Available at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/jff

Lactobacillus fermentum and *Lactobacillus amylovorus* as probiotics alter body adiposity and gut microflora in healthy persons

Jaclyn M. Omar[a], *Yen-Ming Chan*[a], *Mitchell L. Jones*[b,c], *Satya Prakash*[b,c], *Peter J.H. Jones*[a,*]

[a]*Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, MB, Canada*
[b]*Department of Biomedical Engineering, Faculty of Medicine, McGill University, Montreal, Quebec, Canada*
[c]*Micropharma Limited, Montreal, Quebec, Canada*

ARTICLE INFO

Article history:
Received 8 February 2012
Received in revised form
3 September 2012
Accepted 4 September 2012
Available online 9 October 2012

Keywords:
Probiotics
Adiposity
Lactobacillus
Microflora
Energy expenditure

ABSTRACT

Modification of gut microflora has been reported as altering energy and lipid homeostasis, leading to changes in body composition. We evaluated whether consumption of *Lactobacillus amylovorus* (LA) and *Lactobacillus fermentum* (LF) as novel probiotics alters body adiposity through modification of gut microflora. Healthy, but overweight participants ($n = 28$) consumed yogurt containing 1.39×10^9 colony-forming unit (CFU) microencapsulated LA, 1.08×10^9 CFU microencapsulated LF, or a control yogurt using a randomized, double-blind crossover design. Body composition measurements showed that body fat mass was reduced in all treatments, with the greatest reduction from LA consumption. Bacterial distribution of gut microflora determined a significant reduction in the abundance of *Clostridial cluster IV* from LA consumption and significant increases in the abundance of *Lactobacillus* in both LF and LA treatments. The results suggest that modulation of gut microbial composition from probiotic consumption may contribute to altered energy metabolism and body composition.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Obesity exists as a major public health concern in developed countries, as well as increasingly in developing countries, and is a significant risk factor for chronic diseases including diabetes, cardiovascular disease, and cancer (DiBaise et al., 2008). Obesity occurs when energy intake exceeds expenditure, often accompanied by chronic low-grade inflammation and impaired gut hormone signaling related to energy expenditure and body adiposity (Murphy & Bloom, 2006). Recent evidence suggests that gut microflora are directly involved in regulating energy metabolism, and thus changes in the compositional abundance of the gut microflora may confer a modification to energy expenditure (Ley et al., 2005; Raoult, 2008; Turnbaugh, Bäckhed, Fulton, & Gordon, 2008). Animal studies have demonstrated that gut microbial populations and energy metabolism vary substantially between obese mice and their lean counterparts, an observation that suggests a causative association for this dissimilarity in body composition (Turnbaugh et al., 2006). For instance, a study

* *Corresponding author*. Address: Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, 196 Innovation Drive, Winnipeg, MB, Canada R3T 2N2. Tel.: +1 204 474 8883; fax: +1 204 474 7552.
E-mail address: peter_jones@umanitoba.ca (P.J.H. Jones).

Abbreviations: CFU, colony-forming unit; LA, *Lactobacillus amylovorus*; LF, *Lactobacillus fermentum*; FAE, ferulic acid esterase; FA, ferulic acid; APA, alginate-poly-L-lysine-alginate; DXA, dual energy X-ray absorptiometry.

1756-4646/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jff.2012.09.001

with lean gnotobiotic mice inoculated with obese mice gut microbiota developed heightened glycemia, insulinemia and increased fat stores within two weeks (Turnbaugh et al., 2006). The dramatic change in lipid and glucose metabolism observed demonstrates that the gut microbiome may possess properties that determine how efficiently energy is harvested from the diet; and whether it is stored or expended (Turnbaugh et al., 2006).

The link between microbial ecology in the gut and fat storage seen in animal studies appears to also apply to humans. Ley et al. showed in obese individuals that with proportional weight loss, populations of *Firmicutes* bacteria decreased and *Bacteriodetes* increased (Ley, Turnbaugh, Klein, & Gordon, 2006). These findings suggest that the composition as well as the relative abundance of gut microbes affects energy metabolism in humans (Ley et al., 2006).

Bacteria from the genus *Lactobacillus* have been used as probiotics for their capability to lower colonic pH through the production of lactic acid, inhibiting the growth of pathogenic bacteria (Collado, Jalonen, Meriluoto, & Salminen, 2006). Furthermore, a number of various bacterial strains have been used as probiotics since they have demonstrated to confer promising health benefits to the host when administered in adequate amounts. The *Lactobacillus fermentum* (LF) strain of bacteria possesses a high ferulic acid esterase (FAE) activity. In recent years increased interest has occurred in the health benefits of polyphenols, such as ferulic acid (FA), which are found in plant-derived foods. Natural sources of FA, however, are less readily bioavailable and must be enzymatically released to be made bioavailable and absorbable by the human digesta. Thus, orally ingested microencapsulated FAE producing *Lactobacilli* can potentially lead to increased bioavailability of FA and health benefits (Simons, Amansec, & Conway, 2005). In addition, LF has been shown to lower serum lipids and reduce the progression of atherosclerotic lesions in hypercholesterolemic hamsters (Bhathena et al., 2009). The lipid lowering effect of LF seen in hamsters was also tested in humans with elevated cholesterol via capsule administration and was not found to be effective at reducing serum lipids (Bhathena et al., 2009; Simons et al., 2005). As the resistance to host factors, such as digestive acid and enzymes, varies according to the physiology of the bacterial strain, the lipid lowering efficacy of LF may be improved via incorporation into yogurt rather than capsules (Sanders, 2008). Yogurt and other dairy sources have shown to be ideal carriers of probiotics for their proposed buffering capacity against the low pH of gastric acid, which prevent degradation of treatment bacteria and facilitate gut colonization, thus providing an explanation as to the low efficacy of LF to reduce circulating lipids when given in a probiotic capsule (Sanders, 2008). In addition, a current report has shown that LF isolated from fermented milk possesses cholesterol-lowering properties (Pan, Zeng, & Yan, 2011). Therefore, LF was selected for its high FAE activity and ability to reduce serum lipids as a basis to investigate novel properties such as modulation of body adiposity and gut microbial abundance as these elements of this bacteria have yet to be explored in humans. The *Lactobacillus amylovorus* (LA) strain was selected for its purported bile salt hydrolase (BSH) activity enabling deconjugation of bile salts, which is known for its cholesterol-lowering properties. In addition, deconjugated bile salts are less likely to form micelles at the pH of the gut lumen, which may lead to reductions in fat absorption and a possible modification in body adiposity (Grill, Cayeula, Antoine, & Schneider, 2000; Thomas, Pellicciari, Pruzanski, Auwerx, & Schoonjans, 2008). Currently, the two test bacteria strains (LA & LF) are not popular commercially, as they are still considered quite novel in the field of probiotics and functional foods, where current research investigating the viability of these bacterial strains for probiotic consumption is lacking, with the majority of research on these strains focusing on immune properties (Cox, Pyne, Saunders, & Fricker, 2010). However, a current report showed that LF isolated from fermented milk possesses cholesterol-lowering properties (Pan et al., 2011) Furthermore, we wanted to investigate the effect of novel probiotics not typically consumed by humans as probiotics for their digestive health properties, as is the case with *Lactobacillus acidophilus* and *Bifidus bifidobacteria*.

Given these findings, the aim of the current study was to investigate whether the consumption of the probiotics, LF and LA modify the microbial population present in the gut and bring about specific metabolic effects impacting energy storage and body adiposity in healthy humans (DiBaise et al., 2008).

2. Materials and methods

2.1. Participants

The study participants consisted of twenty-eight healthy, but overweight individuals (males = 10; females = 18). Inclusion criteria included a body mass index (BMI) between 25 and 32 kg/m^2 and aged 18–60 for this study and participants diagnosed as having heart disease, kidney disease, diabetes mellitus, liver disease, lactose intolerance or recent major surgery were excluded from the study, as well as any individuals who were taking medications known to affect lipid metabolism (Table 1). In addition, participants underwent a complete physical examination including assessment of medical history and informed consent was obtained by all study participants. The University of Manitoba Biomedical Research Ethics Board approved all study procedures as our institutional review board authority, reference number B2007:070. This clinical study was registered with the United States National Library of Medicine public trials registry record B2007:070.

2.2. Experimental protocol

The study, a placebo controlled, double-blind cross-over clinical investigation using a Latin square sequence design, consisted of three 43 day phases, each separated by a six week wash out period. Participants were randomized to one of three treatment arms per phase: (a) control yogurt; (b) yogurt containing 1.39×10^9 colony-forming units (CFU) of microencapsulated BSH-active LA bacteria; and c) yogurt containing 1.08×10^9 CFU microencapsulated FAE-active LF bacteria. Each participant received all treatments during the study. During each treatment period, participants were provided with diets containing 35% of energy as fat, 50% carbohydrate and 15%

Table 1 – Inclusion and exclusion criteria for study participants.

Inclusion	Exclusion
18–60 years of age	Receiving medications known to affect lipid metabolism
Body mass index (BMI) between 25 and 32 kg/m^2	Diagnosed to have diabetes mellitus, heart disease, liver disease, kidney disease, lactose intolerance
	Recently undergone major surgery

protein (Table 2). All meals were prepared in the metabolic kitchen located at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) using a three-day rotation menu. Control and the two treatment yogurts were consumed with 4 g of wheat bran with supper, which were incorporated to act as prebiotics. Participants were instructed to consume their supper meal in conjunction with one treatment or control yogurt, which was consumed under supervision on a daily basis to monitor compliance and confirm treatment consumption. The remaining meals were packed for take-out. To ensure compliance of consumption of background diet, participants were instructed to return the empty containers. Treatment bacteria intake was confirmed via fecal microbial abundance as an indicator of consumption and degree of colonization. Individual basal energy requirements were determined using the Mifflin equation (Mifflin et al., 1990) and were multiplied by a physical activity factor of 1.7.

2.3. *Yogurt composition and preparation*

Control and treatment yogurts were produced at McGill University, Montreal, Canada. Control yogurts were vanilla-flavored and fermented with *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies bulgaricus* as lactic acid bacteria starters. Control and treatment yogurts contained 100 g of control yogurt; treatment yogurts additionally contained either 10 g of 1.08×10^9 CFU microencapsulated LF or 10 g of 1.39×10^9 CFU microencapsulated LA per container. Once 10 g of treatment was thoroughly incorporated into 100 g of control yogurt, both control and treatment yogurts were packaged into plastic serving containers and stored at 4 °C until consumption. Quality control procedures were performed to ensure consistency between batches.

Table 2 – Average nutrient profile of study diet over a 3 day period.

Diet component	% of energy[a]
Protein	15.0 ± 0.2
Carbohydrate	50.1 ± 0.1
Fat	35.0 ± 0.0
SFA	7.7 ± 0.7
MUFA	14.1 ± 1.3
PUFA	8.8 ± 1.2
Cholesterol (mg/1000 kcal)	78.6 ± 1.3
Fiber (g/1000 kcal)	11.2 ± 0.3

[a] Values are mean ± SE. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA; polyunsaturated fatty acids.

2.4. *Microencapsulation of treatment bacteria*

Alginate-poly-L-lysine-alginate (APA) microcapsules were prepared in compliance with Micropharma Standard Operating Procedure and Quality control procedures. To prepare microcapsules, grown cultures of LF or LA were centrifuged at 3800 rpm (4500 g) for 15 min at 4 °C. After removal of supernatant, the pelleted cell isolate (LF or LA) was re-suspended in sterile saline (0.85% (w/v) NaCl) and gently added to a lightly stirred sterile sodium alginate solution with a final concentration of 1.5% (w/v). In a sterile environment, the bacterial alginate suspension was passed through an Inotech Encapsulator at a payload of 8% (Inotech Biosystems International Inc., Rockville, MD, USA). Encapsulation parameters were as follows: nozzle diameter 300 μm, vibrational frequency 1200 Hz, syringe pump speed 22.0, voltage ~1.90 kV. After extrusion, the droplets were allowed to gel for 10 min in a gently stirred sterile 0.1 M $CaCl_2$ solution. The Ca-alginate beads were sequentially washed with 0.85% (w/v) NaCl for 3 min, coated with 0.1% (w/v) poly-L-lysine for 10 min, washed with 0.85% (w/v) NaCl for 3 min, coated with 0.1% (w/v) sodium alginate for 10 min and washed with 0.85% (w/v) NaCl for 3 min. The resulting APA microcapsules containing either LF or LA were dispensed at 10 g microcapsules in 100 g yogurt and well mixed. The viability of the test bacteria was not tested at the time of consumption; however, faecal microbial abundance of *lactobacillus* was analyzed through quantitative real time-PCR as an indicator of consumption.

2.5. *Body weight and composition assessment*

Body weight of study participants was measured at the beginning and end of each phase to assess whether probiotic yogurt consumption had an effect on overall body weight across treatments. Participants were instructed to maintain normal physical activity to ensure body weight fluctuations were not due to increased activity expenditure, as participant physical activity levels were not measured. Body composition of study participants was measured using dual-energy X-ray absorptiometry (DXA) (General Electric Lunar Digital Prodigy Advance, Madison, WI, USA) at the beginning and end of each phase in order to determine overall total fat mass and total lean mass. Resulting data were analyzed with the corresponding volume integration software (Encore 2005, version 9.30.044; General Electric Lunar Digital Prodigy Advance, Madison, WI, USA).

2.6. *Faecal energy concentration analysis*

Bomb calorimetry (Parr 6300, Moline, IL, USA) was used to determine faecal energy per unit weight (cal/g) and to

quantify energy losses. Two consecutive faecal specimens were collected from each participant at the end of each study phase for a total of three duplicate samples per subject for the entire study. Faecal samples were frozen at −80 °C, freeze dried and ground to produce a homogenous sample.

2.7. DNA extraction and RT-PCR for faecal microbial composition analysis

Faecal samples were thawed, resuspended and washed in phosphate buffered saline (PBS) then centrifuged. DNA was extracted from the pellets by using ZR Faecal DNA Kit (D6010, Zymo Research Corp., Irvine, CA, USA), which included a step for mechanical lysis of the microbial cells. DNA concentration and purity were determined spectrophotometrically by measuring the OD at $A_{260/280}$ (Beckman DU/800, Beckman Coulter Inc., Fullerton, CA, USA). The PCR primers used are listed in Table 3. Primers were assembled from the literature or newly designed and tested for specificity *in silico*. Primers were synthesized by University Core DNA Services (University of Calgary, Calgary, AB, Canada). Real-time PCR was carried out using an AB 7300 system (Applied Biosystems, Foster City, CA, USA) and sequence detection software (Version 1.3; Applied Biosystems, Foster City, CA, USA). Each reaction was run in triplicate. Amplification reactions were carried out with Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) mixed with the selected primer set (Table 5) and 12 ng of genomic DNA. To evaluate the efficiency (E) of amplification of each primer set, DNA templates were pooled. Amplification efficiency was calculated from the slope of the standard curve generated from plotting the threshold cycle (CT) versus logarithmic values of different DNA concentrations using the following equation (Denman & McSweeney, 2006):

$$E = 10^{-1/\text{slope}}$$

Relative quantification followed the mathematical model (Pfaffl, 2001):

$$R_i = [(E_{\text{target}})\Delta CT_{\text{target}}(\text{Control}_i - \text{SARA}_i)]/[(E_{ref})\Delta CT_{\text{ref}}(\text{Control}_i - \text{SARA}_i)]$$

The relative expression ratio of a target gene versus control in a sample was compared to a reference gene. E_{target} is the real-time efficiency of a 16S rDNA target gene transcript; E_{ref} is the real time efficiency of the reference gene *Eubacteria*. ΔCT is the CT deviation of the control vs treatment, i is the period, and R_i is the relative expression ratio of a target gene compared to a reference gene at a specific time point (Denman & McSweeney, 2006).

2.8. Statistical analysis

All data were expressed as mean ± SE. Statistical significance was set at $p < 0.05$ for all analyses. Data that were not normally distributed underwent log transformation. Differences between treatments at baseline and endpoint for body composition were compared by analysis of variance (ANOVA) model for determination of diet effects. When diet effects were found to be significant, Least Squares Means tests were used to identify differences between individual diets. Student's paired t-tests were used to compare diet effects between baseline-endpoint as well as differences of percent changes at endpoint relative to control between LF and LA treatments. The LSD multiple comparison tests were conducted to detect significant differences across treatment groups in gut microbial composition parameters. The sample size of this study was determined based on previous clinical trials investigating similar primary outcomes versus a traditional power analysis. Statistical analysis was conducted with SAS software (version 8.0; SAS Institute Inc, Cary, NC, USA).

3. Results

Twenty-eight participants (10 males and 18 females) completed the entire trial, baseline characteristics of the study are displayed in Table 4.

Table 3 – Primers used for RT-PCR analysis.

Target group	Primer name	Primer sequence	Amplicon size (bp)
Eubacteria	341–357F 518–534R	CCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	189
Lactobacillus spp. (*Lac*)	Ulac16S1F Ulac16S1R	AGCAGTAGGGAATCTTCCA ATTCCACCGCTACACATG	345
Bacteroides spp. (*Bact*)	Bac303F Bfr-Fmrev	GAAGGTCCCCCACATTG CGCKACTTGGCTGGTTCAG	103
Roseburia spp. and *E. rectale* (*Rrec1*)	RrecF Rrec630mR	GCGGTRCGGCAAGTGTGA CCTCCGACACTCTAGTMCGAC	81
Clostridial cluster IV (*Clep*)	Clep866mF Clept1240mR	TTAACACAATAAGTWATCCACCTGG ACCTTCCTCCGTTTTGTCAAC	314
Cluster IV Ruminococcus spp. (*Rum*)	Rflbr730F Clep866mR	GGCGGCYTRCTGGGCTTT CCAGGTGGATWACTTATTGTGTTAA	157
Bifidobacterium adolescentis (*Bad*)	Bif164F BiADO-2	GGGTGGTAATGCCGGATG CGAAGGGCTTGCTCCCAGT	298

Table 4 – Baseline and endpoint for anthropometric measurements, body weight, total fat mass, and total lean mass of participants in response to the consumption of LF-containing yogurt, LA-containing yogurt and control yogurt.

Characteristic	Mean ± SE		
Anthropometric measurements[a]			
N	28		
Age (years)	46.3 ± 2.4		
Height (cm)	164.6 ± 1.8		
Initial body weight (kg)	85.7 ± 2.6		
Initial BMI (kg/m^2)	31.6 ± 0.7		
Parameter	LF	LA	Control
Body weight (kg) [a]			
n	14	14	14
Initial	83.9 ± 3.4	82.7 ± 2.9	82.7 ± 3.2
Final	81.3 ± 3.5	80.9 ± 3.1	80.8 ± 3.2
Total fat mass (kg) [a]			
n	9	10	11
Initial	36.4 ± 2.8	33.3 ± 2.9	29.6 ± 3.1
Final	35.4 ± 2.9	31.9 ± 2.9	29.3 ± 3.1
p-Value[b]	0.05	0.01	0.05
Total lean mass (kg) [a]			
n	10	11	11
Initial	42.8 ± 1.6	45.7 ± 2.2	44.7 ± 1.8
Final	42.5 ± 1.5	45.2 ± 2.2	44.1 ± 1.8
p-Value[b]	0.53	0.43	0.13

[a] Mean ± SE (all such values).
[b] p values obtained by Student paired t-test; significance assessed at $p < 0.05$.

3.1. *Body weight and body compositions in response to treatments*

In terms of body composition analysis, since ethics approval for conducting DXA scans was not granted until midway through the study, only 28 subjects were available to undergo whole body scans over the course of the study. As a result, endpoint scans of all three phases were obtained from a smaller subgroup of subjects ($n = 14$). Among these 14 subjects, eleven subjects received scans at baseline and endpoint for LF treatment; twelve subjects were scanned at baseline and endpoint for LA treatment; and 12 subjects underwent scans at baseline and endpoint for control.

No significant differences in body weight were observed at baseline or endpoint across the three treatments. Body weight fluctuation within each phase was less than 5%. Total lean

Table 5 – DNA normalized by Eubacteria.

Group	Period	Treatment	Target group					
			Lac	Bact	Rrec1	Clep	Rum	Bad
G1	P1	LF	2.964	0.71761	1.20336	0.923	1.17652	1.77268
G1	P2	LA	0.48608	0.70613	1.46935	0.97331	0.76389	1.63384
G1	P3	Con	0.01611	0.55488	0.99122	1.34492	0.72106	1.64537
G2	P1	LF	2.77176	0.82263	0.43008	1.19039	1.85099	0.66458
G2	P3	LA	1.79231	0.49672	0.72656	0.90719	1.39429	0.00598
G2	P2	Con	0.01826	0.63149	0.93282	1.12956	1.70268	3.12246
G3	P2	LF	1.37028	0.93095	1.1523	1.06606	1.26355	0.51896
G3	P1	LA	0.24124	1.58853	0.89581	0.63267	0.77558	0.85963
G3	P3	Con	1.95878	0.54422	0.90364	0.86049	1.53679	5.58516
G4	P3	LF	1.58909	0.54357	1.28923	1.06068	1.21775	0.00119
G4	P1	LA	2.24838	1.2773	0.50941	1.21928	0.95743	0.22046
G4	P2	Con	0.00657	0.87204	1.15523	1.15496	1.66491	0.29461
G5	P2	LF	7.2355	0.80902	1.10284	1.31302	1.45733	1.4671
G5	P3	LA	0.70653	0.8323	1.21252	0.79803	1.28994	0.6909
G5	P1	Con	0.0193	0.73222	1.07927	1.18152	1.24839	0.69805
G6	P3	LF	1.01831	1.33508	1.17913	1.62755	0.8141	1.3161
G6	P2	LA	1.0513	1.61269	0.96006	1.06595	0.76785	0.46941
G6	P1	Con	0.01362	1.22828	0.7308	1.21673	0.84762	0.73871

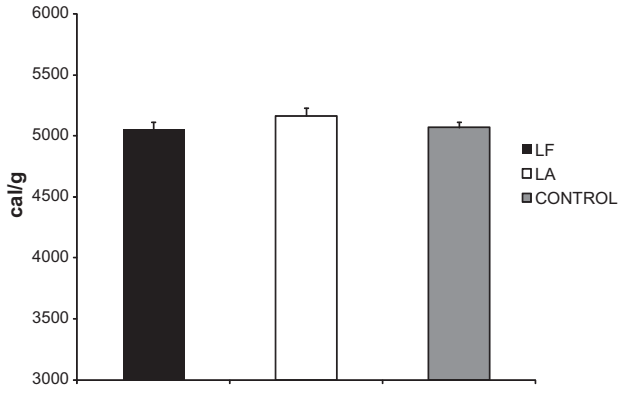

Fig. 1 – Total faecal energy concentrations of all treatment groups at endpoint (week 6).

mass and total fat mass did not differ significantly between treatments at endpoint (Table 4). Over the respective study phase, however, total fat mass decreased by 3% ($p = 0.05$) from baseline in response to LF treatment, while LA feeding reduced total fat mass by 4% ($p = 0.01$) from baseline. In addition, total fat mass was decreased by 1% ($p = 0.05$) from baseline in response to control treatment (Table 4). Although a change in total fat mass was observed within treatments over the study period, total lean mass did not differ significantly in response to treatments (Table 4). These changes in total fat mass occurred despite no statistically significant shift in body weights across treatments (Table 4). Total fat mass and total lean mass failed to change significantly during wash out periods.

3.2. *Faecal energy losses in response to treatments*

Faecal energy contents for LF, LA, and control treatments were 5059 ± 60; 5153 ± 67; and 5049 ± 49 cal/g, respectively. No significant differences in endpoint faecal energy concentrations were observed between treatments indicating that the reduction in total fat mass was not attributed to an increase in faecal energy loss in response to treatment (Fig. 1).

3.3. *Gut microbial abundance in response to treatments*

All RT-PCR data were log-2 normalized and presented relative to control treatment in Table 5. Values are presented relative to the control treatment in finalized, normalized format in Fig. 2. Gut microbial abundances of *Lactobacillus* increased significantly ($p = 0.008$) in response to LF and LA treatments as compared to control yogurt feeding. This increase in the genus *Lactobacillus* validates the consumption of the treatment and viability of the bacteria at consumption in its ability to colonize the intestinal tract. The feeding of LA seemed to exert an inhibitory effect on the *Clostridial cluster IV* (*Clep*) group in the gut as faecal levels of *Clep* exhibited a log2-fold change reduction of 0.318 ($p = 0.038$) in participants administered treatment LA when compared to control. Faecal microbial abundances of *Bacteroides, Bifidobacteria adolescentis, Cluster IV Ruminococcus, Roseburia* spp., *and Eubacterium rectale* failed to show any significant change in response to treatments compared to control (Fig. 2).

4. Discussion

The major finding from this controlled feeding study is that probiotic consumption alters intestinal microflora in a manner that was associated with reduced total body adiposity, an important anthropometric indicator of obesity. A controlled feeding design was essential for treatment administration to ensure study effects were in response to specified treatments and independent of shifts in background diet. Such results support earlier published work in that weight loss modulates gut microbial composition, where the abundance of specific bacterial strains in the gut increase or decrease with weight loss (Santacruz et al., 2009). In the aforementioned study, obese individuals (BMI = 32.8) who exhibited high weight loss (>4.0 kg) showed increased gut bacterial abundance of the genera *Bacteroides*, *Lactobacillus* and *Clep*. This observation presents the question of whether gut colonization of *Bacteroides* and other commensal microbes is solely a reactionary response to weight loss or if populating the gut microflora with specific beneficial bacterial strains through probiotic consumption can directly or indirectly contribute to weight loss. In this probiotic feeding study, participants experienced fat loss across all treatments while body weight did not change significantly. The gut abundance of individual bacterial strains, however, changed in a manner which was dependent on the treatment administered, indicating that the specific probiotic ingested stimulates the proliferation of some bacteria and that gut abundance shifts were not solely due to fat loss or weight change.

Participants lost a total fat mass of 4%, 3% and 1% consuming treatments LA, LF and control, respectively. However, both body weight and composition failed to differ significantly at endpoint amongst treatments. The percentage fat loss observed in participants was not due to diet or faecal caloric density loss as participants consumed identical amounts of calories. Furthermore, faecal energy concentrations were not significantly different across treatments. Such findings further support the rationale that the observed fat loss originated from other metabolic mechanisms related to the corresponding shift in faecal microbial abundance.

LA resulted in the largest fat reduction as well as the largest decrease in prevalence of *Clep*, showing that the decline in the abundance of *Clep* also associates with the greater extent of the diminution of body adiposity in this treatment. Typically, *Clep* is known to be commensal, however, the fact that it belongs to the microbial phylum *Firmicutes* and a reduction in gut bacteria from this family has been shown to facilitate weight loss, however, in this probiotic feeding study, the reduction in levels of this bacteria was shown to coincide with change in body composition (Ley et al., 2006; Santacruz & De Palma, 2008; Zwielehener et al., 2009). A decrease in the abundance of this microbe was observed in the LA treatment, however, the effects that this novel bacterium has on various gut microflora like *Clep* and its direct mechanistic link to total fat loss have not been extensively studied. Participants consuming LF also showed a 3% total fat mass loss suggesting this

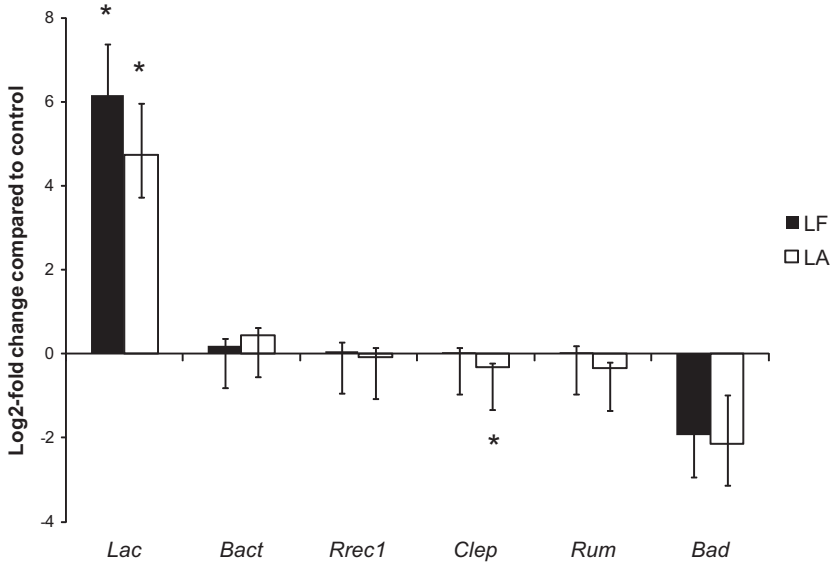

Fig. 2 – RT-PCR data of selected microbial groups as outlined in Table 3. All values are relative to the control. The x-axis are log-2 transformed valued normalized with the eubacterial 16S rRNA gene. *Lactobacillus* spp. (*Lac*); *Bacteroides* spp. (*Bact*); *Roseburia* spp. and *E. rectale* (*Rrec1*); *Clostridial cluster IV* (*Clep*); *Cluster IV Ruminococcus* spp. (*Rum*); *Bifidobacterium adolescentis* (*Bad*). *P < 0.05.

treatment may have also had an effect on energy storage. Contrary to the LA treatment, LF failed to decrease populations of *Clep* bacteria, however, the levels of *Lactobacillus spp.* were higher in both LA and LF than that seen over the control intervention. This result indicates that increasing levels of lactic acid producing bacteria creates a microbial environment that inhibits the growth of pathogenic bacteria and promotes proliferation of beneficial bacteria creating a microbiome ratio favouring *Bacteroides* over *Firmicutes* (Collado et al., 2006).

Current limitations of this study were that gender differences were not distinguished and the female menstrual cycle was not taken into account when determining body composition effects. The differences in body water within the treatment phase may have varied considerably from male to female as well as from female to female, potentially influencing body composition. Although noted, since each phase was six weeks in length rather than four, this body water effect due to the menstrual cycle may have averaged out within the treatment phase and had no significant effect on true body composition results. In addition, only a subset of the total study participants received baseline and endpoint DXA scans for each treatment, which may have reduced the power of the study and the effect of treatment order was not measured. Moreover, waist and hip ratio measurements were not conducted in this study; however, this measurement will be implemented in future studies together with DXA scanning to establish a more comprehensive assessment of body composition. Lastly, although participants were instructed to maintain normal physical activity so as not to skew body weight or composition throughout the study, daily physical activity levels were not recorded and activity energy expenditure cannot be calculated to assess whether total energy expenditure was manipulated or if it contributed to the loss in body fat mass.

In summary, present results suggest that both LF and LA probiotic treatments are capable of inducing shifts in body composition. In addition, the reduction in the gut microbial abundance of *Clep* in response to LA yogurt feeding suggests that fat loss may be facilitated through a decreased gut microbial abundance of *Clep*, as total fat loss was reduced by the greatest degree with LA. Consumption of LF and LA bacteria as probiotics may assist in reducing the development of obesity, since these bacteria may confer modifications to energy handling within the host. This creates a microbiome which favors fat oxidation over fat storage, by populating the gut with protective bacteria and preventing the proliferation of pathogenic bacteria, which may ultimately lead to a reduction in body adiposity and transformation of body composition (Tsai & Coyle, 2009).

Disclosure

Mitchell L. Jones and Satya Prakash acknowledge a conflict of interest as they are co-founders and shareholders of Micropharma Limited. All other authors declare no conflict of interest.

Acknowledgements

The authors acknowledge Dr. Denis Krause as a co-author, in memoriam. The authors acknowledge financial support from Micropharma Limited. The authors acknowledge treatment yogurts were produced at McGill University through a research contract sponsored by Micropharma Limited. The authors would also like to thank the study participants for their cooperation and the invaluable technical assistance from the laboratory staff of the Richardson Centre for Functional Foods and Nutraceuticals and Micropharma Limited.

REFERENCES

Bhathena, J., Martoni, C., Kulamarva, A., Urbanska, A. M., Malhotra, M., & Prakash, S. (2009). Orally delivered microencapsulated live probiotic formulation lowers serum lipids in hypercholesterolemic hamsters. *Journal of Medicinal Food, 2*, 310–319.

Collado, M. C., Jalonen, L., Meriluoto, J., & Salminen, S. (2006). Protection mechanism of probiotic combination against human pathogens: In vitro adhesion to human intestinal mucus. *Asia Pacific Journal of Clinical Nutrition, 15*, 570–575.

Cox, A. J., Pyne, D. B., Saunders, P. U., & Fricker, P. A. (2010). Oral administration of the probiotic *Lactobacillus fermentum* VRI-003 and mucosal immunity in endurance athletes. *British Journal of Sports Medicine, 44*, 222–226.

Denman, S. E., & McSweeney, C. S. (2006). Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. *FEMS Microbiology Ecology, 58*, 572–582.

DiBaise, J. K., Zhang, H., Crowell, M., Krajmalnik-Brown, R., Decker, G. A., & Ritmann, B. E. (2008). Gut microbiota and its possible relationship with obesity. *Mayo Clinic Proceedings, 83*, 460–469.

Grill, J. P., Cayeula, C., Antoine, J. M., & Schneider, F. (2000). Isolation and characterization of a *Lactobacillus amylovorus* mutant depleted in conjugated bile salt hydrolase activity: Relation between activity and bile salt resistance. *Journal of Applied Microbiology, 89*, 553–563.

Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 11070–11075.

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Microbial ecology: Human gut microbes associated with obesity. *Nature, 444*, 1022–1023.

Mifflin, M. D., St-Jeor, S. T., Hill, L. A., Scott, B. J., Daugherty, S. A., & Koh, Y. O. (1990). A new predictive equation for resting energy expenditure in healthy individuals. *American Journal of Clinical Nutrition, 51*, 241–247.

Murphy, K. G., & Bloom, S. R. (2006). Gut hormones and the regulation of energy homeostasis. *Nature, 444*, 854–859.

Pan, D. D., Zeng, X. G., & Yan, Y. T. (2011). Characterisation of *Lactobacillus fermentum* SM-7 isolated from koumiss, a potential probiotic bacterium with cholesterol-lowering effects. *Journal of the Science of Food and Agriculture, 91*(3), 512–518.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research, 2*(9), e45.

Raoult, D. (2008). Obesity pandemics and the modification of digestive bacterial flora. *European Journal of Clinical Microbiology & Infectious Diseases, 27*, 631–634.

Sanders, M. E. (2008). Use of probiotics and yogurt in maintenance of health. *Journal of Clinical Gastroenterology, 42*(2), S71–S74.

Santacruz, A., & De Palma, G. (2008). Insights into the roles of gut microbes in obesity. *Interdisciplinary Perspectives on Infectious Diseases, 2008*, 1–9. http://dx.doi.org/10.1155/2008/829101 [Article ID 829101].

Santacruz, A., Marcos, A., Warnberg, J., Marti, A., Martin-Matillas, M., Campoy, C., Moreno, L. A., Veiga, O., Redondo-Figuero, C., Garagorri, J. M., Azcona, C., Delgado, M., Garcia-Fuentes, M., Collado, M. C., & Sanz, Y. (2009). Interplay between weight loss and gut microbiota composition in overweight adolescents. *Nature, 10*, 1–9.

Simons, L. A., Amansec, S. G., & Conway, P. (2005). Effect of *Lactobacillus fermentum* on serum lipids in subjects with elevated serum cholesterol. *Nutrition, Metabolism & Cardiovascular Diseases, 16*, 531–535.

Thomas, C., Pellicciari, R., Pruzanski, M., Auwerx, J., & Schoonjans, K. (2008). Targeting bile-acid signaling for metabolic diseases. *Nature Reviews Drug Discovery, 7*, 678–693.

Tsai, F., & Coyle, W. J. (2009). The microbiome and obesity: Is obesity linked to our gut flora? *Current Gastroenterology Reports, 11*, 307–313.

Turnbaugh, P. J., Bäckhed, F., Fulton, L., & Gordon, J. I. (2008). Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host & Microbe, 3*, 213–223.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature, 444*, 1027–1031.

Zwielehener, J., Liszt, K., Handschur, M., Lassl, C., Lapin, A., & Haslberger, A. G. (2009). Combined PCR-DGGE fingerprinting and quantitative-PCR indicates shifts in faecal population sizes and diversity of *Bacteroides*, *bifidobacteria* and *Clostridium cluster IV* in institutionalized elderly. *Experimental Gerontology, 44*, 440–446.