Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
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Cite this: *Food Funct.*, 2024, **15**, 8448

Bifidobacterium lactis IDCC 4301 (*B. lactis* Fit™) supplementation effects on body fat, serum triglyceride, and adipokine ratio in obese women: a randomized clinical trial

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Obesity is a common metabolic disease characterized by abnormal fat accumulation. It contributes to health issues, such as type 2 diabetes, cardiovascular disease, and dyslipidemia, necessitating continuous management through diet and physical activity. Probiotics, particularly *Bifidobacterium lactis* IDCC 4301 (*B. lactis* Fit™), have shown promise in positively regulating the gut microbiota. Therefore, this study aimed to evaluate the anti-obesity effect of *B. lactis* IDCC 4301 (*B. lactis* Fit™) in obese women. A randomized, double-blind, placebo-controlled, parallel-arm study was performed in 99 volunteers with a body mass index (BMI) of 25–30 kg m^{-2}. The participants were randomly assigned to probiotics (n = 49, >5.0 × 10^9 CFU day^{-1}) or placebo (n = 50) groups. Body fat, lipid profiles, and adipokine levels were assessed at baseline and at 12 weeks. After 12 weeks, changes in total fat (placebo −0.16 ± 0.83 kg; probiotics −0.45 ± 0.83 kg; p = 0.0407), trunk fat (placebo −0.03 ± 0.50 kg; probiotics −0.22 ± 0.51 kg; p = 0.0200), and serum triglyceride concentration (placebo 13 ± 60 mg dL^{-1}; probiotics −15 ± 62 mg dL^{-1}; p = 0.0088) were significantly different between the groups. The difference in total fat mass change between groups among postmenopausal women was greater than that of all women. A significant positive correlation was found between the change in total fat mass and log leptin/adiponectin ratio (R = 0.371, p = 0.0112) in the probiotics group. In addition, BMI (26.6 ± 1.9 kg m^{-2} to 26.4 ± 2.0 kg m^{-2}, p = 0.0009) and leg fat (42 ± 5% to 41 ± 5%, p = 0.0006) significantly decreased in the probiotics group after 12 weeks, but there was no difference in the placebo group. In conclusion, *B. lactis* IDCC 4301 (*B. lactis* Fit™) may be associated with body fat loss through changes in metabolic health parameters, such as serum triglyceride and adipokine levels. The clinical trial registry number is KCT0007425 (**https://cris.nih.go.kr**).

Received 31st January 2024,
Accepted 31st May 2024

DOI: 10.1039/d4fo00535j

rsc.li/food-function

1. Introduction

Obesity is a metabolic disease characterized by an imbalance in energy metabolism, resulting in abnormal or excessive fat accumulation. The prevalence of overweight/obesity in South Korea is 37.2% in 2022 and has increased rapidly.[1] As obesity can cause type 2 diabetes mellitus, cardiovascular disease (CVD) and dyslipidemia, systematic and continuous management is required.[2] Although the overweight/obesity rate among Korean women is lower than that of men, body fat composition can be affected by hormonal changes after menopause, which can affect obesity.[1,3] Therefore, preventing overweight and obesity through dietary and physical activity interventions is important. These interventions can change gut microbiota through strain diversification, and these changes have been found to be associated with changes in body metabolism and body fat mass.[4]

Probiotics, which can modulate the host immune response and influence various physiological processes, are attracting attention for their potential use in improving obesity.[5,6] Notably, research has indicated significant differences in the gut microbiota composition between obese and lean individuals, sparking interest in strategies that modulate the intestinal microbial environment to prevent obesity.[7] Ingested probiotics contribute to the establishment of a favorable intestinal

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microbial environment, and their metabolites may play a crucial role in regulating energy metabolism by influencing lipid profiles, adipokines, and insulin concentrations.[8–13] Therefore, modifications in the intestinal microbiota composition may be associated with variations in body fat. Recently, metabolites from probiotics have been found to be helpful in reducing inflammation and strengthening immunity and have been emerging under the name of postbiotics.[14]

Most studies on overweight, obesity and probiotics have used *Lactobacillus* or mixed strains; *Bifidobacterium* strains alone are rarely used. However, because using a single strain for each individual can show an independent effect of the strain, this study evaluated the effect of *Bifidobacterium lactis* IDCC 4301 (*B. lactis* Fit™) alone.[15] *Bifidobacterium*, discovered in the 18th century by Tissier in the feces of breast-fed infants, colonizes the colon more effectively and favors intestinal microflora.[16] In a human clinical trial confirming the anti-obesity effect of *Bifidobacterium*, body weight, BMI, and visceral fat area (VFA) significantly decreased after consuming dairy products containing *Bifidobacterium.*[12,17] Based on preliminary studies on *B. lactis*, potential anti-obesity effects of *B. lactis* IDCC 4301 were anticipated.

In preclinical studies, *B. lactis* IDCC 4301 administration decreased body and adipose tissue weight, improved serum lipid levels, and downregulated adipogenic gene expression in mice fed a high-fat diet. Furthermore, among the strains *Lactobacillus lactis*, *Lactobacillus fermentum*, *Streptococcus thermophilus*, *Bifidobacterium breve*, and *B. lactis*, *B. lactis* had a relatively high pancreatic lipase inhibition ability and the highest cholesterol-reducing ability.[18] As there have been no studies evaluating the effect of *B. lactis* IDCC 4301 alone in humans, this study aimed to evaluate the anti-obesity effect of *B. lactis* IDCC 4301 in women with a BMI between 25 and 30 kg m^{-2}.

2. Methods

2.1. Study participants selection and recruitment

In this study, 99 healthy women with a BMI of 25–30 kg m^{-2} was recruited until the target sample size was reached. Individuals willing to participate visited the Kyung Hee University Hospital and were screened according to the participant selection criteria. The inclusion criteria were between 20 and 65 years of age, body mass index (BMI) between 25 and 30 kg m^{-2}, and voluntarily signing an informed consent form.[19] According to World Health Organization Asia Pacific criteria for obesity, a BMI of 23 kg m^{-2} to 24.9 kg m^{-2} is defined as overweight, and a BMI of 25 kg m^{-2} or more is defined as obesity.[20] Exclusion criteria were continuous use of drugs or health functional foods that affect weight or body fat reduction 1 month before the registration date, weight loss of >10% in the 3 months before screening, surgery for weight loss within the past 1 year; participation in a weight loss program within the past 3 months, patients with uncontrolled hypertension and diabetes. Further exclusion criteria were diagnosis and treatment of cancer within the past 5 years, history of surgery within the past 6 months, chronic gastrointestinal disorder, autoimmune disease, thyroid disease, stroke, cardiac disorder, mental illness, central nervous disorder, alcoholism, eating disorder, disability, pregnancy, or lactation.

Participants were randomly classified into the probiotics or placebo groups at the first visit (within 3 weeks of the screening visit). The protocol was approved by the Institutional Review Board of Kyung Hee University Hospital (no. KHUH 2022-01-069), in accordance with the Declaration of Helsinki. This study was registered with the Clinical Research Information Service of the Republic of Korea (KCT0007425).

2.2. Study design

This was a randomized, double-blind, placebo-controlled, parallel-arm clinical trial. The participants were assigned to each group using a block randomization method (placebo : test = 1 : 1, 99 individuals respectively) based on probability, and the randomization was conducted by a third party independent of the study. The primary outcome was body fat change, measured using whole-body dual-energy X-ray absorptiometry scanner (DXA). Secondary outcomes included changes in body composition measured using bioimpedance analysis (BIA), lipid profiles, adipokine level, and waist-hip ratio (WHR).

Laboratory tests were performed at baseline and at 12 weeks, and a pregnancy response test was performed at baseline. Blood pressure and pulse rate were examined to check the vital signs, measured using an automated blood pressure monitor (HEM-7156; Omron, Vietnam) in the sitting position after resting for >15 minutes. These were measured by the same research staff at each visit, using the same equipment and at the same time as possible. Adverse events and side effects were evaluated through interviews conducted during the visit.

2.3. Treatment intervention and compliance

A baseline assessment was performed and 98-day supplies of the investigational product (IP) or placebo were provided to participants. Follow-up visits were conducted 43 (visit 2) and 85 (visit 3) days after the baseline assessment (visit 1). A 7-day visit window was allowed. The participants were instructed to maintain their usual diet and exercise during the study period, banned from consuming drugs or foods that could cause body fat loss and recommended to take the IP at approximately the same time each day. Information regarding all drugs, oriental medicines, and health functional foods, including items or names, doses, and duration of medications taken, was recorded at each visit.

The IP contained *B. lactis* IDCC 4301 as the main ingredient (8.0%), corn starch maltodextrin (69.0%), cassava starch dextrin mixture (20.0%), silicon dioxide (1.5%), and magnesium stearate (1.5%) as excipients at 500 mg per cap. The placebo product contained corn starch maltodextrin (69.0%), cassava starch dextrin mixture (28.0%), silicon dioxide (1.5%), and magnesium stearate (1.5%) as excipients at 500 mg per cap. *B. lactis* IDCC 4301 was anaerobically cultured in commer-

cial medium of Ildong Bioscience containing maltose, yeast extract, and soy peptone at 37 °C for 18 h and subsequently adjusted to a cell density of 10^9 CFU mL^{-1}. The IP contained more than 5.0×10^9 colony-forming units (CFU) of *B. lactis* IDCC 4301 per capsule. Probiotic and placebo capsules were manufactured with similar shapes, sizes, and colors. Probiotic or placebo products were orally administered 1 capsule once daily for 12 weeks. Participants were prescribed 6 weeks dose plus extra products at visits 1 and 2, and were encouraged to continue with the prescribed dose. The remaining unused capsules were returned at visits two and three and counted to evaluate compliance. Compliance was calculated using the following equation: [doses consumed/doses required] × 100. Low-compliance was defined as less than 80% for two consecutive visits.

2.4. Anthropometrics and body composition assessments

As the primary outcome measure, whole-body DXA (Hologic Horizon W instrument, Hologic Inc., USA) was used to assess whole-body fat at baseline and 12 weeks.[21] Body fat mass was measured in the supine position using standard soft-tissue measurement methods. Body fat mass was calculated as the amount of fat present in the section (arm, trunk, and leg) surrounded by the virtual boundary line separating the head and limbs.

Body composition was assessed using BIA (InBody720, Biospace, Seoul, Korea) at baseline and at weeks 6 and 12.[22] Height was measured using a stadiometer (BSM370; Biospace, Seoul, Korea). Participants were asked to fast for 8 h and wear light clothing without socks. Waist circumference (WC) and hip circumferences (HC) were assessed using a flexible tape at baseline and at 12 weeks. WC was measured at the midpoint between the lower margin of the last palpable rib on the mid-axillary line and the top of the iliac crest. HC was measured at the largest hip circumference. The WHR was calculated as WC divided by HC.[23] Obesity degree was calculated by dividing body weight by ideal weight. For Asian women, ideal weight was calculated as (height^2 (m^2) × 21).[24]

2.5. Blood collection and biomarker analyses

Serum lipid profiles (triglyceride [TG], total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C], and high-density lipoprotein cholesterol [HDL-C]), adiponectin, leptin, insulin, and homeostatic model assessment for insulin resistance (HOMA-IR) were assessed at baseline and 12 weeks.[25,26] Blood samples were collected after at least 8 h fast and analyzed in the laboratory. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes and serum separator tubes (SSTs). The SST was left at room temperature for approximately 10 to 30 min, then centrifuged at 4 °C, 3000 rpm for 10 min and stored in a refrigerator along with the hand-mixed EDTA tube. All samples collected from the participants were analyzed at a specialized institution. The atherogenic index of plasma (AIP) was calculated as log (TG/HDL-C) on an empty stomach.[27] The adipokine ratio was calculated as log leptin/adiponectin ratio (LAR).[28]

2.6. Dietary intake and physical activity

To assess daily dietary intake, the participants were required to record a 3-day food diary. The participants were required to record the name of the food, ingredients, and the amount of daily intake on two weekdays and one weekend. The participants were required to maintain their usual dietary patterns during the study period. Daily nutrient intake was analyzed by using a computer-aided nutritional analysis program (CAN-pro 5.0; Korean Nutrition Society, Seoul, Korea).

Physical activity was determined using the Global Physical Activity Questionnaire (GPAQ).[29] The GPAQ records the amount of physical activity by area (occupation, movement from place to place, leisure activities, and sedentary activities). The GPAQ was designed to allow participants to answer how many days (days per week) and hours (hours and minutes) they spent on average in a particular physical activity. High-intensity activities were applied at a level of 8.0 metabolic equivalent (METs), and moderate-intensity activities such as walking and cycling were applied at 4.0 METs. If the amount of exercise regularly practiced per week was 600 metabolic equivalents (METs per week), the participants were classified into the regular physical activity practice group. Moreover, if the amount of exercise regularly practiced per week was 3000 metabolic equivalents (METs per week) per week, it could be classified into active and sufficient physical activity practice groups.

2.7. Statistical analyses

The sample size was calculated in a study by Jung *et al.*, who reported the change in body fat mass of the probiotic (mixture of *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032) and placebo group.[30] A statistical hypothesis test was conducted at a two-sided significance level of 0.05. Continuous values are expressed as mean ± standard deviation (SD), and categorial values are expressed as *n* (%). The primary outcome of this study was the group differences of baseline, 12 weeks and change in body fat. The secondary outcome was the group differences of baseline, 12 weeks and change in blood lipid profiles, adipokines, and glycemic profiles and the baseline and 12 weeks difference of all outcomes within groups. To compare the differences between groups, Student's *t*-test for continuous variables and the Chi-square test or Fisher's exact test for categorial variables were used. Additionally, to assess the differences between the baseline and 12 weeks, paired *t*-test was performed. A general linear model test was applied to adjust for potential confounding factors (menopause, exercise, compliance, energy intake) that may influence changes in body fat as covariates. Pearson's correlation coefficient was used to examine the relationships between variables. The per-protocol set included all participants who completed the study protocol and had no major protocol deviation, this set was used for efficacy analysis. The safety set included all participants who received at least one capsule of IP; this set was used for safety analysis.

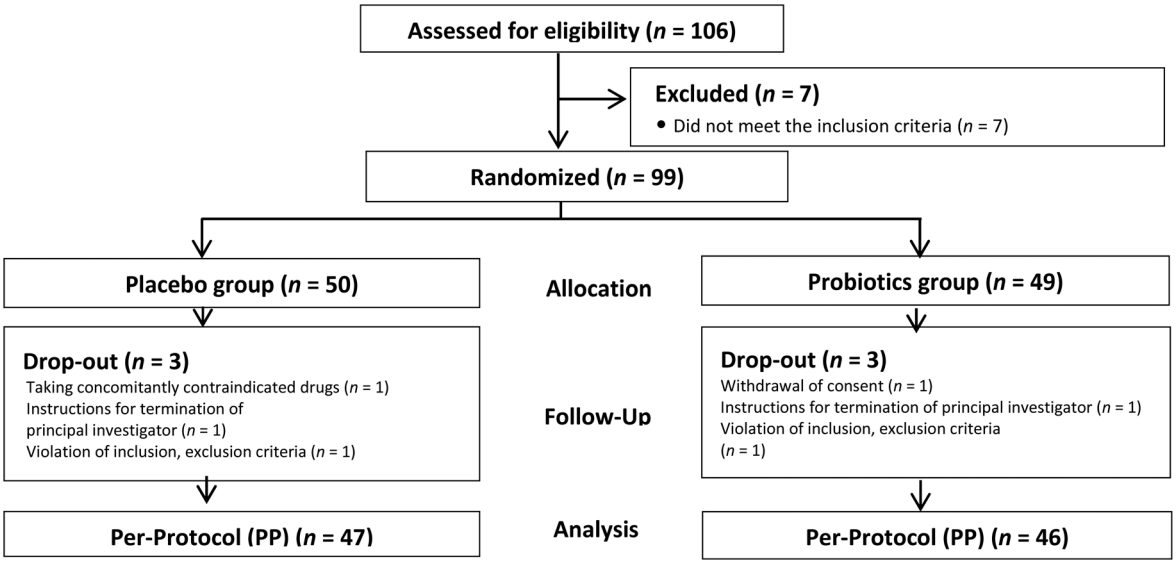

Fig. 1 Flowchart of participant selection.

All statistical analyses were performed using SAS® software (version 9.4, SAS, Cary, NC, USA).

3. Results

3.1. Participant characteristics

The first participant was screened on March 16, 2022, and the last on September 29, 2022. In total, 106 participants were evaluated; among them, 7 participants were excluded. Finally, 99 participants (50 participants in the placebo groups and 49 participants in the probiotics groups) were enrolled. Consequently, three participants from each group (total: six) dropped out; thus, 94 participants (47 in the placebo groups and 46 in the probiotics groups) completed the clinical trial (Fig. 1). There was no significant between-group difference in age (53 ± 10 years in the placebo group *vs.* 54 ± 8 years in the probiotics group, p = 0.5805). There were also no significant between-group differences in alcohol consumption, smoking, regular exercise, or frequency of eating per day (Table 1).

Table 1 Demographic and lifestyle characteristics at baseline of the obese women

Variables	Placebo group (n = 47)	Probiotics group (n = 46)	p-Value
Age (years)	53 ± 10	54 ± 8	0.5805
Alcohol drinking			0.7249
Yes	18 (38)	16 (35)	
No	29 (62)	30 (65)	
Smoking			1.0000
Non-smoker	45 (96)	45 (98)	
Ex-smoker	2 (4)	1 (2)	
Regular exercise			0.2476
Yes	24 (51)	18 (39)	
No	23 (49)	28 (61)	
Frequency of eating/day			
Meal	2.8 ± 0.4	2.9 ± 0.3	0.2980
Snack	2.0 ± 1.0	2.0 ± 0.8	0.7413
Water	5.2 ± 2.1	5.4 ± 2.1	0.7914

Values are expressed as means ± SD or n (%). For alcohol drinking, answer "yes" if the participant consumes alcohol more than once a week, otherwise answer "no". For smoking, non-smoker indicates a person who does not currently smoke. There were no smokers in the groups. For regular exercise, answer "yes" if participants exercised more than once a week, and answer "no" otherwise. No differences were detected between the two groups. p values were obtained from Chi-square test or Fisher's exact test for categorical variables and Student's t-test for continuous variables.

3.2. Anthropometrics and body composition outcomes

Total and trunk body fat masses (kg) decreased more in the probiotics group compared to that in the placebo group (p = 0.0407 and p = 0.0200, respectively). In addition, the total, trunk, and leg body fat mass (kg) in the probiotics group significantly decreased after intake compared to baseline, but in the placebo group, only the leg body fat mass (kg) decreased. Difference in the changes in the degree of obesity, which refers to the current body weight compared with the ideal body weight, between the placebo and probiotics groups were not significant, but it was decreased significantly only in the probiotics group. Difference in the changes in WC, HC, and WHR between the placebo and probiotics groups was not significant, but in both groups, WC, HC, and WHR significantly decreased after intake compared to before intake (Table 2). Additionally, more subjects in the probiotics group had reduced total fat mass (Fig. 2A and B). And looking at the change rate in BMI, there were more subjects with decreased BMI in the probiotics group (Fig. 2C).

Additionally, when comparing the difference in body composition changes of the probiotics group compared to the placebo group of all women (n = 93) and postmenopausal women (n = 69), the differences in weight change (all women −0.42 kg *vs.* postmenopausal women −0.63 kg), BMI (all women −0.16 kg m^{-2} *vs.* postmenopausal women −0.26 kg m^{-2}), total body fat mass (all women −0.29 kg *vs.* postmeno-

Table 2 Anthropometric parameters of the obese women after 12 weeks probiotics intake period

	Placebo group (n = 47)		Probiotics group (n = 46)				
Variables	Baseline	12 weeks	Baseline	12 weeks	p^a	p^b	p^c
Body weight (kg)	64.27 ± 5.56	64.12 ± 5.84	67.06 ± 8.28	66.49 ± 8.38*	0.0609	0.1181	
Change	−0.15 ± 1.46		−0.57 ± 1.10				0.1537
BMI (kg m^{-2})	26.22 ± 1.67	26.15 ± 1.76	26.59 ± 1.87	26.36 ± 1.95*	0.3158	0.5899	
Change	−0.07 ± 0.59		−0.23 ± 0.43				0.1698
Body fat (kg)							
Total	27.01 ± 3.65	26.86 ± 3.64	28.96 ± 5.59	28.51 ± 5.55*	0.0514	0.0950	
Change	−0.16 ± 0.83		−0.45 ± 0.83				0.0407
Trunk	14.15 ± 2.17	14.12 ± 2.19	15.29 ± 3.04	15.06 ± 3.00*	0.0408	0.0883	
Change	−0.03 ± 0.50		−0.22 ± 0.51				0.0200
Arm	3.36 ± 0.59	3.34 ± 0.60	3.50 ± 0.76	3.51 ± 0.75	0.3348	0.2399	
Change	−0.03 ± 0.15		0.00 ± 0.17				0.6350
Leg	8.30 ± 1.53	8.20 ± 1.58*	8.97 ± 2.37	8.74 ± 2.38*	0.1143	0.2023	
Change	−0.11 ± 0.36		−0.23 ± 0.30				0.0782
Body fat (%)							
Total	41.94 ± 3.36	41.80 ± 3.26	42.91 ± 4.00	42.58 ± 3.75	0.2067	0.2843	
Change	−0.14 ± 1.24		−0.33 ± 1.19				0.2016
Trunk	43.30 ± 3.88	43.29 ± 3.78	44.48 ± 4.21	44.29 ± 3.86	0.1631	0.2084	
Change	−0.01 ± 1.48		−0.19 ± 1.44				0.2636
Arm	48.18 ± 4.68	47.87 ± 4.49	48.61 ± 5.40	48.66 ± 5.30	0.6800	0.4392	
Change	−0.31 ± 1.60		0.04 ± 1.83				0.7307
Leg	41.10 ± 4.34	40.76 ± 4.46	41.89 ± 5.42	41.16 ± 5.33*	0.4367	0.6983	
Change	−0.34 ± 1.70		−0.73 ± 1.35				0.1317
Lean mass (kg)							
Total	35.28 ± 3.07	35.29 ± 3.29	36.15 ± 3.67	36.03 ± 3.58	0.2179	0.3008	
Change	0.01 ± 1.32		−0.12 ± 1.08				0.9081
Trunk	17.90 ± 1.71	17.88 ± 1.80	18.34 ± 1.95	18.20 ± 1.93	0.2491	0.4228	
Change	−0.02 ± 0.77		−0.14 ± 0.66				0.6181
Arm	3.34 ± 0.41	3.36 ± 0.42	3.40 ± 0.42	3.39 ± 0.39	0.5310	0.6949	
Change	0.02 ± 0.19		−0.01 ± 0.15				0.9672
Leg	11.12 ± 1.13	11.15 ± 1.26	11.51 ± 1.44	11.54 ± 1.39	0.1444	0.1554	
Change	0.03 ± 0.51		0.03 ± 0.38				0.6616
Obesity degree (%)	126.50 ± 8.03	126.20 ± 8.38	128.30 ± 8.92	127.40 ± 9.42*	0.3046	0.5103	
Change	−0.36 ± 2.75		−0.96 ± 2.14				0.3828
WC (cm)	87.97 ± 5.99	87.10 ± 6.00*	90.10 ± 6.81	88.96 ± 6.70*	0.1128	0.1614	
Change	−0.87 ± 1.30		−1.14 ± 1.18				0.3865
HC (cm)	98.73 ± 4.83	98.32 ± 4.95*	100.30 ± 6.09	99.46 ± 6.04*	0.1657	0.3226	
Change	−0.41 ± 1.32		−0.87 ± 1.05				0.1141
WHR	0.891 ± 0.048	0.886 ± 0.046*	0.899 ± 0.050	0.895 ± 0.049*	0.4209	0.3748	
Change	0.005 ± 0.014		0.004 ± 0.011				0.8394

Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-hip ratio. Values are expressed as means ± SD. * Significant difference between baseline and 12-week data by paired t test at *$p < 0.05$. [a] P-Values derived from independent t-tests at baseline. [b] P-Values derived from independent t-tests at 12 weeks. [c] Group differences of change were calculated using the general linear model (GLM) after adjusting for menopause, exercise, compliance, energy intake.

pausal women −0.40 kg), and trunk body fat mass (all women −0.19 kg *vs.* postmenopausal women −0.25 kg) were larger in postmenopausal women (Table 3).

3.3. Blood biomarkers

Changes in TG concentration and AIP were significantly different between the groups (p = 0.0088 and p = 0.0145, respectively), indicating a lower risk of metabolic diseases. Leptin concentration did not differ between the groups but significantly decreased in both groups (Table 4). No significant differences in the other blood indicators were observed.

3.4. Correlations between changes in total fat mass and adipokines

Fig. 3 shows the correlation between changes in total fat mass and adipokines in the probiotics group after the 12-week probiotics intake. A significant positive correlation was observed between changes in total fat mass and log LAR (R = 0.371, P = 0.0112) (Fig. 3A).

No correlation was found between the changes in total fat mass and adiponectin or leptin levels. Additionally, the same parameters had no correlation in placebo groups. This indicated that changes in adipokines were related to total fat mass in the probiotics group.

3.5. Safety outcomes

Safety outcomes were within the normal range both before and after the study. In the placebo group, three (6%) participants had three adverse events (enteritis [n = 1], corona virus [n = 2]), with no significant differences between groups. In the probiotics group, 3 (6%) participants experienced 3 adverse events

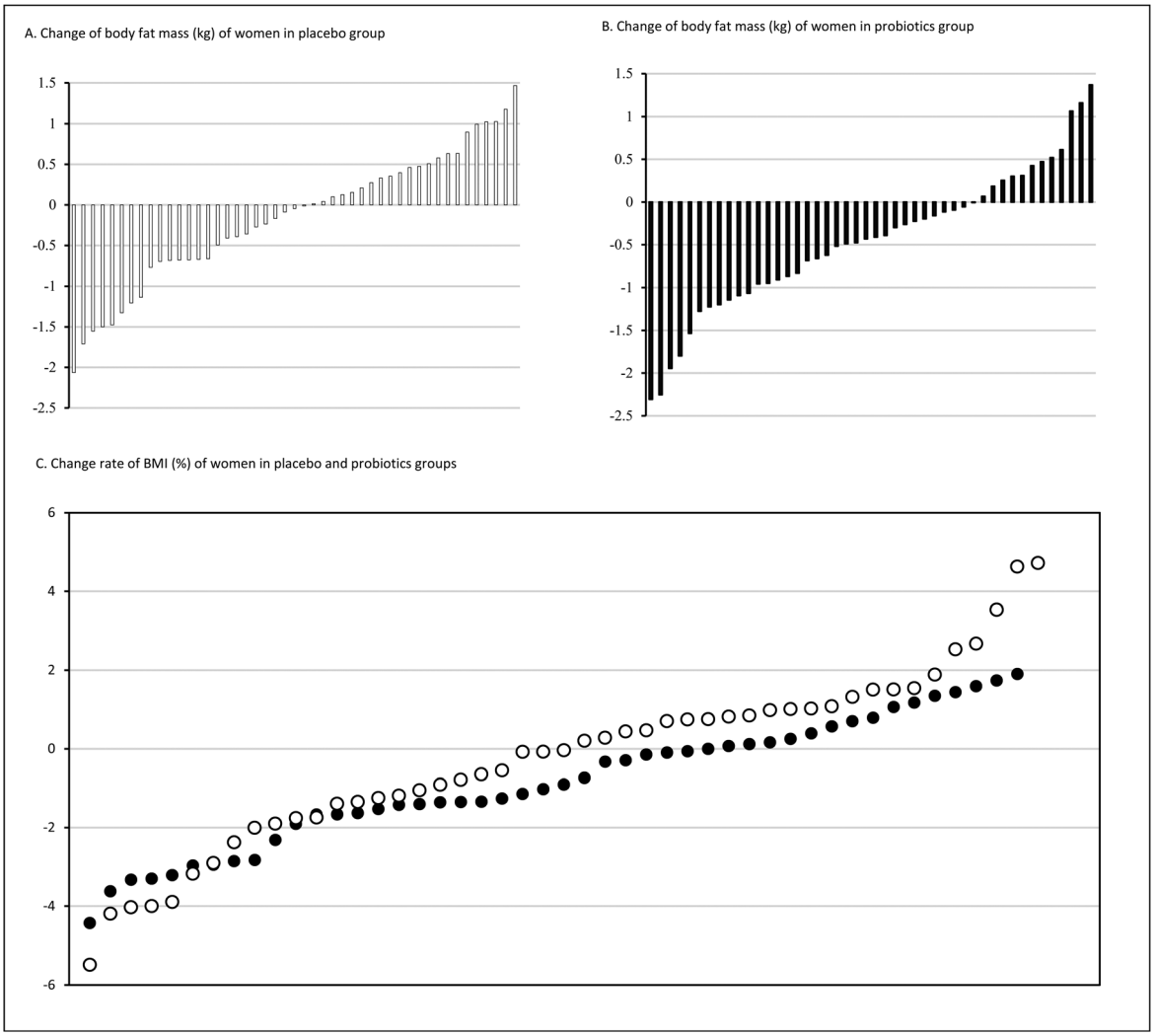

Fig. 2 Inter-group differences of obese women with respect to fat mass and BMI. White is the placebo group and black is the probiotics group. Panel A shows the change of fat mass of women in probiotics group and Panel B shows the same parameter in placebo group. And Panel C shows the change rate of the BMI of women in the probiotics and placebo groups in order. In the probiotics group, number of participants with decreased fat mass and BMI were higher.

(hyperlipidemia [n = 1], corona virus [n = 2]). No adverse events were related to IP and no serious adverse events occurred.

3.6. Dietary intake and physical activity

No significant differences in dietary intake before (0 week) and after (week 12) the intervention. The amount of physical activity increased in both groups after intake compared to that before intake, but there was no significant difference between the groups (Table 5).

4. Discussion

In this trial, we found that 12-week *B. lactis* IDCC 4301 supplementation had anti-obesity effects in obese women. The total fat mass of the probiotics group decreased 2.6 times and the trunk fat mass decreased 7 times compared to those in the placebo group. The difference in total fat mass change between groups among postmenopausal women was 38% greater than that of all women. Changes in TG concentration and AIP levels showed significant differences between the groups, indicating a lower CVD risk in the probiotics group than that in the placebo group. The adipokine ratio was correlated with body fat loss in the probiotics group. These changes are thought to be related to metabolic changes in the body due to changes in the intestinal microorganisms.

Probiotic intake alters intestinal microorganisms and body fat through strain diversification. In a meta-analysis of randomized controlled trials (RCTs) that examined the effects of probiotic supplementation on body composition in BMI 25–30 kg m^{-2} and BMI ≥ 30 kg m^{-2} participants, five studies reported changes in body fat percentage, and the pooled estimate showed that the percent body fat was significantly lower

Table 3 Anthropometric parameters of the postmenopausal women after 12 weeks probiotics intake period

	Placebo group (n = 36)		Probiotics group (n = 33)				
Variables	Baseline	12 weeks	Baseline	12 weeks	p^a	p^b	p^c
Body weight (kg)	63.57 ± 5.74	63.61 ± 6.16	65.62 ± 6.76	65.03 ± 7.10*	0.1771	0.3781	
Change	0.04 ± 1.23		−0.59 ± 1.08				0.0163
BMI (kg m^{-2})	26.07 ± 1.62	26.08 ± 1.78	26.44 ± 1.80	26.20 ± 1.92*	0.3733	0.8041	
Change	0.01 ± 0.50		−0.25 ± 0.44				0.0160
Body fat (kg)							
Total	26.89 ± 3.89	26.77 ± 3.92	28.18 ± 4.85	27.65 ± 4.85*	0.2249	0.4050	
Change	−0.13 ± 0.84		−0.53 ± 0.91				0.0383
Trunk	14.23 ± 2.19	14.21 ± 2.25	15.22 ± 2.71	14.96 ± 2.68*	0.0962	0.2138	
Change	−0.02 ± 0.52		−0.27 ± 0.55				0.0278
Arm	3.32 ± 0.62	3.32 ± 0.65	3.46 ± 0.75	3.45 ± 0.76	0.4211	0.4326	
Change	0.00 ± 0.15		0.00 ± 0.17				0.8515
Leg	8.16 ± 1.57	8.05 ± 1.59	8.31 ± 1.87	8.05 ± 1.85*	0.7252	0.9987	
Change	−0.12 ± 0.37		−0.26 ± 0.34				0.1054

Abbreviations: BMI, body mass index; WHR, waist-hip ratio. Values are expressed as means ± SD. * Significant difference between baseline and 12-week data by paired t test at *$p < 0.05$. [a] P-Values derived from independent t-tests at baseline. [b] P-Values derived from independent t-tests at 12 weeks. [c] Group differences of change were calculated using the general linear model (GLM) after adjusting for menopause, exercise, compliance, energy intake.

Table 4 Blood lipid profiles, adipokines, and glycemic profiles of obese women after 12 weeks of probiotic use

	Placebo group (n = 47)		Probiotics group (n = 46)				
Variables	Baseline	12 weeks	Baseline	12 weeks	p^a	p^b	p^c
Triglyceride (mg dL^{-1})	119.20 ± 84.78	132.00 ± 78.50	131.50 ± 80.76	116.40 ± 63.30	0.4754	0.2972	
Change	12.74 ± 60.07		−15.09 ± 61.60				0.0088
Total cholesterol (mg dL^{-1})	191.50 ± 43.04	194.00 ± 42.00	208.40 ± 43.54	205.80 ± 32.71	0.0630	0.1352	
Change	2.53 ± 20.72		−2.59 ± 28.29				0.1482
HDL-C (mg dL^{-1})	54.21 ± 10.92	54.55 ± 12.75	54.89 ± 10.75	55.04 ± 10.90	0.7634	0.8426	
Change	0.34 ± 6.82		0.15 ± 7.86				0.8912
LDL-C (mg dL^{-1})	108.00 ± 32.61	110.10 ± 34.67	126.50 ± 39.89	125.40 ± 30.66	0.0160	0.0270	
Change	2.13 ± 17.74		−1.13 ± 25.44				0.4350
Atherogenic index of plasma	0.27 ± 0.31	0.33 ± 0.30*	0.32 ± 0.30	0.28 ± 0.27	0.3757	0.3881	
Change	0.07 ± 0.22		−0.04 ± 0.23				0.0145
Adiponectin (μg mL^{-1})	7.00 ± 2.03	7.02 ± 2.38	7.09 ± 2.41	7.38 ± 2.53	0.8509	0.4876	
Change	0.02 ± 1.40		0.29 ± 1.31				0.4898
Leptin (ng mL^{-1})	49.42 ± 18.82	39.05 ± 22.82*	51.76 ± 20.40	38.90 ± 22.86*	0.5660	0.9759	
Change	−10.37 ± 24.32		−12.86 ± 25.19				0.6353
Glucose (mg dL^{-1})	97.72 ± 8.27	97.04 ± 7.91	98.96 ± 8.13	97.28 ± 8.88	0.4704	0.8907	
Change	−0.68 ± 6.57		−1.67 ± 7.96				0.6017
Insulin (μU mL^{-1})	8.25 ± 3.67	8.38 ± 3.61	9.82 ± 4.49	9.21 ± 4.23	0.0679	0.3146	
Change	0.13 ± 3.62		−0.62 ± 3.73				0.2554
HOMA-IR	2.00 ± 0.93	2.04 ± 1.00	2.41 ± 1.12	2.23 ± 1.08	0.0550	0.3796	
Change	0.04 ± 0.93		−0.18 ± 0.98				0.2152

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglyceride; HOMA-IR, homeostatic model assessment for insulin resistance. Values are expressed as means ± SD. * Significant difference between baseline and 12-week data by paired t test at *$p < 0.05$. [a] P-Values derived from independent t-tests at baseline. [b] P-Values derived from independent t-tests at 12 weeks. [c] Group differences of change were calculated using the general linear model (GLM) after adjusting for menopause, exercise, compliance, energy intake.

in the intervention groups than that in the placebo groups, with low heterogeneity among the studies.[31,32] Takahashi *et al.* investigated the effects of *B. lactis* GCL2505 ingestion for 12 weeks in 137 overweight and slightly obese Japanese adults.[17] The VFA significantly decreased by 6.4 cm^2 in the test group compared to increase of 2.2 cm^2 in the placebo group. The total number of fecal bifidobacteria significantly increased in the probiotics group. Although the number of fecal *Bifidobacterium* could not be confirmed in this study, it could be inferred that the anti-obesity effect due to the *Bifidobacterium* increase was based on a previous study. Sung *et al.* demonstrated a significant decrease in body fat (of 587.05 g in obese adults) after consuming *Bifidobacterium breve* B-3 for 12 weeks.[33] Furthermore, in postmenopausal women, estrogen levels and sex hormone-binding globulins are known to decline and free testosterone levels are known to increase after menopause, and this is thought to induce central obesity.[34] In a study conducted by Szulińska *et al.*,

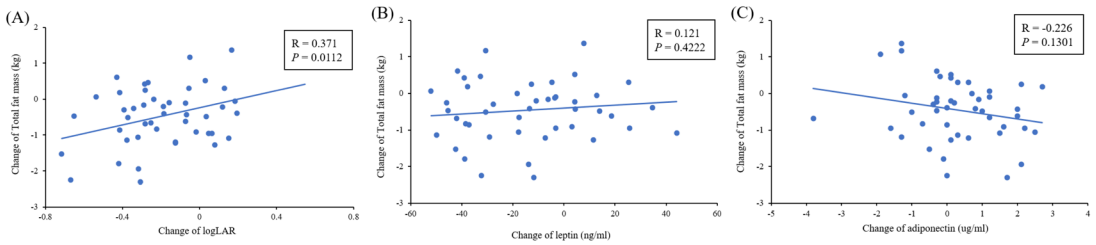

Fig. 3 Correlations between changes in total fat mass and adipokines in the probiotics group after the 12-week probiotics intake period. Panel A shows the correlation between changes in total fat mass and log leptin/adiponectin ratio (LAR). Panel B shows the correlation between changes in total fat mass and leptin concentration. Panel C shows the correlation between changes in total fat mass and adiponectin concentration. There were no significant correlations with the same parameters in placebo groups.

Table 5 Changes in dietary intake and physical activity of the obese women after 12 weeks probiotics intake period

	Placebo group (n = 47)		Probiotics group (n = 46)				
Variables	Baseline	12 weeks	Baseline	12 weeks	p^a	p^b	p^c
Energy (kcal)	1629.7 ± 373.8	1570.5 ± 427.8	1620.6 ± 440.8	1615.0 ± 436.7	0.9144	0.6208	
Change	−59.3 ± 388.3		−5.6 ± 349.9				0.4862
Energy intake per body weight (kcal kg^{-1})	25.1 ± 6.2	24.2 ± 7.1	23.9 ± 6.0	24.1 ± 6.5	0.3452	0.9052	
Change	−0.8 ± 6.1		0.2 ± 5.2				0.3827
Carbohydrate (g)	222.3 ± 60.8	212.5 ± 63.4	215.0 ± 51.0	214.9 ± 47.5	0.5278	0.8378	
Change	−9.8 ± 53.5		−0.1 ± 48.2				0.3577
Dietary fiber (g)	19.9 ± 7.3	19.5 ± 8.5	21.3 ± 5.6	20.3 ± 6.3	0.3149	0.5972	
Change	−0.4 ± 7.7		−1.0 ± 6.8				0.7194
Protein (g)	63.4 ± 16.2	60.9 ± 18.0	64.7 ± 21.1	66.3 ± 19.8	0.7427	0.1741	
Change	−2.5 ± 17.2		1.6 ± 18.3				0.2692
Fat (g)	49.1 ± 16.2	47.8 ± 18.8	49.8 ± 17.8	47.6 ± 18.9	0.8386	0.9525	
Change	−1.3 ± 20.0		−2.3 ± 20.6				0.8212
Cholesterol (mg)	309.7 ± 147.9	307.3 ± 154.6	337.9 ± 157.1	370.7 ± 142.4	0.3750	0.0427	
Change	−2.4 ± 172.3		32.7 ± 172.0				0.3273
Saturated fatty acids (g)	9.3 ± 5.6	8.6 ± 5.5	9.0 ± 4.6	8.4 ± 4.1	0.7386	0.8765	
Change	−0.8 ± 7.1		−0.6 ± 5.1				0.8772
Monounsaturated fatty acids (g)	11.4 ± 6.8	11.0 ± 6.7	11.6 ± 5.9	10.5 ± 5.1	0.8775	0.7011	
Change	−0.4 ± 8.4		−1.1 ± 7.4				0.6793
Polyunsaturated fatty acids (g)	10.4 ± 5.2	9.5 ± 5.3	10.5 ± 4.8	9.4 ± 3.0	0.9055	0.9255	
Change	−0.9 ± 6.4		−1.1 ± 5.7				0.8693
EPA (g)	1.6 ± 3.2	1.0 ± 1.7	1.1 ± 2.0	1.8 ± 3.9	0.3358	0.1754	
Change	−0.7 ± 3.5		0.7 ± 4.6				0.1039
DHA (g)	2.8 ± 4.8	2.0 ± 2.8	2.1 ± 4.0	2.9 ± 5.4	0.4496	0.2831	
Change	−0.9 ± 5.7		0.8 ± 7.0				0.2162
Total METs (METs per min per week)	3865.5 ± 2737.2	4859.6 ± 2788.9*	3604.3 ± 2700.5	4597.0 ± 3252.3*	0.6444	0.6767	
Change	994.0 ± 2843.1		992.6 ± 2391.0				0.9979

MET is defined as the metabolic equivalent of task. If the Total MET score is over 600 METs per min per week, the group is classified as regular physical activity, and if >3000 METs per min per week, is classified as active and sufficient physical activity. Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; METs, metabolic equivalents. Values are expressed as means ± SD. * Significant difference between baseline and 12-week data by paired t test at $*p < 0.05$. [a] P-Values derived from independent t-tests at baseline. [b] P-Values derived from independent t-tests at 12 weeks. [c] Group differences of change were calculated using the general linear model (GLM) after adjusting for menopause, exercise, compliance, energy intake.

the consumption of high-dose supplementation (1×10^{10} CFU) of preparations containing several probiotic strains in postmenopausal women (*Bifidobacterium bifidum W23*, *B. lactis W51*, *B. lactis W52*, *Lactobacillus acidophilus W37*, *L. brevis W63*, *L. casei W56*, *L. salivarius W24*, *Lactococcus lactis W19*, and *Lactococcus lactis W58*) for 12 weeks led to reductions in waist circumference, fat percentage, visceral fat, glucose, lipopolysaccharides, total cholesterol, and insulin not body weight.[35] However, significant improvements in both body fat and body weight were confirmed in this study.

In this study, the serum TG concentration was lower in the probiotics group than that in the placebo group. If the TG concentration is high, it is stored as an energy source in the body and the weight of adipose tissue increases; therefore, lowering the TG concentration can affect the reduction of body fat.[36] AIP, a cardiovascular risk indicator, was also significantly lower in the probiotics group, indicating that CVD risk in the

probiotics group was reduced by.[27] Li *et al.* demonstrated that 8 weeks of *B. lactis* supplementation combined with training may help improve lipid metabolism, including triglyceride and sports performance, by increasing the abundance of *Bifidobacterium*, which can promote the generation of short-chain fatty acids and unsaturated fatty acids and inhibit the synthesis of bile acids.[37] There was no difference in the amount of physical activity between the groups; however, it can be assumed that the intake of *B. lactis* IDCC 4301 affected fat metabolism in the body by altering the composition of intestinal microorganisms.

Adipocytes are a source of adipokines, and adipokine levels correlate with the number of adipocytes.[38] Adipokines levels decrease as adipocytes decrease.[39] Our results showed that leptin and adiponectin ratios were correlated with body fat loss in the probiotics group, whereas no correlation was observed in the placebo group. According to the Ely and European Group for the Study of Insulin Resistance Relationship between Insulin Sensitivity and Cardiovascular Risk study, when the LAR of 2097 people was examined, the results showed that as LAR increased, HOMA-derived insulin sensitivity (HOMA-S) decreased.[28] As HOMA-S decreases, it may have an impact on body fat gain.[40] Additionally, pre-clinical study have shown that a mixture of *Bifidobacterium animalis subsp. lactis* CP-9 and *Lactobacillus rhamnosus* bv-77 suppresses the increase in leptin concentration and increases adiponectin concentration in the blood of obese mice, preventing excessive intake of energy from accumulating in the adipose tissue, resulting in a decrease in body fat mass.[41] Although a single strain was used in this study, LAR was associated with changes in body fat in the probiotics group. Leptin levels also decreased in the placebo group, but this is presumed to reflect the psychological effect of the placebo and the characteristics of leptin, which vary greatly depending on the circadian cycle.[42,43] Therefore, the changes in adipokine levels following *B. lactis* IDCC 4301 intake affect adipocytes and reduce body fat.

Our study has several limitations. First, dietary intake was self-reported. However, measurement errors from self-reported dietary intake and lifestyle variables have been demonstrated to be relatively small.[44] Second, we focused on Korean participants with obese. Therefore, our data cannot be generalized to other ethnic groups. Third, following the protocol, we aimed to sequentially recruit adults with a BMI between 25 and 30 kg m^{-2}, and only one male participant was recruited. Therefore, the number of male participants was very small, and only women are presented in this paper. Fourth, for the purposes of the study, we did not assess the gut microbiome, but measuring it would have strengthened the study. Despite these limitations, compared with the placebo group, supplementation with *B. lactis* IDCC 4301 for 12 weeks in obese participants led to a significant improvement in body fat mass and metabolic health. No adverse events related to the probiotics were observed. These results suggest a beneficial effect of supplementation with *B. lactis* IDCC 4301 on body fat, serum triglyceride, and adipokine levels in obese women.

5. Conclusions

When obese women consumed *B. lactis* IDCC 4301 at a concentration of $>5 \times 10^9$ CFU day^{-1} for 12 weeks, decrease in body fat was greater in the probiotics group than in the placebo group. In the probiotics group, body weight, BMI, and the degree of obesity significantly decreased. Furthermore, difference in the serum TG concentration and AIP levels between the groups was significant, indicating that the probiotics group had a lower CVD risk. Adipokine ratio was also associated with body fat loss in the probiotics group. Therefore, this study determined that the consumption of *B. lactis* IDCC 4301 (*B. lactis* Fit™) can lead to anti-obesity effects in obese women.

Author contributions

ML: study concept, design, data analysis, writing the draft of the manuscript, editing and revising. MKB: supplementation study and sample collections. KS: study concept, design, manuscript editing. ML, HMP, JY: study concept, manuscript review and revising. HL: study concept, design, administration, manuscript review and editing, revising and funding acquisition. All authors agreed to their individual contributions. All authors read and approved the final manuscript.

Conflicts of interest

HL, ML, MKB, KS declared they had no conflicts of interest. ML, HMP is an employee of ILDONG Bioscience Co., Ltd. JY was an ex-employee of ILDONG Bioscience Co., Ltd.

Note added after first publication

This article replaces the version published on 25th July 2024, which contained errors within Table 2.

Acknowledgements

This research was funded by ILDONG Bioscience Co., Ltd. We thank the volunteers for taking the time to participate in this study.

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