The effects of probiotic bacteria on glycaemic control in overweight men and women: a randomised controlled trial.

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Running head: Probiotics and glycaemic control.

ABSTRACT

Background: Evidence from animal and *in vitro* models suggest a role of probiotic bacteria in improving glycaemic control and delaying the onset of type 2 diabetes. However, the evidence from controlled trials in humans is limited.

Objective: To determine if the probiotic bacteria *L. acidophilus* La5 and *B. animalis* subsp *lactis* Bb12, supplemented in a whole food (yoghurt) or isolated (capsules) form, can improve biomarkers of glycaemic control.

Subjects and methods: Following a 2-week washout period, 156 overweight men and women over 55 y (mean age: 67 ± 8 years; mean BMI: 31 ± 4 kg/m^2) were randomized to a 6-week double-blinded parallel study. The four intervention groups were: A) probiotic yoghurt plus probiotic capsules; B) probiotic yoghurt plus placebo capsules; C) control milk plus probiotic capsules; and D) control milk plus placebo capsules. Outcome measurements including fasting glucose, insulin, glycated haemoglobin and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), were performed at baseline and week 6.

Results: Relative to the milk control group, probiotic yoghurt resulted in a significantly higher HOMA-IR (0.32 ± 0.15, P=0.038), but did not have a significant effect on the other three measures of glycaemic control (P>0.05). Relative to placebo capsules, probiotic capsules resulted in a significantly higher fasting glucose (0.15 ± 0.07 mmol/L, P=0.037), with no significant effect on the other three measures of glycaemic control (P>0.05). Further analyses did not identify other variables as contributing to these adverse findings.

Conclusions: Data from this study does not support the hypothesis that *L. acidophilus* La5 and *B. animalis* subsp *lactis* Bb12, either in isolated form or as part of a whole food, benefit short-term glycaemic control. Indeed, there is weak data for an adverse effect of these strains on glucose homeostasis.

Keywords: Probiotics; yoghurt; dairy products; blood glucose; insulin resistance.

INTRODUCTION

At a population level, increased glycaemia is associated with increased risk of micro- and macro-vascular disease (1-4), even in the non-diabetic range (5). Thus population based approaches to improve glycaemia may reduce adverse vascular outcomes. The pathogenesis of impaired glucose tolerance and insulin resistance is complex and multifaceted. In addition to non-modifiable risk factors such as age, genetics and ethnicity, the worldwide epidemic of excessive body fat due to over-nutrition and physical underactivity, substantially contributes to type two diabetes prevalence (6-9). Interactions between nutrition and the relative abundances of genera comprising the over 100 trillion microorganisms residing in the gastrointestinal tract (10) have also been associated with type two diabetes and related risk factors (11-17).

Recent experimental data provides impetus for further investigation into the role probiotic bacteria can play in improving insulin sensitivity and glucose tolerance (18). Probiotic bacteria are microorganisms which, when administered in adequate amounts, as either isolated bacteria or in food products, confer a health benefit to the host (19). The most commonly investigated and verified health benefits of probiotics is their beneficial effect on gastrointestinal outcomes (20). However, recently the effect of probiotic bacteria on metabolic outcomes has been studied (21-24).

The role of probiotics in improving glycaemic control has been explored in a RCT of probiotic supplementation and dietary education in normoglycaemic pregnant women (25). This study found that in addition to dietary counselling, probiotic supplementation resulted in significantly lower glucose concentrations and reduced risk of elevated blood glucose level. Similarly, probiotic supplementation delayed the onset of glucose intolerance, hyperglycaemia, and hyperinsulinaemia in fructose induced type 2 diabetic rats (26), and improved long-term glycaemic control in streptozotocin-induced diabetic rats (27). The glycaemic benefits of probiotics have been attributed to metabolites of these bacteria are which have been shown to affecting biological signalling pathways, modulate genes involved in ubiquitination and proteasomal processes, and alter autonomic nerve activity. (28-33).

Overall the evidence from animal models suggests that probiotics may be useful in improving glycaemic control and delaying onset of type 2 diabetes. However, there is little data to confirm whether these effects are seen in humans. The proposed study aimed to investigate

57 the effects of *L. acidophilus* La5 and *B. animalis* subsp *lactis* Bb12, provided in either
58 yoghurt or capsules, on biomarkers of glycaemic control in overweight men and women.

METHODS

Subjects

Between February 2012 and February 2013, 156 men and women were recruited using a population-based approach. A random selection of 8,000 men and women aged above 55 years, who were registered on the Western Australian electoral roll, received a letter inviting them to join the study.

Inclusion criteria included minimal usual probiotic intake (consuming less than 400 g yoghurt per week, and not taking probiotic supplements), body mass index (BMI) $\geq$ 25 kg/m^2, elevated waist circumference ($\geq$ 94 cm in men and $\geq$ 80cm in women) and an office blood pressure $\geq$ 120/80 mmHg. Exclusion criteria included: inability to complete the study, intolerance to dairy foods, and the use of antibiotics, immunosuppressive treatments or hypoglycaemic treatments. Of the 887 respondants screened, 156 were considered eligible and were randomised into the study (Figure 1). Prespecified sample size calculations concluded this sample was sufficient to detect a 5% change in fasting glucose concentrations, with 80% power at P=0.05.

Intervention

Participants were asked to cease consumption of all foods and products containing probiotic bacteria during both the 3-week washout and 6-week intervention periods. Following washout, subjects were allocated to 1 of 4 study treatments via block randomization using computer-generated random numbers, devised by a statistician. Participants were assigned to receive either: A) probiotic yoghurt plus probiotic capsules; B) probiotic yoghurt plus placebo capsules; C) control milk plus probiotic capsules; or D) control milk plus placebo capsules. Dairy products and capsules were consumed once daily, 30 minutes prior to the first meal of the day.

Both the probiotic yoghurt and probiotic capsules provided a minimum *Lactobacillus acidophilus* La5 and *Bifidobacterium animalis* subsp *lactis* Bb12 dose of 3.0 x 10^9 CFU/d. All capsules were identical in appearance, size, and colour and were prepared by Chr Hansen (Australia). The probiotic yoghurt (prepared by Casa Dairy Products, Australia) and control milk (prepared by Harvey Fresh, Australia) were similar in their nutritional composition. Participants in the control milk group received 8 g protein, 720 kJ, 4 g saturated fat, 12 g carbohydrate. Similarly, participants in the probiotic yoghurt group received 9 g protein, 650 kJ, 4 g saturated fat, 9 g carbohydrate from yoghurt per day.

Written informed consent was obtained in 100% of participants, and the Human Research Ethics Committee of the University of Western Australia, Perth, Australia, approved the study. The study was carried out in accordance with the World Medical Association Declaration of Helsinki, and was registered with the Australian New Zealand Clinical Trials Registry prior to recruitment (ACTRN12612000033842). All data was collected at Sir Charles Gairdner Hospital, Perth, Australia.

Compliance

Compliance was assessed by counting remaining capsules and weighing remaining dairy product at the completion of the study. Adherence was further assessed by a compliance diary whereby participants kept a daily log of test article consumption throughout the intervention period.

Baseline measurements

At the end of the washout (baseline) standing height was measured by a wall-mounted stadiometer to the nearest 0.1cm, and body weight was measured by an electronic scale to the nearest 0.1 kg. Body mass index was calculated in kg/m^2. Waist circumference was measured by a tape measure to the nearest 0.1 cm at the narrowest part of the torso from the ventral view.

Dietary intake was assessed by a validated semi-quantitative food frequency questionnaire developed by the Anti-Cancer Council of Victoria (34). Energy and nutrient intakes were estimated based on frequency of consumption and an overall estimate of usual portion size (35), and the glycaemic load of the diet was estimated based on published values (36). The international Physical Activity Questionnaire was used to estimate the weekly energy expended in physical tasks, as represented by the metabolic equivalent of task (MET) score (37).

Measurements of glycaemic control

Fasting blood glucose, insulin and glycated haemoglobin (HbA1c) concentrations were assessed at the end of the washout (baseline) and at end of the 6-week intervention period (week 6).

In order to determine effects of the intervention on longer term glycaemic control (38), HbA1c was measured by the Tina-quant Haemoglobin A1c Gen2 whole blood application (Roche Diagnostics for Integra 800 - [A1C-W, 2007-01, V 3]).

Serum glucose was measured by the Architect c16000 Analyser and serum insulin was measured on the Architect i2000SR Analyser. Glucose and insulin reagents were obtained from Abbott Diagnostics (Abbott Laboratories, Abbott Park, IL 60064, USA). In order to determine the effect of the intervention on the responsiveness of peripheral tissues to insulin action, the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated with the following formula: fasting serum insulin (mU/ml) x fasting plasma glucose (mmol/l) / 22.5 (39).

Blinding and statistical analysis

Participants were allocated to a study treatment via block randomization, using computer-generated random numbers (generated by a biostatistician who was not involved in the conduct of the study) sealed in opaque envelopes. All study personnel and participants were blinded to treatment assignment for the duration of the study. A senior investigator not involved in trial implementation held the randomisation code in a password protected folder, which was not broken until the trial had been completed and the analytical protocol had been finalised. All data was analysed according to a pre-specified protocol using SPSS (PASW version 18; IBM Corp., New York, NY, USA).

The week-6 fasting glucose, insulin, glycated haemoglobin and HOMA-IR were compared across intervention groups using a multivariable regression model, with adjustment for the baseline levels of each outcome, and for the effect of the other intervention (40).

As a secondary analysis, the interaction between probiotic yoghurt and probiotic capsules was explored. Further multivariable regression analyses, adjusting for changes (week-6 – Baseline) in BMI, waist circumference, physical activity level, glycaemic load, and intakes of energy, fat, carbohydrate and protein, were undertaken in order to explore factors which may contribute to the findings.

RESULTS

Participant characteristics and compliance

A total of 60 women and 96 men were randomised (**Figure 1**), with a mean age of 67 ± 8 years and a mean BMI of 31 ± 4 kg/m^2. During the 6-week intervention, 5 participants withdrew from the study: 2 due to a death in the family, and 3 due to illnesses which did not appear to be as a result of the dairy products or capsules. Throughout the study period, all participants remained free of hypoglycaemic agent use, and median compliance was 100%.

Treatment groups were well matched at baseline (**Table 1**), and there were no significant differences between groups for age, sex, BMI, waist circumference, physical activity level, and dietary intake variables (P>0.05). Similarly, the biomarkers of glycaemic control (**Table 2**) were not different between intervention groups at baseline (P>0.05). A total of 5 (3%) of participants had a HbA1c value greater than 6.5 % at baseline.

Effect of intervention on biomarkers of glycaemic control

Probiotics from yoghurt or capsules did not significantly alter concentrations of either glycated haemoglobin or insulin relative to control treatments (**Table 3**). Probiotic yoghurt resulted in higher HOMA-IR (**Table 3**), whilst probiotic capsules did not significantly alter HOMA-IR (**Table 4**). Probiotic capsules resulted in higher fasting glucose concentration (**Table 4**), whereas probiotic yoghurt did not significantly alter fasting glucose (**Table 3**).

Exploratory analyses

The interaction between the interventions was investigated as a secondary analysis, and was found to be non-significant: interaction coefficient (HbA1c) = 0.001 (P=0.978); interaction coefficient (glucose) = 0.027 (P=0.871); interaction coefficient (insulin) = 0.684 (P=0.410); interaction coefficient (HOMA-IR) = 0.443 (P=0.507). As such, the observed effects of probiotic yoghurt and probiotic capsules did not appear to be influenced by the presence or absence of the other probiotic test article.

In order to assess how overall glycaemic control at baseline, as assessed by HbA1c, affects responsiveness to the probiotic interventions, we explored the interaction between the interventions and HbA1c. Inclusion of baseline HbA1c in the multivariable regression models did not alter interpretation of results (data not shown).

In order to identify factors which may explain observed results, the degree in which the hyperglycaemia risk factors changed during the intervention period were adjusted for in

multivariable regression analyses. Inclusion of these variables in the models did not ameliorate or exacerbate the effect of the interventions on glycaemic outcomes. Furthermore, we did not observe any significant difference across treatment groups in change (Week 6 – Baseline) of the modifiable risk factors outlined in Table 1 (data not shown).

DISCUSSION

Although data from animal studies suggest mechanisms whereby probiotics may benefit glycaemic control and insulin sensitivity (28-30), the present study suggests that supplementation with *L. acidophilus* La5 and *B. animalis* subsp *lactis* Bb12 does not improve glycaemic control, and may indeed have a slight detrimental effect.

When compared to the appropriate control, capsules containing *L. acidophilus* La5 and *B. animalis* subsp *lactis* Bb12 marginally increased glycaemia, probiotic yoghurt increased insulin resistance, and no statistically significant effect on other biomarkers of glycaemic control was observed. These results are in contrast to the animal studies and other human trial data that suggest an acute and long-term hypoglycaemic effect of probiotic bacteria (25-27). However, our results are in keeping with data from other human randomised controlled trials demonstrating no effect of probiotic bacteria on glycaemia (41-43). The discrepancy between findings from human and animal trials may be due to innate biological differences between species, and the subsequent differences in maintenance of glucose homeostasis.

In this regard, the complexity of beneficial and detrimental probiotic-microbiome-host interactions should be recognised (44). In addition to increasing probiotic levels in the gastrointestinal tract, supplementation of probiotic bacteria can also result in proportional reductions in other genera (45). Furthermore, the activities of probiotic bacteria are highly variable and influenced by numerous factors. Gene expression of probiotic bacteria is not only affected by interactions with other bacteria residing in the gastrointestinal tract, but by the genotype of the host (46). This metabolic variance is further complicated by the effect host diet has on probiotic metabolism (47). The numerous factors affecting probiotic metabolism and activity, and the numerous factors probiotic bacteria impact on are not yet fully understood, and may explain why in this study, *L. acidophilus* La5 and *B. animalis* subsp *lactis* Bb12 did not exert the hypothesised effects.

Another explanation for the discordance between findings from this and other studies may lie with the variations in study design. Studies observing glycaemic benefits of probiotic supplementation used models of induced diabetes or naturally occurring insulin resistance during pregnancy (25-27). Gestational state, although associated with insulin resistance, is almost certainly fundamentally different in physiology compared to non-pregnant individuals, primarily due to the variety of differences in hormonal status (48-50). In concert with the other negative probiotic studies (41-43), this cohort largely exhibited good glycaemic control.

Therefore, despite not observing a mediating association of baseline long term glycaemic control in this primarily healthy population, we hypothesise that the beneficial effects of probiotics may be limited to pathological states of insulin resistance or type 2 diabetes.

An important but often overlooked factor affecting both metabolic outcomes and ability of the bacteria colonise the gastrointestinal tract, is the bacterial strain and combinations of strains used in probiotic products (51). To date, all the animal and human studies of probiotics on glycaemia have used different strains, combinations of strains or doses of probiotic bacteria, which may help explain the variation in reported glycaemic effects. A strength of this study design is that in addition to being commonly used in the yoghurt and supplement industries, the strains *L. acidophilus* La5 and *B. animalis* subsp *lactis* Bb12 used in this study were chosen due to their demonstrated capacity to survive the harsh environment of the human gastrointestinal tract (52-54), adhere to hydrocarbons (55, 56), and exert metabolic benefits (23). However, despite this, glycaemic benefits of these strains were not observed.

We found that probiotic capsules and probiotic yoghurt had different effects on glycaemic biomarkers. The fasting glucose concentration was significantly higher in the participants taking probiotic capsules, but not the probiotic yoghurt group. The HOMA-IR was significantly higher in the participants consuming probiotic yoghurt group, but not the probiotic capsules group. This apparent discrepancy in effects of probiotic yoghurt and capsules may be due to either a Type I or type II statistical error. A post-hoc power calculation showed that the study had only 9% power to detect the observed difference in HOMA-IR for probiotic capsules, and 22% power to detect the observed difference in fasting glucose for probiotic yoghurt. Thus, it may be that there is a negative effect of probiotics on glycaemia, but the effect size is so small that we were underpowered to detect it.

In conclusion, data from this study does not support the hypothesis that *L. acidophilus* La5 and *B. animalis* subsp *lactis* Bb12, either in isolated form or incorporated into a whole food, benefit short-term glycaemic control in men and women. The effect of probiotic bacteria on metabolism is complex due to both the complexity of host-microbiome interactions and the complexity of strains of probiotic bacteria. Future replication studies, particularly in diabetic patients, are indicated in order to clarify the role of probiotic strains on glycaemic control.

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CONFLICT OF INTEREST

No conflicts of interest were reported.

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Figure 1: Trial profile.

Table 1: Baseline characteristics by treatment group.

Dairy test article	**Probiotic yoghurt**		**Control milk**	
Capsule test article	**Probiotic**	**Placebo**	**Probiotic**	**Placebo**
	Group A	**Group B**	**Group C**	**Group D**
Number	40	37	39	40
Age (years)	68.4 ± 7.8	68.4 ± 8.7	64.7 ± 7.1	65.4 ± 8.4
Gender (M:F)	25:15	25:12	23:16	23:17
BMI (kg/m^2)	30.6 ± 3.8	30.2 ± 4.3	30.8 ± 3.5	30.8 ± 3.5
Waist circumference (cm)	103 ± 10	101 ± 12	100 ± 9	100 ± 9
Physical activity (MET)	111 ± 7	109 ± 8	109 ± 8	111 ± 6
Energy intake (kJ/d)	7590 ± 2649	7473 ± 2433	8199 ± 2505	7367 ± 2299
Glycaemic load	83 ± 35	87 ± 33	94 ± 33	81 ± 34
Fat intake (g/d)	69 ± 26	73 ± 29	81 ± 29	72 ± 27
Carbohydrate intake (g/d)	169 ± 66	171 ± 60	187 ± 59	166 ± 64
Protein intake (g/d)	87 ± 31	94 ± 29	94 ± 35	85 ± 29

[1] Results are mean ± SD or n where appropriate.

No significant between group differences were identified by ANOVA (P>0.05).

Table 2: Treatment group summary statistics of glycaemic parameters at baseline and 6-weeks.

Dairy test article	**Probiotic yoghurt**		**Control milk**	
Capsule test article	**Probiotic**	**Placebo**	**Probiotic**	**Placebo**
	Group A	**Group B**	**Group C**	**Group D**
Number	40	37	39	40
Fasting glucose				
Baseline (mmol/L)	5.53 ± 0.57	5.64 ± 1.01	5.59 ± 1.15	5.36 ± 0.55
Week 6 (mmol/L)	5.62 ± 0.65	5.47 ± 0.73	5.58 ± 1.29	5.18 ± 0.65
Change (mmol/L)	0.08 ± 0.39	-0.07 ± 0.43	-0.04 ± 0.36	-0.17 ± 0.50
Fasting insulin				
Baseline (mU/ml)	9.93 ± 4.75	9.63 ± 4.82	9.77 ± 4.59	9.99 ± 4.49
Week 6 (mU/ml)	11.20 ± 5.27	10.59 ± 6.74	9.74 ± 4.56	10.18 ± 5.36
Change (mmol/L)	1.32 ± 2.76	0.63 ± 3.83	-0.18 ± 3.33	0.18 ± 4.22
HOMA-IR				
Baseline	2.47 ± 1.27	2.48 ± 1.45	2.45 ± 1.18	2.44 ± 1.29
Week 6	2.85 ± 1.52	2.63 ± 1.91	2.41 ± 1.17	2.38 ± 1.39
Change (mmol/L)	0.39 ± 0.75	0.15 ± 1.04	-0.05 ± 0.87	-0.05 ± 1.011
HbA1c				
Baseline (%)	5.74 ± 0.41	5.86 ± 0.65	5.83 ± 0.67	5.56 ± 0.36
Week 6 (%)	5.69 ± 0.33	5.74 ± 3.19	5.78 ± 0.64	5.60 ± 0.34
Change (mmol/L)	-0.05 ± 0.28	-0.04 ± 0.23	-0.05 ± 0.28	0.28 ± 0.28

Results are mean ± SD.
No significant between group differences were identified by ANOVA (P>0.05).

Table 3: Main effect model of probiotic yoghurt supplementation on biomarkers of glycaemic control at 6-weeks.

	Probiotic yoghurt[1]		Baseline adjusted difference[2]	P value
	No	**Yes**		
Number	79	77		
HbA1c (%)	5.71 ± 0.03	5.69 ± 0.03	-0.02 ± 0.04	0.710
Glucose (mmol/L)	5.40 ± 0.05	5.52 ± 0.05	0.12 ± 0.07	0.094
Insulin (mU/ml)	9.97 ± 0.40	10.92 ± 0.42	0.95 ± 0.58	0.106
HOMA-IR	2.43 ± 0.10	2.75 ± 0.11	0.32 ± 0.15	**0.038**

[1] Results are week 6 mean (± SE), adjusted for baseline values and treatment.
[2] Mean difference (± SE) between yes and no.
The interaction between the interventions was found to be non-significant (P>0.05).

Table 4: Main effect model of probiotic capsule supplementation on biomarkers of glycaemic control at week 6.

	Probiotic capsule[1]		Baseline adjusted difference[2]	P value
	No	**Yes**		
Number	77	79		
HbA1c (%)	5.71 ± 0.03	5.69 ± 0.03	-0.02 ± 0.04	0.705
Glucose (mmol/L)	5.39 ± 0.05	5.54 ± 0.05	0.15 ± 0.07	**0.037**
Insulin (mU/ml)	10.37 ± 0.42	10.52 ± 0.40	0.15 ± 0.58	0.796
HOMA-IR	2.53 ± 0.11	2.65 ± 0.10	0.12 ± 0.15	0.419

[1] Results are week 6 mean (± SE), adjusted for baseline values and treatment.
[2] Mean difference (± SE) between yes and no.
The interaction between the interventions was found to be non-significant (P>0.05).