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A combination of various functional food ingredients as a weight management program: randomized, placebo-controlled, and double-blind human clinical studies

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ABSTRACT:

Background: *Lycium barbarum* increased the postprandial energy expenditure (PPEE). Negative energy balance caused by the systematic procedure (TAI*slim*® System), including increasing metabolic rate through physical activity, use of *Lycium barbarum*-containing TAI*slim* (Product A), and decreasing caloric intake by consuming a chewable confection (TAI*slim* SKINNY=Product B), and a meal replacement shake (TAI*slim* SHAKE=Product C), would be successful for weight loss.

Methods: We examined TAI*slim* System on anthropometrics, appetite in Study 1 and PPEE in Study 2, both in a randomized, placebo-controlled, double-blind manner. **1**) A total of 67 participants were randomized into 2 groups (placebo or TAI*slim* System). Intake procedures were: Product A, 60 ml (20 kcal) b.i.d. immediately before breakfast and lunch, Product B, 1 chew (20 kcal) t.i.d. between meals and after dinner; Product C, 40.5 g (158 kcal) as breakfast. A calorie-restricted diet with multi-vitamin supplementation and daily exercise was required. Anthropometric parameters were assessed at baseline, 4, 8, and 12 w. **2**) Appetite was measured using a subjective visual analog scale during the initial 3-7 days of intake. **3**) For PPEE evaluation, 12 participants consumed a single bout of TAI*slim* System products or placebo, and took part in 6 study sessions. EE was measured by an indirect calorimeter immediately before (baseline) and at 1, 2, and 4 h post-intake of samples.

Results: 1) Body weight was significantly reduced by 6.2±0.7%, compared to pre-intervention with TAI*slim* System ($P<0.01$). Waist circumference, total body fat, blood pressure, and fasting blood glucose levels were also significantly reduced by TAI*slim* System, in a range of 3.8-9.9%. TAI*slim* System was significantly more effective than the placebo ($P<0.05$). The placebo group showed -0.1-3.9% reduction from pre-intervention with no significant difference. **2**) TAI*slim*

System also significantly suppressed appetite, by 39%, compared to the baseline and placebo ($P<0.05$) (11% reduction in the placebo). **3**) PPEE was significantly increased by TAI*slim* System compared to placebo and baseline levels. Compared to the baseline EE, placebo increased only by 0.8±0.9%, but 7.2±1.2 % with TAI*slim* System ($P<0.01$).

Conclusions: It is suggested that TAI*slim* System exhibits significant weight loss and stimulating effects on caloric expenditure, and thus may be a useful and effective weight loss program.

Keywords: *Lycium barbarum*, Goji, Fiber, Phenylalanine, N-Acetyl-L-Tyrosine, Tea, Polyphenols, Human clinical trial, Energy expenditure, Resting metabolic rate, Waist circumference, Appetite, Body mass index, TAI*slim*.

BACKGROUND:

Obesity is a major problem in our modern society. In the US, over 65% of the population is either overweight or obese [1]. Functional foods or dietary supplements that help promote a negative energy balance could have an impact on the weight trajectories of a large proportion of the population. Functional foods are foods that have health benefits above and beyond their nutritional value. For weight management, this may be enhancement of postprandial thermogenesis or satiety relative to similar food products or ingredients. Although numerous functional food ingredients have been individually studied, it is not known whether the combined intake of various functional foods leads to greater effectiveness than the sum of their individual effects.

Our recent clinical trials have shown that consumption of *Lycium barbarum* (goji) fruit juice (GoChi®) standardized for bioactive polysaccharides (LBP), stimulated postprandial energy expenditure (PPEE) through adrenocortical hormone control [2-4]. The rise in PPEE following *Lycium barbarum* consumption may in part explain the improvements in anthropometric parameters - especially waist circumference - observed in our clinical trials [2-4]. *Lycium barbarum* is a Solanaceous defoliated shrubbery and the fruit is a well-known traditional medicine in Asian countries where it has been used for medicinal purposes and as a functional food for over 2,500 years [5]. In support of its traditional use, *Lycium barbarum* is known to increase energy metabolism [6], improve glucose control and other symptoms in diabetics [7,8], and to exhibit anti-oxidant properties [9], immune modulation [10] and anti-inflammatory effects [11].

To strengthen the effects of *Lycium barbarum* on central adiposity and further expand its effects for better body weight control, we have developed a liquid dietary supplement, TAI*slim*®, by combining *Lycium barbarum* with additional functional food ingredients such as soluble indigestible maltodextrin dietary fiber, appetite-suppressing amino acids, a special blend of green, black, oolong, and white tea extracts standardized with polyphenols including epigallocatechin gallate (EGCG) and caffeine. Based on the potential effects of these ingredients [12-31], this product is designed to increase thermogenesis and fat oxidation [12-15], decrease absorption of dietary fats and starches by inhibiting lipase and amylase enzymes [12-28], improve insulin

sensitivity [16,17,19], suppress appetite [29-31], reduce fasting lipids [20,27], improve serum glucose control [16,17,19], and improve the balance of intestinal flora [25-28]. In our previous double-blind, placebo-controlled human clinical study, TAI*slim* intake was shown to cause a statistically significant reduction in anthropometric parameters including body weight, BMI, total body fat, blood pressures, pulse, and fasting glucose levels during the 30 to 90 day consumption with a moderate exercise and diet control [32].

Furthermore, to make TAI*slim* work well and help individuals achieve negative caloric balance on a daily basis, we also developed two additional products to take in combination with the TAI*slim* liquid supplement: a meal replacement product (TAI*slim* SHAKE) based on meta analysis of partial meal replacement [33]; and a confectionary chew snack (TAI*slim* SKINNY), which contains indigestible soluble glucomannan fiber based on its effects in weight management area [23-28]. The total fiber content of all 3 products of the TAI*slim* Total Body Weight System (TAI*slim* System) is 23 g/day, when used as directed. Along with regular exercise and a healthy diet, the TAI*slim* System is expected to help individuals reach and maintain a healthy weight.

This is the first 12-week clinical study using all 3 products as TAI*slim* System to investigate effects of the System on the body weight, and related anthropometric parameters, appetite, energy metabolism which are related to body weight control evaluated by in healthy overweight human adults under calorie restriction and exercise program.

MATERIALS AND METHODS:

Test Product preparations. FreeLife International located in Phoenix, Arizona supplied all products and placebo samples in TAI*slim* System. TAI*slim* System was a combination of liquid dietary supplement (TAI*slim*=Product A) with chewable confection (TAI*slim* SKINNY=Product B) and meal replacement shake (TAI*slim* SHAKE=Product C). Test and placebo products were isocaloric/isoenergetic, identical in color, viscosity, texture, taste and energy content and differed only in the presence or absence of active ingredients.

TAI*slim* (Product A, Lot No. APP1063X) contained various functional food ingredients, such as *Lycium barbarum*, appetite-suppressing amino acids, a special blend of green, black, oolong, and white tea extracts that achieves a total of 200 mg of tea polyphenols, including 90 mg of EGCG and 100 mg of caffeine, and modified soluble indigestible maltodextrin dietary fiber blend as listed in Figure 1. *Lycium barbarum* fruit juice (GoChi®) was produced from fresh ripe fruit and standardized with LBP. Description and standardization procedures of GoChi were previously described [34]. One serving (60 ml) of caloric amount of TAI*slim* was 20 kcal. Placebo control material (Lot No. APP1063A) matched the color, flavor and taste of TAI*slim*, and packaged in the same type of container; however, it provided no active ingredients, such as *Lycium barbarum*, amino acids, tea blend and modified dextrin dietary fiber blend.

TAI*slim* SKINNY (Product B, Lot No. PCC10060X) was a confectionary chew and contained ingredients listed in Figure 1. The placebo chew (Lot No. PCC10060A) was also supplied by FreeLife International and had same flavor, taste and texture, but did not contain any active material, such as glucomannnan fiber.

TAI*slim* SHAKE (Product C, Lot No. 003390X) was a meal replacement powder shake, and its ingredients were listed in Figure 1. The placebo powder (Lot No. 003390A) had same flavor, taste and texture, but did not contain any active material, such as enzyme-activated fatty acids and medium chain triglycerides from coconut, canola and sunflower oils, or glucomannnan fiber.

TAI*slim* (Product A)

Supplement Facts

Serving Size: 2 fl. oz. (60 mL)
Servings Per Container: Approx. 16

	Amount Per Serving	% Daily Value
Calories	20	
Total Carbohydrates	7 g	2†
Dietary Fiber	5g	20†
Sugar	2 g	*
Sodium	20 mg	1%
Potassium	15 mg	<!%
Appitol Plus™ (appetite controlling complex) L-Phenylalanine N-Acetyl-L-tyrosine	700 mg	*
Lipitol Ultra™ (fat burning complex) Standardized extracts of green, black, oolong and white tea leaf providing 200 mg polyphenols (90 mg as EGCG) and 100 mg caffeine	330 mg	*
GojiSol™ (advanced soluble fiber complex) Modified dextrin with whole fruit goji fiber	5.7 g	*
GoChi® HIMALAYAN GOJI® Juice (reconstituted goji juice from fresh whole *Lycium barbarum* fruit utilizing our exclusive Spectral Signature LBP Process™**), white grape juice concentrate, red grape juice concentrate, pomegranate juice concentrate, and natural flavor)	0.5 fl. oz. (15 mL)	*

†Daily Values based on a 2000 calorie diet.
*Daily Value not established.

Other ingredients: Ultra-purified reverse osmosis water, natural flavors, potassium sorbate and sodium benzoate (to maintain freshness), malic acid (for tartness) and sucralose.

TAI*slim* SKINNY (Product B)

Supplement Facts

Serving Size: 1 SKINNY
Servings Per Container: 60

	Amount Per Serving	% Daily Value
Calories	**20**	
Calories from Fat	**5**	
Total Fat	**1 g**	**2%***
Saturated Fat	1g	5%*
Trans Fat	0g	
Cholesterol	**0mg**	
Sodium	**10mg**	**<1%***
Potassium	**15mg**	**<1%***
Total Carbohydrate	**5g**	**2%***
Dietary Fiber	1g	4%*
Sugars	0g	
Protein	0g	
GojiMannan Proprietary blend of patented, clinically tested Glucomannan fiber with standardized goji berry (*Lycium barbarum*) polysaccharide extract.	1g	

†Daily Values based on a 2000 calorie diet.
*Daily Value not established.

Other ingredients: Palm fruit oil, cocoa powder, natural flavor, cacao mass (pure liquid chocolate), vegetable-source emulsifiers (soybean oil mono- and diglycerides, soybean lecithin) and salt, in a natural-source sugar-free base of polyglycitol syrup, isomalt, and rebaudioside-A (purified stevia extract).

TAIs*lim* SHAKE (Product C)

Nutrition Facts

Serving Size: 1 scoop (40.5 g)

Servings Per Container: 15

Amount Per Serving	
Calories	156
Calories from Fat	40
% Daily Value**	
Total Fat 4 g*	6%
Saturated Fat (from MCT/EFA complex) 1 g*	5%
Trans Fat 0 g	
Cholesterol 29mg*	10%
Sodium 173 mg*	7%
Potassium 1000mg*	29%
Total Carbohydrate 19 g*	6%
Dietary Fiber 5 g*	20%
Sugars 14 g*	
Protein 10 g	20%
Vitamin A	35%
Vitamin C	35%
Calcium	30%
Iron	17%
Vitamin D	35%
Vitamin E	35%
Thiamin	35%
Riboflavin	35%
Niacin	35%
Vitamin B6	35%
Folate	35%
Vitamin B12	33%
Biotin	35%
Pantothenic acid	35%
Phosphorus	20%
Iodine	35%
Magnesium	35%
Zinc	33%
Selenium	25%
Copper	25%
Manganese	50%
Chromium	28%
Molybdenum	35%

*Amount in Mix. Skim milk provides additional nutrients.
**Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

INGREDIENTS: Micro-filtered cold-processed whey protein concentrate, cane juice crystals, MCT/EFA Complex (enzyme-activated fatty acids and medium chain triglycerides from coconut, canola and sunflower oils), GojiMannan™ Fiber Blend [microcrystalline cellulose, patented glucomannan fiber, standardized goji berry (*Lycium barbarum*), cellulose gum, xanthan gum and carrageenan], potassium citrate, natural vanilla with other natural flavors, dibasic potassium phosphate, calcium citrate, calcium carbonate, salt, magnesium oxide, soy lecithin, ferrous gluconate, ascorbic acid, Atlantic kelp (*Ascophyllum nodosum*), zinc oxide, niacinamide, vitamin E acetate, manganese carbonate, calcium pantothenate, pyridoxine hydrochloride, cupric oxide, riboflavin, thiamine mononitrate, vitamin A palmitate, sodium selenate, chromic chloride, folic acid, sodium molybdate, biotin, cholecalciferol and cyanocobalamin.

Figure 1. Label description (Supplement Fact Information) of TAI*slim*® (Product A), TAI*slim* SKINNY (Product B) and TAI*slim* SHAKE (Product C).

Study population. In all present clinical studies, all participants were recruited based on the same criteria but then provided separate protocols for each study. Randomized participants in the 12 w intervention (Study 1) was n=67, and in the EE testing (Study 2) was n=12 (Figure 2). Participants in Study 2 were independent from Study 1 and not a subgroup.

All participants were healthy men and women, age 18 years and older. Recruitment was conducted to ensure that participants were serious about participating in these studies and well aware of its demands. Participants were excluded from the study if they exhibited any evidence of heart, liver, lung, or kidney disease, had known allergies to the ingredients in TAI*slim* System products, were pregnant or breast feeding, were under anticoagulant therapy, such as Coumadin® (warfarin), were using any fiber materials, medication or supplements for weight loss, weight

control purpose, and/or appetite suppression, had gastrointestinal disease or problems including chronic symptoms, had cardiac problems, were in a weight control diet program, exhibited unstable body weight (more than 2% loss/gain over the previous 3 months), or had any acute or chronic medical or psychiatric condition. All participants were fully informed of the purpose of the study, and signed the Human Participants Informed Consent forms approved by the Internal Review Board organized at FreeLife International under the Helsinki Declaration. In the background of the participants, groups did not differ in pre-study diet on the parameters of dietary intake, average *Lycium barbarum* consumption history, and consumption patterns for other beverages such as sweetened beverages (soda), coffee, tea and alcoholic beverages, or smoking history.

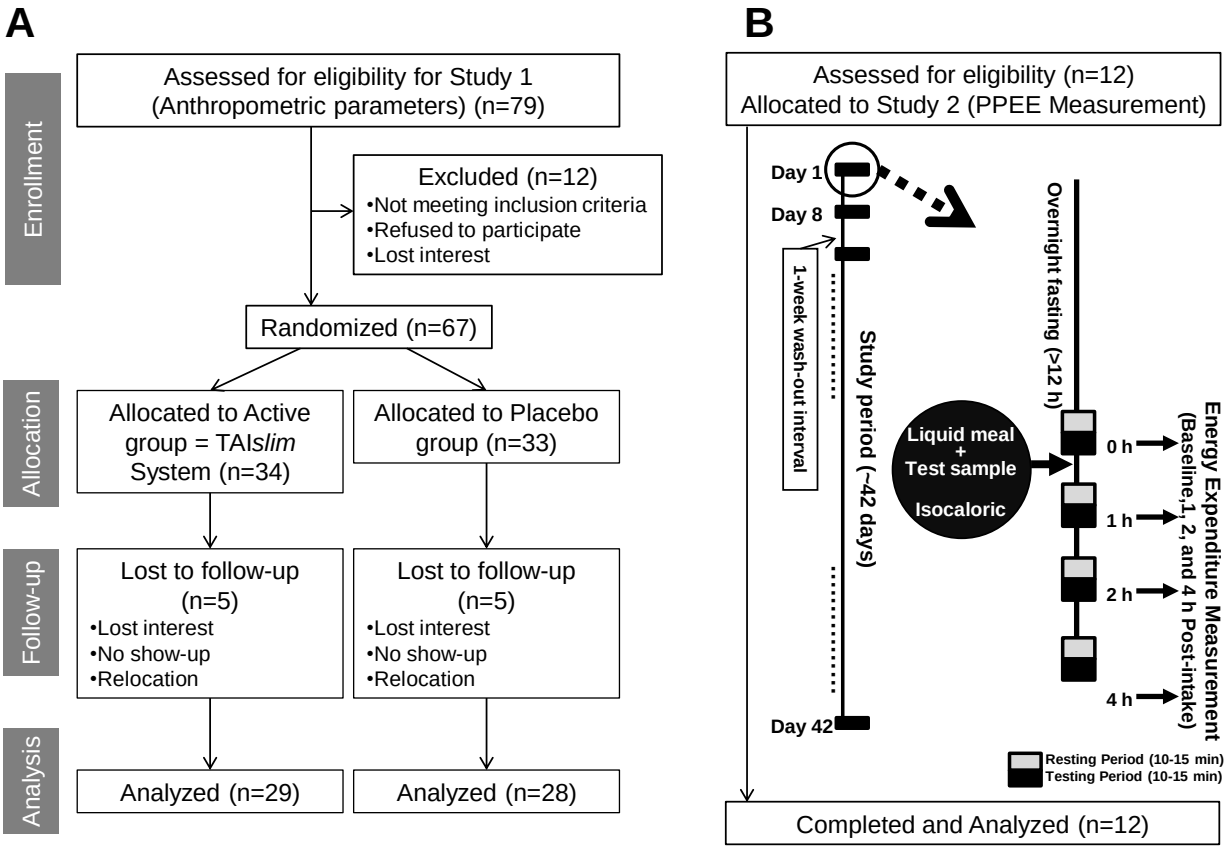

Figure 2. (**A**) CONSORT flow diagram of a randomized, double-blind, placebo-controlled human clinical study (Study 1) of the impact of TAI*slim* System (a combination of TAI*slim*+SKINNY+SHAKE/Product A+B+C) on anthroparametric parameters including body weight (n=67). The diagram includes detailed information on the excluded participants. (**B**) Test procedure of kinetic analysis 0 through 4 hour post-intake of TAI*slim* System on breath oxygen volume (VO_2)/postprandial energy expenditure (PPEE) measured by a hand-held indirect calorimeter (n=12). TAI*slim* System products or placebo control (placebos of all products) were taken orally after greater than 12 h fasting. The test was performed at weekly intervals in a randomized order of the sample intake with triplicate manner throughout up to 42 days.

Research Protocol. Following enrollment, in all present studies, all participants completed wash-out period at least two weeks during which time they were to discontinue use of any weight-loss or weight-control products, any fiber, *Lycium barbarum* or *Lycium barbarum*-containing foods, any dietary supplements, energy drinks, caffeinated beverages or green tea. This was continued throughout the study based upon the self-declaration in the daily dietary diary and verbal confirmation. The pre-intervention period ensured that participants in the study were motivated to continue the study for its entire duration.

1) ***Anthropometric changes***. TAI*slim* System was studied in a randomized, placebo-controlled, double-blind manner to evaluate its effects relative to placebos on the anthropometric parameters (Study 1). A total of 67 male and female adults were randomized into either one of the two treatment groups followed by at least 2 weeks pre-intervention period (Figure 2A and Table 1). These were the TAI*slim* System (Product A+B+C) treatment group, or placebo control with the use of a random digit table followed by the pre-intervention period. Men and women were randomized separately to ensure an equal number of men and women in each treatment group. The participants and all investigators and staff involved in this study were blinded to the subject's treatment assignment. Tested products were assigned a number or letter code. This code remained unrevealed to the investigators involved in the study until after completion of the data analyses. Upon randomization, participants started the 12-week weight loss period. The weight loss diet emphasized consumption of low-calorie, nutrient-dense foods such as fruits and vegetables, complex carbohydrates and lean proteins. The interventions were isocaloric/isoenergetic. All participants were given a medical exam, and physical anthropometric measurements were collected at the pre-, middle-, and post-intervention period following an overnight 12 h fast and included: body weight, BMI (Seca 703, Hamburg, Germany), waist circumference at umbilical level [35,36], and total body fat (Tanita BF-679W, Tokyo, Japan). All participants were monitored daily to ensure full compliance with the protocol including sample consumption and restriction of dietary intake. In the intervention trial, we evaluated the effect of TAI*slim* System or placebo samples under free-living conditions. Based upon our previous preliminary studies [2-4,9,10,34], a sample size of 57 participants was deemed to be sufficient to detect effectiveness of Product A alone with 95% confidence and 80% power. Daily intake procedures of all test samples are shown in Table 1B. A calorie-restricted isocaloric diet with multi-vitamin supplementation and daily exercise was required. Participants were encouraged to keep food records as a means of controlling food intake. Participants were counseled to reduce their energy intake to about 1,200 kcal/d including study samples, which supplied approximately 420 kcal/d. Testing products completely replaced breakfast but a healthy lunch and dinner were recommended. The remaining calories were consumed in the form of healthy snacks, such as fruit or yogurt. Sample menus for lunch and examples of healthy snacks were discussed during the counseling sessions. Participants were also requested not to eat after dinner within 3 hours of bedtime.

2) Products were dispensed to the participants on a weekly basis. At week 0 (baseline), participants were given a 14-day supply of products to ensure that participants had enough products in the event that they must miss a counseling session. Should a subject miss a weekly counseling

session, the extra products were used and extra products to cover 1 week were given at the subsequent session. This ensured that each subject always had a 1-week back-up supply of samples. Participants started taking the TAI*slim* System or placebo products after baseline measurements were taken. Empty containers were returned to the researcher at the following counseling session for compliance check. Anthropometric parameters, fasting blood glucose level (mg/dl) measured by conventional test kit (True track™, Code# 2612, Home Diagnostics, Ft. Lauderdale, FL) and subjective gastrointestinal questionnaire were assessed in the fasted state at baseline, 4, 8 and 12 w (Table 1).

Table 1. (**A**) Test procedures of the Study for Anthropometric changes by TAI*slim* System and (**B**) Study samples and intake procedures.

A

	Washout/Pre-intervention period*		**Intervention Period**											
WEEK	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12
Dietary counseling	X*		X		X		X		X		X		X		X
Body weight	X		X				X				X				X
Waist circumference			X				X				X				X
Hip circumference			X				X				X				X
Blood pressure			X				X				X				X
Heart rate			X				X				X				X
Fasting glucose level			X				X				X				X
Satiety			X	X	X		X				X				X
General health	X		X				X				X				X

*monitor dietary background only, no dietary consulting restriction, or exercise

B

Study product	**Breakfast**	**Snacks**	**Lunch**	**Snacks**	**Dinner**	**Snacks**
TAI*slim* (Product A) [a]	60 mL		60 mL			
SKINNY (Product B) [b]		1 chew		1 chew		1 chew as needed
SHAKE (Product C) [c]	1 scoop with 240 ml of water					
Self-selected meal		Per counseling	Per counseling	Per counseling	Per counseling	

[a] 60 ml with the SHAKE for breakfast, 60 ml at lunch immediately before meal, taken with 240 ml of water. Placebo for TAI*slim* contained 20 kcal/serving same with the active, but provided no active ingredients.

[b] One chew in the mid-morning (10-11 am), and 1 mid-afternoon (3-4 pm), approximately 1-2 hours before meals with 240 ml of water. Another 1 chew taken with 240 ml of water during the evening, if hungry and desired as a snack, but not immediately before bedtime. Placebo for the SKINNY (Chew) contained 20 kcal/serving same with the active, but provided no active ingredients.

[c] One serving (40.5 g) mixed with 240 ml of water, once a day as a meal replacement for breakfast. Placebo for the SHAKE contained 158 kcal/serving same with the active, but provided no active ingredients.

At each testing day and endpoint of the weight loss period (weeks 0, 4, 8 and 12), participants were weighed without shoes and had their resting blood pressure and heart rate measured. Participants were instructed to consume no food or beverage, except for water, for 12 h before their scheduled test. These measurements were taken with the subject seated and after a 3- to 5-minute rest period. Waist and hip circumferences were also measured by a single investigator. Waist circumference was measured on the skin at the umbilical level. Hip circumference was measured at the level of the widest circumference over the buttocks. Subjective gastrointestinal indices were assessed by ranked scale questionnaire (0-10) for flatulence, rumbling sounds caused by gas moving through the intestines, abdominal distension, abdominal pain, loose stools and sum of these symptoms, and fecal conditions [37]. Dietary background was collected from food diary for energy intake (kcal/day), fat intake (g/day), carbohydrate intake (g/day), and protein intake (g/day). Five participants dropped out from each group, but none of them were related to the samples or adverse effects (Figure 2A). However, there was no statistical difference demographically in those that completed the study between both groups.

3) ***Appetite suppression measurement.*** In several randomized, double-blind, placebo-controlled human clinical studies, satiety was assessed in the initial 3 days through first 2 weeks using a visual analog scale (VAS) [38,39]. Participants were asked to rate their general feelings of appetite and satiety, desire to eat and hunger over the previous week. Questions asked included: Over the past 7 days, 1) how hungry do you feel?; 2) how satisfied do you feel?; 3) how full do you feel?; 4) how much do you think you can eat?; 5) how energetic do you feel?; and 6) how sluggish do you feel? Participants rated their feelings on a 100-mm scale, with 0 being "not at all" and 100 being "very much so". An open-ended question on general feelings of health was answered. We also asked participants to provide information about their general health, side effects, and medication use on a bi-weekly basis. Adverse events were to be recorded on a separate form should any event occur.

4) ***Resting Metabolic Rate (RMR) and PPEE measurement (Study 2).*** Energy/caloric metabolism activity indicated by RMR and PPEE was measured by breath oxygen volume (VO_2; ml/min)

using hand-held indirect calorimeter (MedGem® test kit, Microlife®, Golden, CO) [40] in a separate independent study from Study 1. Test procedures are shown in Figure 2B.

A total of 12 healthy adult participants (21-51 y, age=34.5±7y, body mass index (BMI=28.2±2 kg/m^2) were enrolled to a randomized, double-blind, placebo-controlled study and took part in 6 study sessions (Figure 2B). All test participants randomly consumed all test sample preparations on a separate day. The tests were repeated with a same sample in a triplicate manner. Test days were held at least 1 week apart. After basal RMR was measured, participants consumed isocaloric total 198 kcal consisted with a single bout of consuming one serving of Product A (60 ml) (20 kcal) + 1 chew of Product B (20 kcal) and 158 kcal of Product C, or all of these placebo with liquid meal (Boost Plus®, Nestle Healthcare Nutrition Inc., Minneapolis, MN). RMR and PPEE were measured immediately before (baseline), 1, 2 and 4 h post-sample intake after at least 12 h fast [41-43].

Statistical analyses. In the 12-week intervention study (Study 1), all data obtained at pre-intervention, baseline, week 4, 8 and 12 (endpoint) were used in the statistical analyses. This ensured standardized conditions between time points (all data obtained on those days fasted). We keep data from participants who completed the study. Data were analyzed using mixed models analysis of variance with age, race, gender, baseline body weight (or appropriate body composition compartment) as variables in the model. Any non-significant variables were removed from the models. The main variables for inclusion in all models were treatment (TAI*slim* System or placebo, in blinded fashion), time (week -2, 0, 4, 8 and 12), and treatment-by-time interaction. The treatment-by-time interaction was also tested as a 3-way interaction with gender to test whether men and women might respond differently to our intervention. The treatment-by-time interaction was the main variable of interest. This statistical analysis plan was performed on absolute and percent change in body composition variables from the measurements, body weight, BMI, total body fat, waist circumference, hip circumference, blood pressures, and fasting blood glucose levels. The percentage of participants losing at least 5% of their initial body weight was also to be used as efficacy data and was compared between groups using unpaired t-test. Descriptive statistics were calculated for pre-intervention and each measurement period for all dependent measures and summarized as means and standard errors. For all clinical symptom questions under all categories (appetite, GI condition, and others), each question was graded and the scores analyzed for changes between pre-intervention and each measurement with the nonparametric Wilcoxon matched pairs tests. Differences were considered significant at $P<0.05$.

RESULTS:

1) *Anthropometric changes*.

At the 12 w post-intervention, TAI*slim* System group showed 84.6±1.5 kg, which was significantly better reduction in body weight than placebo group (88.1±1.4 kg) ($P<0.05$) (Table 2). Compared to the pre-intervention baseline level, it was also significantly lowered by taking TAI*slim* System products (Product A+B+C) by 6.3±1.0 kg ($P<0.05$), which is about 6.2±0.7% (Figure 3). Placebo group showed no significant difference. Body weight change in the placebo

group was lowered by 1.5±0.4% compared to the pre-intervention at 12 w post-intervention, but no significant difference was detected.

Table 2. Impact of TAI*slim* System on various anthropometric parameters, such as body weight, body mass index (BMI), total body fat, waist circumference, hip circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), and fasting blood glucose level in the overweight human participants in a randomized, placebo-controlled, double-blind, human clinical study. Each value indicates mean±SEM. a, b, and ab indicate P<0.05, and (b) indicates P<0.10 compared to the pre-intervention and placebo control, respectively analyzed by ANOVA.

	Placebo (n=28)				**TAI*slim* System (n=29)**			
	0 w	**4 w**	**8 w**	**12 w**	**0 w**	**4 w**	**8 w**	**12 w**
Body weight (kg)	89.5 ± 2.8	88.3 ± 2.6	88.4 ± 2.7	88.1 ± 1.4	90.9 ± 3.7	87.0 ± 3.5	85.8 ± 3.3	84.6 ± 1.5[ab]
BMI (kg/m^2)	30.9 ± 0.8	30.7 ± 0.7	30.8 ± 0.8	31.0 ± 0.8	32.3 ± 1.2	31.2 ± 1.2	31.0 ± 1.1	30.7 ± 1.0
Total body fat (%)	37.3 ± 1.0	37.0 ± 1.0	36.7 ± 1.0	36.7 ± 1.0	37.9 ± 1.3	36.8 ± 1.2	36.4 ± 1.3	35.6 ± 1.3
Waist circumference (cm)	99.3 ± 3.3	100.1 ± 1.6	99.7 ± 1.4	98.1 ± 1.3	108.7 ± 2.8 [(b)]	105.3 ± 2.5	103.4 ± 2.3	101.0 ± 2.4 [a]
Hip circumference (cm)	110.4 ± 1.9	109.4 ± 2.0	109.6 ± 1.9	110.1 ± 2.2	114.8 ± 2.8	112.1 ± 2.4	111.2 ± 2.4	110.1 ± 2.4
Systolic blood pressure (mmHg)	121.3 ± 2.2	115.5 ± 1.6	117.6 ± 2.0	115.7 ± 1.8	112.6 ± 1.9	105.4 ± 1.9 [ab]	108.4 ± 1.5	105.5 ± 1.3 [ab]
Diastolic blood pressure (mmHg)	79.1 ± 1.6	75.9 ± 1.3	75.8 ± 1.7	75.6 ± 1.5	72.4 ± 1.3	67.1 ± 1.6 [ab]	69.7 ± 1.8	65.4 ± 1.6 [ab]
Fasting glucose level (mg/dl)	106.3 ± 2.2	105.9 ± 2.4	102.2 ± 1.3	103.8 ± 1.8	103.2 ± 1.6	101.5 ± 1.8	95.1 ± 1.8 [ab]	96.0 ± 2.0 [ab]

As shown in Table 2 and Figure 3B, other anthropometric parameters related to the metabolic syndrome, such as BMI, waist and hip circumference, total body fat, fasting glucose level, and blood pressure (SBP, DBP) in the TAI*slim* System group were significantly reduced from the pre-intervention by 4.3%, 6.6%, 3.8%, 6.3%, 4.9%, 5.7% and 9.9%, respectively compared to the pre-intervention at the 12 w post-intervention (P<0.05). In the placebo group, the changes were by 0.02%, 1.2%, 0.4%, 0.8%, 1.9%, 3.2% and 3.9%, respectively compared to the pre-intervention, but no statistically significant difference was found.

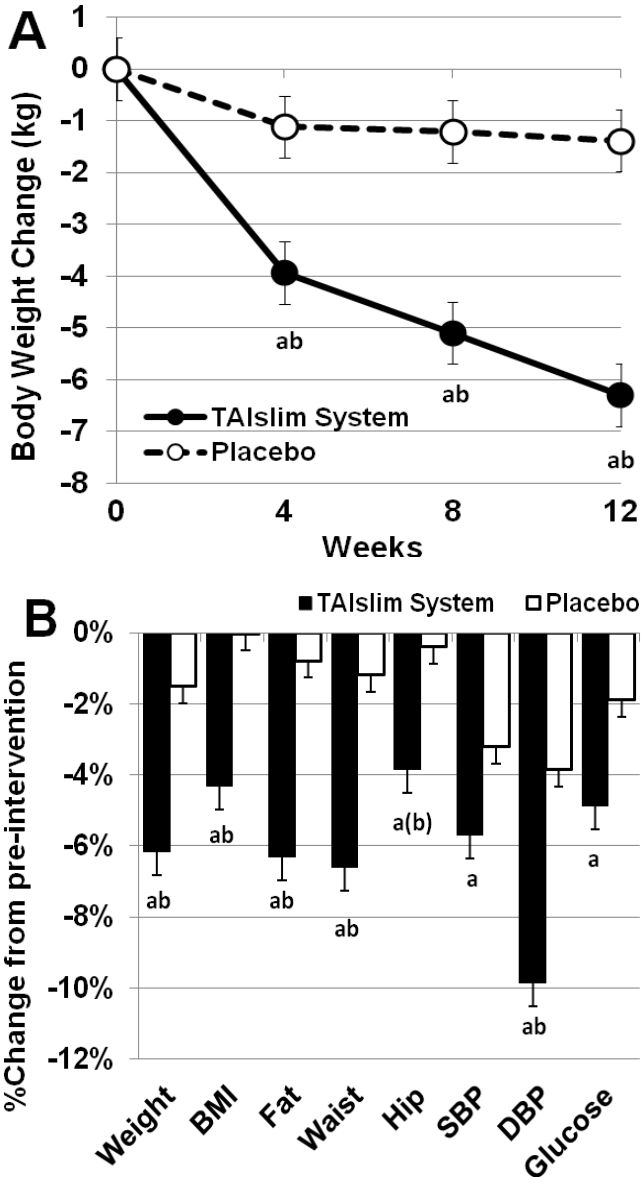

Figure 3. (**A**) Impact of TAI*slim* System on body weight change, and (**B**) percent changes from the pre-intervention in body mass index (BMI), total body fat, waist circumference, hip circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), and fasting blood glucose level in the overweight human participants in a randomized, placebo-controlled, double-blind, human clinical study. Each value indicates mean±SEM. a, b, and ab indicate $P<0.05$, and (b) indicates $P<0.10$ compared to the pre-intervention and placebo control, respectively analyzed by ANOVA.

Regarding gastrointestinal conditions, some participants in the TAI*slim* System group noticed abdominal rumbling, soft stool or slight constipation at the beginning of the intervention, but these effects were minor with no need to stop taking the TAI*slim* System products. No severe conditions were found in either group.

2) ***Appetite suppression.*** Feeling of appetite in both groups was lowered about a half hour after the breakfast nutritional beverage and/or products compared to the starting point/pre-intervention

(Figure 4A). However, the degree of the effects by the products was different. TAI*slim* System was the most effective to suppress appetite among the treatments (Figure 4A). On average, appetite was significantly reduced by 38.6±6.5% compared to the baseline level by TAI*slim* System, better than meal alone or placebo ($P<0.05$). In the placebo group, 11% reduction was seen but no statistical difference was detected (Figure 4B). TAI*slim* System significantly delayed time of the meal intake and population of delayed participants ($P<0.05$) (data not shown).

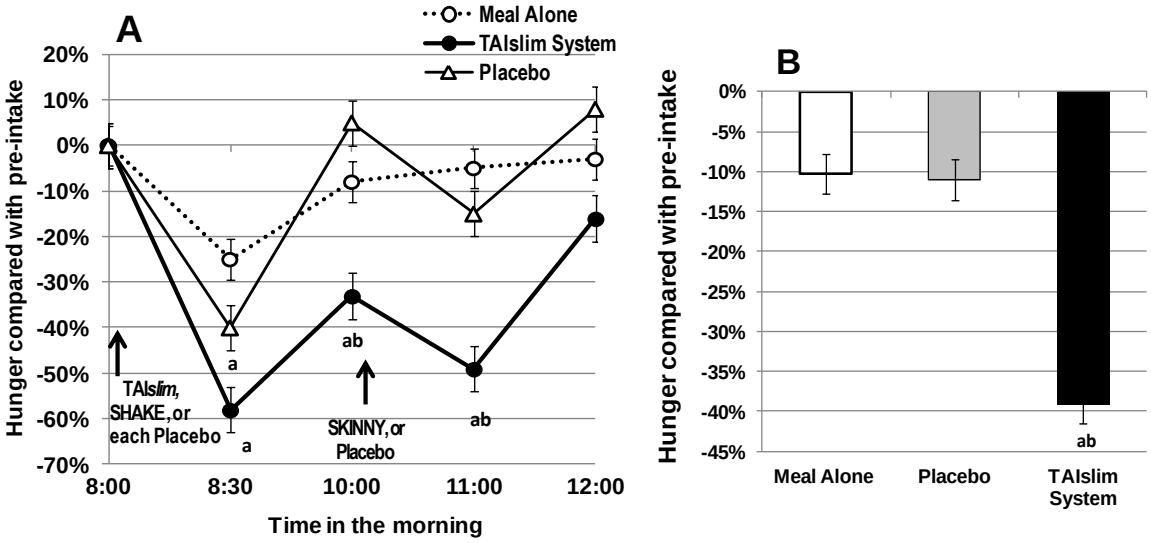

Figure 4. Effect of TAI*slim* System, placebo and meal alone on appetite evaluated by subjective questionnaire (0-10 visual analogue scale, VAS) in (**A**) the course of intake and (**B**) average.

Each value was obtained from the average of 4-times repeatedly asked VAS for the first 3-7 days of the studies, and indicates mean±SE.

3) Energy Expenditure stimulation. Kinetic behavior of TAI*slim* System on breath oxygen volume (VO_2) or PPEE, and area under the curve (AUC) 0 through 4 h after the consumption measured by the indirect calorimeter were all significantly increased by single bolus intake of TAI*slim* System products. The baseline RMR level in average VO_2 of all participants before intake of samples was 230.5 ± 9.3 ml/min, which was equivalent to 1,605 ± 66 kcal/d. TAI*slim* System was compared to this placebo baseline level. Placebo PPEE at 1 h post-intake after over 12 h overnight fasting was significantly increased by 254.5 ± 8.7 ml/min due to the nutritional beverage intake of 158 kcal, which is the control of the Product C (Figure 5A). However, PPEE in the placebo control returned to baseline within 2 h. Conversely, PPEE was significantly increased by TAI*slim* System compared to the placebo control and baseline levels for more than 4 h post-intake. PPEE at 1 h post-intake were significantly increased by 269.1 ± 9.1 ml/min with TAI*slim* System, which was statistically significantly higher than baseline level (Figure 5A). PPEE at 2 and 4 h post-intake were significantly increased by 252.4 ± 7.7 and 254.3 ± 8.9, respectively with TAI*slim* System, which were statistically significantly higher than placebo group and baseline level, representing statistically higher levels than the control at all times ($P<0.05$) (Figure 5A).

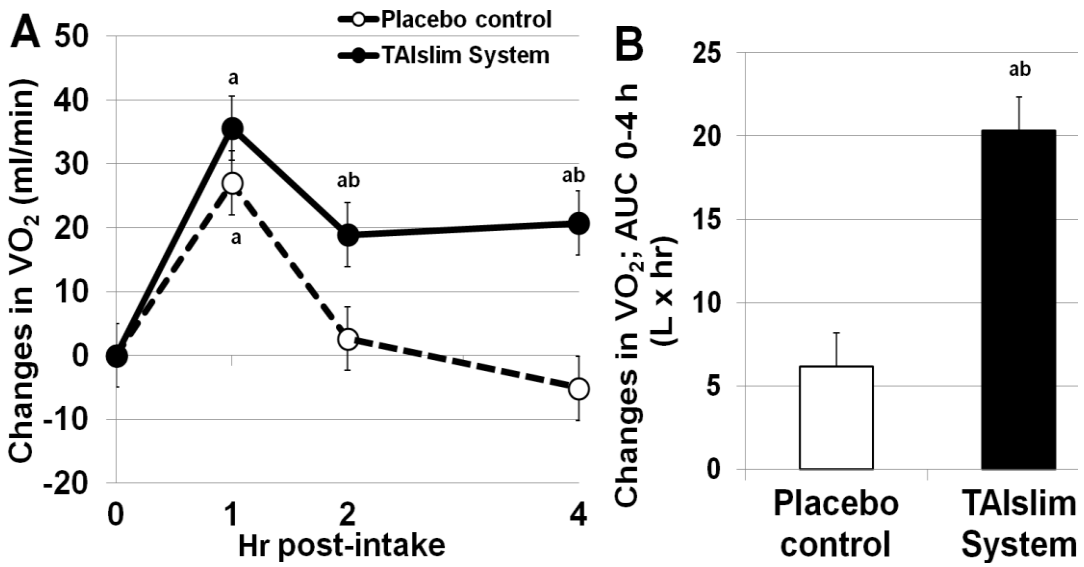

Figure 5. (**A**) Kinetic analysis and (**B**) area under the curve (AUC) 0 through 4 h post-intake of TAI*slim* System (TAI*slim* + SKINNY + SHAKE/Product A+B+C) on breath oxygen volume (VO_2) [resting metabolic rate (RMR)] measured by the hand-held indirect calorimeter. Product A+B+C or control (nutritional beverage only (158kcal) equivalent calorie to Product C plus each placebo) was taken orally after over 12 h fasting. Each value indicates mean±SEM. a, ab indicate $P<0.05$ vs Control and baseline level analyzed by ANOVA.

Area under the curve (AUC) during the 4 h study was shown in Figure 5B. AUC of the PPEE throughout 0-4 h post-consumption was increased by 7.2±1.2 % in TAI*slim* System group, which is significantly higher than the control group (0.8±0.9%) (Figure 5B). Placebo control did not show any significant increases over the baseline.

This acute PPEE increase was kept at the similar level throughout the 12-w intervention period in Study 1, and baseline RMR has not been statistically changed in 12 w by TAI*slim* System intake (data not shown).

DISCUSSION:

The present preliminary small scale pilot trials have shown that negative caloric intake by taking TAI*slim* System products may be effective to reduce the body weight and other anthropometric parameters related to metabolic syndrome. These results suggest that TAI*slim* System may control appetite and help to reduce caloric intake and stimulate energy expenditure.

Tea preparations have been reported to show fat oxidation effect in humans [12-15]. Dietary fiber used in TAI*slim* System has been reported to reduce visceral fat in the humans [16-28]. *L. barbarum* has also been shown reduce waist circumference [2-4]. As visceral fat is thought to be a major cause of metabolic syndrome, a combination of these food ingredients may be considered to be beneficial for prevention of metabolic syndrome based on their reported effects [2-4, 16-28]. The present trials were the first clinical studies to assess efficacies of a combination of these dietary ingredients. Functional food ingredients have been studied individually and synergistic effects were expected, but they have not been studied in a systematic

way. In the present studies, several functional food ingredients which have reported positive effects on weight management were combined to achieve negative caloric balance. This may be useful for weight management from various aspects based on their mechanisms of action. Daily consumption of this combination has been shown in the present studies to increase energy expenditure, which may be caused by improving caloric metabolism, As a result, body weight, waist circumference and other anthropometric parameters seem to be significantly reduced. These results suggest that a combination of the ingredients in TAI*slim* System may work in collaboration to increase PPEE, with effects lasting until the next meal time. Based upon the reported effects of the formulated individual ingredients [2-4, 12-24] and current study results, it is suggested to provide increased metabolism/thermogenesis. Although these effects may be exhibited by the hypothesis that TAI*slim* increased fat burning, decreased absorption of dietary fats and starches by inhibiting lipase and amylase enzymes, improved insulin sensitivity, appetite suppression, blood lipid reduction, blood glucose control and remodeling of intestinal flora to eliminate those implicated in obesity, it is left to a future studies to show such effects, as we have not measured these blood markers including cholesterol, triglycerides, insulin, the satiety-inducing gut hormones and visceral fat content, except for fasting blood glucose level. It is assumed that these additional parameters might be influenced by taking TAI*slim* System products based on various previously published studies [2-4, 12-28] for each ingredient. Due to the limitation of the current pilot trials, extended scale of the study is necessary to confirm the effects found in the current trials, and furthermore, more detailed analysis of several key blood biochemical markers, such as CCK, adiponectin, blood lipids, and other related indications are needed to confirm the above hypothesis and the mechanism of the actions.

PPEE was significantly increased by TAI*slim* System intake shown in the present studies. Since one serving of TAI*slim* (60 ml) is only about 20 kcal, an insufficient calorie level to exhibit observed level of PPEE increase in the present studies, it may not explain the significant and extended increase of PPEE. Since *Lycium barbarum* has been shown to increase PPEE in our previous studies [2-4], it was expected that we would see PPEE increase. However, a dose of *Lycium barbarum* in TAI*slim* (15 ml) is about 12.5% of previously tested highest dose of *Lycium barbarum* (120 ml) [3]. Therefore, the dose of *Lycium barbarum* in TAI*slim* may not be sufficient to significantly increase PPEE shown in the present studies. Thus, there may be a synergistic effect with other ingredients. Furthermore, duration of the PPEE stimulation was extended compared to high dose of *Lycium barbarum* alone. A high dose of *Lycium barbarum* (120 ml) increased 32 ml/min of VO_2 in 4 h after intake shown in the previous study [3]. At 4 h post-intake of TAI*slim*, 29 ml/min of VO_2 increase over baseline level was detected, which was about 76% of peak value of 1 h post-intake. Extending the increased level of PPEE throughout 4 h post-intake of TAI*slim* was unexpectedly long and may be caused by a combination effect with these other ingredients [44]. Since indigestible dietary fiber has been known to increase metabolism, postpone gastric emptying time and have other various impacts in the GI tract [24], the PPEE extension through 4 h post-intake found in the present study may be caused in part by the indigestible dietary fiber as an additional unexpected effect [44]. Further analysis using different types of dietary fibers would be interesting to study to clarify if this PPEE prolongation effect is also caused by other kinds of fibers.

For the energy expenditure study, due to the limitation of the subject numbers, we enrolled crossover design for the present pilot trials. It is well-known, the primary strengths of the repeated measures design is that it makes a study more efficient and helps keep the variability low. This helps to keep the validity of the results higher, while still allowing for smaller than usual subject groups. On the other hand, a disadvantage to the repeated measures design is that it may not be possible for each participant to be in all conditions of the experiment, due to time constraints, location of experiment, and others. Thus, we tested single bolus intake of test samples repeatedly in the different time at the same location. While it has not been established well for *Lycium barbarum* or TAI*slim*, we assumed that 1-week wash-out interval between the single-bolus intake of TAI*slim* may be sufficient based on the previous clinical studies [2-4, 9, 10, 32, 34, 44], as these previous studies suggest 2 weeks to 1 month may be sufficient to wash-out many of the significant effects of *Lycium barbarum* indicated by various general well-being scores. We have also confirmed that the baseline RMR was stable and consistent in all kinetic analyses. Further future studies to clarify any useful biomarkers of the duration of the effects of TAI*slim* System would be helpful.

Considering the overall effects of TAI*slim* System, it seems that this combination may be useful for a weight control program when combined with exercise and diet control program.

CONCLUSIONS:

The present studies suggest that TAI*slim* System controls appetite and PPEE, which may be caused by improving caloric metabolism and as a result, body weight, waist circumference and other anthropometric parameters, which seem to be reduced significantly from the pre-intervention. It is suggested that combining these products may be useful as part of a weight loss program. Detailed analysis of biochemical markers in the blood may clarify the mechanism of the actions of TAI*slim* System.

List of abbreviations: Body Mass Index (BMI), Epigallocatechin Gallate (EGCG), *Lycium barbarum* Polysaccharide (LBP), Postprandial Energy Expenditure (PPEE), Resting Metabolic Rate (RMR)

Competing interests: Corresponding author is an employee of FreeLife International.

Author's contributions: Author is the principle investigator for these studies providing oversight and contributed to all research activities, preparing manuscripts.

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