figure 1:** Differences in fibre intake assessed by two researchers from 35 randomly selected food diaries plotted against the average of each paired assessment. The mean difference is shown by the solid line and limits of agreement by the dashed lines.