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A randomised controlled trial of a probiotic and a prebiotic examining metabolic and mental health outcomes in adults with pre-diabetes

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ABSTRACT

Aims To evaluate the effect of the probiotic *Lactobacillus rhamnosus* HN001 and/or cereal enriched with oat-derived beta-glucan (OBG) on metabolic and mental health outcomes when administered to adults with pre-diabetes.

Design 2×2 factorial design randomised, parallel-groups placebo-controlled; double-blinded for probiotic, single-blinded for cereals.

Participants Community-dwelling adults aged 18–80 years with pre-diabetes: glycated haemoglobin (HbA_{1c}) 41–49 mmol/mol.

Interventions Capsules containing *Lactobacillus rhamnosus* (HN001) (6×10^9 colony-forming units/day), or placebo capsules; and cereal containing 4 g/day OBG or calorie-matched control cereal, taken daily, for 6 months. Study groups were: (A) HN001 capsules+OBG cereal; (B) HN001 capsules+control cereal; (C) placebo capsules+OBG cereal and (D) placebo capsules+control cereal.

Outcome measures Primary outcome: HbA_{1c} at 6 months. Secondary outcomes: fasting plasma glucose, fasting insulin, homeostatic model assessment of insulin resistance, fasting lipids, blood pressure, body weight, waist circumference, body mass index and mental well-being.

Results 153 participants were randomised. There was complete HbA_{1c} outcome data available for 129 participants. At 6 months the mean (SD) HbA_{1c} was 45.9 (4.4) mmol/mol, n=66 for HN001, and 46.7 (4.3) mmol/mol, n=63 for placebo capsules; 46.5 (4.0) mmol/mol, n=67 for OBG and 46.0 (4.6) mmol/mol n=62 for control cereal. The estimated difference between HN001-placebo capsules was −0.83, 95% CI −1.93 to 0.27 mmol/mol, p=0.63, and between OBG-control cereals −0.17, 95% CI −1.28 to 0.94 mmol/mol, p=0.76. There was no significant interaction between treatments p=0.79. There were no differences between groups or significant interactions between treatments for any of the secondary outcomes.

Conclusions This study found no evidence of clinical benefit from the supplementation with either HN001 and/or cereal containing 4 g OBG on HbA_{1c} and all secondary outcomes relevant to adults with pre-diabetes.

Trial registration number Australian New Zealand Clincial Trials Registry number ACTRN12617000990325

Strengths and limitations of this study

- This is the first study to combine the probiotic, *Lactobacillus rhamnosus* HN001 with the prebiotic, oat-derived beta glucan.
- The factorial design enabled evaluation of the single or combined effect of two potentially synergistic interventions (probiotic and prebiotic) on a wide range of outcomes clinically relevant to those with pre-diabetes.
- In contrast to many probiotic studies conducted in populations with established diabetes, our study population had pre-diabetes with no exposure to glucose-lowering medications.
- This randomised controlled trial was conducted according to a predefined published protocol and used intention-to-treat analysis.
- An unexpected number of participants were taking statins and antihypertensive medications, and this may have altered results for some secondary outcomes.

INTRODUCTION

Multiple evidence-based strategies, including population and individual level interventions, will be needed to reduce, and ultimately reverse the rapidly growing rates of type 2 diabetes worldwide[1] and within New Zealand (NZ).[2 3] Pre-diabetes is associated with nephropathy, neuropathy, and increased risk of macrovascular disease[4] and those who progress to type 2 diabetes require extensive healthcare,[3] face multiple comorbidities including poorer mental health,[5] and 2–3 fold increase in all-cause mortality.[6] To date, the strongest evidence for prevention of the progress of pre-diabetes to type 2 diabetes is for lifestyle interventions, including modification of dietary intake and exercise levels, with the goal of a net energy deficit and weight reduction.[7–15] However, translation of this evidence on a large scale into practice is expensive, complex to implement and does

not always achieve the outcomes reported in published research.

There is evidence that the gut microbiome can influence metabolic[16] and mental health.[17] Therefore, the manipulation of gut microbes by probiotic and prebiotic supplements may present an additional, and potentially complementary strategy to lifestyle modification alone for diabetes prevention. We have reported that the probiotic *Lactobacillus rhamnosus* HN001 (HN001) (which Zheng *et al*[18] have recently proposed be renamed as *Lactocaseibacillus rhamnosus* HN001), administered at a dose of 6×10^9 colony-forming units per day (cfu/day), from 14 to 16 weeks gestation, reduced gestational diabetes, from 6.5% to 2.1%, a relative rate of 0.32.[19] In addition, participants who took the probiotic had lower postpartum depression and anxiety scores.[20] This suggests that HN001 may reduce the risk of progression to diabetes and improve mental well-being in other clinical populations, such as those with pre-diabetes.

Prebiotics are non-digestible fibres, which can be fermented by gut microbiota to potentially provide health benefits to the host.[21] Oat-derived beta glucans (OBG) are high molecular weight soluble polysaccharides[22] which act as prebiotics, have been extensively researched for their health effects, and are endorsed by the European Food Safety Authority for reduction of lipid levels and postprandial blood glucose.[23] We hypothesised that the combined use of OBG and HN001 might give greater effects than using HN001 alone. In addition, in vitro evidence suggests that HN001 growth is supported by the presence of barley derived beta glucan,[24] and while not known we anticipated that OBG may also support the growth of HN001.

Aim of study

The aim of this study was to investigate the metabolic and mental health effects of 6 months supplementation with probiotic HN001 with or without a cereal enriched with OBG in adults with pre-diabetes.

METHODS

Patient and public involvement statement

Consultation with high-risk ethnic groups (Māori, Pacific Peoples, and Indian/South Asians) was held during the initial phases of study setup. This informed refinements to study procedures: information giving and consent processes; and modifications to delivery methods for study interventions. An embedded qualitative study ascertained participants' experience of taking study interventions.

Study design

A comprehensive description of study design, protocols, outcomes and planned statistical analysis is published elsewhere.[25] A brief description is given below.

This study was a 2×2 factorial design, randomised parallel group superiority trial with a 6-month intervention period and follow-up 3 months after interventions were discontinued. Participants were recruited from the community in the Wellington region of NZ. All participants gave informed written consent.

Participants

Participants were adults aged 18–80 years with pre-diabetes: glycated haemoglobin (HbA_{1c}) 41–49 mmol/mol, (5.9%–6.6%), as defined by the NZ criteria.[26] Key exclusion criteria were reported previously.[25]

Randomisation and masking

Non-stratified block randomisation performed independently by Fonterra Co-Operative, and undertaken with blocks of eight using previously described methods.[27] Study participants were allocated to one of four groups, each group receiving both a capsule and cereal intervention (see figure 1) with an allocation ratio of 1:1:1:1. Both capsule and cereal supplies were packaged using sequentially numbered containers matching the randomisation schedule. The study was double blinded for capsules. Cereal interventions were not able to be blinded, however study participants were not informed of the full content of cereal packages, the rationale for cereal choices or which cereal was hypothesised to be efficacious.

Study interventions and procedures

Probiotic capsules, supplied by Fonterra Co-operative Group, contained HN001 (6×10^9 cfu) and 140 mg corn-derived maltodextrin. Identical appearance placebo capsules contained 150 mg corn-derived maltodextrin. (For further detail, see reference[25]). Capsules were supplied in 3-month allocations. Throughout the study Fonterra tested the viability of a selection of unused capsules. With very few exceptions, the viability was higher than the minimum required or within the limit of uncertainty of the counting method.

Cereals were packed in individual daily single serve portions by HealthPak, Auckland NZ. The active cereal contained 4 g OBG obtained from 40 g Uncle Toby's Flemings Rolled Oats, (Nestle Australia) and 8 g of OatWell 28XF oatbran (DSM Nutritional Products Ltd, Switzerland). The calorie matched control cereal consisted of 35 g cornflakes (Sanitarium Health and Wellbeing, Auckland, NZ) and 8 g non-dairy creamer (C35) (Shantou City Chenghai District Wen-hui Food, China). (For further cereal details, see supplemental files for the published paper.[25])

Baseline demographic data and health history data were collected at enrolment (time point 0). At all study time points additional questionnaire data covering a range of variables including potential confounders and effect modifiers were collected. This included 3-day food diaries to assess caloric and fibre intakes, measures of physical activity using the Stanford Leisure-Time Activity Categorical Item questionnaire (L-Cat 2.2),[28] medication and supplement use, side effects of interventions and adverse event data. Food diary data were analysed using Foodworks 9 (Xyris Software Australia) using both the NZ

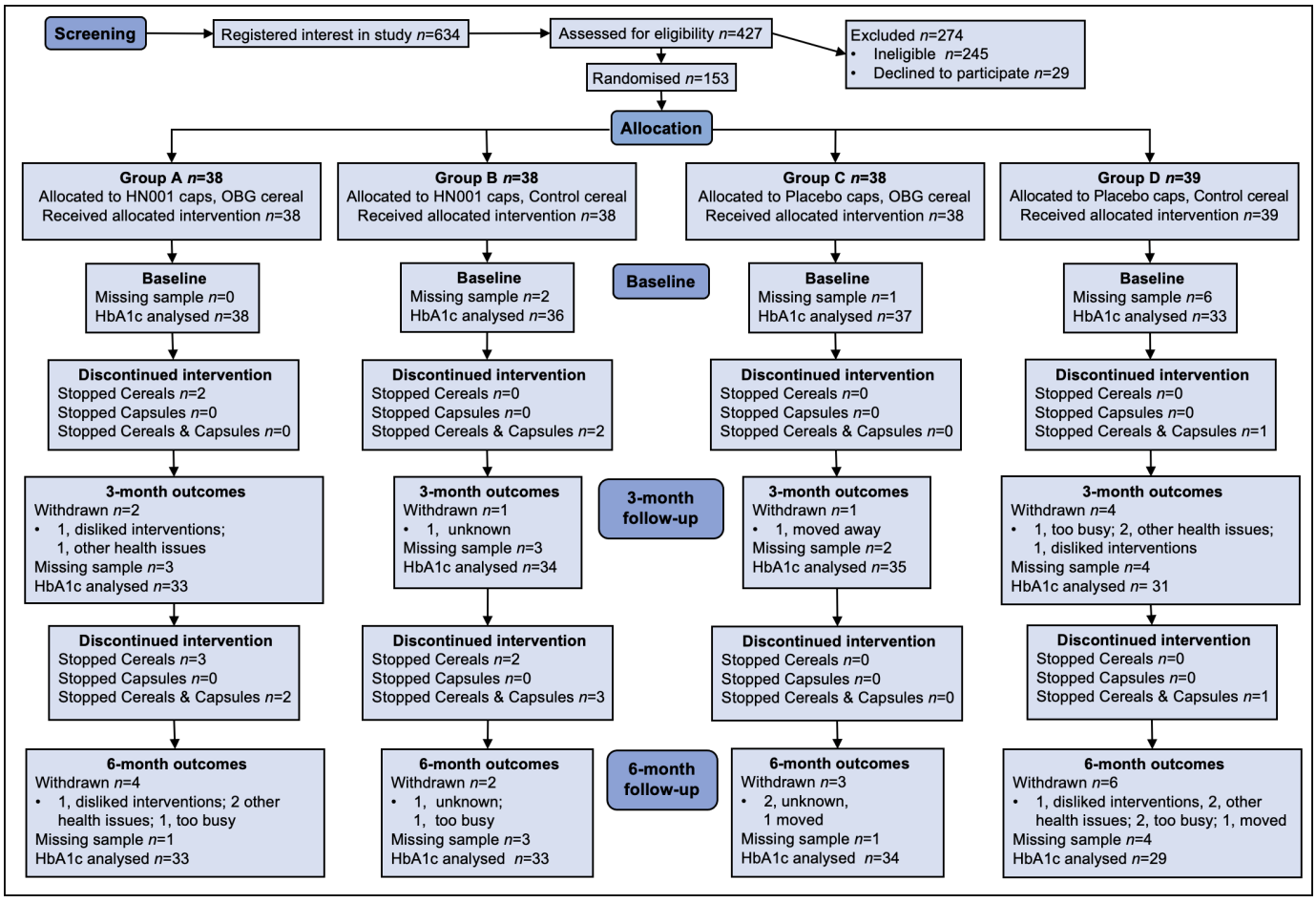

Figure 1 CONSORT flow diagram of the study HN001, *Lactobacillus rhamnosus* strain HN001. HbA_{1c}, glycated haemoglobin; OBG, oat-derived beta-glucan. CONSORT, consolidated standards of reporting trials.

and Australian food databases. Anthropometric data were collected as specified previously.[25]

Study interventions were allocated to participants after all baseline measures were collected with staff allocating interventions matching the next number in the randomisation schedule. Participants were instructed to take one study capsule and one portion of cereal daily, using the portion of cereal in place of a similar component of their usual daily dietary intake and apart from this continue with their usual dietary and exercise routines. All unused capsules and cereals were collected and counted to assess adherence.

Blood sample biochemical analyses were performed in research laboratories using standardised procedures. HbA_{1c}, fasting plasma glucose (FPG) and lipids were analysed on Cobas c331, and insulin was analysed by ELISA assay (online supplemental table 1).

Outcomes

The primary outcome was HbA_{1c} measured at 6 months. Secondary outcomes included: HbA_{1c} at 3 months; other biological markers and physical measures including FPG, homeostatic model assessment of insulin resistance (HOMA-IR),[29] fasting lipid profiles; mean systolic (SBP) and diastolic (DBP) blood pressure and anthropometric measures (waist circumference, body weight and body mass index (BMI)) at 3, 6 and 9 months; and measures of psychological symptoms stress, anxiety, depression and health-related quality of life assessed by the Short-Form Health Survey version 2 for NZ/Australia (SF-36)[30] and Depression Anxiety Stress Scale (DASS 21)[31] at 6 and 9 months. Other variables included adherence to study interventions measured as percentage of interventions taken based on number taken/time, and side effects of interventions covering a range of gastrointestinal and bowel symptoms measured by a Likert-type scale.[32]

Statistical analysis

Statistical analysis was by a prespecified analysis plan.[25] Main analyses followed an intention-to-treat framework and the study statistician was masked as to treatment allocation. The primary outcome variable HbA_{1c} and all other continuous variables were analysed using analysis of covariance (ANCOVA) with adjustment for baseline measures. Main effects and an interaction between the two randomised treatments were calculated. Where the interaction term was $p>0.05$, the main effects comparisons were estimated. If the interaction term is $p<0.05$ then the comparison of the main effects within each category of other main effect was calculated. The sensitivity analyses

for the primary outcome variable, HbA_{1c}, were to explore if confounding by important covariates; prespecified as BMI, level of exercise and energy intake had occurred; and subgroup analyses were also explored; prespecified effect modifying variables were ethnicity, sex and socioeconomic status as summarised by self-reported personal income. Finally, a mixed linear model was used to assess if there was a difference in HbA_{1c} between treatments in relation to the two times outcomes were measured; at three and 6 months using the individual participant as a random effect. Ordinal variables: ratings of adverse effects on nausea, pain, bloating, and bowel function; were analysed by ordinal regression. All statistical analysis was conducted in SAS V.9.4.

The sample size of the study was based on the ability to detect a clinically important difference in HbA_{1c} of 3.8 mmol/mol (2.5%) with an SD of 6% and 90% power. Allowing for a 25% drop-out rate, this required 152 participants to be enrolled. Further details of these calculations are in the protocol paper.[25]

Data monitoring committee

Internal data monitoring occurred throughout the study, with investigators reviewing any adverse events and need for protocol amendments. No interim analysis of study outcomes was performed. For further details refer to the protocol paper.[25]

Changes to protocol after trial commencement

Minor amendments were made to inclusion criteria after the study commenced. These included (1) extension of the upper age from <70 years to <80 years with the addition of screening questions to ensure all participants were in generally good health and (2) a change from the requirement for screening HbA_{1c} tests to be done in the 3 months before study enrolment which was modified to enrolment within: 4 months for those with screening HbA_{1c} of 41–44 mmol/mol (5.9%–6.2%); and 1 year for those with screening HbA_{1c} of 45–49 mmol/mol (6.3%–6.6%). The rationale being that those with HbA_{1c} in the lower group were more likely to regress to normal than those in the higher range, and a shorter time frame between screening and enrolment would reduce the likelihood of this occurrence. Both amendments were made to facilitate study recruitment in a timely manner, and were agreed on by the study monitoring committee, and notified and accepted by the ethics committee and clinical trials register.

RESULTS

The consolidated standards of reporting trials (CONSORT) flow diagram of participants in the study is shown in figure 1. A total of 153 participants were enrolled between 19 February 2018 and 29 March 2019 and data collection was completed on 19 December 2019. The study participants are described in table 1 and online supplemental table 2.

Primary outcome

The mean (SD) HbA_{1c} after 6 months was 45.9 (4.4) mmol/mol (6.3 (0.4)%), n=66 for HN001 and 46.7 (4.3) mmol/mol, (6.4 (0.4)%), n=63 for placebo capsules; 46.5 (4.0) mmol/mol, (6.4 (0.4)%), n=67 for OBG and 46.0 (4.6) mmol/mol (6.4 (0.4)%), n=62 for control cereal. The mean difference, adjusted for baseline HN001-placebo capsules was −0.83, 95% CI −1.93 to 0.27 mmol/mol, (−0.1, 95% CI −0.2 to 0.0%), p=0.63, and for OBG-control cereal was −0.17, 95% CI −1.28 to 0.94 mmol/mol (0.0, 95% CI −0.1 to 0.1%), p=0.76. There was no statistically significant interaction between treatments p=0.79 (table 2). There was no important difference after adjustment for prespecified confounders (online supplemental table 3), or in a mixed linear model (online supplemental table 4). There were no differences in treatments at 3 months (table 2). There was no evidence of any subgroup effects; sex, ethnicity and income (online supplemental figures 1 and 2).

Secondary outcomes

The mean (SD) FPG after 6 months was, 6.9 (1.2) mmol/L, n=70 for HN001 and 6.9 (1.1) mmol/L, n=68 for placebo capsules; 7.1 (1.3) mmol/L, n=71 for OBG and, 6.7 (0.9) mmol/L, n=69 for control cereal. The mean difference, adjusted for baseline for HN001-placebo capsules, was −0.04 mmol/L (95% CI −0.35 to 0.27), p=0.80, and for OBG-control cereal was 0.08 mmol/L (95% CI −0.23 to 0.40), p=0.60. There was no significant interaction between treatments p=0.16. There were no important differences in FPG for any of the other timepoints (table 3, online supplemental table 5). Similarly, there were no important differences in HOMA-IR at any time point.

The mean (SD) total cholesterol (TC) after 6 months was, 4.8 (1.2) mmol/L, n=69 for HN001 and 4.8 (1.1) mmol/L, n=68 for placebo capsules; 4.6 (1.2) mmol/L, n=69 for OBG and, 4.9 (1.2) mmol/L, n=68 for control cereal. The mean difference, adjusted for baseline HN001-placebo capsules, was 0.04 mmol/L (95% CI −0.23 to 0.31), p=0.78, and for OBG-control cereal was −0.13 mmol/L (95% CI −0.40 to 0.15), p=0.36. There was no significant interaction between treatments p=0.99. There were no significant interactions or differences between groups for TC for three or 9 months, or for high density lipoprotein (HDL), low density lipoprotein (LDL) or triglycerides (TG) at any timepoint (table 3, online supplemental table 5).

There were no differences for anthropometric measures including weight, BMI and waist circumference, or SBP and DBP (table 3, online supplemental table 5). Mental well-being outcomes measured by DASS 21 and SF-36 showed no significant differences (table 3 and online supplemental table 6).

Other outcomes

Adherence to study interventions was high with mean adherence for all interventions and at all timepoints ≥84% (online supplemental table 7). A subgroup analysis of HbA_{1c} outcome at 6 months, defined as adherence to

Table 1 Baseline description of study participants by factorial group

	HN001 Capsule OBG Cereal	HN001 Capsule Control cereal	Placebo capsule OBG Cereal	Placebo capsule Control cereal
n*	n=38	n=38	n=38	n=39
Demographic characteristics	**Mean (IQR) Range**	**Mean (IQR) Range**	**Mean (IQR) Range**	**Mean (IQR) Range**
Age, years	60.4 (55.7 to 67.4) 39.1 to 78	60 (52.1 to 66.5) 44.1 to 80.3	58.3 (50.9 to 65.4) 37.5 to 70.8	59.9 (55.3 to 67) 38.8 to 74.6
	n (%)	**n (%)**	**n (%)**	**n (%)**
Gender, male	20 (52.6)	16 (42.1)	24 (63.2)	20 (51.3)
Household income, NZ$				n=38
NZ$0–NZ$49 000	8 (21.1)	3 (7.9)	7 (18.4)	7 (18.0)
NZ$50–NZ$99 000	16 (42.2)	17 (44.8)	18 (47.4)	12 (30.8)
NZ$100–NZ$149 000	10 (26.4)	13 (34.2)	7 (18.4)	9 (23.1)
NZ$150 000+	4 (10.5)	5 (13.2)	6 (15.8)	10 (25.6)
History of comorbid conditions				
Hypertension	24 (63.2)	20 (52.6)	21 (55.3)	14 (35.9)
Hyperlipidaemia	19 (50.0)	25 (65.8)	21 (55.3)	17 (43.6)
Depression†	6 (15.8)	6 (15.8)	9 (23.7)	7 (18.0)
Anxiety‡	4 (10.5)	3 (7.9)	3 (7.9)	2 (5.1)
Dietary intake	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Total calorie intake, kJ§	8651 (2648)	8590.5 (2097)	8530 (2401)	8724 (2200)
Fibre intake§, g	23.8 (7.2)	26.7 (8.5)	27.0 (9.8)	24.9 (8.9)
Smoking	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Current smoker	2 (5.3)	3 (7.9)	5 (13.2)	5 (12.8)
Prescribed and OTT medications				
Antihypertensives/diuretics¶	23 (60.5)	14 (36.8)	18 (47.4)	15 (38.5)
Lipid lowering medications¶	14 (36.8)	14 (36.8)	10 (26.3)	12 (30.8)
Antidepressant/anxiolytic¶	3 (7.9)	2 (5.3)	7 (18.4)	3 (7.7)
Glucoregulatory markers	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
HbA_{1c}, mmol/mol	46.8 (4.4)	45.3 (3.9) n=36	45.9 (3.5) n=37	45.8 (4.2) n=33
HbA_{1c}, %	6.4 (0.4)	6.3 (0.4) n=36	6.3 (0.3) n=37	6.3 (0.4) n=33
Fasting serum glucose, mmol/L	7.1 (1.5) n=37	6.5 (1.5)	6.8 (1.0) n=37	6.5 (0.9)
Insulin, pmol/L	113.9 (129.9)	95.3 (66.4)	113.5 (87.8)	94.1 (53.1)
HOMA-IR	5.2 (6.4) n=37	4.1 (3)	4.9 (4.0) n=37	4 (2.4)
Fasting lipids				
Total cholesterol, mmol/L	4.9 (1.4)	5.1 (1.2)	5.1 (1.2)	5.1 (1.4)
HDL, mmol/L	1.2 (0.3)	1.2 (0.4)	1.2 (0.3)	1.2 (0.3)
LDL, mmol/L	3 (1.1)	3.3 (1.1)	3.3 (1.1)	3.3 (1.3)
Triglycerides, mmol/L	1.4 (0.6)	1.4 (0.6)	1.4 (0.6)	1.3 (0.5)
Anthropometry				
Weight, kg	87.7 (17.4)	84.6 (17.2)	88.2 (28.2)	83.3 (18.9)
BMI, kg/m^2	31.7 (5.6)	30.2 (5)	30.5 (7.6)	29.3 (5.4)
Waist circumference, cm	105.2 (12.7)	101.5 (12.2)	102.8 (19.7)	101.2 (13.8)
Blood pressure				

Continued

Table 1 Continued

	HN001 Capsule OBG Cereal	HN001 Capsule Control cereal	Placebo capsule OBG Cereal	Placebo capsule Control cereal
n*	n=38	n=38	n=38	n=39
Systolic, mm Hg	135.9 (11.5)	140.7 (17.7)	133.7 (18.3)	134.4 (15.3)
Diastolic, mm Hg	81.5 (8.9)	83.2 (13.5)	79.3 (11.7)	80.5 (10.6)
Mental health outcome measures				
DASS 21				
Total score	11.9 (10.4)	13.9 (14.0)	11.9 (13.1)	14.1 (11.6)
SF-36				
Physical Component Summary	52.3 (6.9)	51.6 (6)	52.7 (6.9)	51.7 (6.2)
Mental Component Summary	52.9 (6.9)	52.8 (8.8)	54.5 (6.4)	55.1 (6.2)

*n applies for all variables unless otherwise specified.
†Depression defined participant ever told by a health professional that they were depressed or needed antidepressant medication.
‡Anxiety defined as participant ever told by a health professional that they were anxious or needed treatment for anxiety.
§Estimated from 3-day food diary.
¶Used in the last month.
BMI, body mass index; DASS 21, Depression Anxiety Stress Scale; HbA1c, glycated haemoglobinn; HDL, high density lipoprotein; HOMA-IR, homoeostatic model assessment of insulin resistance; LDL, low density lipoprotein; OBG, oat-derived beta glucan; OTT, over the counter; SF-36, Short-Form Health Survey.

capsules ≥75% and adherence to cereals ≥75% did not alter study outcomes (online supplemental figures 3 and 4). There were no meaningful differences in the prevalence of gastrointestinal symptoms for capsules or cereals at 6 months (online supplemental table 8) or other time points (data not shown).

We performed additional exploratory subgroup analysis post-hoc to examine the effect of age, BMI and baseline HbA_{1c} level on HbA_{1c} at 6 months which showed no statistically significant evidence of any subgroup effect on any of these variables (online supplemental figures 3 and 4).

DISCUSSION

This factorial-design randomised controlled trial (RCT) assessed the impact of 6 months supplementation with the probiotic HN001 (6×10^9 cfu/day) and/or 4 g/day OBG on a range of metabolic and mental health outcome in adults with pre-diabetes. No effect was found for HN001 alone, for OBG alone or the combination of HN001 with OBG for any of the outcomes measured at any time point. Based on these findings, there is no evidence to support the use of these interventions in their current form and dose in those with pre-diabetes.

Our study was adequately powered to detect clinically important differences the primary outcome of HbA_{1c} at 6 months as demonstrated by the confidence bounds for the comparison of treatments being well within the prespecified smallest clinically important difference of 3.8 mmol/mol. In addition, the duration of intervention and follow-up of 6 months are more than adequate to determine changes in HbA_{1c}.[16] Our results concur with the recently performed PROFAST feasibility study using HN001 or placebo in conjunction with intermittent fasting in obese adults with pre-diabetes. That study also found no effect on HbA_{1c}, FPG, insulin, TC, LDL, HDL or TG attributable to the probiotic.[33] As far as we are aware to date this is the only other study using HN001 in a population with pre-diabetes.

In comparison, our previous work with using HN001 in pregnant women, found reduced incidence of gestational diabetes mellitus (GDM) (as assessed by oral glucose tolerance test and the NZ diagnostic criteria) 2.1% (95% CI 0.6% to 5.2%) in the HN001 group, 6.5% (95% CI 3.5% to 10.9%) in the placebo group (p=0.03), and a slightly lowered fasting glucose levels for the probiotic group (mean difference −0.08 mmol/L (95% CI −0.15 to 0.00), p=0.048), and stronger effects in the subgroups of women who had a history of gestational diabetes and those older than 35 years.[19] In contrast, a more recent four-arm placebo controlled RCT evaluating HN001 and *Bifidobacterium animalis ssp. lactis* 420, (10^{10} cfu each) with or without fish oil in a higher risk group including overweight or obese pregnant women found no differences in the prevalence of GDM (according to the International Association of Diabetes and Pregnancy Study Groups criteria), change of glucose, insulin or HOMA-IR between the groups (p>0.05).[34] The lack of data comparing GDM based on NZ cut points, different risk profiles of study populations as well as the use of single versus dual probiotic interventions mean these pregnancy study outcomes are not directly comparable.

Research is limited in the use of probiotics in those with pre-diabetes, however numerous probiotic intervention studies have been undertaken in those with established

Table 2 The effects of HN001 and OBG and their interaction on HbA_{1c} adjusted for study time point and baseline values of HbA_{1c}

	Probiotic				Prebiotic				
	HN001 capsule	Placebo capsule	Difference (95% CI)	P value	OBG cereal	Control cereal	Difference (95% CI)	P value	P interaction
Primary outcome	n=66	n=63			n=67	n=62			
HbA_{1c} at 6 months, mmol/mol	45.9 (4.4)	46.7 (4.3)	−0.83 (−1.93 to 0.27)	0.63	46.5 (4.0)	46.0 (4.6)	−0.17 (−1.28 to 0.94)	0.76	0.79
HbA_{1c} at 6 months, %	6.3 (0.4)	6.4 (0.4)	−0.1 (-0.2 to 0.0)		6.4 (0.4)	6.4 (0.4)	0.0 (−0.1 to 0.1)		
Secondary outcomes	n=67	n=66			n=68	n=65			
HbA_{1c} at 3 months, mmol/mol	46.1 (4.5)	46.3 (4.5)	−0.24 (−1.22 to 0.75)	0.63	46.7 (4.7)	45.6 (4.4)	0.32 (−0.68 to 1.31)	0.53	0.84
HbA_{1c} at 3 months, %	6.4 (0.4)	6.4 (0.4)	0.0 (-0.1 to 0.1)		6.4 (0.4)	6.3 (0.4)	0.0 (−0.1 to 0.1)		

Data are expressed as mean (SD).
HbA_{1c}, glycated haemoglobin; HN001, *Lactobacillus rhamnosus* HN001; OBG, oat-derived beta glucan.

type 2 diabetes or more general population groups examining outcomes related to glycaemia, dyslipidaemia, hypertension, anthropometry and, to a lesser extent mental health. Several meta-analyses include significant improvements in some of these outcomes, but these findings are often inconsistent.[16 35–41] The clinical utility of these reviews is limited due to inclusion of multiple different probiotic species and strains being used singularly or combination with each other being combined in a meta-analysis, however, it is useful to consider the findings more broadly as they relate to our results. Consistent findings appear to be that multistrain probiotic interventions appear to be more effective than single strain interventions.[35–37] In addition, the magnitude of effects can vary according to participant characteristics such as: lower versus higher BMI[35 36 38]; age, with younger groups generally achieving more benefit[35 36 38]; higher baseline values of the outcome measures[39]; country of origin, suggesting genetic and dietary influences[38]; established diabetes vs high risk groups[39] probiotic dose[41]; medium for the delivery of the probiotic such as in food versus as a capsule or powder supplement[38 39 41] and duration of treatment[38 40 41] all potentially impacting on the outcomes. Several of these factors might be relevant in this study. It is well known that probiotic species and strains have specific effects, and cross-talk between organisms, and or host can alter their effects.[42] Our study used a single probiotic strain with a dose of 6×10^9 cfu/day. In contrast, multistrain interventions may provide more benefit through interaction between probiotic organisms, and/or a generally higher total dose of probiotic organisms being administered.[38] Profiles of gut microbiota differ according to gender[43] and this could moderate the response to the probiotic intervention. Our prespecified subgroup analysis did not find a statistically significant difference according to gender, however, it appears that HN001 may be more beneficial in males, populations including other Asians and higher income groups (online supplemental figure 1) . One possible explanation for some of these differences may relate to the HbA_{1c} glycation gap.[44] Therefore, detailed consideration of these potential subgroup differences in future studies may be valuable. Our population had a higher mean age (59.6 years) and BMI (30.4 kg/m^2) profile than meta-analyses reporting more benefits for younger[36 38 45] and lower BMI (<30 kg/m^2)[38] subgroups. Gut microbiota profiles are known to differ according to age,[46] and obesity,[47] and therefore, we speculate that our findings may not be replicable in a younger and less obese population with pre-diabetes. The lower baseline measures for HbA_{1c} and other outcomes examined in our population with pre-diabetes may have meant there was little room for biological markers to shift when compared with a population with established diabetes.[39] While the post hoc analysis did not show any statistically significant evidence of subgroups affects it appears that HN001 may be more effective in those with BMI less than 30 kg/m^2 (online supplemental figures 3 and 4), and this should also be examined in further studies.

Table 3 Baseline and 6-month values for secondary outcomes according to probiotic or prebiotic allocation

Other secondary metabolic outcomes	HN001 Capsule		Placebo capsule		OBG cereal		Control cereal	
	Baseline	6 months	Baseline	6 months	Baseline	6 months	Baseline	6 months
Glucoregulatory markers, n*	n=76	n=70	n=77	n=68	n=76	n=69	n=77	n=69
Fasting glucose, mmol/L	6.8 (1.5) n=75	6.9 (1.2)	6.7 (1) n=76	6.9 (1.1)	6.9 (1.3) n=74	7.1 (1.3)	6.5 (1.2)	6.7 (0.9)
Insulin, pmol/L	104.6 (102.9)	105.6 (87.7)	103.7 (72.5)	116.5 (118.3)	113.7 (110.1)	123.2 (135.3)	94.7 (59.6)	98.7 (55.1)
HOMA-IR	4.6 (5) n=74	4.7 (4.3)	4.5 (3.3)	5.4 (6.5)	5.1 (5.3)	5.8 (7.2) n=69	4 (2.7)	4.3 (2.7)
Fasting lipids, n*	n=76	n=69	n=77	n=68	n=76	n=69	n=77	n=68
Total cholesterol, mmol/L	5.0 (1.3)	4.8 (1.2)	5.1 (1.3)	4.8 (1.1)	5.0 (1.3)	4.6 (1.2)	5.1 (1.3)	4.9 (1.2)
HDL, mmol/L	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	1.1 (0.3)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)
LDL, mmol/L	3.1 (1.1)	3.0 (1.1)	3.3 (1.2)	3.0 (1.0) n=67	1.2 (0.3)	2.9 (1.0) n=68	1.2 (0.3)	3.2 (1.0)
Triglycerides, mmol/L	1.4 (0.6)	1.4 (0.6)	1.3 (0.6)	1.3 (0.7)	3.1 (1.1)	1.4 (0.8)	3.3 (1.2)	1.4 (0.6)
Blood pressure, n*	n=76	n=70	n=77	n=68	n=76	n=69	n=76	n=69
Systolic, mm Hg	138.3 (15)	137.7 (17.9)	134.1 (16.8)	133.1 (15.8)	134.8 (15.3)	134.8 (16.1)	137.6 (16.8)	136.1 (18)
Diastolic, mm Hg	82.3 (11.4)	81.1 (9.9)	79.9 (11.1)	78.4 (11.1)	80.4 (10.4)	79.7 (9.2)	81.8 (12.1)	79.8 (11.9)
Anthropometry, n*	n=76	n=70	n=77	n=68	n=76	n=69	n=77	n=69
Weight, kg	86.2 (17.2)	86.2 (16.7)	85.7 (23.9)	84.8 (23.4)	87.9 (23.3)	87.9 (23.4)	84 (18)	83.1 (16.2)
BMI, kg/m^2	30.9 (5.3)	30.9 (5.3)	29.9 (6.6)	29.6 (6.3)	31.1 (6.7)	31 (6.8)	29.7 (5.2)	29.5 (4.7)
Waist circumference, cm	103.4 (12.5)	103.3 (12.3)	102 (16.8)	101 (16.5)	104 (16.5)	103.2 (16.5)	101.4 (13)	101.1 (12.2)
Mental health and well-being outcomes, n*	n=76	n=70	n=77	n=68	n=76	n=69	n=77	n=69
DASS 21								
Total score	12.9 (12.3)	9.2 (10.2)	13.0 (12.3)	10.9 (11.5)	11.9 (11.8)	9.2 (9.7)	14.0 (12.7)	10.8 (11.9)
SF-36								
Physical Component Summary	51.9 (6.4)	51.7 (7.8)	52.2 (6.5)	53.3 (5.4)	52.5 (6.8)	52.8 (6.3)	51.7 (6.1)	52.2 (7.2)
Mental Component Summary	52.9 (7.8)	55.1 (7.1)	54.8 (6.2)	54.7 (5.9)	53.7 (6.6)	55.5 (5.9)	54 (7.6)	54.4 (7.1)

Data are expressed as mean (SD).
*n applies unless otherwise specified.
BMI, body mass index; DASS, Depression Anxiety Stress Scale; HDL, high density lipoprotein; HOMA-IR, homoeostatic model assessment of insulin resistance; LDL, low density lipoprotein; OBG, oat-derived beta glucans; SF-36, Short-Form Health Survey.

The lack of benefits from HN001 administration for lipid outcomes are consistent with our previous pregnancy study[48] and the PROFAST study.[33] However, the lack of effect of OBG on lipids is surprising given the strong evidence for beta glucans in improving lipids profiles, especially TC and LDL. The Food and Drug Administration recommends a dose of 3 g/day beta glucan for health benefits.[49] This dosage is supported by Whitehead *et al*[50] who undertook a meta-analysis of high molecular weight (100 kDa) OBG intervention studies in adults with normal, or high cholesterol, including lean, overweight and obese, and some individuals with type 2 diabetes. Their analysis was confined to studies with doses ≥3 g/day and found OBG reduced TC and LDL by 0.30 mmol/L (95% CI 0.24 to 0.35), p=0.0001 and 0.25 mmol/L (95% CI 0.20 to 0.30), p=0.0001 relative to control, respectively, but found no effect on HDL or TG. That study found greater effect in those with higher baseline LDL, or those with type 2 diabetes. Our study used a 4 g/day dose comprising of oats and a high molecular weight OBG extract, which we anticipated would be sufficient to see benefits. One-third of our study participants were receiving statin therapy which can exert potent effects on lipid profiles, with reductions of 20%–50% in LDL depending on the class of statin used.[51] Therefore, statin consumption may have obscured the effects of OBG on lipids.

We found no effect on blood pressure at any timepoint, however, 46% of participants in this study were receiving antihypertensive therapy, with uneven distribution between groups at baseline (table 1). These factors may have influenced these outcomes.

There were no changes in mental well-being when measured on the DASS 21 and SF-36. This is in contrast to our previous work in pregnant woman using HN001 which found significantly lower depression and anxiety scores postpartum with effect sizes of −1.2 (95% CI −2.3 to −0.1), p=0.037, and −1.0 (95% CI −1.9 to −0.2), p=0.014 for depression and anxiety measured on the Edinburgh Postnatal Depression Scale and State Trait Anxiety Inventory respectively.[20] The difference in populations including age, underlying physiology of pregnancy and stress levels, as well as different tools for assessing change may explain these differences. At baseline, our study population had low scores for all components of the DASS 21. The baseline mean (SD) scores for the total study population were: depression 3.7 (4.6); anxiety 3.3 (4.1); and stress 5.9 (5.4) (data not shown). In a normative sample Ronk *et al*[52] established that minimum changes of 3.9, 3.6 and 4.9 are required for the depression, anxiety and stress scales respectively to determine a reliable changes on these scales. In both the depression and anxiety scales these changes are greater than the baseline mean scores of our study population. Population norm means for SF-36 mental and physical component scores are set at 50 with an SD of 10,[53] with higher scores reflecting better health. Baseline mean (SD) of these components for our total population were 54 (7.2) and 51.8 (6.5), respectively (data not shown), again indicating a predominantly mentally healthy population. Where populations are principally healthy the sensitivity of the SF-36 to detect change between groups is limited.[54] Consequently, in our study population there was little room for detectable improvement on the outcome measures used.

A major strength of this study is the factorial design enabling examination of the health effects of the single or combined use of daily HN001 and 4 g OBG to be tested on the clinically relevant primary outcome of HbA_{1c} with a 6-month intervention period. Furthermore, we examined a wide range of metabolic and mental health secondary outcomes relevant to those with pre-diabetes. To our knowledge, this is the first study to examine the effect of HN001 in conjunction with OBG. Few probiotic studies have studied populations with pre-diabetes, and in contrast to those performed among those with established type 2 diabetes where some participants may be on glucose lowering medications our population was naïve to diabetes medication. Therefore, the potential for confounding by medication on glucoregulatory markers results is lower in our study than may be found in those with established diabetes.[16]

CONCLUSIONS

This study does not support the use of HN001 (6×10^9 cfu/day) and/or 4 g/day OBG in the forms used within this study to improve HbA_{1c}, other metabolic and mental health outcomes in those with pre-diabetes. It is possible that future studies in populations with established diabetes may be fruitful and that other probiotics with or without a prebiotic may benefit those with pre-diabetes. Any future studies should evaluate possible differential effects on subgroups according to BMI, gender, ethnicity and socioeconomic status.

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Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval Approval for the study was obtained from the Central Health and Disability Ethics Committee, New Zealand (17/CEN/88).

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