A randomized, double-blind, placebo-controlled trial to evaluate cholesterol-lowering effect of BBR 4401 in adults with moderate hypercholesterolemia

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Supplementary Materials

Table S1. Composition of BBR 4401 and placebo products

(Composition: %)

Raw material	Placebo	BBR-L	BBR-H
Bifidobacterium breve IDCC 4401 tyndallizate	0	8.00	74.10
Maltodextrin	97.80	89.90	24.60
Gardenia Yellow color powder	0.80	0.73	0.20
Caramel color powder	0.40	0.37	0.10
Magnesium Stearate	1.00	1.00	1.00

Table S2. Baseline characteristics[1)]

Variables	Placebo (n=21)				BBR-L (n=20)				BBR-H(n=20)				P-value[2)]
	Mean ± SE	Min	Median	Max	Mean ± SE	Min	Median	Max	Mean ± SE	Min	Median	Max	
Age (yr)	52.1 ± 2.9	24.0	52.0	76.0	51.5 ± 1.6	42.0	52.0	67.0	50.8 ± 2.9	24.0	52.0	76.0	0.925
Gender (male / female)	5 / 16				4 / 16				5 / 15				1.000
Menstruation (Y/N/NA)	6 / 10 / 5				9 / 7 / 4				9 / 6 / 5				0.753
Post-menopausalperiod(month)	120.3 ± 30.6	23.0	96.0	312.0	75.4 ± 14.5	36.0	60.0	132.0	104.0 ± 30.6	23.0	96.0	312.0	0.528
Alcohol drinker (Y/N)	6 / 15				8 / 12				10 / 10				0.372
Alcohol amount (g/wk)	21.8 ± 17.6	0.0	0.0	370.8	18.4 ± 7.8	0.0	0.0	132.0	55.0 ± 17.6	0.0	0.0	370.8	0.401
Smoker (Y/N)	0 / 21				0 / 20				1 / 19				0.322
Smoking amount (cigarettes/d)	0.0 ± 0.0	0.0	0.0	0.0	0.0 ± 0.0	0.0	0.0	0.0	0.5 ± 0.0	0.0	0.0	0.0	0.365
Physical activity (MET-min/wk)	2897.4 ± 623.0	149.0	2079.0	14238.0	1586.6 ± 227.7	99.0	1206.5	4105.0	2310.4 ± 623.0	149.0	2079.0	14238.0	0.118
Body weight (kg)	63.1 ± 2.1	49.0	61.5	81.5	60.0 ± 2.3	47.0	58.5	83.8	62.8 ± 2.1	49.0	61.5	81.5	0.520
BMI (kg/m^2)	24.3 ± 0.6	19.7	23.7	31.0	22.8 ± 0.7	16.8	22.2	31.6	24.4 ± 0.6	19.7	23.7	31.0	0.179
Waist circumference (cm)	81.2 ± 1.8	67.0	80.0	96.0	76.8 ± 1.8	63.5	76.0	95.0	81.2 ± 1.8	67.0	80.0	96.0	0.140
SBP (mmHg)	122.8 ± 2.4	107.0	120.0	153.0	116.0 ± 2.3	100.0	114.5	136.0	118.9 ± 2.4	107.0	120.0	153.0	0.161
DBP (mmHg)	79.7 ± 2.1	64.0	77.0	97.0	75.9 ± 2.0	60.0	74.0	93.0	78.4 ± 2.1	64.0	77.0	97.0	0.458
LDL-C (mg/dL)	124.7 ± 2.0	102.0	128.0	138.0	131.1 ± 2.8	109.0	131.5	149.0	127.4 ± 2.0	102.0	128.0	138.0	0.227
TG (mg/dL)	123.4 ± 11.4	58.0	108.0	217.0	119.5 ± 17.0	53.0	85.0	313.0	101.8 ± 11.4	58.0	108.0	217.0	0.532
TC (mg/dL)	201.0 ± 2.4	176.0	201.0	226.0	212.8 ± 4.2	182.0	213.0	255.0	207.8 ± 2.4	176.0	201.0	226.0	0.156
HDL-C (mg/dL)	58.5 ± 2.2	39.0	60.0	76.0	62.0 ± 3.3	44.0	58.0	96.0	63.3 ± 2.2	39.0	60.0	76.0	0.523
VLDL-C (mg/dL)	24.7 ± 2.3	11.6	21.6	43.4	23.9 ± 3.4	10.6	17.0	62.6	20.4 ± 2.3	11.6	21.6	43.4	0.532
Non HDL-C (mg/dL)	142.6 ± 3.0	121.0	142.0	175.0	150.8 ± 4.9	110.0	146.0	191.0	144.6 ± 3.0	121.0	142.0	175.0	0.449
Fasting blood glucose (mg/dL)	96.0 ± 1.6	78.0	96.0	108.0	98.5 ± 2.2	87.0	97.0	127.0	97.5 ± 1.6	78.0	96.0	108.0	0.621
AST (IU/L)	21.4 ± 1.0	15.0	21.0	31.0	22.1 ± 2.5	12.0	19.0	63.0	21.0 ± 1.0	15.0	21.0	31.0	0.912
ALT (IU/L)	20.4 ± 2.4	12.0	16.0	53.0	22.7 ± 4.9	9.0	16.5	107.0	19.2 ± 2.4	12.0	16.0	53.0	0.764
Creatinine (mg/dL)	0.8 ± 0.0	0.5	0.7	1.1	0.8 ± 0.0	0.5	0.7	1.1	0.8 ± 0.0	0.5	0.7	1.1	0.943

1) BBR-L, low dose of Bifidobacterium breve IDCC 4401; BBR-H, high dose of Bifidobacterium breve IDCC 4401. Min, minimum; Max, maximum; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low density lipoprotein cholesterol; TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; VLDL-C, very low density lipoprotein; AST, aspartate aminotransferase; ALT, alanine transaminase.

2) ANOVA for continuous variables and Chi-square or Fisher's exact test for categorical variables were used to compare the difference among the groups.

Table S3. Compliance[1)]

(unit: %)

Week	Placebo (n=21)	BBR-L (n=20)	BBR-H (n=20)	*p*-value[2)]
Week 4	99.1 ± 0.5	98.5 ± 0.9	97.0 ± 1.4	0.280
Week 8	98.8 ± 0.5	98.5 ± 1.0	97.1 ± 1.1	0.362
Week 12	98.7 ± 0.5	96.8 ± 1.1	97.8 ± 1.0	0.327
Total	98.9 ± 0.4	97.7 ± 0.8	97.3 ± 0.8	0.268

1) Mean±SE (all such values). BBR-L, low dose of *Bifidobacterium breve* IDCC 4401; BBR-H, high dose of *Bifidobacterium breve* IDCC 4401.
2) ANOVA was used to compare the difference among the groups.

Table S4. Dietary intake and physical activity[1)]

Variables	Placebo (n=21)			BBR-L (n=20)			BBR-H (n=20)			*p*-value[2)]	*p*-value[3)]
Dietary intake											
Energy (Kcal/d)											
Week 0	1615.2	±	108.8	1679.5	±	111.5	1786.9	±	111.5		
Week 4	1651.9	±	108.8	1647.9	±	111.5	1671.7	±	111.5	0.586	0.226
Week 12	1560.2	±	108.8	1542.4	±	112.8	1553.1	±	111.5	0.516	0.155
Carbohydrate (g/d)											
Week 0	237.3	±	17.1	234.3	±	17.5	245.8	±	17.5		
Week 4	239.3	±	17.1	247.0	±	17.5	233.9	±	17.5	0.572	0.460
Week 12	235.1	±	17.1	227.0	±	17.7	217.9	±	17.5	0.787	0.174
Protein (g/d)											
Week 0	61.5	±	5.0	64.7	±	5.2	66.4	±	5.2		
Week 4	65.3	±	5.0	61.0	±	5.2	70.8	±	5.2	0.278	0.938
Week 12	61.5	±	5.0	59.3	±	5.2	62.9	±	5.2	0.442	0.614
Fat (g/d)											
Week 0	44.0	±	4.4	49.9	±	4.5	50.8	±	4.5		
Week 4	45.8	±	4.4	44.9	±	4.5	46.8	±	4.5	0.272	0.352
Week 12	40.6	±	4.4	43.0	±	4.6	44.0	±	4.5	0.581	0.584
Cholesterol (mg/d)											
Week 0	243.2	±	31.1	252.2	±	31.9	279.6	±	31.9		
Week 4	253.6	±	31.1	297.6	±	31.9	242.1	±	31.9	0.434	0.282
Week 12	273.5	±	31.1	236.1	±	32.4	283.4	±	31.9	0.303	0.551
SFA (g/d)											
Week 0	10.9	±	1.4	12.7	±	1.5	10.6	±	1.5		
Week 4	8.7	±	1.4	9.8	±	1.5	8.3	±	1.5	0.731	0.954
Week 12	8.1	±	1.4	10.6	±	1.5	9.5	±	1.5	0.702	0.405
MUFA (g/d)											
Week 0	12.7	±	1.9	14.7	±	1.9	13.1	±	1.9		
Week 4	12.1	±	1.9	11.9	±	1.9	9.5	±	1.9	0.452	0.304
Week 12	9.1	±	1.9	13.5	±	2.0	12.6	±	1.9	0.407	0.304
PUFA (g/d)											
Week 0	10.8	±	1.5	10.9	±	1.5	10.1	±	1.5		
Week 4	11.4	±	1.5	9.5	±	1.5	13.3	±	1.5	0.422	0.321
Week 12	10.1	±	1.5	11.2	±	1.5	13.0	±	1.5	0.707	0.158
Sodium (mg/d)											
Week 0	3156.3	±	292.0	3360.9	±	299.3	3376.2	±	299.3		
Week 4	3266.1	±	292.0	3490.3	±	299.3	3547.1	±	299.3	0.962	0.883
Week 12	3098.3	±	292.0	3286.0	±	304.4	3810.9	±	299.3	0.968	0.236
Physical activity (MET-min/wk)											
Week 0	2897.4	±	492.7	1586.6	±	504.9	2310.4	±	504.9		
Week 4	3208.7	±	492.7	1993.4	±	504.9	2739.9	±	504.9	0.886	0.859
Week 12	2809.9	±	492.7	1779.9	±	512.8	2139.3	±	504.9	0.675	0.900

1) LSmean ± SE (all such values). BBR-L, low dose of *Bifidobacterium breve* IDCC 4401; BBR-H, high dose of *Bifidobacterium breve* IDCC 4401; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.
2) Linear mixed-effect model was used to analyze group*time effect between placebo and low-dose groups.
3) Linear mixed-effect model was used to analyze group*time effect between placebo and high-dose groups.

Table S5. Adverse event[1)]

Item	Placebo (n=22)	BBR-L (n=22)	BBR-H (n=22)	*p*-value[2)]
Events				
Adverse event(AE)	1 (4.6%) / 1	4 (18.2%) / 4	4 (18.2%) / 5	0.366
Serious adverse event(SAE)	0 (0.0%) / 0	0 (0.0%) / 0	0 (0.0%) / 0	-
Dropout due to adverse reactions	0 (0.0%) / 0	0 (0.0%) / 0	0 (0.0%) / 0	-
Types				
Gassy	0 (0.0%) / 0	0 (0.0%) / 0	1 (4.6%) / 2	1.000
Loose stool	0 (0.0%) / 0	1 (4.6%) / 1	0 (0.0%) / 0	1.000
Diarrhea	1 (4.6%) / 1	0 (0.0%) / 0	0 (0.0%) / 0	1.000
Muscle pain	0 (0.0%) / 0	1 (4.6%) / 1	0 (0.0%) / 0	1.000
Leg numbness	0 (0.0%) / 0	0 (0.0%) / 0	1 (4.6%) / 1	1.000
Common cold	0 (0.0%) / 0	0 (0.0%) / 0	1 (4.6%) / 1	1.000
Headache	0 (0.0%) / 0	1 (4.6%) / 1	0 (0.0%) / 0	1.000
Urticaria	0 (0.0%) / 0	1 (4.6%) / 1	0 (0.0%) / 0	1.000
Herpes zoster	0 (0.0%) / 0	0 (0.0%) / 0	1 (4.6%) / 1	1.000
Symptom Intensity				
Mild	1 (4.6%) / 1	4 (18.2%) / 4	4 (18.2%) / 5	0.366
Moderate	0 (0.0%) / 0	0 (0.0%) / 0	0 (0.0%) / 0	-
Severe	0 (0.0%) / 0	0 (0.0%) / 0	0 (0.0%) / 0	-
Relevance				
Definitely related	0 (0.0%) / 0	0 (0.0%) / 0	0 (0.0%) / 0	-
Probably related	1 (4.6%) / 1	0 (0.0%) / 0	1 (4.6%) / 2	1.000
Possibly related	0 (0.0%) / 0	2 (9.1%) / 2	0 (0.0%) / 0	0.323
Probably not related	0 (0.0%) / 0	2 (9.1%) / 2	3 (13.6%) / 3	0.356
Definitely not related	0 (0.0%) / 0	0 (0.0%) / 0	0 (0.0%) / 0	-
Unknown	0 (0.0%) / 0	0 (0.0%) / 0	0 (0.0%) / 0	-

1) Number of subjects (percent of subjects) / number of cases. BBR-L, low dose of *Bifidobacterium breve* IDCC 4401; BBR-H, high dose of *Bifidobacterium breve* IDCC 4401.
2) Fisher's exact test was used to compare the difference of numbers of subjects among the groups.