**The effect of yoghurt and its probiotics on blood pressure and serum lipid profile; a
randomised controlled trial**

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Running head: yoghurt and cholesterol

ABSTRACT

Background: Despite strong mechanistic data, and promising results from *in vitro* and animal studies, the ability of probiotic bacteria to improve blood pressure and serum lipid concentrations in humans remains uncertain.

Objective: To determine the effect of *Lactobacillus acidophilus* La5 and *Bifidobacterium animalis* subsp *lactis* Bb12, provided in either yoghurt or capsule form, on home blood pressure and serum lipid profile.

Subjects and methods: Following a 3-week washout period, 156 overweight men and women over 55 y were randomized to a 6-week double-blinded, factorial, 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. Each probiotic test article provided a minimum *Lactobacillus acidophilus* La5 and *Bifidobacterium animalis* subsp. *lactis* Bb12 dose of 3.0×10^9 CFU/d. Home blood pressure monitoring, consisting of 7-day bi-daily repeat measurements, were collected at baseline and week 6. Fasting total cholesterol, low density lipoprotein cholesterol (LDLC), high density lipoprotein cholesterol (HDLC), and serum triglyceride were performed at baseline and week 6.

Results: When compared to control milk, probiotic yoghurt did not significantly alter blood pressure, heart rate or serum lipid concentrations ($P > 0.05$). Similarly, when compared to placebo capsules, supplementation with probiotic capsules did not alter blood pressure or concentrations of total cholesterol LDLC, HDLC, or triglycerides ($P > 0.05$).

Conclusions: The probiotic strains *L. acidophilus* La5 and *B. animalis* subsp. *lactis* Bb12 did not improve cardiovascular risk factors.

24 **Keywords:** Probiotics; yoghurt; dairy; blood pressure; cholesterol; lipid.

INTRODUCTION

Features of the metabolic syndrome (MetS) include increased waist circumference, elevated triglycerides, lowered high density lipoprotein cholesterol (HDL), and elevated blood pressure¹. In 2010, 23% of US adults had MetS⁶. Compared to their non-MetS counterparts, people with MetS are over 4.6 times more likely to have insulin resistance and 5.5 times more likely to have cardiovascular disease⁷. Therefore it is important to identify effective, non-pharmacological, population-based approaches for prevention of MetS and its co-morbidities.

Yoghurt is a complex functional food produced by the probiotic bacterial fermentation of milk. The World Health Organisation¹⁸ defines probiotics as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. Interestingly, this definition does not stipulate that probiotics improve colonic microflora composition, suggesting that metabolites of probiotics may exert health benefits, independent of gastrointestinal colonisation. The cardiovascular benefits of yoghurt have been investigated since the early 1970s²⁰, with evidence of beneficial effects^{12; 2}. Despite this, there remains little understanding of the role of the whole food (yoghurt) in these relationships, and the role probiotic bacteria can play in improving blood pressure and serum lipid concentrations.

Yoghurt contains biologically active peptides produced in the bacterial fermentation of milk. Some evidence suggests these bioactive peptides have ACE-inhibitory and antithrombotic activity that may be responsible for the beneficial effects on features of MetS, including reduction in blood pressure^{12; 22; 27; 16}. An alternative mode of action of probiotic bacteria is through direct colonisation of the gastrointestinal tract. In this regard several mechanisms have been advanced to explain the hypocholesterolemic effect of probiotics including the role of probiotic bacteria in increasing: 1) bile acid deconjugation through the action of bile salt hydrolase^{8; 25; 26; 21}; 2) cholesterol and fatty acid assimilation into probiotic bacteria

49 membranes ¹⁹; and 3) conversion of cholesterol to coprostanol in the gastrointestinal tract ¹⁹.
50 Despite promising and consistent results from *in vitro* and animal models ^{8; 25; 26; 21; 19}, the
51 hypocholesterolemic effect of probiotics in humans remains uncertain ^{12; 10; 23}. The major
52 limitation of previous trials is the lack of power to detect small but clinically important
53 effects on serum lipids.

54 This appropriately powered study aims to determine the effect of *Lactobacillus acidophilus*
55 La5 and *Bifidobacterium animalis* subsp *lactis* Bb12, provided in either yoghurt or capsule
56 form, on home blood pressure and serum lipid profile men and women with features of the
57 metabolic syndrome.

METHODS

Subjects

Participants were randomly recruited from the Perth general population aged over 55 y, using the Western Australian Electoral Roll ¹⁵. Inclusion criteria included: minimal usual probiotic intake (consuming < 400 g yoghurt / week, and not taking probiotic supplements), body mass index $\geq 25 \text{ kg/m}^2$, elevated waist circumference ($\geq 94 \text{ cm}$ in men and $\geq 80 \text{ cm}$ in women), and an office blood pressure $\geq 120/80 \text{ mmHg}$. Exclusion criteria included: inability to complete the study, intolerance to dairy foods, and the use of antibiotics, immunosuppressive treatments or hypoglycaemic agents.

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).

Study design

A six week randomized, controlled, parallel, double blind, factorial study was performed. Participants were asked to refrain from consumption of all foods and products containing probiotic bacteria from three weeks prior to their baseline visit, and for the duration of the study.

At baseline, participants were randomly assigned by computer-generated random numbers to 1 of 4 intervention groups. 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. During the 6 week intervention

period, 5 participants withdrew from the study due to personal reasons (n = 2) and illnesses unrelated to the test articles (n = 3); A = 1, B = 3, and C = 1¹⁵.

Dairy products and capsules were consumed once daily for 6 weeks, 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 10⁹ CFU/d. All capsules were identical in appearance, size, and colour and were prepared by Chr. Hansen (Australia).

The unflavoured, unsweetened probiotic yoghurt (prepared by Casa Dairy Products, Australia) provided 650 kJ, 9 g protein, 4 g saturated fat, 9 g carbohydrate per day. The unflavoured, unsweetened control milk (prepared by Harvey Fresh, Australia) provided 720 kJ, 8 g protein, 4 g saturated fat, and 12 g carbohydrate per day.

Baseline and week 6 measurements

Data pertaining to past medical history, medication use and history of cigarette smoking were collected at recruitment, and updated at the 6 clinic visits throughout the 9 week study period. Data on use of cholesterol lowering supplements, which included fish oil and omega 3, was also collected at baseline and throughout the study. Estimates of energy and nutrient consumption were based on frequency of consumption and an overall estimate of usual portion size obtained from a validated semi-quantitative food frequency questionnaire^{14; 13}.

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/m². 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) score⁹.

104 *Home blood pressure monitoring*

105 Participants performed bi-daily home blood pressure measurements during the 7 days
106 preceding both the baseline and week 6 visits. A fully automated home blood pressure
107 monitor (UA-767PC, A&D, Japan) was provided to each participant at visits held one week
108 prior to baseline, and one week prior to week 6. During these visits, participants were guided
109 through the correct measurement procedure and were asked to perform a blood pressure
110 reading in front of the study coordinator. This was to ensure the reliability of the blood
111 pressure data from home monitoring.

112 Participants recorded blood pressure twice daily; approximately 1 hour after breakfast and
113 approximately 1 hour after the evening meal. In a quiet room, participants relaxed in a seated
114 position for 5 minutes, and then performed 2 measurements over the following 5 minutes.

115 The blood pressure cuff was positioned on bare skin, and located 2-3 cm above the
116 antecubital position, and the arm was rested in a lateral position with the cuff in line with the
117 heart.

118 Systolic blood pressure, diastolic blood pressure, pulse pressure and heart rate measurements
119 were automatically recorded on the home blood pressure monitor, and were downloaded
120 when monitors were returned to the study centre at the baseline and week 6 visits. In addition,
121 participants also kept a diary where they manually recorded results of each home blood
122 pressure measurement. In the case of missing or excess data recorded on the device,
123 electronically recorded values were cross referenced with the manual records.

Measurements of serum lipid profile

At baseline and at 6 weeks, blood samples were collected from the antecubital fossa vein after an overnight fast. All biochemical analyses were performed in the PathWest laboratory at the Royal Perth Hospital, Western Australia.

Serum was analysed for total cholesterol, HDLC and triglycerides using routine analysis based on an enzymatic colorimetric test using the fully automated Architect c16000 Analyser (Abbott Diagnostics, Abbott Laboratories, Abbott Park, IL 60064, USA). Low density lipoprotein cholesterol (LDLC) concentrations were calculated using the Friedewald formula¹¹.

Statistical analysis

A pre-specified sample size calculation determined that 68 participants in each main effect treatment arm (probiotic yoghurt or probiotic capsules) would be sufficient to detect a 3.6% change in LDLC (~0.13 mmol/L), with 80% power at P=0.05. Given the factorial study design and analysis approach adopted by this study, this sample size equated to 34 participants in each of the 4 intervention groups. With P=0.05, 68 participants per main effect treatment arm was also calculated to provide >80% power to detect a 3.5 mmHg difference in mean systolic blood pressure (measured twice daily over 7 days). This number was increased to allow for a predicted drop-out from treatment of around 10%.

All data was analysed according to a pre-specified protocol using SPSS (PASW version 18; IBM Corp., New York, NY, USA). Outcome measurements 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 intervention²⁴. As a secondary analysis, the interaction between probiotic yoghurt and probiotic capsules was explored.

RESULTS

Baseline cohort characteristics

A total of 60 women and 96 men were randomised, with a mean age of 67 ± 8 years and a mean BMI of 31 ± 4 kg/m². The mean baseline total cholesterol of the cohort was $5.33 (\pm 1.15)$ mmol/L. Mean 7-day home systolic and diastolic blood pressures at baseline were $131 (\pm 12)$ and $74 (\pm 9)$ mmHg, respectively.

Treatment groups were well matched, and there were no significant differences between groups for baseline characteristics ($P > 0.05$); **Table 1**. Similarly, the blood pressure, heart rate (**Table 2**), and serum lipid profile (**Table 3**) of participants were similar between intervention groups at baseline ($P > 0.05$).

Physical activity, energy and nutrient intakes

The level of physical activity as well as intakes of energy and nutrients were similar between the intervention groups at baseline (**Table 1**) and week 6 ($P > 0.05$). Furthermore, we did not observe any significant ($P > 0.05$) differences across treatment groups in change (Week 6 – Baseline) of these measures (data not shown).

Effect of probiotics on blood pressure

When compared to control milk, probiotic yoghurt did not significantly alter blood pressure or heart rate ($P > 0.05$). Similarly, when compared to placebo capsules, supplementation with probiotic capsules did not alter blood pressure or heart rate ($P > 0.05$); **Table 4**.

Effect of probiotics on serum lipid profile

When compared to control milk, probiotic yoghurt did not significantly alter lipid concentrations ($P > 0.05$). Similarly, when compared to placebo capsules, supplementation

169 with probiotic capsules did not alter concentrations of total cholesterol LDLC, HDLC, or
170 triglycerides ($P > 0.05$); **Table 5**.

171 *Secondary analysis*

172 In a secondary analysis, we found no evidence for an interaction between probiotic yoghurt
173 and probiotic capsules ($P > 0.05$). As such, the lack of significant effects of probiotic yoghurt
174 and probiotic capsules did not appear to be influenced by the presence or absence of the other
175 probiotic test article.

DISCUSSION

We assessed the effects of 6 week probiotic supplementation on blood pressure and serum lipid profile in 156 overweight or obese men and women. There was no evidence that blood pressure, heart rate, or lipid concentrations were altered as a result of consuming whole-fat yoghurt or capsules containing the probiotic strains *L. acidophilus* La5 and *B. animalis* subsp. *lactis* Bb12.

We found that combination therapy of *L. acidophilus* La5 and *B. animalis* subsp. *lactis* Bb12 had no effect on blood pressure. Our findings of lack of effect are supported by results of other randomised controlled trials which supplemented with the same combination of probiotic strains^{4; 29}. As the effects of probiotic bacteria are highly strain specific¹⁷, it is not surprising that studies which supplemented with different probiotic strains and species found different results. Specifically, numerous randomised controlled trials of *L. helveticus* have demonstrated that supplementation with this species has an antihypertensive effect in humans^{12; 27; 16}. As such, the results of our study support the strain specificity of probiotic actions by confirming that unlike other probiotic preparations, *L. acidophilus* La5 and *B. animalis* subsp. *lactis* Bb12 co-therapy does not improve blood pressure.

Similar to results from other clinical trials^{12; 10; 23}, our study found that daily probiotic supplementation had no effect on serum lipid concentrations. However, these findings are in contrast to other human trial data demonstrating that probiotic supplementation improves serum lipid concentrations^{3; 5}. Our results are further opposed by strong *in vitro* and animal model data which clearly define mechanisms supporting the hypocholesterolaemic effect of probiotics^{8; 25; 26; 21; 19}.

The lack of cholesterol lowering effect in our, and other studies, may be explained by subject parameters. The mean baseline cholesterol concentration in our cohort cholesterol was 5

200 mmol/L. The mean baseline total cholesterol concentration of participants in the other
201 randomised controlled trials that did not observe a beneficial effect was less than 5.4 mmol/L
202 ^{12; 10; 23}. Conversely, the mean baseline total cholesterol concentration of participants in trials
203 which observed a hypocholesterolaemic effect of probiotics was ≥ 5.7 mmol/L ^{3; 5}. These
204 findings suggest that the hypocholesterolaemic benefits of probiotics may be limited to
205 populations with borderline high / high baseline total cholesterol levels.

206 It is conceivable that the lack of significant results in this study may be attributed to a lack of
207 power to detect observed associations. This may occur if the treatment effect was smaller
208 than the hypothesised 3.6% improvement in LDLC that was used as the basis of our
209 prespecified power calculations. It is important to note, however, intervention effect sizes
210 smaller than this may be unlikely to be of clinical relevance in terms of cardiovascular
211 disease prevention ²⁸.

212 In conclusion, the probiotic strains *L. acidophilus* La5 and *B. animalis* subsp. *lactis* Bb12 did
213 not improve 7-day home blood pressure. The lack of antihypertensive effect is likely due to
214 the strain specificity of probiotic actions. Furthermore, probiotic supplementation did not
215 improve serum lipid parameters. This lack of effect may be a result of the relatively good
216 baseline cholesterol levels of the cohort. As such, future replication studies supplementing
217 with *L. acidophilus* La5 and *B. animalis* subsp. *lactis* Bb12 in hypercholesterolemic subjects
218 are indicated.

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Table 1: Baseline characteristics of participants by treatment group

Dairy test article Capsule test article	Probiotic yoghurt		Control milk	
	Probiotic	Placebo	Probiotic	Placebo
	Group A	Group B	Group C	Group D
Number	40	37	39	40
Baseline characteristics				
Age (years)	68 ± 8	68 ± 8.7	65 ± 7	65 ± 8
Gender (M:F)	25:15	25:12	23:16	23:17
Body mass index (kg/m ²)	31 ± 4	30 ± 4	31 ± 4	31 ± 4
Hypolipidaemic medication use [n(%)]	25 (62)	21 (57)	20 (51)	27 (68)
Hypolipidaemic supplement use ¹ [n(%)]	14 (35)	14 (36)	11 (28)	16 (40)
History of Cigarette smoking [n(%)]	21 (52)	21 (57)	21 (46)	21 (52)
Prevalent cardiovascular disease [n(%)]	12 (30)	13 (35)	16 (41)	18 (45)
Physical activity (MET)	111 ± 7	109 ± 8	109 ± 8	111 ± 6
Dietary intake				
Energy (kJ/d)	7.6 ± 2.6	7.4 ± 2.4	8.2 ± 2.5	7.4 ± 2.3
Saturated fat (g/d)	27 ± 12	29 ± 12	33 ± 14	30 ± 13
Cholesterol (g/d)	303 ± 133	297 ± 122	335 ± 159	308 ± 134
Fibre (g/d)	20 ± 8	21 ± 8	22 ± 8	20 ± 7
Sugar (g/d)	80 ± 34	74 ± 26	82 ± 23	73 ± 31

Results are mean ± SD or n (%) where appropriate.

No significant between group differences were identified by ANOVA or chi squared test, where appropriate ($P > 0.05$).

¹ Hypolipidaemic supplements included fish oil and omega 3 supplements

Table 2: Seven day home blood pressure parameters stratified 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
Systolic blood pressure				
Baseline (mmHg)	131 ± 13	130 ± 12	132 ± 12	130 ± 12
Week 6 (mmHg)	131 ± 14	129 ± 11	130 ± 12	129 ± 13
Diastolic blood pressure				
Baseline (mmHg)	74 ± 11	74 ± 7	76 ± 10	74 ± 7
Week 6 (mmHg)	74 ± 10	75 ± 7	75 ± 9	73 ± 8
Pulse pressure				
Baseline (mmHg)	57 ± 12	57 ± 11	56 ± 14	56 ± 12
Week 6 (mmHg)	57 ± 12	55 ± 10	56 ± 13	56 ± 14
Heart rate				
Baseline (bpm)	71 ± 9	71 ± 9	70 ± 14	72 ± 12
Week 6 (bpm)	70 ± 9	70 ± 9	70 ± 14	72 ± 11

Results are mean ± SD.

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

Table 3: Serum lipid concentrations stratified by treatment group

Dairy test article Capsule test article	Probiotic yoghurt		Control milk	
	Probiotic	Placebo	Probiotic	Placebo
	Group A	Group B	Group C	Group D
Number	40	37	39	40
Total cholesterol				
Baseline (mmol/L)	5.52 ± 0.95	5.35 ± 1.40	5.20 ± 1.12	5.22 ± 1.12
Week 6 (mmol/L)	5.37 ± 0.88	5.32 ± 1.33	5.15 ± 1.10	5.21 ± 1.09
LDLC				
Baseline (mmol/L)	3.31 ± 0.77	3.22 ± 1.16	3.07 ± 0.92	3.10 ± 0.95
Week 6 (mmol/L)	3.18 ± 0.75	3.20 ± 1.18	3.03 ± 0.87	3.10 ± 0.95
HDLC				
Baseline (mmol/L)	1.42 ± 0.37	1.36 ± 0.32	1.41 ± 0.33	1.42 ± 0.35
Week 6 (mmol/L)	1.40 ± 0.34	1.36 ± 0.32	1.39 ± 0.33	1.44 ± 0.36
Triglycerides				
Baseline (mmol/L)	1.70 ± 0.77	1.70 ± 0.94	1.58 ± 0.74	1.53 ± 0.79
Week 6 (mmol/L)	1.71 ± 0.75	1.65 ± 0.77	1.59 ± 0.82	1.47 ± 0.62

Results are mean ± SD.

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

Table 4: Main effect model of yoghurt and probiotic supplementation on home blood pressure parameters

	Treatment arm ¹		Baseline adjusted difference ²	P value
	Yoghurt supplementation			
	No	Yes		
Systolic blood pressure (<i>mmHg</i>)	129 ± 1	130 ± 1	1.1 ± 1.8	0.548
Diastolic blood pressure (<i>mmHg</i>)	74 ± 1	75 ± 1	1.1 ± 1.3	0.422
Pulse pressure (<i>mmHg</i>)	56 ± 1	56 ± 1	-0.1 ± 1.8	0.933
Heart rate (bpm)	71 ± 1	70 ± 1	-0.6 ± 1.7	0.703
	Probiotic supplementation			
	No	Yes		
Systolic blood pressure (<i>mmHg</i>)	129 ± 1	130 ± 1	0.9 ± 1.8	0.601
Diastolic blood pressure (<i>mmHg</i>)	74 ± 1	75 ± 1	0.8 ± 1.3	0.543
Pulse pressure (<i>mmHg</i>)	56 ± 1	56 ± 1	0.9 ± 1.8	0.615
Heart rate (bpm)	70 ± 1	70 ± 1	-0.1 ± 1.7	0.950

¹ Baseline and treatment adjusted week 6 values ($\pm$ SE).

² Mean difference between yes and no ($\pm$ SE).

Table 5: Main effect model of yoghurt and probiotic supplementation on serum lipid parameters

	Treatment arm ¹		Baseline adjusted difference ²	P value
	Yoghurt supplementation			
	No	Yes		
Total cholesterol (mmol/L)	5.29 ± 0.06	5.23 ± 0.06	-0.06 ± 0.09	0.477
LDLC (mmol/L)	3.16 ± 0.05	3.10 ± 0.05	-0.06 ± 0.07	0.410
HDLC (mmol/L)	1.42 ± 0.02	1.38 ± 0.02	-0.04 ± 0.02	0.126
Triglyceride (mmol/L)	1.58 ± 0.05	1.63 ± 0.06	0.05 ± 0.08	0.490
	Probiotic supplementation			
	No	Yes		
Total cholesterol (mmol/L)	5.29 ± 0.06	5.24 ± 0.06	-0.06 ± 0.08	0.513
LDLC (mmol/L)	3.16 ± 0.05	3.10 ± 0.05	-0.06 ± 0.07	0.377
HDLC (mmol/L)	1.41 ± 0.02	1.39 ± 0.02	-0.02 ± 0.02	0.356
Triglyceride (mmol/L)	1.57 ± 0.06	1.64 ± 0.05	0.07 ± 0.08	0.376

¹ Baseline and treatment adjusted week 6 values (±SE).

² Mean difference between yes and no (±SE).