Journal of Food and Nutrition Research, 2020, Vol. 8, No. 6, 279-287
Available online at http://pubs.sciepub.com/jfnr/8/6/6
Published by Science and Education Publishing
DOI:10.12691/jfnr-8-6-6

A Clinical Investigation: A Weight Loss Kit with a Prebiotic Formula for Weight Management and Metabolic Improvement

Hui-Chun Chang[1], Bo-Han Wu[2], Yen-Ju Lin[3], Yung-Hao Lin[3], Yung-Hsiang Lin[1], Chen-Meng Kuan[1,*]

[1]Research & Design Center, TCI CO., Ltd., Taipei, Taiwan
[2]Department of Recreation Sport and Health Promotion, Pingtung University of Science and Technology, Pingtung 912, Taiwan
[3]Research & Design Center, TCI Gene Inc., Taipei 114, Taiwan
[4]Global Business Center, TCI CO., Ltd., Taipei, Taiwan
*Corresponding author: Chenmeng.Kuan@tci-bio.com

Received June 06, 2020; Revised July 07, 2020; Accepted July 15, 2020

Abstract This preliminary clinical study reveals the possibilities of a weight loss kit with a prebiotic formula for weight management and metabolic improvement in volunteers. Considering the convenience, passive diets are a popular choice for some people for weight management. However, the synergic effect between passive diet kits and prebiotics has seldom been reported. 90 volunteers were enrolled in this two-month study and assigned them to normal diet (ND), weight loss program kit (WLPK), or WLPK with the prebiotic formula (WLPK-AKK) group based on purposive sampling. Anthropometric measurements, biochemical analysis, and fecal microbiome analysis were conducted at the baseline and 1 and 2 months. The prebiotic formula could reduce weight mass, fat mass, visceral fat, body fat percentage, triglyceride, total cholesterol, and LDL cholesterol of the subjects by 0.5 kg, 0.7 kg, 9 cm2, 0.6%, 2%, 1.6%, 1.2%, respectively. Also, it improved the abundance of *A. muciniphila* by 250% in comparison with the control group. In short, this study demonstrates that the weight loss kit with the prebiotic formula enables to facilitate the abundance of probiotics in the human intestines and comprehensively improve body weight and metabolic disorders.

Keywords: *prebiotic, obesity, overweight, weight loss, metabolic improvement*

Cite This Article: Hui-Chun Chang, Bo-Han Wu, Yen-Ju Lin, Yung-Hao Lin, Yung-Hsiang Lin, and Chen-Meng Kuan, "A Clinical Investigation: A Weight Loss Kit with a Prebiotic Formula for Weight Management and Metabolic Improvement." *Journal of Food and Nutrition Research*, vol. 8, no. 6 (2020): 279-287. doi: 10.12691/jfnr-8-6-6.

1. Introduction

According to the estimates of World Health Organization, over 1.9 billion adults were overweight and approximately 13% of the worldwide adult population was obese in 2016 [1]. Overweight and obesity impose catastrophic disasters on contemporary human civilization as well as lead to the prevalence of non-communicable diseases (NCDs) - 35% of ischemic heart disease, 55% of hypertension, and 80% of type 2 diabetes mellitus are associated with obesity [2,3]. The obesity-associated diseases cost around USD 2 trillion of global healthcare expenditure (2.8% of the global gross domestic product) in 2014, and the medical spending will continue to upsurge annually [4]. For example, the obesity-related healthcare cost in the USA will increase by USD 48-66 billion per year by 2030 [5]. Therefore, the management of obesity is a critical public health issue for every nation considering allocation of limited healthcare resources. The modern obesity results from an imbalance between energy intake and expenditure [6]. The appropriate strategy to manage overweighs and obesity with minimum efforts relies on abstinence from inappropriate diet habits, moderate exercise, and behavior modifications [7]. Nonetheless, it is challenging for most people to implement such self-aware actions and change their habits. Therefore, passive diet becomes a popular option for some populations who plan to undertake a series of weight loss programs.

Recent studies have unveiled that gut microbiota plays a vital role in the development of several diseases (e.g., Alzheimer's disease, cancer, inflammatory bowel disease) and metabolic disorders (e.g., obesity, diabetes mellitus) [8,9]. Proboitcs and prebiotics are recognized as potent strategies to alter the balance of human microbiome and/or enrich the gut microbiota [10,11]. Especially, the development of probiotic foods or supplements has recently advanced on improving obesity and lowering cardiovascular risk factors (such as low density lipoprotein (LDL) cholesterol, total cholesterol, hypertension, and insulin sensitivity) [12,13,14]. Note that *Lactobacillus* and *Bifidobacterium* species are the most common bacteria in probiotic development [15-19]. Moreover, an insight into the efficacy

of *Akkermansia muciniphila*, an emerging probiotic, for improvement of obesity has been recently uncovered in a preliminary clinical research [20]. The abundance of *A. muciniphila* is inversely correlated with overweight, obese, hyperglycemia, insulin resistance, and type 2 diabetes in the rodent models [21-25]. On the other hand, prebiotics (e.g., fructo-oligosaccharides and galacto-oligosaccharides) have also exerted the beneficial effects on the increase of the abundance of gut microbiota and improvements in gastrointestinal disorders (e.g., diarrhea, irritable bowel syndrome) and metabolic syndrome [26-31]. To the best of our knowledge, the synergistic effect between a real weight loss kit and a prebiotic formula has never been reported. In this study, we attempted to explore the clinical efficacy for weight management and enrichment of probiotics by a weight loss program kit (WLPK) with a prebiotic formula, consisting mainly of banana enzyme, xylooligosaccharide, and galactooligosaccharide.

2. Material and Methods

2.1. Participants and Study Design

This study was designed as a controlled and parallel study. This clinical study was approved by the ethics committee of the Antai Medical Care Corporation Antai Tian-Sheng Memorial Hospital (IRB No. 19-062-A).

90 subjects were recruited in this study and were assigned to normal diet (ND) group, weight loss program kit (WLPK) group, or weight loss program kit with a prebiotic formula (AKK Formula^{TM}) (WLPK-AKK) group with the purposive sampling approach. All of them returned the written consent forms before the study. Eligible subjects were determined by the following inclusion criteria: i) male or female aged between 20 and 50 years old; ii) body mass index (BMI) ≥ 22 (kg/m^2) or body fat mass: male > 25%, female > 30% (by the definition); iii) prohibitions on other nutritional supplements (probiotics and prebiotics) before two weeks of the study; and, iv) abidance by the similar diet and exercise habits during the study. The exclusion criteria were as follows: i) pregnant or breastfeeding woman; ii) menopausal woman; iii) diabetes mellitus; iv) implementation in weight loss programs before a half year of this study; v) metabolic disorders; vi) kidney diseases; vii) liver diseases; viii) cardiovascular diseases; ix) nervous system diseases; x) gastrointestinal diseases; and, xi) heavy drinking or constant drug use. The blood samples (before meal) and stool samples were collected at 0, 1 and 2 months. Anthropometric measurements through a body composition analyzer (InBody770) were assessed by body mass index (BMI), body mass, body fat mass, and body fat percentage, and visceral fat.

Participants in WLPK and WLPK-AKK groups were required to adhere to the eating plan (Table S1). WLPK (Table S2-S11) contained four flavors of collagen drinks [i.e., Caviar Collagen Enzyme Drink, Collagen Enzyme Drinks (Peach Taste), Collagen Enzyme Drinks (Grape Fruit Taste), Saussurea involucrata Enzyme Drink (Litchi Taste)], six flavors of mike shakes (i.e., Corn Soup Mike Shake, Strawberry Mike Shake, Chocolate Mike Shake, Vanilla Mike Shake, Banana Mike Shakes, Matcha Mike Shakes), Fruit and Vegetable Congee, Botanical Liquid Sachet, and Probiotics Liquid Sachet. Note that participants would have different flavors of collagen drinks and mike shakes every week. Apart from the above kit components, participants in WLPK-AKK group took two capsule (400 mg; banana enzyme: 160 mg, xylooligosaccharide: 120 mg, galactooligosaccharide: 120 mg) per day.

Table S1. Eating plan

Time	Mon.	Tue.	Wed.	Thu.	Fri.	Sat.	Sun.
Before breakfirst	Probiotics Liquid Sachet (25 mL)	Probiotics Liquid Sachet (25 mL)	Probiotics Liquid Sachet (25 mL)	Probiotics Liquid Sachet (25 mL)	Probiotics Liquid Sachet (25 mL)	Probiotics Liquid Sachet (25 mL)	Probiotics Liquid Sachet (25 mL)
09:00-10:00	Collagen Enzyme Drink * (150 mL), AKK Formula§ (400 mg)	Mike Skake† (25g), AKK Formula§ (400 mg)	Mike Skake† (25g), AKK Formula§ (400 mg)	Mike Skake† (25g), AKK Formula§ (400 mg)	Mike Skake† (25g), AKK Formula§ (400 mg)	Mike Skake† (25g), AKK Formula§ (400 mg)	Mike Skake† (25g), AKK Formula§ (400 mg)
10:00-12:00	Collagen Enzyme Drink* (50 mL)						
12:00-13:00	Collagen Enzyme Drink* (200 mL)	Moringa Leaf Compound Powder (6g), Normal Diet	Moringa Leaf Compound Powder (6g), Normal Diet	Moringa Leaf Compound Powder (6g), Normal Diet	Moringa Leaf Compound Powder (6g), Normal Diet	Moringa Leaf Compound Powder (6g), Normal Diet	Moringa Leaf Compound Powder (6g), Normal Diet
16:00-17:00	Botanical Liquid Sachet (25 mL)	Botanical Liquid Sachet (25 mL)	Botanical Liquid Sachet (25 mL)	Botanical Liquid Sachet (25 mL)	Botanical Liquid Sachet (25 mL)	Botanical Liquid Sachet (25 mL)	Botanical Liquid Sachet (25 mL)
17:00-18:00	Collagen Enzyme Drink* (100 mL)						
18:00-19:00	Collagen Enzyme Drink* (200 mL), AKK Formula§ (400 mg)	Mike Skake† (25g)	Moringa Leaf Compound Powder (6g), Normal Diet, AKK Formula§ (400 mg)	Mike Skake† (25g), AKK Formula§ (400 mg)	Moringa Leaf Compound Powder (6g), Normal Diet, AKK Formula§ (400 mg)	Mike Skake† (25g) AKK Formula§ (400 mg)	Moringa Leaf Compound Powder (6g), Normal Diet, AKK Formula§ (400 mg)
22:00-23:00	Collagen Enzyme Drink* (50 mL)						

* Subjects consumed one flavor of collagen enzyme drinks (Table S2-5)
†Subjects consumed one flavor of mike shake drinks (Table S6, 7)
§WLPK-AKK group only

Table S2. Collagen Enzyme Drink (peach flavor)

Ingredient	Percentage
Soy peptide	> 3%
Indigestible maltode	> 3%
B-Complex vitamins	< 1%
Ascorbic acid	< 1%
O'Young® Broccoli	< 1%
Noni condensed powder	< 1%
Apple vinegar	< 1%
Noni juice	< 1%
Potassium sorbate	< 1%
Citric acid	1%
Sucralose	< 1%
HM pectin	< 1%
Fructose F55	< 1%
Peach flovor	< 1%
Milk flavor	< 1%
Water	54%
Collagen peptide-D	< 3%
Collagen peptide	< 3%
SuperX-Reshi extract	> 3%
Apple juice	> 3%

Table S3. *Saussurea involucrata* Enzyme Drink (litchi flavor)

Ingredient	Percentage
Soy peptide	> 3%
Indigestible maltode	> 3%
B-Complex vitamins	< 1%
Ascorbic acid	< 1%
O'Young® Broccoli	< 1%
Cell Young® Snow Lotus	< 1%
Noni condensed powder	< 1%
Apple vinegar	< 1%
Noni juice	< 1%
Potassium sorbate	< 1%
Citric acid	1%
Sucralose	< 1%
HM pectin	< 1%
Fructose F55	< 1%
Litchi flavors	< 1%
Water	54%
Collagen peptide-D	< 3%
Collagen peptide	< 3%
SuperX-Reshi extract	> 3%
Litchi juice	< 1%

Table S4. Collagen Enzyme Drink (grape fruit flavor)

Ingredient	Percentage
Soy peptide	> 3%
Indigestible maltode	> 3%
B-Complex vitamins	< 1%
Ascorbic acid	< 1%
O'Young® Broccoli	< 1%
Orange Juice	> 3%
Noni condensed powder	< 1%
Apple vinegar	< 1%
Noni juice	< 1%
Potassium sorbate	< 1%
Citric acid	1%
Sucralose	< 1%
HM pectin	< 1%
Fructose F55	< 1%
Grape fruit flavors	< 1%
Water	52%
Collagen peptide-D	< 3%
Collagen peptide	< 3%
SuperX-Reshi extract	21%
Polydextrose	< 3%

Table S5. Caviar Collagen Enzyme Drink

Ingredient	Percentage
Collagen(fish)	> 3%
Nippi Fish Collagen	> 3%
Mango unripe fruit extract	< 1%
Music enzyme	> 3%
Apple Juice	> 3%
Soy Peptide	< 3%
Indigestible maltode	> 3%
Irvingia gabonensis	< 1%
Ascorbic acid	< 1%
O'Young® Broccoli	< 1%
Bioperine® (Piperine)	1%
Jasmine green tea extract	< 1%
DNA-Na	< 1%
Yeast powder	< 1%
Apple vinegar	< 1%
Noni juice	< 1%
Potassium sorbate	< 3%
Citric acid	< 3%
Sucralose	< 1%
HM pectin	< 1%
Mango flavor	< 1%
Pineapple flavor	< 1%
Water	0.67000

Table S6. Corn Soup Mike Shake

Ingredient	Percentage
Whey protein powder	> 6%
Soy peptide	> 6%
creamer	> 6%
Sucralose	< 1%
Refined Salt	> 2%
Sweet corn powder	> 6%
Skim milk powder	< 2%
Onion	> 6%
Psyllium husk	> 6%
Guar(bean)gum	< 2%
β- carotene	< 1%
Yeast extract	< 1%
Maltodextrin	> 6%
Silicon dioxide	< 1%
Calcium phosphate	< 1%
Cron flavor	> 1%
Thiamine mononitrate	< 1%
Riboflavin	< 1%
Niacinamide	< 1%
Calcium Pantothenate	< 1%
Pyridoxine Hydrochlo	< 1%
Ascorbic acid	< 1%
Ferric pyrophosphate	< 1%
Zinc Gluconate	< 1%
Tara gum	> 2%
Lactic acid bacteria	< 1%
Indigestible maltode	> 6%

Table S7. Strawberry/Chocolate/Vanilla/Banana/Matcha Mike Shake

Ingredient	Percentage
Soy peptide	> 11%
Indigestible maltode	< 1%
Lecithin (Soy)	< 1%
Tara gum powder	< 1%
Fructose	> 11%
Sucralose	< 1%
Strawberry/Chocolate/Vanilla /Banana/Matcha flavor	< 1%
Garden beet extract	< 1%
Creamer	< 11%
Refined Salt	< 1%
Silicon Dioxide	> 1%
Maltodextrin	> 11%
Casein phosphate	< 1%
Inulin	< 1%
Riboflavin	< 1%
Niacinamide	< 1%
Calcium Pantothenate	< 1%
Pyridoxine hydrochlo	< 1%
Ascorbic acid	< 1%
Calcium carbonate	< 1%
Ferric pyrophosphate	< 1%
Zinc cluconate	< 1%
Thiamine mononitrate	< 1%
Lactic acid bacteria	< 1%

Table S8. Moringa leaf compound powder

Ingredient	Percentage
Cleaner J ™ Flammuli	> 10%
Sugarlock® Peanut sk	> 2%
Indigestible maltode	< 1%
Citri-Fi® 100FG	< 1%
Green tea extract	< 1%
Gum acacia quick gum	> 10%
Moringa leaf extract	> 2%
Citric acid	10%
Sucralose	< 1%
Orange flavor	< 1%
Lime flavor	< 1%
Maltodextrin	> 10%
Silicon dioxide	< 1%
Calcium phosphate	< 1%

Table S9. Fruit and Vegetable Congee

Ingredient	Percentage
Vegetable extracts	< 1%
Brown rice	> 4%
Oat bran powder	> 4%
Seafood flavor	> 4%
Clam extract	< 4%
Katsuo extract	< 4%
Yeast extract	< 4%
Refined salt	< 4%
Guar(bean)gum	< 4%
Sugar	< 4%
Crab flavor	< 4%
Kelp sprout	< 4%
Dehydrated chive	< 4%
Maltodextrin	> 4%
Calcium phosphate	< 4%
Silicon dioxide	< 4%

Table S10. Botanical Liquid Sachet

Ingredient	Percentage
CitriSlim® Ponkan Unripe Fruit Extract Liquid	> 2%
Red wine extract	< 1%
Orange peel enzymes	> 5%
Green coffee bean extract 45%	< 1%
Orange flavor	< 1%
Steviol glycoside	< 1%
Maltodextrin	> 5%
L-Leucine	< 1%
L-Isoleucine	< 1%
L-Valine	< 1%
Red reet root powder	< 1%
LC-40K	< 1%
Ascorbic acid	< 1%
B-Complex vitamins	< 1%
Apple juice	> 5%
HM Pectin	< 1%
Citric acid	< 1%
Sucralose	< 1%
Lemon flavor	< 1%
Potassium sorbate	< 1%
Water	45%

Table S11. Probiotics Liquid Sachet

Ingredient	Percentage
TCI378 nextbiotis (*Lactobacillus plantarum* fermentation liquid)	> 10%
Ginger extract	< 1%
Polydextrose	> 10%
Stachyose	< 1%
Banana enzymes	> 1%
Xylooligosaccharide	< 1%
Galactooligosaccharide	1.00%
Sorbitol	> 10%
Citric acid	< 1%
Fruit flavor	< 1%
Water	34.00%
Potassium sorbate	< 1%
Isomaltooligosacchai	1.00000
Fructooligosaccharid	< 1%
Lactitol	> 10%

2.2. Biochemical Analysis

The biochemical analysis included the following items: fasting glycemia, aspartato aminotransferasi (AST), alanine aminotransferase (ALT), albumin, blood urea nitrogen, creatine, uric acid, white blood cell (WBC), triglyceride, total cholesterol, high density lipoprotein (HDL)-cholesterol, and low density lipoprotein (LDL)-cholesterol.

2.3. Fecal Microbiome Analysis

Fecal samples were collected by a stool collection kit (BIOTOOLS CO., LTD.) and stored frozen (-80°C)

before genomic DNA extraction. The genomic DNA extraction and purification were used the EasyPrep Stool Genomic Kit (BIOTOOLS CO., LTD.), and Firmicutes, Bacteroidetes, *Lactobucillius*, and *A. muciniphila* were quantified by quantitative PCR.

2.4. Statistical Analysis

Results of anthropometric measurements and biochemical analyses were first analyzed by the analysis of covariance (ANCOVA) to compare the differential values in three groups. When the whole analysis had a significant level, least-significant difference (LSD) was used to compare the statistical difference between two groups, with $p < 0.05$ considered significant. The statistical analyses were based on SPSS v.14.0 (IBM Corporation). Results of fecal microbiome analysis were analyzed by two-tailed t-test in Excel (Microsoft), with $p < 0.05$ considered significant.

3. Results and Discussion

3.1. Subjects

This research aims to investigate the volunteers with ND, WLPK, or WLPK-AKK for assessing the effect of weight loss in connection with the abundance of probiotics in the human intestines [32]. Given that more and more modern people rely on passive diets for weight management, we incorporated a weight loss program kit into the research to evaluate its influence of the probiotic levels in the human intestines. Also, we assumed that the combination of weight loss program kit and a prebiotic formula can gain maximum benefit of weight control. 90 volunteers were recruited in this clinical study and assigned to normal diet, WLPK, or WLPK-AKK group, and 88 subjects accomplished the 2-month trial (Figure 1). There was no any adverse health effect reported in the study. Table 1 shows the demographic and anthropometric measurement results at 0, 1, and 2 months. The average ages of the subjects in three groups were in the range of 34.1-37.0 year olds, so the basal metabolic rates should not contribute too much bias to this study. Compared with ND group, WLPK and WLPK-AKK groups acquired significant weight loss results after the trial though BMI results did not show obvious improvements. The mean differences of body weight between baseline and month 2 for ND, WLPK, and WLPK-AKK groups were -0.1 kg, -1.7 kg, and -2.2 kg, respectively. In addition, WLPK-AKK also reached a superior improvement by -1 kg in one month in comparison with +0.3 kg (ND) and -0.2 kg (WLPK). The prominent improvement effect of WLPK-AKK was also available for reduction of body fat mass, visceral fat, and body fat percentage after the study. The mean differences of body fat mass (visceral fat) between baseline and month 2 for ND, WLPK, and WLPK-AKK groups were -0.6 kg (-4 cm^2), -1.7 kg (-6 cm^2), and -2.4 kg (-15 cm^2), respectively. The mean differences of body fat percentage between baseline and month 2 for ND, WLPK, and WLPK-AKK groups were -0.8%, -1.7%, and -2.3%, respectively.

According to the anthropometric measurement results, we proved that WLPK and WLPK-AKK indeed could be beneficial for weight loss and body fat reduction. WLPK and WLPK-AKK persisted in improving body weight over the study, while the measurement values of ND group fluctuated over the study. Namely, WLPK and WLPK-AKK were able to stably improve human metabolism. Mean BMI values of three groups after the trial resembled those at the baseline, which is subject to the short term investigation period. WLPK-AKK exerted the most prominent improvement in decreasing body weight, but the degree of improvement between WLPK and WLPK-AKK did not show distinctive. In addition, we discovered that WLPK-AKK conferred a better effect on fat reduction than WLPK on the basis of the results of body fat mass and visceral fat.

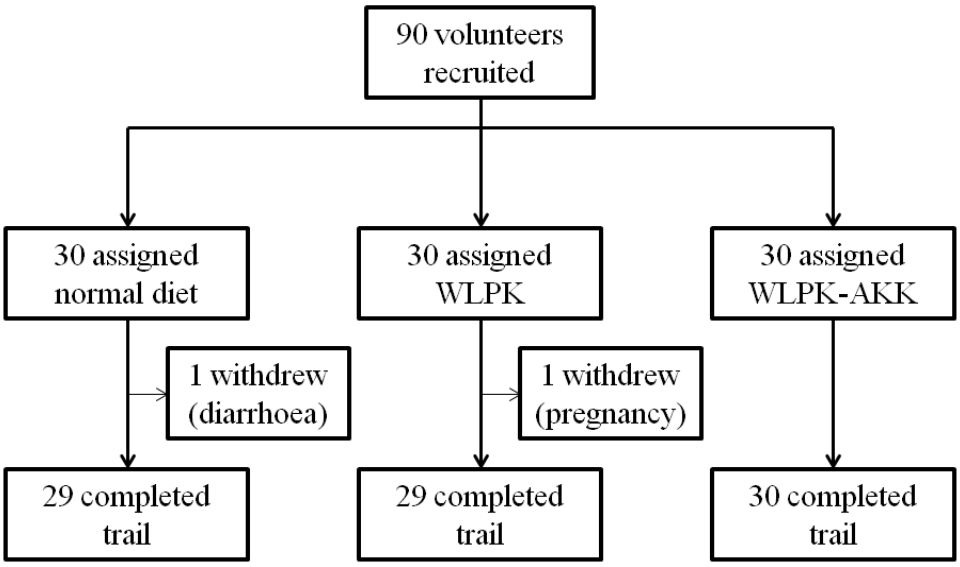

Figure 1. Trial profile

Table 1. Demographic characteristics and anthropometric measurement results of all volunteers

	ND (n = 29)	WLPK (n = 29)	WLPK-AKK (n = 30)	*P* value[a,b,c]
Age (years)	37.0 ± 7.3	34.1 ± 6.7	34.5 ± 9.7	
Male, Female	13, 16	8, 21	12, 18	
BMI (kg/m^2)				
Baseline	26.9 ± 7.3	25.8 ± 5.9	27.9 ± 7.5	
Month 1	27.5 ± 7.7	25.6 ± 5.9	27.8 ± 7.3	0.128, 0.012, < 0.001
Month 2	26.9 ± 7.2	25.8 ± 6.0	28.0 ± 7.1	< 0.001, 0.168, < 0.001
Body mass (kg)				
Baseline	69.4 ± 15.6	72.4 ± 13.5	74.5 ± 15.1	
Month 1	69.7 ± 16.1	72.2 ± 14.0	73.5 ± 14.9	0.119, 0.005, < 0.001
Month 2	69.5 ± 16.2	70.7 ± 13.7	72.3 ± 14.7	< 0.001, 0.99, < 0.001
Body fat mass (kg)				
Baseline	21.0 ± 7.7	25.7 ± 6.2	24.6 ± 6.8	
Month 1	20.2 ± 7.6	25.5 ± 6.5	23.8 ± 6.9	0.140, 0.135, 0.961
Month 2	20.4 ± 7.7	24.0 ± 6.3	22.2 ± 6.4	0.007, 0.041, < 0.001
Visceral fat (10 cm^2)				
Baseline	90 ± 40	115 ± 36	107 ± 37	
Month 1	84 ± 37	117 ± 38	106 ± 39	0.012, 0.360, 0.086
Month 2	8 6± 35	109 ± 34	92 ± 32	0.477,0.011, 0.072
Body fat percentage (%)				
Baseline	30.1 ± 7.9	35.5 ± 5.5	33.3 ± 6.6	
Month 1	29.0 ± 7.7	35.2 ± 5.9	32.6 ± 6.7	0.067, 0.312, 0.357
Month 2	29.3 ± 7.4	33.8 ± 5.7	31.0 ± 6.5	0.320, 0.107, 0.010

Values were shown as mean value ± SEM a: Statistical analysis between ND and WLPK. b: Statistical analysis between WLPK and WLPK-AKK. c: Statistical analysis between ND and WLPK-AKK.

Table 2. Biochemical parameters for lipid metabolism

	ND (n = 29)	WLPK (n = 29)	WLPK-AKK (n = 30)	*P* value[a,b,c]
Triglyceride (mg/ dL)				
Baseline	98.7 ± 39.0	121.8 ± 100.0	101.5 ± 72.8	
Month 1	99.8 ± 55.4	100.4 ± 67.5	108.1 ± 112.6	0.166, 0.690, 0.665
Month 2	103.8 ± 50.3	119.6 ± 86.5	97.6 ± 61.9	0.857, 0.500, 0.389
Total cholestrol(mg/ dL)				
Baseline	188.3 ± 37.3	188.4 ± 36.7	187.5 ± 36.1	
Month 1	182.6 ± 36.1	181.3 ± 35.0	178.3 ± 36.3	0.758, 0.588, 0.394
Month 2	183.0 ± 32.5	177.9 ± 30.9	174.0 ± 31.4	0.122, 0.340, 0.013
HDL-cholestrol (mg/ dL)				
Baseline	56.6 ± 14.8	54.1 ± 11.4	52.7 ± 11.5	
Month 1	52.9 ± 16.6	52.8 ± 10.4	49.8 ± 10.8	0.309, 0.343, 0.936
Month 2	52 ± 13.2	52.3 ± 9.9	52.7 ± 12.9	0.156, 0.449, 0.031
LDL-cholestrol (mg/ dL)				
Baseline	128.6 ± 33.0	129.7 ± 38.5	131.0 ± 35.9	
Month 1	122.9 ± 32.4	128.5 ± 38.2	125.0 ± 35.8	0.362, 0.218, 0.752
Month 2	125.8 ± 33.7	120.2 ± 36.5	119.9 ± 33.2	0.038, 0.634, 0.011

Values were shown as mean value ± SEM a: Statistical analysis between ND and WLPK. b: Statistical analysis between WLPK and WLPK-AKK. c: Statistical analysis between ND and WLPK-AKK.

3.2. Biochemical Analysis

Taking safety issue into account, we monitored some biochemical parameters regarding kidney and liver functions (e.g., AST, ALT, BUN, creatine, and WBC) over the trial, the monitoring parameters were in the normal reference ranges without abnormality. Table 2 shows biochemical parameters for lipid metabolism in the study. The triglyceride improvements in ND, WLPK, and WLPK-AKK groups after the trial were +5.2%, -1.8%, and -3.8%. Although WLPK-AKK could decrease triglyceride values, it did not have a significant improvement effect on triglyceride modulation as compared with WLPK group. The improvements of total cholesterol (LDL-cholesterol) for ND, WLPK, and WLPK-AKK groups after the trial were -2.8% (-2.2%), -5.6% (-7.3%), and -7.2% (-8.5%), whereas only WLPK-AKK could provide noticeable improvement effect on total cholesterol and LDL-cholesterol modulations. In addition, regarding HDL cholesterol metabolism, WLPK-AKK stabilized the HDL cholesterol level after the trial but the other groups showed negative impacts on HDL cholesterol modulation as evidenced by -8.1% (ND) and -3.3% (WLPK).

The biochemical parameters in the clinical reference ranges supported the safety of the prebiotic formula. Several clinical studies have verified that the elevated ALT and AST activities are associated with hepatic inflammation, insulin resistance, and the development of metabolic syndromes (e.g., obesity, type 2 diabetes, nonalcoholic fatty liver disease) [33,34,35]. In this research, we did not observe the improvements for the values of ALT and AST. Moreover, WBC level is also regarded as an indicator for the risk of type 2 diabetes as

for the clinical relevance in the difference range of 300-1000 cells/μL [36]. The WBC count differences in WLPK (200 cells/μL) and WLPK-AKK (100 cells/μL) groups did not reach the meaningful difference range. Nevertheless, we believe that this disparaging outcome can be overcome by extending the study period. Despite no significant improvement in triglyceride level, WLPK, and WLPK-AKK still conferred a positive effect on triglyceride modulation in contrast to the consistent increase of triglyceride values in ND group. As compared with the results of ND and WLPK groups, WLPK-AKK imparted the better improvements of the total cholesterol, LDL- and HDL-cholesterol.

3.3. Enhancement of the Levels of Probiotics in the Intestines

Figure 2 shows the relative levels of *A. muciniphila*, *Bifidobacterium* spp., Firmicutes, and Bacteroidetes in the faecal samples of the participants. The analytical results were comparing with the baseline results. The relative levels of *A. muciniphila* in ND, WLPK, and WLPK-AKK groups were increased by 4.7, 4.3, and 16.5 folds after the study (Figure 2A). In accordance with the result, *A. muciniphila* prebiotics could boost the growth of *A. muciniphila* by a 250% of improvement in comparison with ND group. The levels of Firmicutes bacteria for three groups after the study increased in the range of 0.4-0.5 fold without obvious changes (Figure 2B). The relative abundance results of Bacteroidetes bacteria of WLPK and WLPK-AKK groups were changed by +2% and -6.7%, respectively, as compared with ND group (Figure 2C). Regarding the probiotic *Bifidobacterium* spp., only WLPK group got much improved in the level of *Bifidobacterium* spp. after the trial by +565.9% in ccomparison with ND group (Figure 2D).

Prebiotic supplements are able to improve the abundance of *A. muciniphila*, a degree of weight loss, insulin resistance, and steatosis in rodents [22,38,39]. The results of *A. muciniphila* indicate that the prebiotic formula successfully promoted the levels of *A. muciniphila* in the intestines and consistently remained a certain level over the trial. The outcome also coincided with the encouraging progresses of weight loss, fat reduction, and cholesterol metabolism for WLPK-AKK group. In addition, several studies have proved that the abundance of *Bifidobacterium* spp. is inversely correlated with fat mass and body weight [40]. The gut microbiota in overweight and prediabetic individuals can be readjusted by an increase of Bifidobacteria and reduction of Bacteroides after treatment with a prebiotic supplement [41]. In our experiment, the obvious increase of *Bifidobacterium* spp. occurred on the WLPK group rather than WLPK-AKK group. Thus, we deduced that the effect of weight loss for WLPK may be attributed to the abundance of Bifidobacteria. Bacteroidetes and Firmicutes account for approximate 90% of human gut bacteria; it is common to find that high Firmicutes/Bacteroidetes ratios in obsess adults [42,43]. When participants were intervened by a diet therapy, the levels of Firmicutes and Bacteroidetes correspondingly decreased and increased. Our Firmicutes results indicate that the levels of Firmicutes in three groups were similar without differential effects. The prebiotic formula slightly reduced the abundance of Bacteroidetesm, but its influence did not reach a significant impact as compared to the result of ND group. As a matter of the fact, our study is not leveraging phylogenetic analysis of the gut microbiota, so it is hard to elaborate the detailed correlations among the bacteria from a fair angle. Despite lack of meticulous gut bacteria information, we minimally confirmed that the prebiotic formula might enhance the abundance of *A. muciniphila* in the intestines and exert the effect of weight management as well as improve the efficiency of weight loss for the normal weight loss kit.

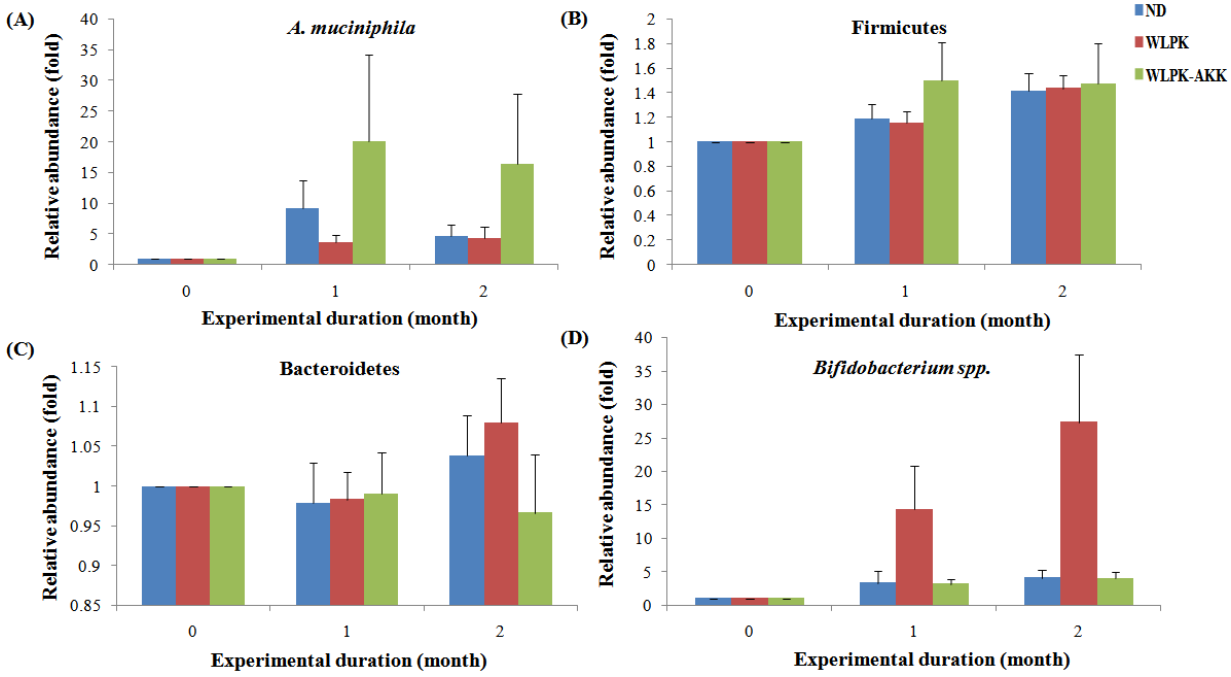

Figure 2. Relative abundance of bacteria in human intestines. (A) *A. muciniphila* (WLPK-AKK: n = 30; ND/WLPK: n = 29; mean value ± S.D.). (B) Firmicutes (WLPK-AKK: n = 30; ND/WLPK: n = 29; mean value ± S.D.). (C) Bacteroidetes (n = 29; mean value ± S.D.). (D) *Bifidobacterium.* spp. (WlPK-AKK /ND: n = 29; WLPK: n = 28; mean value ± S.D.)

4. Conclusion

In summary, this clinical study successfully demonstrates the utilization of the WLPK, and WLPK-AKK for weight management and improvement in cholesterol metabolism in subjects. Strikingly, the prebiotic formula could reduce weight mass, fat mass, visceral fat, body fat percentage, triglyceride, total cholesterol, and LDL cholesterol of the subjects by more 0.5 kg, 0.7kg, 9 cm^2, 0.6%, 2%, 1.6%, 1.2%, respectively, on the basis of WLPK. Also, the prebiotic formula improved the relative levels of *A. muciniphila* in human intestines by 283.7% in comparison with WLPK group. However, this research is subject to the deficiency of phylogenetic analysis of the gut microbiota, so it is difficult to illustrate the meticulous relationship between *A. muciniphila* and other bacteria and the influence of the prebiotic formula. In brief, the weight loss kit with the prebiotic formula can substantially impart the metabolic improvements in lipid metabolism and basal metabolic rate.

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