

## Effects of a Multi-Strain Supplement Combination *Lactobacillus* and *Bifidobacterium* on Anthropometric Measurements and Body Fat Percentage in Adults with Overweight and Obesity

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### Abstract

**Objective:** Probiotics are living microorganisms that play a part in energy balance. One of the most important causes of obesity is dysbiosis in the intestinal microbiota composition. This study aimed to determine the effects of a multi-strain probiotic supplement containing *Lactobacillus* and *Bifidobacterium* on anthropometric measurements and body fat percentage in adults with overweight and obesity.

**Materials and Methods:** This was a randomized trial including 74 overweight and obese adults referring to two private clinics who entered the study after signing a written informed consent. Participants were randomly assigned to either group and received either  $1 \times 10^9$  CFU/day probiotic capsule or a placebo for 12 weeks. They were advised not to change their usual diet or physical activity during the study period. Anthropometric measurements, body fat percentage, dietary intake, and physical activity were assessed both at first and 12 weeks of the treatment.

**Results:** The intervention group showed significant differences in the mean body weight (-1.46 kg), body mass index (-0.54 kg/m<sup>2</sup>), waist circumference (-1.24cm), hip circumference (-0.42cm), waist-to-hip ratio (-0.01), and fat percentage (-0.66%) compared to their baseline values (all  $P < 0.001$ ), but there were no significant differences in comparison with the placebo group ( $P > 0.05$ ). In the placebo group there were negligible non-significant effects on body weight, body mass index and waist circumference.

**Conclusion:** The findings of this study showed that probiotic supplementation containing several strains of *Lactobacillus* and *Bifidobacterium* can improve anthropometric measurements and body fat percentage in adults.


**Keywords:** Probiotic, Obesity, Lactobacillus, Bifidobacterium, Body fat.

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## Introduction

Obesity is a chronic and progressive disease, and its main cause is an imbalance between the energy received and consumed, which gradually leads to abnormal and excessive fat accumulation in the body and increases the risk of non-communicable diseases (1,2).

Body mass index (BMI) is a criterion for defining overweight and obesity, such that an individual with a BMI of 25-29.9 kg/m<sup>2</sup> is considered overweight, while a BMI of  $\geq 30$  kg/m<sup>2</sup> is considered obese. The World Health Organization reported that in 2022, about 16% of the world's adult population was obese and 43% were overweight (2). The prevalence of obesity and overweight among Iranian adults, men and women was 40.3% and 29.2%, respectively (3). Health organizations try to train people to switch to a balanced diet and continuous physical activity in order to prevent obesity. Although overweight and obese people primarily need to lose weight by modifying their lifestyle, diet, and physical activity (4). Other adjuvant methods are also necessary to manage obesity.

The gut microbiota with a role in energy balance regulation has recently been highly regarded (5-7). The composition of the gut microbiota of obese people is qualitatively and quantitatively different from that of lean people (8). Gut dysbiosis (imbalance in the gut microbiota) caused by the westernization of eating habits (high intake of processed foods, rich in sugar and saturated fat) (9) is also associated with obesity-related inflammation (10). Probiotics have the potential to correct gut dysbiosis (11) and can contribute to the health of the gut microbiota. Probiotics are live microorganisms, consuming sufficient amounts of which can have health benefits for the host (12). Probiotics produce short-chain fatty acids that can regulate the expression of genes associated with inflammation and obesity (13). Taking probiotics is a good way to balance the composition of the gut microbiota for weight loss. Consumption of

Lactobacillus strains (*Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*) can suppress weight gain and reduce adipose tissue accumulation in mice fed a high-fat diet and help regulate lipid metabolism, decrease leptin levels, and increase serum adiponectin (14-16). Some Bifidobacterium strains are able to reduce obesity, lean body mass accretion, and improve insulin sensitivity (17-19).

The anti-obesity effects by decreasing body weight and fat mass are mostly related to Lactobacillus and Bifidobacterium strains (20,21). In this study, mixtures of several probiotic agents that are common in obesity studies were used. Hence the objective of this research was to investigate the effects of several probiotic species on anthropometric indices in overweight and obese adults.

## Material and Methods

This triple-blinded, parallel, randomized, placebo-controlled clinical trial was conducted in two clinics in Rasht, Iran, during 2022-2023.

Overweight or obese individuals (BMI  $\geq 25$ ), aged 18 to 60 years, were included and who provided informed written consent. Criteria included serious conditions such as cardiovascular, liver, kidney, thyroid, cancer and endocrine diseases; allergies; pregnancy; lactation; having special conditions such as immune system problems and AIDS, psychiatric disorders, and surgery for weight loss, as well as weight loss of more than 5%, taking antibiotics, anti-diabetes drugs, probiotics, and other supplements in the past 3 months; use of weight loss and appetite reduction drugs, capsule consumption less than 80% and other conditions that might affect the study results were also considered. Participants could withdraw from the study whenever they wanted and for any reason.

In order to determine the difference in weight in 12 weeks, considering  $\alpha = 0.05$ ;  $\beta =$

0.20; and 80% power, the sample size was calculated to be 31 participants for each group. Taking into account the probable 20% loss of samples, the sample size was increased by 20%, resulting in 37 participants in each group.

A total of 74 eligible subjects entered in the study and were randomly divided into two groups using stratified sampling method; and they were given either a probiotic capsule or a placebo daily for 12 weeks. The enrollment period lasted about 6 months. Random allocation, assigned of interventions were done by the researcher.

In this study, probiotic and placebo supplement boxes were labeled with A or B code by the manufacturer so that neither the participants, researcher, nor the statistician knew about the type of intervention. After the statistical analysis, the group type (probiotic or placebo) was determined.

The probiotic supplements used in this study (FamiLact®), containing 500 mg, included 9 beneficial, live and active strains of bacteria, live and active strains of bacteria (*Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Bifidobacterium lactis*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*,  $1 \times 10^9$  cfu) and other ingredients such as Fructo-oligosaccharide, Microcrystalline cellulose, Lactose, Magnesium stearate, Talc, Silicon dioxide, Maltodextrin, Sodium starch. The placebo was quite similar to the probiotic supplement but without microorganisms, and both supplement and placebo were manufactured by Zist Takhmir Company, Tehran, Iran. To increase the shelf-life of probiotic supplementations, volunteers were advised to keep capsules in the refrigerator. To remind participants to use the capsules during the study, the participants were contacted by telephone once a week on the average.

General characteristics of the participants including age, gender and other demographic

factors were collected at the beginning of the study through an interviews.

Anthropometric data including body weight, BMI, waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR), and body fat percentage (BF or BF%) were assessed at baseline and the end of week 12. The weight of the subjects was measured in light clothing and without shoes using the SECA® scale. The wall stadiometer (SECA®-206, Germany) was used to measure their height in the standing position with feet attached to the wall without shoes, and looking forward. BMI was calculated from division of weight in kilograms by height squared in meters. Waist circumference was measured without imposing any pressure on the body in the narrowest area between the last rib and upper iliac crown at the end of exhalation, and the most prominent part of the hip was considered for hip circumference, both of which were done by SECA® -201. BF% was calculated by Durenberg's formula.

$BF = (1.20 \times BMI) + (0.23 \times Age) - (10.8 \times sex) - 5.4$

Sex: Male= 1, Female= 0

Participants' food intake was recorded both at the beginning and the end of the study using a 24-hour dietary recall questionnaire including two consecutive days a week and one weekend day, and data were analyzed using Nutritionist IV software. The subjects' Physical Activity was measured using the International Short Form of Physical Activity (IPAQ) (22) by an interview both at weeks 0 and 12 of the study. For the mentioned physical activities, metabolic equivalents (MET) were calculated. Data were recorded as MET-min/week.

The analysis was done using SPSS, v. 26 and R Core Team, R 4.2.3. Differences in demographic data of the two groups were determined using chi-square and t-test. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine the normality of data distribution and were used after paired T-test and Wilcoxon test. In order to investigate the trend of mean changes between the two groups

and the time period of drug use, repeated measures ANOVA and post hoc Bonferroni test were used (Confidence interval: 95% [CI])

### Ethical considerations

This study was reviewed by the Ethics Committee of Islamic Azad University, Science and Research Branch, Tehran, Iran and approved with the code IR.IAU.SRB.REC.1401.134. It was also registered on the Iranian Clinical Trials website ([www.irct.ir](http://www.irct.ir)) with the ID number IRCT20220425054653N1. Informed consent was obtained from all participants in order to comply with ethical principles and they could withdraw from the study at any time. The supplements used were provided to the volunteers without any side effects.

### Results

In the study, 86 men and women volunteered to participate, and 74 eligible participants were included in the research process. For a 12-

week intervention during 2022-2023, 37 people were assigned to either group (control group OR probiotic group), a total of 68 people completed the study, while 6 participants were excluded due to unwillingness to cooperate, taking antibiotics, changing the treatment group, changing diet, and not taking enough capsules (Figure.1).

### Baseline characteristics

There were no statistically significant differences in the general characteristics (Table 1). Most of the participants were women (57.4%), and the mean age for all in the placebo group was  $44.15 \pm 11.8$  years while in the intervention group it was  $45.12 \pm 11.38$  years, which did not differ significantly ( $P > 0.05$ ). Participants did not report any side effects during the study.

### Food intake and physical activity

Participants' food intake and physical activity were analyzed both before and after

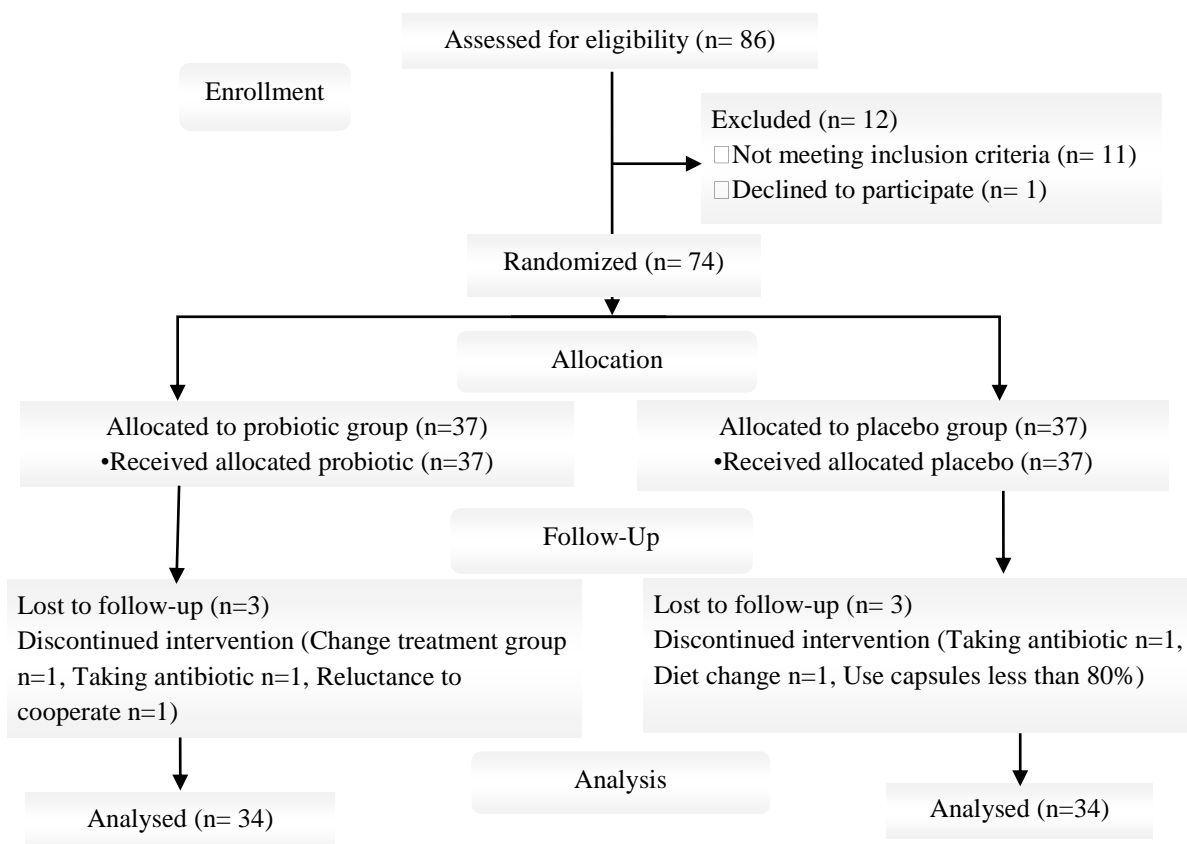


Figure 1. CONSORT Flow Diagram of the study

the intervention by repeated measures analysis of variance. There was no statistically significant difference between the interaction of time and group on energy, macronutrients, fiber intake, and physical activity in the two groups ( $P > 0.05$ ).

### Anthropometric indices and Body fat percentage

Table 2 shows the results on anthropometric indicators and BF%. The results of repeated measures analysis of variance showed that the interactions between time and group on body

weight, BMI, WC, HC, WHR, and BF% were significant ( $P < 0.05$ ). In order to investigate whether there was a significant difference in which groups at different times and which times in different groups, Bonferroni post hoc test was used and observed that there was a no significant difference between the two groups in the parameters of weight, WC, and WHR and, there is a significant difference between the two groups in the parameters of BMI, HC and BF at the baseline and end of the study. There were significant reductions in body weight (-1.46kg), BMI (-0.55 kg/m<sup>2</sup>), WC (-

**Table 1. Baseline characteristics of participants in the probiotic and placebo groups**

Characteristics	Probiotic group	Placebo group	P-value
Age† (Year)	45.12 (±11.38)	44.15 (±11.8)	0.73 *
Height† (m)	1.626 (±0.085)	1.644 (±0.079)	0.36 *
Sex, n (%)			
Female	20 (59%)	19 (56%)	0.80 **
Male	14 (41%)	15 (44%)	
Marital status, n (%)			
Single	8 (23%)	11 (32%)	0.41 **
Married	26 (77%)	23 (68%)	
Level of education, n (%)			
Diploma and under diploma	22 (65%)	17 (50%)	0.274 **
Bachelor	5 (15%)	12 (35%)	
Masters and above	7 (20%)	5 (15%)	
Economic level, n (%)			
Poor	10 (29.5%)	11 (32.5%)	0.745 **
Average	17 (50%)	14 (41%)	
Good	7 (20.5%)	9 (26.5)	

†Mean, ±SD

\*Paired-samples T-test, \*\*Chi-square correlation test

**Table 2. Anthropometric indicators at baseline and after 12 weeks**

Parameters		Probiotic	Placebo	P-value*	P-value***	Effect size
Weight (kg)	pre	84.155 (±13.651)	83.191 (±11.394)	0.753	<0.001	0.292
	post	82.695 (±13.252)	83.039 (±11.217)	0.908		
	P**	<0.001	0.397			
BMI (kg/m <sup>2</sup> )	pre	31.845 (±4.722)	30.632 (±3.061)	<0.001	<0.001	0.28
	post	31.296 (±4.527)	30.559 (±3.039)	<0.001		
	P**	<0.001	0.553			
WC (cm)	pre	98.109 (±8.607)	96.059 (±7.024)	0.286	<0.001	0.177
	post	96.871 (± 8.018)	95.844 (±6.901)	0.573		
	P**	<0.001	0.268			
HC (cm)	pre	108.688 (±8.738)	106.571 (±4.881)	<0.001	0.008	0.10
	post	108.265 (±8.440)	106.444 (±4.777)	<0.001		
	P**	<0.001	0.977			
WHR	pre	0.905 (±0.061)	0.901 (±0.048)	0.760	0.002	0.134
	post	0.894 (±0.059)	0.900 (±0.047)	0.613		
	P**	<0.001	0.912			
Body fat (%)	pre	38.753 (±9.078)	36.444 (±6.417)	<0.001	<0.001	0.28
	post	38.093 (±8.889)	36.383 (± 6.442)	<0.001		
	P**	<0.001	0.800			

\*Between groups; \*\* within group (post hoc Bonferroni test)

\*\*\*Related to the interaction of the two groups and time, repeated measures ANOVA

P-value < 0.05 = significant; Confidence interval: 95%

BMI, Body Mass Index; WC, Waist circumference; HC, Hip Circumference; WHR, Waist to hip ratio.

1.24 cm), HC (-0.55 cm), BF (-0.66%), WHR (-0.11) relative to baseline values ( $P < 0.001$ , 95%CI) only in the probiotic group after the intervention. The placebo group showed a very small reduction in mean body weight (-0.288 kg,  $P = 0.397$ ), BMI (-0.073 kg/m<sup>2</sup>,  $P = 0.553$ ), WC (-0.21cm,  $P = 0.268$ ), HC (-0.127cm,  $P = 0.977$ ), WHR (-0.001,  $P = 0.912$ ), BF (-0.06%,  $P = 0.8$ ) relative to their baseline values, but this was not significant (95%CI).

## Discussion

Obesity is increasing in the world, and one of the environmental factors that plays an important role in obesity is the gut microbiota (23). The composition of the microbiota of obese adults and those of normal weight varies (24). Obese people have less bacterial diversity and gain more weight than those with more diverse gut bacteria (25). Considering that adjustment in the gut microbiota leads to changes in weight, BMI, and WC, probiotics have been proposed as a treatment approach for obesity prevention and treatment (26). The exact mechanism of probiotics' effects on obesity is not yet fully understood and it seems that the gut microbiota can be effective in weight control through the stimulation of satiety hormones, effects on insulin sensitivity, improvement of intestinal barrier function, and regulation of fat metabolism in adipose tissue (27,28).

Studies have shown that the reduction or increase in body weight depends on a particular strain of probiotics, and only some species of Bifidobacterium and Lactobacillus are effective, while the use of other strains can be harmful (29). In this study, the effects of a mixture of probiotic strains on anthropometric parameters among overweight and obese Iranians were evaluated.

The number of people excluded from this study was low, and this may be due to the lack of side effects of capsules. There was a slight decrease in the average dietary intake in the probiotic group, but it was not significant. It seems that the strains used in this study have no effect on dietary intake and appetite, and

the beneficial effect of these strains on obesity indicators may have led to a reduction in calories absorbed from food through inhibiting the absorption of dietary fat.

The results of this study show that taking a probiotic supplement, especially a combination of Lactobacillus and Bifidobacterium strains for 12 weeks, has the potential to reduce anthropometric indicators and BF% in adults with overweight and obesity.

In the probiotic group statistically significant weight loss (about 2 kg on the average, with weight loss <5%) occurred compared to baseline. Although weight loss below 5% is not clinically significant; however, this amount of weight loss and the ability of the strains used to prevent weight gain can still be considered a way to fight obesity. The placebo group showed a slight reduction in mean weight, BMI, WC, although the differences were not statistically significant. In obesity studies, the effect of placebo has become one of the recognized characteristics in these studies (30,31).

The findings of a meta-analysis reported reductions in weight, BMI, and WC following taking probiotics. However, the results showed no significant effects on HC, WHR, and body fat (32). Also, in a review of clinical trials, weight loss and BMI were more pronounced on those who were treated with probiotics for longer periods (33). However, there was no consensus, and other studies have not shown any significant effect of probiotics on body weight and BMI (34). In another study, the intervention with probiotics for 3 months did not work, but in those who took higher doses and longer periods of time, weight loss and BMI loss were observed (35). The reason for the difference between the findings of this study and other studies may be due to the duration of the intervention, the probiotic dose, and bacterial strain, as not all strains may show the same results. This may be an important confounding factor in probiotics studies.

One of the strengths in this study was blinding the study, and as a result, it reduced the risk of bias. In a randomized clinical trial that has not been blinded, the potential of an increased bias may lead to inaccurate results. Additionally, in this study there was no adjustment in diet or physical activity program, and only the specific effect of probiotic was measured without low-calorie diet or increased physical activity, which makes the findings of this study applicable. Also, people who took antibiotics were excluded from the study. So similar conditions for probiotics were guaranteed compared to placebo. However, changes in gut microbiota were not investigated in this study, and the major limitation of this study included the duration of the intervention and larger amounts of supplementation.

It seems that the combined use of *Lactobacillus* and *Bifidobacterium* strains along with caloric restriction and increased physical activity is useful for effective weight loss.

## Conclusion

The findings of this research suggest that a multi-strain combination of *Lactobacillus* and *Bifidobacterium* can improve anthropometric measurements and body fat content in obese and overweight individuals aged 18 to 60 years. To confirm the effectiveness of probiotics further studies are recommended to

determine the appropriate dose and duration of intervention.

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## Conflict of Interest

None.

## Authors' contributions

M.I.: Conceptualization; validation; data curation; investigation; methodology; formal analysis; writing- original draft; writing, review ; editing and interpretation of data.

A.D.: Conceptualization; supervision; project administration; writing, review; editing and interpretation of data.

SA.K.: Methodology; supervision; writing, review and editing.

All the authors critically revised the manuscript, agree to be fully accountable for the integrity and accuracy of the study, and read and approved the final manuscript.

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