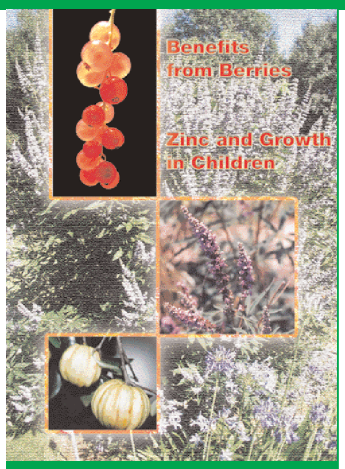

A new class of phytonutrients for body weight management*

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SUMMARY

A botanical weight management formula (BWMF) composed of hydroxycitric acid (HCA), garcinol, diterpene forskolin and alkaloid piperine was developed. The composition of BWMF is aimed at overcoming the shortcomings of the individual botanical ingredients, facilitating their actions and improved bioavailability to the target tissues and cells.
BWMF was evaluated in a double blind, randomized, placebo controlled 12 week study. The 500 mg of BWMF or placebo capsules were taken twice daily by 50 subjects, consisting of 25-55 year old male and female participants. Both groups combined treatments with a healthy and active lifestyle and a balanced diet. The BWMF group responded with 7.5% total body weight loss, while placebo group responded with 1.1% total body weight loss after 12 weeks on the study regimen. The weight loss in the placebo group was attributed to exercise and diet regimen. The BWMF group responded over 12 weeks with a significant reduction in body fat, and an increase in lean body mass (LBM). Not serious side effects were reported by the participants and supervising physicians and 75% of participants rated BWMF as a successful treatment. The unique combination of ingredients offers a viable weight management formula.

INTRODUCTION

The cumulative scientific and clinical evidence supports change in the direction for weight loss regimens that would address growing urgency for safe, practical and effective solutions in body weight management. This urgency is obvious in view of growing number of cases of well recognized and new disease conditions linked with obesity, e.g. recently discussed the pre-diabetic state (**1**,**2**), and potential link between faulty insulin signaling mechanism, carbohydrate metabolism and the pathogenesis of Alzheimer's disease (AD), nicknamed diabetes type 3 (**3**). Interestingly the dramatic increase in obese population worldwide coincides with increase in the pre-Alzheimer's condition and the full-blown Alzheimer's disease cases worldwide. The growing trend in obesity calls on health professionals to rethink the current approach to treat obesity and offer a new class of treatments.

To address the critical issues of safety and efficacy in prevention and treatment of obesity this article discusses an example of a new class of weight-management supplement that would: (a) modify eating habits, (b) prevent loss of LBM, and (c) would not adversely affect the digestive and metabolic processes, nor would it impact negatively the physiology of body organs and systems.

What and how much we eat impacts our body composition and body weight more than the levels of physical exercise. The available weigh loss diets demonstrate that low-carbohydrate and high-protein diets are in general more effective than low-fat diets in reducing weight and cardiovascular disease risk in overweight and obese populations (**4**). It has also been found that calorie restriction diets produce many of the positive metabolic adjustments including decreased metabolic, hormonal, and inflammatory risk factors for diabetes, cardiovascular disease, and cancer (**5**).
Based on current research, the purpose of an opti-

mal weight-management program could be stated as adapting the body to thrive on restricted calorie intake attained preferably by restricting carbohydrates.

The importance of healthy body composition, especially in maintaining or regaining LBM, has recently come to light for two reasons. First is the increased recognition that preserving LBM plays a vital role in any successful weight management regimen. Second, there is a growing awareness that LBM is proportionate to the overall health of an individual (**6**).

LBM consists of muscles, vital organs, bone and bone marrow, connective tissue and most (over 70%) of the body water. The proportion of LBM to body fat clearly determines our aesthetic looks, but more importantly it determines physical fitness, health status and the risk of morbidity and premature mortality. Most of us, especially in the course of aging, are deficient in LBM. Osteoporosis and sarcopenia may exemplify two conditions related to loss of LBM in the course of aging. The aging of LBM starts early in life: an average adult loses 0.2kg of muscle and bone mass each year after age 25 and gains 0.45 kg of body fat per year after age 25.

This paper will discuss the rational behind the formulation of BWMF, which consists of hydroxycitric acid (HCA), garcinol, diterpene forskolin and alkaloid piperine .
The present clinical study was carried out to determine the clinical efficacy and safety of BWMF in view of the preclinical and clinical findings on its individual ingredients as described in ***Appendix I***.

MATERIALS AND METHODS

Formulation of the capsules

The components of BWMF are hydroxycitric acid (HCA), garcinol (both compounds extracted from rinds of *Garcinia cambogia* fruit), diterpene forskolin (extracted from roots of *Coleus forskohlii)* and alkaloid piperine (a spice principle extracted from fruits of *Piper nigrum*) (***Table 1***).

Table 1 *Composition of BWMF*

	Ingredients	Dose per serving
1	Hydroxycitric acid calcium salt	250 mg
2	Garcinol (poly-isoprinolated benzophenone)[a]	5 mg
3	Forskolin 10% extract[b] (Diterpene)	250 mg
4	Piperine 95% extract[c] (Alkaloid)	5 mg

Components of LeanGard® are [a]Garcitrin®, [b]Forslean®, [c]Bioperine® and are registered trademarks of *Sabinsa Corporation*, NJ-USA

Study design

The institutional board review approval and the execution of the methodology such as selection of participants, location, duration, study design, body composition monitoring were all obtained as per standardized clinical procedures and carried out at Bangalore, South India under the clinical research organization, ClinWorld, Inc.

Subjects

The study population consisted of 50 obese (BMI above 30 kg/m^2) healthy subjects, 24 men and 26 women, ranging from 25 to 55 years of age. Approximately 87% of the subjects were non-vegetarians, 13% were vegetarians. Based on socio-economic status, 62% of subjects were from middle class, 18% from lower class with 20% from upper class. The 82% of study population came from suburban areas, 11% from rural areas and 7% from urban areas. The participants were asked to limit their daily food and drink intake to 1200-1500 calories and keep daily-menu diaries (***Table 2***). The participants were also advised about the need of regular daily exercise, e.g. 30 min a day of recreational walking. The information on physical exercise was collected during the scheduled visits to the study center.

Table 2 *Example of recommended 1200 kcalories diet*

BREAKFAST*
Idli or bread 3 nos
Dosa or chapatti 2 nos
Lime rice/Upma/bise bele bath/avalakki 1½ cups
Akki rotti 1½ nos

BRUNCH
fruit or vegetable salads or butter milk or lime juice or tomato juice
1 (Any) Coffee/tea 1 cup

LUNCH*
Rice or chapatti or Ragi ball or rice cooked with rasam, sambar, salads, vegetables curry unlimited.1½ cups/pieces
Curds ½ cup

AFTERNOON TEA
coffee/ tea 1 Cup
Idli or bread or 3-biscuits 2 nos/slices
Dosa or chapatti 1 no
Lime rice/upma/bise belebath/avalakki ½ cup

DINNER*
same as lunch

*The food products mentioned above are derived from rice, wheat and lentil and are processed in either fermented or non-fermented form.

Treatment protocol
The trial was carried out at a single center over a period of 6 months with each subject involved in the trial for a period of 12 weeks. Study design consisted of controlled, randomized, double blind and parallel-groups. After screening for eligibility, subjects were assigned either to active group receiving BWMF, 500 mg bid, or identical placebo, 500 mg bid. Five visits to the clinic were scheduled after the initial screening visit which included the baseline visit on day 0 and four visits in the active trial period on weeks 3, 6, 9 and 12.

Clinical observations and measurements
Anthropometric measurements included waist, hip, mid-arm circumference, neck circumference and calf circumference. Clinical examination included blood pressure, heart rate and ECG. Body composition monitoring included a number of parameters, such as total body water (TBW), basal metabolic rate (BMR), body mass index (BMI), waist to hip ratio (WHR), body fat percentage (BF%) and lean body mass percentage (LBM%). All subjects were prescribed a balanced diet. All were encouraged to exercise 30 min a day, most days of the week. Subjects underwent the following laboratory investigations: complete blood count [CBC], erythrocyte sedimentation rate [ESR], lipid profile, liver function test, renal function test, thyroid panel, estrogen and testosterone levels. The estimation of biological markers such as leptin, adiponectin and TNF alpha was carried out. Subjects were instructed to take assigned intervention twice daily before food and to record the date and time of each swallowing, concomitant medication use and any adverse events on their diary cards.

Data and statistical analysis
The data were plotted based on the Deurenberg formula, Watson's formula, Harris Benedict formula, BMI formula and LBM formula as described in the supplemental data sheet (***see Appendix II***). The data obtained from each visit was compared with the initial value and the differences were tested using the paired sample t-test (two tailed); and the differences between groups were analyzed by Student's t-tests (unpaired sample, two tailed). One-way ANOVA analysis was performed using the Kruskal-Wallis test and Dunn's Multiple Comparison test to establish statistical significance of treatment regimen between placebo vs. BWMF. The primary endpoints were analyzed using an Analysis of Covariance (ANCOVA) model, an approach which is often more practical, having high statistical power and adjusts each subject's follow-up measurement according to their baseline value. The analysis of covariance provides a way of measuring and removing the effects of the initial differences between the samples. The ANCOVA assessed the simultaneous effects of diet arm, prior weight change upon week 12 mean body weight, after adjustment for baseline body weight as a covariate. In this way, treatment differences in adjusted mean weight at week 12 correctly estimate the change over time, after adjusting for differences in baseline weight. The accepted level of statistical significance was $p < 0.05$, and the overall significance level was set at 5% ($p<0.05$). The statistical program, SPSS software program version 11.0 (SPSS, Chicago, IL USA) was used in data analysis.

RESULTS

Of the 50 participants, 46 successfully completed the 12 week trial and were included in the statistical evaluation. Three subjects dropped out due to reasons unrelated to BWMF treatment, i.e. due to urinary tract infection, eye laser surgery and one to non compliance. One patient dropped out due to gastrointestinal discomfort and heartburn which could be related to intake of BWMF. There were no additional subjective or objective side effects in the active or placebo receiving groups in the course of the study. The systolic and diastolic blood pressure changes as well as changes in the pulse rate over 12 weeks were not significant as compared to the baseline readings.
The blood biochemistry and hematological evaluation at day 0 and week 12 included the liver, blood lipid and thyroid panels as well as testosterone levels, estradiol levels and biomarkers leptin, adiponectin and tumor necrosis factor alpha – all the listed parameters scored within normal value range in both treatment groups at the baseline and at week 12 (***Table 3***).

In both treatment groups a significant ($p<0.05$) reduction in food calorie intake values were recorded at the 1st through 4th visits. However, both placebo and BWMF groups were not able to attain the target daily calorie intake, 1200-1500 kcal.
For the BWMF group, the caloric intake ranged from 2828.6 ± 297.84 kcal at baseline to 2671.43 ± 277.99 kcal on week 12, a decrease of 5.6% in calorie intake; for the placebo group the corresponding values were 2884 ± 270.8 at baseline, 2676 ± 210.3 on week 12, a decrease of 7.21% in calorie intake .
The change in body weight and body composition parameters with BWMF occurred in the first three weeks (first visit) and within nine or twelve weeks for the placebo group (third or fourth visit) (***Tables 4,5***). The following clinical parameters evaluate the weight management potential of BWMF vs. placebo and the values are based on comparison between values at the baseline and values at week 12.

Table 3 *Baseline and week-12 laboratory data in the placebo and BWMF groups*

Parameters	Placebo		BWMF		
	Baseline†	Final†	Baseline†	Final†	Normal Range
Hb (g/dL)	13.86 ± 0.48	14.46 ± 0.51	14.04 ± 0.43	14.46 ± 0.44	M:13.5 -17.5
					F: 12.5 to 15
PCV (%)	39.89 ± 1.97	42.56 ± 1.37	41.41 ± 1.12	42.07 ± 1.21	M: 40 to 54
					F: 36 to 46
MCH (pg)	26.38 ± 0.68	26.85 ± 0.75	28.38 ± 0.62	28.79 ± 0.61	27 to 32
MCHC (%)	33.48 ± 0.19	33.58 ± 0.22	33.81 ± 0.22	34.10 ± 0.22	31 to 36
MCV (fL)	78.86 ± 1.65	79.24 ± 1.86	83.92 ± 1.46	84.13 ± 1.52	78 to 98
RBC (million/cumm)	5.26 ± 0.13	5.32 ± 0.11	4.94 ± 0.11	4.83 ± 0.19	4 to 5.5
WBC (x1000/mm^3)	8.43 ± 0.36	8.72 ± 0.30	8.29 ± 0.41	8.21± 0.43	4.0 to 10.5
Platelet Count (x1000/mm^3)	280.68 ± 11.87	304.56 ± 10.40	306.86 ± 14.67	325.58 ± 15.66	120 to 400
ESR (mm/hr)	8.52 ± 1.29	6.56 ± 0.74	8.38 ± 1.12	6.86 ± 0.74	0 to 10
Liver Function test					
Total Protein (g/dL)	7.29 ± 0.14	7.78 ± 0.10	7.38 ± 0.11	7.58 ± 0.11	6 to 8.3
Albumin (g/dL)	4.36 ± 0.07	4.55 ± 0.09	4.35 ± 0.07	4.40 ± 0.09	3.5 to 4.8
Total Bilirubin (mg/dL)	0.63 ± 0.04	0.58 ± 0.05	0.81± 0.09	0.62 ± 0.05	0.2 to 1.5
SGPT (U/L)	32.65 ± 3.25	28.19 ± 2.70	23.09 ± 1.58	21.79 ± 1.48	5 to 34
SGOT (U/L)	42.92 ± 6.31	37.46 ± 5.28	25.18 ± 2.83	22.01 ± 1.86	10 to 42
GGT (U/L)	44.04 ± 4.96	39.20 ± 2.93	30.82 ± 3.62	28.18 ± 2.41	0 to 50
ALP (U/L)	127.62 ± 6.71	94.68 ± 5.18	132.82 ± 6.43	88.98 ± 5.59	42 to 128
Renal Function test					
Creatinine (mg/ dL)	0.95 ± 0.03	0.92 ± 0.03	0.84 ± 0.04	0.86 ± 0.04	0.6 to 1.2
Urea (mg/ dL)	11.29 ± 0.59	10.28 ± 0.60	9.98 ± 0.64	11.19 ± 0.74	7 to 18
Uric Acid (mg/ dL)	5.91 ± 0.28	5.88 ± 0.26	5.76 ± 0.37	5.47 ± 0.42	2 to 7
Lipid profile parameters					
TC (mg/dL)	174.17 ± 3.95	178.77 ± 5.39	167.70 ± 5.37	175.97 ± 7.01	50 to 200
LDL (mg/dL)	110.00 ± 3.80	106.99 ± 5.03	101.82 ± 6.31	106.10 ± 5.44	50 to 150
VLDL (mg/dL)	40.61 ± 3.72	38.49 ± 3.39	36.45 ± 5.42	36.65 ± 6.02	10 to 40
TRI (mg/dL)	203.17 ± 18.56	192.60 ± 16.93	182.10 ± 27.03	185.09 ± 29.76	44 to 200
HDL (mg/dL)	36.93 ± 1.01	40.37 ± 1.36	35.25 ± 1.18	37.87 ± 1.15	35 to 65
Thyroid parameters					
TSH (μIU/ml)	1.89 ± 0.27	1.71± 0.29	2.13 ± 0.24	2.15 ± 0.32	0.3 to 4.5
T3 (ng/ dL)	154.19 ± 5.71	118.20 ± 6.58	170.29 ± 6.11	134.16 ± 6.04	70 to 200
T4 (μg/ dL)	7.54 ± 0.31	8.24 ± 0.39	8.65 ± 0.45	9.65 ± 0.54	4.5 to 13
Hormones					
Testosterone (ng/ dL)	323.73 ± 16.66	363.62 ± 42.55	313.60 ± 35.77	347.17 ± 18.55	M: 241 to 1000
	24.13 ± 5.40	22.20 ± 4.63	29.54 ± 4.47	20.44 ± 3.04	F: 14 to 76
Estradiol (pg/ml)	34.21 ± 3.29	31.14 ± 3.11	29.42 ± 2.76	27.92 ± 2.16	M: up to 56
	108.19 ± 24.84	109.58 ± 25.37	117.56 ± 29.13	92.78 ± 21.38	F: *
Biomarkers					
Leptin (ng/ml)	24.39 ± 2.77	48.16 ± 6.59	23.62 ± 2.59	48.57 ± 7.40	23 - 50 ng/ml
Adiponectin (μg/ml)	13.83 ± 0.68	14.68 ± 0.36	13.90 ± 0.61	14.25 ± 0.73	8.5 - 15.0 μg/ml
TNF-α (pg/ml)	5.80 ± 1.41	4.99 ± 1.34	4.92 ± 2.29	9.03 ± 3.69	2.5 ± 10.5 pg/ml

† Mean ± SEM;

* Menstruating Women: Follicular Phase- up to 160;

† Follicular Phase (2-3d)- up to 84; Mid-cycle peak- 34-400; Luteal Phase - 27-246.

Menopausal Women: Untreated menopause - up to 30; Treated menopause - up to 93.

Females on medication: On oral contraceptives: up to 102; on conjugated estrogen: 16-90; on estradiol valerate: 60-177

The BWMF group had reduced body weight over 12 weeks by 7.5% (6.42±0.36 kg) and the placebo group reduced body weight by 1.1% (0.97±0.26 kg) (***Tables 4, 5***). The body weight reduction was significant ($p<0.05$) for the BWMF group comparing to the baseline already after the first three weeks (first visit) on the formula.

The weight reduction in the placebo group occurred only after 9 weeks (third visit), was statistically not significant and most likely was a result of combined effects of calorie restriction diet and benefits of regular exercise.

The inter group variation for weight loss was found to be statistically significant from Visit 1 onward ($p<0.05$), with highly statistically significant difference between groups observed ($F=89.92$; df (1, 43), $p<0.0001$) when data were evaluated using ANCOVA.

The 95.23% and 42.85% of study subjects on BWMF lost more than 5% and 8% of body weight, respectively, as compared to placebo group which lost less than 5% of body weight (***Tables 4, 5***).

Table 4 *Weight change in placebo and BWMF-treated groups (500 mg bid) on week 12*

Characteristics	Placebo (Mean ± SEM) (95% CI)	BWMF (Mean ± SEM) (95% CI)	Δ
(n)	25	21	
Baseline (kg)	88.43 ± 2.22 (84.07 - 92.78)	85.82 ± 2.14 (81.62 - 90.01)	—
Change (kg)	-0.97 ± 0.26 (0.45 - 1.49)	-6.42 ± 0.36 (5.73 - 7.12)	-5.46
Change from Baseline (%)	-1.09 ± 0.28 (0.53 - 1.65)	-7.50 ± 0.41 (6.70 - 8.31)	-6.41
Patients ≥ 5% loss in weight (%)	0 (0/25)	95.23 (20/21)	—
Patients ≥ 8% loss in weight (%)	0 (0/25)	42.85 (9/21)	—

BWMF decreased waist circumference and waist: hip ratio (WHR) by 6.43% ($p<0.05$) whereas placebo had actually increased waist circumference and WHR by 2.16% over the 12 weeks (***Table 6***).

The reduction in WHR for BWMF group was statistically significant ($p<0.05$) from the second visit onward. A significant decrease in circumference of upper mid arm was observed in BWMF group, i.e. 5.33% when compared with placebo group 1.00%, (t test: $p<0.05$, ANOVA: $p<0.01$ and ANCOVA, F value=37.25, $p<0.0001$, df: 1, 43). The reduction in upper mid arm circumference was statistically significant ($p<0.05$) from the second visit onward for the BWMF group. Reduction in calf circumference was 4.15% and 0.84% in BWMF and placebo groups, respectively. The reduction was statistically significant in BWMF treated group (t test: $p<0.05$ on Visit 4, ANOVA: $p<0.01$, ANCOVA: F value=16.15, $p=0.00023$, df: 1, 43). Similarly a decrease of 2.50% and 0.71% was observed in the neck measurement in BWMF and placebo treated groups respectively. The reduction in neck circumference was statistically significant in BWMF group (significant with ANCOVA analysis, F value: 24.52, $p=0.000012$, df: 1, 43).

Body Mass Index (BMI) in BWMF group was reduced significantly ($p<0.05$) by 7.45%, as compared to 1.09% reduction with placebo ($p<0.05$) (***Table 6***). Body Fat% was reduced significantly ($p<0.05$) by 8.15% as compared to 1.18% with placebo. LBM increased by 4.36% in the BWMF group vs. 0.67% increase in placebo group ($p<0.05$).

Interestingly, the Basal Metabolic Rate (BMR) decreased in BWMF group significantly ($p<0.05$) by 4.5% by week 12 comparing to the baseline; BMR in placebo group decreased only by 0.64% during the same time. The significant body weight loss combined with an apparent lower energy requirement, as assessed by the BMR, in BWMF group can be possibly explained by the observation that individuals with lower body weight require less energy to maintain each kilogram or pound of body weight. Total body water (TBW) decreased significantly by

Table 5 *Changes in body weight over a 12-week period of treatment with BWMF (500 mg bid or placebo)*

Treatment	Baseline	Visit (n)							
		1		2		3		4	
	Body Wt (kg)	Body Wt (kg)	% change	Body Wt (kg)	% change	Body Wt(kg)	% change	Body Wt (kg)	% change
Placebo	88.43± 2.22	88.41 ± 2.20	0.00	88.07 ± 2.24	0.42	87.80±2.26	0.74	87.46±2.21	1.09
BWMF	85.82± 2.14	83.20 ± 2.17	π 3.10	81.22±2.16	π 5.41*	80.35±2.05	π 6.38*	79.40±2.05	π 7.50*‡

†Data are the mean ± SEM

*P<0.05 as compared with placebo group as determined by Student's t test

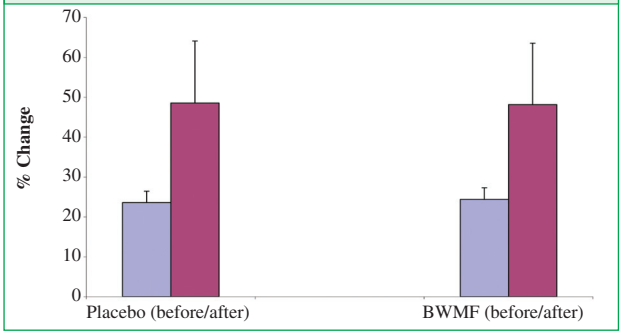
‡P<0.05 (Evaluation of Intra group variation) – Student's t test

πp<0.0001, F=162.71, df (1,43) with ANCOVA analysis

4.66% (p<0.05) in BWMF group vs. 0.66% decrease in placebo group. The significant decrease of water content with BWMF can be rationalized since this group lost more body water than placebo due to larger than placebo loss of body fat which contains approximately 30% of body water (***Table 6***).
The biomarkers of obesity were evaluated in both treatment groups. The statistically significant changes were observed only in values of leptin for both groups. In the BWMF group 23.62 ± 11.6 at baseline increased significantly (p<0.05) to 48.57 ± 33.1 at week 12, an increase of 105. 58%. In placebo group corresponding values were 24.39 ± 13.5 at baseline and 48.16 ± 32.26 at week 12, an increase of 97.48% (p<0.05). These results may indicate that change in leptin values in both study groups may be due to lower calorie intake combined with the exercise regimen (***Fig 1***). Adiponectin levels did not differ at any point of measurement within placebo and BWMF groups. Levels of TNF-alpha are commonly found elevated in obese individuals. In the present study, TNF-alpha levels were also found to be higher than normal values. However, no significant change in levels of this biomarker was observed in either placebo or BWMF groups. Therefore, changes in weight and related body composition parameters observed in the present study were independent of leptin, adiponectin and TNF-alpha levels (***Table 3***).

The usefulness of treatment was evaluated by a supervising physician and patients. In the BWMF group, 33.33% patients rated the treatment as producing 50% improvement vs. the baseline, and 61.90% patients rated received treatment as producing 75% improvement comparing to the baseline. The Global Evaluation of the treatments by supervising physician rated 100% and 92% patients for BWMF and placebo groups respectively as scoring 5 points or more for efficacy.

Figure 1 *Leptin profile before and after 12 weeks of intake of placebo or BWMF*



DISCUSSION AND CONCLUSIONS

There are relatively few pharmaceutical compounds available for body weight management.
Dexfenfluramine was originally developed to increase the brain levels of serotonin, a neurotransmitter and neurohormone that quells the appetite. However, this drug is no longer approved by the FDA due to the serious cardiovascular side effects (**7**). Sibutramine also increases the levels of serotonin, as well as noradrenaline, and works to quell the appetite. However, this drug still needs a longterm evaluation for its efficacy and cardiovascular side effects (**8**). Rimonabant is a selective blocker of cannabinoid receptors in the brain which is responsible for the drug's anorectic and metabolic actions. Use of this drug is limited since it can produce mental depression which should be given spe-

Table 6 *Body composition and basic metabolic rate at baseline vs. 12 weeks on BWMF (500 mg bid or placebo)*

Parameter	Placebo		BWMF		ANCOVA analysis	
	Baseline Visit 0	Final Visit 4	Baseline Visit 0	Final Visit 4	F	p value
WHR	0.97 ± 0.02	0.99 ± 0.01	0.99 ± 0.01	*0.92 ± 0.01‡	89.92	<0.0001
BMI (kg/m2)	32.55 ± 0.59	32.20 ± 0.59	31.77 ± 0.58	*29.41 ± 0.60‡	164.40	<0.0001
BF (%)	36.15 ± 1.54	35.73 ± 1.53	34.82 ± 1.63	31.98 ± 1.67	165.45	<0.0001
BMR (kcal/day)	1766.77 ± 47.45	1755.49 ± 47.04	1748.93 ± 51.60	1670.20 ± 47.50	129.36	<0.0001
TBW (liters)	42.58 ± 1.48	42.30 ± 1.47	42.03 ± 1.64	40.08 ± 1.53	143.03	<0.0001
LBM (%)	63.85 ± 1.54	64.27 ± 1.53	65.18 ± 1.63	68.02 ± 1.67	165.45	<0.0001
Neck (cm)	39.09 ± 0.75	38.81 ± 0.75	38.57 ± 0.81	37.60 ± 0.78	24.52	<0.000012
Upper mid arm (cm)	32.79 ± 0.49	32.46 ± 0.48	31.91 ± 0.47	*30.20 ± 0.47‡	37.25	<0.0001
Calf (cm)	38.72 ± 0.51	38.40 ± 0.55	38.05 ± 0.53	*36.45 ± 0.52‡	16.15	<0.00023

Data are the mean ± SEM

*P<0.05 as compared with placebo group-Student's t test

‡P<0.05 (Evaluation of Intra group variation) – Student's t test

cial considerations in view of the recent FDA finding of increased risk of suicide during treatment with Rimonabant (**9**).

Orlistat and its OTC version 'Alli' interfere with pancreatic lipase, which results in inhibition of absorption of dietary fat and the weight loss effect. Due to this specific mechanism of action, the undigested fat may result in gastrointestinal side effects and poor absorption of fat soluble nutrients, e.g. vitamins A, D, E and K. The inhibition of intestinal lipase by Orlistat has produced the undesired effect of acutely increasing appetite for food (**10-12**).

The apparent lack of acceptable weight management compounds and problem of obesity of pandemic proportions calls for intensified research to identify products that would combine efficacy with safety. The natural products with a history of traditional use and preferably food use in indigenous traditions offer a promise of new drug discovery useful in bariatric medicine. In fact, all the botanicals in BWMF have well established tradition of use as common food. In addition, some of them, such as the ethanolic extract of black pepper fruits containing piperine have the US FDA recognized GRAS (Generally recognized as safe) status (21 CFR 100,01 182,10 182-20).

The present results of a clinical trial of BWMF qualify this multicomponent nutraceutical as an emerging natural weight loss formula.

The good compliance and safety of BWMF 500 mg bid in a broad range of ages and both genders confirms that the formula meets hypothesized requirements for the new class of nutritional regimen, i.e. (a) to assist a meaningful lifestyle modification to normalize and maintain healthy total body weight, (b) help improve the healthy body composition thus preventing the loss of LBM, and (c) would not interfere with the digestive and metabolic processes and adversely impact the major body organs and systems. It is difficult to explain the mechanism of the formula based on the present clinical and laboratory data. To gain better understanding of BWMF in clinical practice, the pharmacokinetic parameters of the lead ingredients (i.e. hydroxycitric acid and forskolin together with biomarkers) will be evaluated. In the interim and based on the presented results, BWMF meets the criteria for a safe and effective weight loss formula which together with balanced diet and physical exercise can be part of a comprehensive weight management program.

Appendix I Components of BWMF

Hydroxycitric acid

Hydroxycitric acid is an alpha-hydroxy tribasic acid (1,2-dihydroxypropane-1,2,3-tricarboxylic acid) with two asymmetric centers which result in formation of two pairs of diasteroisomers or four different isomers (**13**,**14**). The (-) hydroxycitric acid (HCA) isomer is found in the rind of *Garcinia cambogia* fruit (*fam*. *Clusiaceae*) which is commonly used in South Indian daily food (**13**,**14**). This isomer has been shown to be a potent linear competitive inhibitor of ATP citrate lyase enzyme, *in vitro*, demonstrating a much greater affinity for the purified enzyme than its natural substrate citrate as well as the other stereoisomers of hydroxycitric acid (**13**,**14**). The biological importance of ATP citrate lyase is as a citrate cleavage enzyme which catalyzes the extra-mitochondrial cleavage of citrate to acetyl CoA and oxaloacetate, and facilitates the biosynthesis of fatty acids. The reversible inhibition of citrate lyase by (-) HCA may lead to the reduction of fatty acids synthesis and lipogenesis. These effects have been measured and demonstrated *in vivo* following the oral, intravenous or intraperitoneal administration of (-)hydroxycitrate to experimental animals (**9**). When (-) HCA was given orally before the feeding period, the animals fed (-) HCA consumed less food and their hepatic synthesis of fatty acids and cholesterol was significantly diminished as compared to the untreated controls (**15**,**16**). The observed decrease in food intake may be only one of the factors responsible for the (-) HCA promoted weight loss, because experimentation with rats fed (-) HCA showed weight loss with no decrease in cumulative food intake (**17**,**18**). Although the potential of (-)HCA as a weight lowering compound has been recognized since the 1970's, relatively few clinical studies have been conducted with this compound (**19**-**25**). These few studies examining HCA-mediated prevention of excess body fat resulted in contradictory results, most likely due to HCA being poorly bioavailable in the cytosol of a target cell. The issue of bioavailability becomes critical with HCA because its reported efficacy in inhibiting the intracellular enzyme, adenosine triphosphate (ATP)-citrate-lyase, depends entirely on the presence of HCA inside the target cell. Searching for a more bioavailable form of HCA, we have developed a water soluble potassium salt of HCA (**26**). Although the potassium salt of HCA has considerably improved bioavailability, in comparison to calcium salt of HCA, its bioavailability was still relatively inefficient. For example, an *in vitro* study done on hepatic cells, indicates that 5 mM of extracellular potassium HCA could inhibit ATP citrate lyase. However, only 0.5 mM of potassium HCA is actually needed in the cytosol to effectively inhibit ATP citrate lyase. Therefore, a 10-fold excess amount of potassium HCA is needed outside of the target cell in order to achieve a concentration of 1/10 that amount in the cytosol (**25**).

Garcinol and HCA

We have further isolated from rinds of Garcinia sp. fruit, the source of HCA, a compound known as polyisoprenylated benzophenone, garcinol. Garcinol exhibits anti-oxidant, chemoprotective and antibacterial properties (**27**-**29**). Administration of garcinol and HCA to two strains of mice, SKH-1 and CF-1, respectively, resulted in significantly less total body weight and abdominal fat gain, as compared to control, chow-receiving animals and the groups of animals receiving either garcinol or HCA alone (V. Badmaev, personal communication). In addition, dietary administration

of garcinol caused significant reduction in aberrant colonic crypt formation (ACF) in CF-1 mice as compared to the animals fed control diet or diet containing HCA or garcinol alone. Interestingly, the weight-gain preventive effect of the garcinol and HCA combination was accomplished despite the fact that the garcinol plus HCA animals had higher food and water consumption than the control, garcinol and HCA groups.

The combination consisting of 500 mg of the calcium salt of HCA and 25 mg of garcinol (Garcitrin®) was evaluated in a double-blind, 12 weeks clinical study against the formula containing 500 mg of calcium salt HCA (Citrin®). The study was performed on 46 overweight female volunteers (BMI greater than 25 kg/m^2). Participants were instructed to take one capsule of either Garcitrin or Citrin formula three times a day, half an hour before a meal. Participants were asked to maintain their previous daily physical exercise and eating habits. In addition, physical activity was monitored based on a questionnaire before and during the trial. The 12 weeks trial, the mean values in Garcitrin group for body weight and fat content significantly decreased whereas LBM and total body water significantly increased as compared to Citrin group ($p \leq 0.05$). The appetite levels were significantly less in the Garcitrin group than in the Citrin group, ($p \leq 0.05$), whereas energy levels were equally increased in both study groups as compared to the baseline. No subjective or objective adverse effects were reported in the course of this study. The Garcitrin was approximately 30% more effective in body weight management than the Citrin (Badmaev V, personal communication). HCA has received the self-affirmed status as a US FDA recognized GRAS food supplement in the US (**30**).

Diterpene forskolin

Coleus forskohlii (*Fam*. *Labiatae*) is a mint family plant that is native to India. The roots of C. forskohlii traditionally have been used as a pickle and spice and gained prominence as a unique source of diterpene forskolin. Forskolin is known from literature as a compound with versatile biological actions based on its ability to generate adenylate cyclase, an enzyme that splits a high energy molecule of ATP to yield cyclic AMP (cAMP). AMP, a secondary messenger, is part of signal transduction cascade for a primary messenger, i.e. a hormone or a bioactive substance. The secondary messenger facilitates the action of primary messenger. The attachment of a hormone to the receptor changes the receptor and causes it to expose a binding site for a G-protein coupling. The activated G-protein or 'transducer' generates a cAMP to help express biological action of a hormone or biological molecule. cAMP may facilitate food induced thermogenesis and enhance body metabolism, and theoretically provide a mechanism(s) for preserving LBM and promoting healthy body composition.

In Hilton Head study, ForsLean was tested in an open-field protocol for changes in weight loss and LBM (**31**). Six overweight (BMI $\geq$ 25 kg/m^2), but otherwise healthy, women were selected for the trial. ForsLean was prepared in the form of two-piece hard shell capsules. Each capsule contained 250 mg of the extract standardized for 10% forskolin. Participants were instructed to take one capsule in the morning and one in the evening, half an hour before a meal. Participants were asked to maintain their previous daily physical exercise and eating habits. In addition, physical activity was monitored based on a questionnaire before and during the trial. During the eight weeks trial the mean values for body weight, and fat content were significantly decreased, whereas LBM was significantly increased as compared to the baseline ($p \leq 0.05$). The eight-weeks therapy with 50 mg (500 mg 10% extract) of forskolin per day did not produce side effects or adversely affect the systolic/diastolic blood pressure or the pulse rate.

A 12-weeks randomized, double blind, placebo controlled clinical study of Forslean was performed to evaluate forskolin's effect on body composition, testosterone, metabolic rate, and blood pressure in overweight and obese (BMI $\geq$ 26 kg/m^2) young men (**32**). Thirty subjects were investigated in a randomized, double blind, and placebo-controlled study for a period of 12 weeks receiving 250 mg of Forslean twice a day. Forskolin was shown to elicit favorable changes in body composition as determined by DEXA compared with the placebo group ($p \leq 0.05$). The study showed a significant total increase in LBM, including an increase in bone mass in the ForsLean group. Results also showed a significant decrease in body fat. The placebo group showed no statistically significant change in LBM, bone mass or body fat. Serum free testosterone levels were significantly increased in the forskolin group compared with the placebo group (≤ 0.05). The results indicate that forskolin is a possible therapeutic agent for the management and treatment of obesity. No statistically significant changes were reported in blood pressure or blood chemistry and no subjective or objective adverse effects were reported. To date eight clinical studies were performed with 10% standardized extract of *Coleus forskohlii* in the form of Forslean, which cumulative experience confirms the usefulness of Forslean as a safe and effective body weight management (**31-38**). In the course of the studies there were no serious side effects reported either by study participants or by study supervising health professionals. No clinically significant interactions were seen in metabolic markers, blood lipids, muscle and liver enzymes, electrolytes, red cells, white cells, hormones (insulin, TSH, T3, and T4), heart rate, blood pressure, or weekly reports of side effects. Interestingly, the Forslean regimen may increase the serum HDL levels and significantly decrease total cholesterol/HDL ratio as compared to the control group.

Piperine

Regulation and enhancement of the nutrient and drug delivery to targeted tissues and cells (bioavailability) has become important in preventive health care, especially in aging organism with dwindling abilities of the gastrointestinal tract to absorb micronutrients from food and food supplements. An emerging new absorption and bioavailability enhancer is piperine, a pungent principle from fruits of black pepper, *Piper nigrum* (*fam*. *Piperaceae*). The addition of 5 mg of extract of black pepper (extract from black pepper fruits standardized for 95% alkaloid piperine) per serving is aimed at increased absorption and bioavailability of active principles of the BWMF formula. The nutritional application of piperine is characterized by use of low dose piperine in enhancing nutrient absorption (**39**-**42**). The "low dose" is defined as under 20 mg of piperine per person per day administered concomitantly with a supplemented nutrient. Piperine in low dose has been shown to increase absorption of diversified nutrients including the fat soluble beta carotene, water soluble vitamin B6, vitamin C, coenzyme Q10, the mineral selenium in form of L-selenomethionine and botanical phenolics (**39**-**42**).

Appendix II Supplemental data sheet

Body composition was monitored using number of derived parameters encompassing Total Body Water (TBW), Basal Metabolic Rate (BMR), Body Mass Index (BMI), Waist to Hip Ratio (WHR), Body Fat percentage (BF%) and LBM percentage (LBM%).

The weight (kg) of the subjects was measured using a standard weighing scale at each visit; the height (cm) was measured at the baseline visit. The following formulae were used to calculate the values. An excel sheet with the following formulae entered into the sheet was provided at the site. At each visit, the study coordinator entered these values to obtain results of the body composition parameters:

1	BF% (by Deurenberg Formula): (For males, the value is 1 and for females, 0)	Adult BF% = (1.20 x BMI) + (0.23 x age in years) - (10.8 x gender) - 5.4
2.	TBW (by Watson's Formula):	Male TBW = 2.447 - (0.09156 x age) + (0.1074 x height) + (0.3362 x weight) Female TBW = -2.097 + (0.1069 x height) + (0.2466 x weight)
3.	BMR (by Harris-Benedict Formula):	Men: BMR = 66 + (13.7 x weight) + (5 x height) - (6.8 x age) Women: BMR = 655 + (9.6 x weight) + (1.8 x height) - (4.7 x age)
4.	BMI: (or Quetelet index)	$BMI = \frac{weight}{(height)^2}$
5.	LBM%	LBM%=100-BF%

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