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RESEARCH ARTICLE

The effects of synbiotic supplementation on some cardio-metabolic risk factors in overweight and obese children: a randomized triple-masked controlled trial

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Abstract

Recent studies have suggested some beneficial effects of probiotics on controlling excess weight in adults; such experience is limited in the pediatric age group. This study aimed to assess the anti-obesity and lipid-lowering effects of a synbiotic supplement among children and adolescents. We conducted a randomized triple-masked controlled trial among 70 participants aged 6–18 years with body mass index (BMI) equal or higher than 85th percentile. They were randomly assigned to two groups of equal number to receive synbiotic or placebo for 8 weeks. At the end of the trial, decrease in BMI Z-score, waist circumference, and waist-to-hip ratio were significantly higher in the synbiotic group than in the placebo group. Likewise, synbiotic group had significant decrease in serum triglycerides, total- and low density lipoprotein-cholesterol levels. The beneficial effects of a synbiotic supplement on controlling excess weight and some cardio-metabolic risk factors among children and adolescents can be considered in clinical practice.

Keywords

Children and adolescents, lipid profile, obesity, synbiotic, trial

History

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Introduction

The ongoing rise in the prevalence of overweight and obesity among children is a major public health problem (Ebbeling et al., 2002). It is demonstrated that the worldwide prevalence of excess weight in children and adolescents is approximately 10% (Oude Luttikhuis et al., 2009). The corresponding figure is reported to be13.4% among Iranian children and adolescents (Kelishadi et al., 2007).

Childhood overweight leads to various adverse health effects as hyperlipidaemia, hypertension, insulin resistance and abnormal glucose tolerance (Montero et al., 2012; Weiss et al., 2009).

Both genetic and environmental factors affect the development of obesity, the exact pathways remain to be determined. Recent studies underscored the role of gut microbiota as an environmental factor involved in the development of obesity (Tilg et al., 2009).

Backhed et al. (2004) found that conventionally raised mice contained 40% higher total body fat and 47% higher gonadal fat content than germ-free mice did, although germ-free mice had higher food intake.

Turnbaugh et al. (2006) showed that colonization of germ-free wild-type mice with gut microbiota harvested from ob/ob mice leads to a significantly greater increase in total body fat when compared to colonization with microbiota from lean donors (4768.3 versus 2763.6% increase or 1.360.2 versus 0.8660.1 g fat).

Likewise, another study demonstrated that transplantation of a diet-induced obesity gut microbiota to lean germ-free recipient mice promoted greater fat deposition than transplantation from lean donor mice (Turnbaugh et al., 2008).

Thus, according to evidences, it might be suggested that regulating gut microbiota, for instance with probiotics, may be an appropriate strategy to control obesity and its related disorders.

According to the currently adopted definition by the Food Agriculture Organization/World Health Organization (FAO/WHO), probiotics are: ''Live microorganisms, when administered in adequate amounts, confer a health benefit on the host'' (FAO/WHO 2001).

Animal studies showed the effect of some bacterial strains on controlling obesity (Hamad et al., 2009; Lee et al., 2006, 2007; Ohnuki et al., 2001; Sato et al., 2008; Tanida et al., 2008).

For example, in the study of Takemura et al. (2010), administrating *Lactobacillus plantarum* tended to reduce average adipocyte size in mice (2056 ± 477 in HFD versus 1450 ± 265 in LP14). Sato et al. (2008) demonstrated the 22% reduction effect of fermented skimmed milk containing *L. gasseri SBT2055* on the size of adipocytes in visceral adipose tissues of rats. In a human study, Kadooka et al. (2010) found that the intake of *L. gasseri SBT2055* was effective in reducing abdominal visceral (−5.8%) and subcutaneous fat areas (−7.4%), body weight (−1.1%), body mass index (BMI) (−0.4%), waist and hip circumference (−0.004).

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Lee et al. (2006, 2007) reported that administration of *L. rhamnosus PL60* and *L. plantarum PL62* reduced body fat (16%) in diet-induced obese mice, maybe due to producing CLAs. In the study by Ma et al. (2008), VSL no. 3, which contains lyophilized *bifidobacteria*, *lactobacilli* and *Streptococcus thermophilus*, improved diet-induced obesity.

Moreover, *Bifidobacterium breve* administration in mouse model with high fat diet suppresses the increase of body weight and epididymal fat with improved serum level of total cholesterol (TC; $1450 + 265$ in control group versus $1450 + 265$ in active group) (Kondo et al., 2010). Furthermore, probiotics may have a role in improvement of serum lipid profile; in an experimental study, administrating four *Bifidobacteria* strains led to significant decrease in serum triglycerides (TG) levels compared with the control group (1.59 ± 0.73, 1.54 ± 0.30, 1.23 ± 0.65, 1.47 ± 0.70 versus 2.23 ± 0.76), moreover TC level in serum and liver were obviously lower than in model group (1.11 ± 0.18, 1.37 ± 0.26 versus 1.72 ± 0.38, and 1.27 ± 0.08, 0.62 ± 0.6 versus 1.47 ± 0.05). This study concluded that the response of energy metabolism to administration of probiotics is strain dependent (Yin et al., 2010).

Some studies showed that probiotics, including *Bifidobacterium longum* (in Bifidobacterium group 243.9 ± 15.2 at the beginning of study versus 237.3 ± 15.9 at the end of study) (Xiao et al., 2003) and *Lactobacillus acidophilus* (25%) (Park et al., 2007) had cholesterol reducing effects in animal and human studies.

The study of *Bifidobacterium* spp. in high fat diet-induced obese rats revealed that lactic acid bacteria (LAB) administration reduced body weight gain and fat weight.

Likewise, serum TC levels decreased in high fat diet rats (TC, 89.70 ± 17.33 in HFD-LAB group versus 88.20 ± 12.24 in standard diet group), high-density lipoprotein-cholesterol (HDL-C, 46.09 ± 7.25in HFD-LAB group versus 54.40 ± 9.25in standard diet group) and low-density lipoprotein-cholesterol (LDL-C, 15.22 ± 4.40 in HFD-LAB group versus 19.15 ± 2.96 in standard diet group) (An et al., 2011).

In individuals with type 2 diabetes mellitus, the consumption of probiotic yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* is shown to significantly decrease TC(4.54%), LDL-C(7.45%), TC:HDL-C ratio and LDL-C:HDL-C ratio compared with the conventional yogurt (Ejtahed et al., 2011).

These data suggest that some bacterial strains may be useful probiotics to prevent or control obesity and its related disorders.

This study aimed to assess the effects of probiotic supplementation on anthropometric indexes and cardio-metabolic risk factors in obese children and adolescents.

Methods

This randomized triple-masked controlled trial was conducted from September to November 2011 in Isfahan University of Medical Sciences (IUMS), Isfahan, Iran. The study protocol was in accordance with the Declaration of Helsinki, and was approved by the Research and Ethics Committee of IUMS. The trial is registered with the Trial registry code: IRCT201103081434N4.

After providing detailed information, we obtained written informed consent from parents and oral assent from participants. The sample size was calculated as 50, but considering possible attrition in the follow up, we increased it to 70.

Participants

This trial comprised 70 healthy-looking children and adolescents, aged 6–18 years, with a BMI equal to or higher than the age- and sex-specific 85th percentile according to the charts of the Centers for Disease Control and Prevention (Kuczmarski et al., 2000),which are confirmed to be appropriate for Iranian children and adolescents (Kelishadi et al., 2008). They were selected by random sampling from among overweight or obese children who were referred to the Pediatric Obesity and Metabolic Syndrome Clinic, Child Growth and Development Research Center, IUMS. Children with syndromal obesity, endocrine disorders, any physical disability, history of chronic medication use, use of mineral and/or vitamin supplements, history of any chronic diseases and/or chronic medication use or those under special diets were not included in the study.

Physical examination

The age and birth date of participants were recorded. All anthropometric measurements were made by the same trained person and under the supervision of the same pediatrician. Physical examination was conducted under standard protocols by using calibrated instruments at the beginning and end of the study.

Body weight was measured (digital floor scale; Seca, Hamburg, Germany) with 100 g accuracy without shoes and with minimum clothing. Height was measured, with 1 mm accuracy, with non-stretchable tape (Seca, Hamburg, Germany).

Waist, hip and neck circumferences were measured with a non-elastic tape. Waist circumference was measured at a point midway between the lower border of the rib cage and the iliac crest at the end of normal expiration.

Hip circumference was measured at the maximum girth of the buttocks

Blood pressure (BP) was measured using mercury sphygmomanometers after 5 min of rest in the sitting position. The subjects were seated with the heart, cuff and zero-indicator on the manometer at the level of the observer's eye. Appropriate size cuffs were used with cuff-width 40% of mid-arm circumference, and cuff bladders covering 80–100% of the arm circumference and approximately two thirds of the length of the upper arm without overlapping. The readings at the first and the fifth Korotkoff phase were taken as systolic and diastolic BP (SBP and DBP), respectively.

Biochemical measurements

The biochemical factors were measured in Pars laboratory with adherence to external national and international quality control. Participants were instructed to fast for 12 h before blood sampling. With one of the parents accompanying his/her child, blood samples were taken from the antecubital vein between 08:00 and 09:30 am. After collecting blood samples, the participants were served a healthy snack provided by the project team. Fasting plasma glucose, TG, TC, LDL-C and HDL-C were measured enzymatically by auto-analyzer (Pars Azmoun, Tehran, Iran). TC and LDL-C were measured as well.

Synbiotic administration

We used synbiotic capsules (Protexin company, London, England) containing a combination of viable freeze-dried *Lactobacillus Casei*, *Lactobacillus Rhamnosus*, *Streptococcus Thermophilus*, *Bifidobacterium Breve*, *Lactobacillus Acidophilus*, *Bifidobacterium Longum* and *Lactobacillus Bulgaricus* of human origin with prebiotics (frocto oligosaccharides), Vitamin E, Vitamin A and Vitamin C. Each capsule contained 2.0×10^8 colony-forming units (CFU) daily. Each individual was instructed to take one capsule once a day before a main meal for 8 weeks.

The placebo was prepared in the Pharmaceutics Department, Faculty of Pharmacy, IUMS. It contained maltodextrine and

consisted of capsules with shape, taste and smell identical to synbiotic capsules. The medication adherence was tracked by weekly phone call to participants and regular stool examination for bacterial count.

Stool sample collection

Stool samples were collected from both case and control groups on the first, 15th and 60th days of the study. Samples were kept refrigerated in the thigh plastic containers for less than 6 h until they were transferred to the laboratory, where they were examined at the earliest possible time.

Media

MRS agar (Merck, Darmstadt, Germany, pH = 5.7) and MRS agar (Merck, pH = 5.7) added with 1% muprocin (Sigma, St. Louis, MO) and 0.5% systein hydrochloride (Sigma) were used for enumeration of Lactobacillus and Bifidobacterium colonies, respectively.

Bacterial counts

Each fecal sample (0.5 g) was placed in the sterilized tube mixed with 5 ml sterile normal saline, mixed thoroughly and centrifuged for 5 min at 100 rpm. One ml of the upper phase was serially 10-fold diluted to 10^{-7} dilution.

One hundred micro liter of the proper dilution was surface cultured on both types of plates. Plates were incubated anaerobically 5% Co2, using Co2 injected incubator. MRS plates were kept at 37–38 °C for 48 h and the plates containing MRS-muprocin-hydrochloride were kept at the same temperature for 72 h. Colony counting was done by expert eyes and expressed as a log of the CFU per gram of fresh feces.

Dietary records

Nutrient intakes were estimated using 24 h dietary recall at the beginning and at the end of the study for 3 d. Participants were asked to write down the type and amount of food eaten, using scales or household measures to gauge portion sizes where possible. Three day averages of energy and macronutrient intakes were analyzed by the Nutritionist 4 software (First Databank Inc., Hearst Corp., San Bruno, CA). Data entry was performed by a trained dietitian. If a participant ate a food not included in the database, a food with very similar nutrient composition was selected. Nutrient information was also obtained through food labels or recipes from participants. Participants were encouraged to keep their diet intakes and exercise patterns followed by Physical Activity Questionnaire for Older Children unchanged during the experiment.

Statistical analysis

We used the SPSS for Windows software (version 16:00; SPSS, Chicago, IL) for statistical analysis. Descriptive data are expressed as mean ± standard deviation (SD). After assessment of the normal distribution by the Kolmogorov–Smirnov test, within group changes were compared by the paired *t*-test. For comparison of data between the two groups, we used independent *t*-test for data with normal distribution. A *p* value of less than 0.05 was considered as statistically significant.

Results

Baseline characteristics

Among 70 participants who took part in the study, 56 individual completed it and 14 children stopped consuming the capsules during the study. Participants demonstrated good compliance with the supplement consumption and no adverse effects or symptoms were reported. Subjects' demographics and physical characteristics at the beginning of the study did not differ significantly between the two groups (Table 1). The intake of energy and nutrient, as well as the levels of physical activity in the two groups resembled each other and during the study none of them showed significant alteration (Table 2).

Table 1. Baseline characteristics* of participants in the two groups studied.

Variables	Synbiotic group	Placebo group	*p* Value
N	29	27	
Age (yEArs)	10.75 ± 2.49	10.09 ± 1.93	
BMI Z-score	1.79 ± 0.50	1.67 ± 0.39	0.34
Waist circumference (cm)	84.22 ± 15.36	76.53 ± 9.92	0.32
Waist-to-hip ratio	0.36 ± 0.09	0.41 ± 0.42	0.53

*Values are mean ± SD.

The mean (SE) of anthropometric measures before and after receiving synbiotic supplement and placebo are presented in Table 3. The percent change in BMI Z-score was significantly higher in the synbiotic than in the placebo group (−5.5% versus 1.19%, respectively, $p = 0.002$). Similar results were obtained for the percent change of waist circumference (−1.57% versus 0.4%, respectively, $p < 0.0001$). Likewise, the percent change of the waist-to-hip ratio was = −2.2% ($p < 0.0001$). The corresponding figure was not significant for other variables.

Serum biochemical analysis

Comparison of the effects of probiotic and placebo consumption on serum biochemical factors are presented in Table 4. Compared with the placebo group, the supplement group after adjusting for weight reduction had significant decrease in TC ($p < 0.0001$, percentage reduction in synbiotic group = −2.3%, percentage reduction in placebo group = 0.24%), LDL-C ($p = 0.01$, percentage reduction in synbiotic group = −1.07%, percentage reduction in placebo group = 0.38%) and TG ($p = 0.00$, percentage reduction in synbiotic group = −1.32%, percentage reduction in placebo group = 0.0%) levels, whereas there was no significant alteration in HDL-C and FBG.

Fecal total LAB counts

Fecal total LAB counts are shown in Figure 1. Over the experimental period, the fecal total LAB counts increased significantly in the probiotic group compared to the placebo group.

Discussion

To the best of our knowledge, this is the first study of its kind to investigate the effect of synbiotic supplementation on cardio-metabolic risk factors in obese and overweight children. Although many studies have reported anti-obesity effects of some bacterial strains such as *Lactobacillus* spp. and *Bifidobacterium* spp. in our synbiotic capsules (Lee et al., 2006; Ma et al., 2008; Takemura et al., 2010; Yin et al., 2010), but such experience was not documented in the pediatric age group.

In this study the intake of synbiotic resulted in a significant reduction in BMI-Z score and waist circumference, as well as in some cardio-metabolic risk factors as TC, LDL-C and TG. Of special concern is the reduction in BMI-Z score and waist circumference stand out, which are the main cause of cardio-metabolic abnormalities (Després et al., 2008).

Table 2. Dietary intake and physical activity level of participants throughout the study.

	Stage	Synbiotic group ($n=29$) $\overline{X} \pm$ SE	Placebo group ($n=27$) $\overline{X} \pm$ SE	p
Physical activity (PA)	Before	2.31 ± 0.47	2.44 ± 0.50	0.30
	After	2.41 ± 0.51	2.44 ± 0.57	0.83
	Dif	-0.10 ± 0.61	0.00 ± 0.67	0.55
p**		0.37	1.00	
Energy (kcal)	Before	1508 ± 44.69	1454 ± 44.69	0.35
	After	1516 ± 33.83	1433 ± 35.06	0.09
	Dif	-7.90 ± 34.36	20.90 ± 28.61	0.52
p**		0.82	0.41	
Protein (g)	Before	59.40 ± 2.48	64.20 ± 2.93	0.21
	After	55.22 ± 1.88	58.14 ± 1.96	0.28
	Dif	8.87 ± 4.72	6.05 ± 3.50	0.66
p**		0.12	0.09	
Carbohydrate (g)	Before	187.05 ± 6.39	179.24 ± 6.91	0.41
	After	189.68 ± 6.94	183.05 ± 7.31	0.51
	Dif	-2.63 ± 6.07	-3.80 ± 7.12	0.90
p**		0.66	0.59	
Total fat (g)	Before	65.08 ± 4.54	58.05 ± 3.76	0.24
	After	65.39 ± 8.83	61.62 ± 1.21	0.07
	Dif	-0.31 ± 5.06	-3.57 ± 3.55	0.60
p**		0.95	0.32	
Saturated fat (g)	Before	17.93 ± 0.80	17.14 ± 0.74	0.47
	After	18.61 ± 0.90	17.54 ± 0.71	0.37
	Dif	-0.66 ± 1.03	-0.43 ± 0.81	0.85
p**		0.51	0.59	
Mono unsaturated fat (g)	Before	16.92 ± 0.78	17.02 ± 0.87	0.93
	After	17.97 ± 0.47	16.79 ± 0.59	0.12
	Dif	-1.05 ± 0.83	0.23 ± 0.84	0.28
p**		0.21	0.78	
Poly unsaturated fat (g)	Before	13.71 ± 0.69	13.15 ± 0.69	0.53
	After	14.23 ± 0.45	13.49 ± 0.39	0.22
	Dif	-0.52 ± 0.49	-0.33 ± 0.33	0.79
p**		0.30	0.49	
Cholesterol (g)	Before	257.44 ± 13.58	233.11 ± 8.12	0.13
	After	257.26 ± 9.08	241.72 ± 10.52	0.26
	Dif	0.17 ± 14.45	-8.60 ± 9.64	0.61
p**		0.99	0.38	
Dietary fiber (g)	Before	12.21 ± 0.84	14.00 ± 0.72	0.11
	After	11.14 ± 0.70	12.37 ± 0.51	0.16
	Dif	1.07 ± 0.79	1.62 ± 0.96	0.66
p**		0.18	0.10	

Data are presented as means $\pm$ SE for nutrient.
Data are presented as means $\pm$ SD for physical activity.
*p Value resulted from independent t-test for difference between probiotic and placebo groups after intervention ($p<0.05$).
**p Value resulted from paired t-test for difference within groups throughout the study ($p<0.05$).

During the study period, the energy and nutrient intake and the level of physical activities did not have any significant change within each group (Table 2), besides increase in concentrations of protective bacteria, as shown by the microbiological data (Figure 1), and their metabolic activities suggest that the improvement in anthropometric measures and cardiometabolic risk factors may be due to the synbiotic supplementation.

Such effect of probiotic supplementation has been shown in animal studies (An et al., 2011; Kondo et al., 2010; Lee et al., 2006, 2007; Sato et al., 2008; Takemura et al., 2010; Tanida et al., 2008; Yin et al., 2010)

Lee et al. (2006, 2007) reported that the administration of *L. rhamnosus PL60* and *L. plantarum PL62* reduced fat storage in diet-induced obese mice, and they demonstrated that the production of CLAs by administered lactobacilli mediate the anti-obesity effects.

However, Hamad et al. (2009) reported that the reduction of adipocyte size due to the administration of fermented skimmed milk containing *L. gasseri SBT2055* was accompanied by the increased fecal excretion of fatty acids, and by the reduced maximal transport rate of TAG and phospholipids in the thoracic duct lymph in rats. They concluded that the reduction in fat storage has been because of the inhibition of dietary fat absorption.

Sato et al. (2008) also pointed to this mechanism for the reduction in adipocyte size in WATs of Sprague–Dawley rats after the administration of fermented skimmed milk containing *L. gasseri SBT2055*.

In a trial conducted by Kadooka group, among adults with obesity tendencies, probiotic supplementation was effective in the reduction of abdominal fat, body weight, BMI, waist and hip circumferences and body fat mass. The author concluded that the anti obesity effect is due to the inhibition of lipid absorption (Kondo et al., 2010).

Furthermore in our study, due to a significant reduction in serum TG (percentage reduction in synbiotic group $= -1.32\%$ without any change in the placebo group), it seems that the reduction in BMI-z score (percent change of -5.5% in synbiotic group versus 1.19% in the placebo group) and waist circumference (percent change of -1.57%, in synbiotic group versus 0.4% in placebo group) is because of the reduction in lipid absorption. In addition to the pointed mechanisms, Turnbaugh et al. (2006) presented evidence that the distal gut microbiota of genetically obese mice has an increased capacity to harvest energy from diet. The result identifies the gut microbiota as an additional factor contributing to the pathophysiology of obesity.

One pathway to help the host to harvest energy from food is by processing indigestible polysaccharides to short chain fatty acids, which are an important energy source for our body; also short chain fatty acids influence the gut motility and intestinal transit rate (Samuel et al., 2008).

Due to Figure 1, the survival of lactoacid bacterial was concluded during supplementation, and the short chain fatty acids production by these LABs may influence gut motility and intestinal transit rate too.

In addition, Backhed et al. (2004) demonstrated that reduced expression of Fiaf, a circulating LPL inhibitor, in the intestine is essential for the commensal gut microbiota-related fat storage in mice. However, we did not examine Fiaf mRNA levels in the intestinal mucosa.

Toll-like receptors (TLRs) are expressed in many types of immune and non-immune cells and play a critical role in the activation of innate immune responses in mammals by recognizing conserved pathogen-associated molecular patterns (Akira et al., 2006). TLR4 can be activated by lipopolysaccharide, a major cell wall component of Gram-negative bacteria, and also by non-bacterial ligands such as saturated fatty acids (Akira et al., 2006; Lee et al., 2003). Recent studies have reported that mice deficient in TLR4 or its co-receptor CD14 are protected against diet-induced obesity, suggesting that the TLR4-dependent mechanism contributes to the onset and progression of obesity (Cani et al., 2007; Davis et al., 2008; Tsukumo et al., 2007). Thus, the suppression of TLR4 activation may contribute to the prevention and treatment of obesity.

Some bacterial strains reportedly inhibit TLR4 expression and/or activation in the intestinal epithelial cells. It will be of interest to test whether or not synbiotic affects TLR4 expression and/or activation (Takemura et al., 2010).

In our study, fecal LAB counts were clearly increased in the synbiotic-fed groups compared to those in the control group. Thus, it can suggest that after oral feeding, LAB survive passes through the upper gastrointestinal tract, and ingested LAB affects the intestinal environment to favor LAB colonization (Pochart et al., 1992).

Table 3. Effects of probiotic and placebo consumption on anthropometric parameters and BP of participants.

	Stage	Synbiotic group ($n=29$) $\overline{X} \pm$ SE	Placebo group ($n=27$) $\overline{X} \pm$ SE	p^1	p^3 Time * group	p Time[4]	p Group[5]
BMI Z-score	Before	1.79 ± 0.09	1.67 ± 0.07	0.34	0.002	0.00	0.52
	After	1.69 ± 0.09	1.65 ± 0.07	0.74			
	Dif	0.09 ± 0.01	0.02 ± 0.01	0.002			
p^2		<0.0001	0.38				
WC1 (cm)	Before	84.22 ± 2.85	76.53 ± 1.91	0.32	0.00	0.00	0.05
	After	82.89 ± 2.82	76.85 ± 1.92	0.08			
	Dif	-1.32 ± 0.66	0.31 ± 0.88	<0.0001			
p^2		<0.0001	0.73				
WHR	Before	0.36 ± 0.01	0.41 ± 0.08	0.53	0.43	0.33	0.67
	After	0.35 ± 0.01	0.34 ± 0.01	0.49			
	Dif	0.008 ± 0.009	0.073 ± 0.44	0.43			
p^2		<0.0001	4.00				
SBP (mmHg)	Before	166.55 ± 2.09	116.85 ± 1.98	0.81	1.00	1.00	0.79
	After	116.55 ± 2.08	116.85 ± 1.98	0.80			
	Dif	0.00 ± 4.00	0.00 ± 3.66	1.00			
p^2		1.00	1.00				
DBP (mmHg)	Before	66.20 ± 0.97	65.92 ± 0.99	0.78	0.73	0.10	0.71
	After	66.72 ± 0.98	66.29 ± 0.99	0.66			
	Dif	-0.51 ± 2.04	-0.37 ± 1.92	0.78			
p^2		0.18	0.32				

All values are mean $\pm$ SE.
[1]p Values present comparison baseline and end point values between two groups (computed by independent samples t test).
[2]p Values present comparison baseline and end point values within each group (computed by paired sample t test).
[3]p Values represent the time*group interaction (computed by analysis of the covariance).
[4]p Values demonstrate the effect of time (computed by analysis of the covariance).
[5]p Values represent the effect of grouping (computed by analysis of the covariance).
WC: waist circumference; WHR: waist-to-hip ratio.

Table 4. Effects of probiotic and placebo consumption on serum biochemical factors.

	Stage	Synbiotic group ($n=29$) $\overline{X} \pm$ SD	Placebo group ($n=27$) $\overline{X} \pm$ SD	p^1	p^3 time * group	p Time[4]	p Group[5]
HDL-C (mmol/l)	Before	1.02 ± 0.03	1.09 ± 0.04	0.18	0.31	0.74	0.22
	After	1.03 ± 0.09	1.09 ± 0.10	0.27			
	Dif	-0.005 ± 0.01	0.002 ± 0.04	0.31			
p^2		0.08	0.71				
LDL-C (mmol/l)	Before	2.80 ± 0.03	2.57 ± 0.02	0.17	0.01	0.18	0.21
	After	2.77 ± 0.01	2.58 ± 0.01	0.01			
p^2		0.005	0.39				
Adjusted[6]		2.65 ± 0.01	2.70 ± 0.01	0.01			
TG (mmol/l)	Before	1.51 ± 0.09	1.30 ± 0.08	0.46	0.001	0.02	0.12
	After	1.49 ± 0.08	1.30 ± 0.06	0.00			
p^2		0.001	0.29				
Adjusted[6]		1.38 ± 0.00	1.41 ± 0.00	0.00			
TC (mmol/l)	Before	4.32 ± 0.06	4.15 ± 0.01	0.32	0.001	0.02	0.12
	After	4.22 ± 0.06	4.16 ± 0.07	0.00			
p^2		<0.0001	0.41				
Adjusted[6]		4.13 ± 0.01	4.25 ± 0.01	0.00			
FBS	Before	85.75 ± 2.05	86.48 ± 2.04	0.70	0.62	0.33	0.92
	After	85.17 ± 2.07	84.77 ± 2.02	0.87			
	Dif	0.57 ± 0.44	1.70 ± 0.89	0.66			
p^2		0.75	0.27				

All values are mean $\pm$ SE.
[1]p Values present comparison baseline (computed by independent samples t test) and end point values (computed by ANCOVA) between two groups.
[2]p Values present comparison baseline and end point values within each group (computed by paired sample t test).
[3]p Values represent the time*group interaction (computed by analysis of the covariance).
[4]p Values demonstrate the effect of time (computed by analysis of the covariance).
[5]p Values represent the effect of grouping (computed by analysis of the covariance).
[6]Adjusted for weight.
FBS: fasting blood sugar.

Several studies have shown that these specific bacteria reduce the intestinal endotoxin levels and improve mucosal barrier function (Griffiths et al., 2004; Wang et al., 2004, 2006). Furthermore, LABs have anti-tumor effects and block harmful intestinal enzyme activities, a recognized risk factor for colon cancer (Goldin et al., 1996; Lee et al., 2008).

Besides, in our study, we found favorable changes in serum lipid profile, i.e. the serum levels of TC (percent change of −2.3%

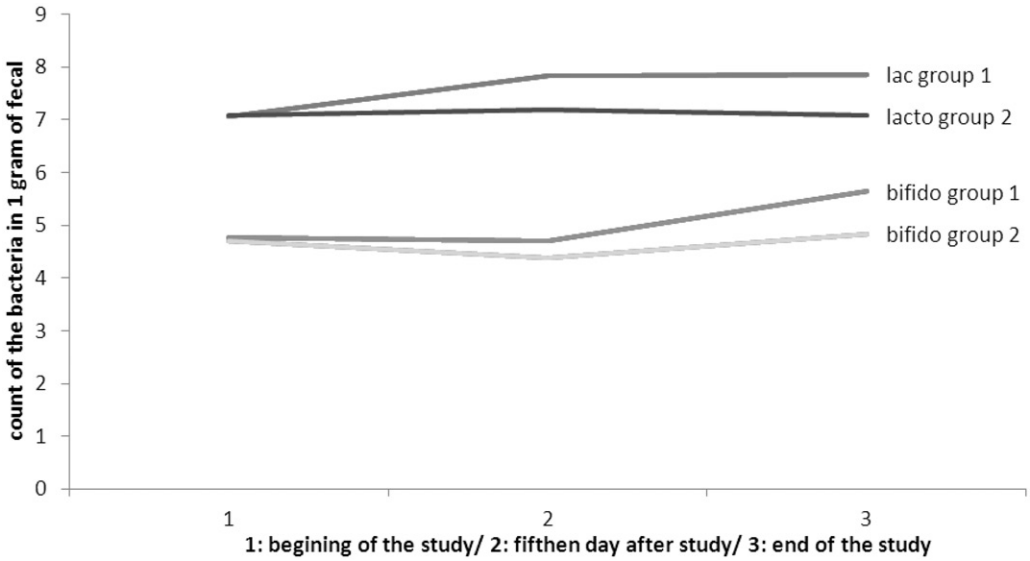

Figure 1. Bacterial count in stool at three times during the trial.

in synbiotic group versus 0.24% in placebo group), LDL-C (percent change of −1.07% in synbiotic group versus 0.38% in placebo group) and TG (percent change of −1.32% in synbiotic group without any change in placebo group), decreased significantly. Studies on *Bifidobacterium longum* (Xiao et al., 2003) and *Lactobacillus acidophilus* (Park et al., 2007) demonstrated hypocholesteremic effects in both experimental and human studies. The mechanisms which explain this observation may be as follows (Ejtahed et al., 2011): (1) fermentation products of LAB inhibit cholesterol synthesis enzymes and thus reduce cholesterol production; (2) the bacteria facilitate the elimination of cholesterol in feces; (3) the bacteria inhibit the absorption of cholesterol back into the body by binding with cholesterol; (4) the bacteria interfere with the recycling of bile salt (a metabolic product of cholesterol) and facilitate its elimination, which raises the demand for bile salt made from cholesterol and thus results in body cholesterol consumption and (5) the assimilation of lactic acid.

Conclusion

The findings of this trial suggest beneficial effects of a synbiotic supplement on controlling excess weight and some cardiometabolic risk factors among children and adolescents. More studies with longer follow-up should be conducted in this regard.

Declaration of interest

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