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Effects of tea combined with high-protein meal replacement shakes on anthropometric measurements, lipid profiles, cellular biochemistry, neurochemistry, and microbial metabolism: a prospective observational study

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Abstract

Objective: The purpose of this study was to report preliminary data on the effects of tea and high-protein meal replacement shakes on weight loss, waist-to-hip ratios, and lipid profiles in healthy subjects. Secondary analyses of urine samples assessed pre-post changes in cellular biochemistry, neurochemistry, and microbial metabolism.

Methods: This study used a pre-post intervention design without a control group. Thirty healthy subjects (20-60 years of age; 23 women and 7 men) participated in a 28-day diet intervention program consisting of a cleansing day and 6 restricted diet days per week. On cleansing days, the subjects drank 4 oz of tea 4 times per day with a recommendation to drink at least 64 oz of filtered water. On the restricted diet days, the subjects drank 2 high-protein meal replacement shakes, consumed one 400- to 600-cal (1674.3-2511.5 joules) meal consisting of low–glycemic index foods, and drank at least 64 oz of filtered water.

Results: Multiple paired *t* tests detected reductions in weight (6.4 lb), waist (1.9 in), and hip (1.1 in) measurements and in total cholesterol (13.3 mg/dL) and low-density lipoprotein cholesterol (11.4 mg/dL) ($P < .05$). Multiple paired *t* tests detected significant increases in energy metabolism from carbohydrates and amino acids and concomitant increases in oxidative stress ($P < .05$).

Conclusion: The data support the concept that a low–glycemic load diet intervention incorporating tea and high-protein meal replacement shakes may cause weight loss and

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improve lipid profiles. The significant physiologic changes from the urine samples did not reflect meaningful metabolic effects.

Introduction

National statistics currently suggest that 33% to 36% of the US population is obese.[1] The emphasis of most weight management programs include energy (calorie) restriction and increased physical activity, with recent programs addressing the role of dietary macronutrients.[2-5] The outcomes of these studies indicate that calorie restriction is the critical element in weight management programs regardless of dietary macronutrients.[2-5] More importantly, evidence is emerging that diets emphasizing calorie restriction, without malnutrition of essential nutrients, are promoting healthy weight loss, improving lipid profiles and fasting insulin levels, inducing metabolic adaptations, and reducing oxidative stress.[2,5-7] Weight management programs that emphasize the use of meal replacement products to restrict calories and provide essential nutrients may promote clinically meaningful weight loss and improve health status.

Evidence is accumulating that a meal replacement strategy as part of a hypocaloric diet plan is a safe and effective intervention for weight loss, weight loss maintenance, and improvements in a number of health-related parameters.[8-11] However, accurate information about weight loss and safety among commercially available meal replacement products is still emerging.[8,12-14] Recommendations of safe, evidence-based commercial weight management programs are essential in the treatment of overweight and obese patients, as these programs can address time constraints, cost-effectiveness, and training of primary care providers with respect to providing effective nutritional intervention services for disease prevention.[13,15-17] Although sufficient evidence is lacking to support a causal relationship between increasing body mass index and low back pain or on the effectiveness of weight loss in reducing low back pain and increasing function, overweight and obese patients with low back pain are often advised by health care professional to lose weight.[16] Providing primary care providers with evidence-based weight loss options from commercially available products is beneficial to their clinical practices because they are easy to administer and supervise while simultaneously promoting health benefits, for example, reduction of cardiovascular disease risk factors or low back pain, and providing their patients with meal replacement options that fit their lifestyle, nutritional preferences or eating habits, and economic status.

Besides calorie restriction, many weight management programs include cleansing products. Many of these cleansing products include herbs, which may have beneficial effects on the microbial ecology of the gut by increasing motility and reducing transit time of waste materials through the gastrointestinal tract. The cleansing products also contain ingredients, such as fructo-oligosaccharide derivatives or various forms of galacto-oligosaccharides, that meet the criteria to be classified as prebiotics.[18] Recommendations for dietary interventions with probiotic and/or prebiotic nutritional components suggest that alleviating dysbiosis, an imbalance of intestinal bacteria and/or elevated levels of fungi, would restore the optimal microbial ecology of the gut.[18-20] Recent evidence is beginning to indicate a relationship between the microbial ecology of the gut and obesity.[20-22] Prebiotics may have significant health benefits on lipid metabolism, mineral absorption, and immune function via their beneficial influences on microbial ecology of the gut.[18,20,23,24] However, there are limited clinical data on the role of microflora management interventions on weight loss and improved health status.[25-27]

Reliable and valid methodologies to measure microbial ecology of the gut are essential to determine the effectiveness of microflora management interventions, that is, probiotics and prebiotics.[28] In clinical trials, microbiological and biochemical analyses of fecal materials are the accepted methodologies to measure the microbial ecology of the gut.[21,29-31] Multiple endoscopic biopsies during a colonoscopy are necessary to determine the mucosal microflora ecology of the different regions within the gut.[32] Based upon various disease conditions and theoretical models, the specificity and sensitivity of organic acid markers in urine samples to predict the presence of dysbiosis have been suggested.[33] Similarly, the levels of specific organic acids in urine samples have been suggested as markers of the inhibition of detoxification processes in the liver; and their presence in urine samples reflect the accumulation of exogenous and endogenous toxins remaining within the body, that is, harmful impurities.[34]

Prevention of weight gain and morbidity is important for adults who are normal weight, overweight, and

obese, with an added emphasis on weight loss and weight loss maintenance for overweight and obese adults.[8,35] The primary purpose of this study was to report preliminary data on the effects of a commercially available cleansing tea and high-protein meal replacement shakes on weight loss, waist-to-hip ratios, and lipid profiles in healthy subjects. The secondary purpose of the study was to assess changes in cellular biochemistry, neurochemistry, and microbial metabolism after the weight loss program using a commercially available urine analysis home kit.[33,34] Although the reliability and validity of the levels of specific organic acids in urine samples are based upon clinical observations and theoretical models, these secondary outcomes were exploratory; and the use of urine samples facilitated data collection and subject compliance as compared with using stool samples.

Methods

Study design

The study design was an observational, pre-post intervention, without a control group or blinding (n = 30). A sample size of 30 subjects was deemed appropriate based upon the assumption of normality and the central limit theorem underlying the mathematics of inferential statistics.[36] All 30 subjects received 2 bottles of cleansing tea (Cleanse for Life; Isagenix International, Chandler, AZ) and 2 containers of the high-protein meal replacement shakes (IsaLean Shake; Isagenix International) and followed the product directions for the 28-day diet intervention. Outcome assessments were performed at baseline (preintervention) and after the 28-day diet intervention (postintervention).

Study population

The Institutional Review Board of New York Chiropractic College approved this study. Thirty subjects were recruited from the faculty and staff at a professional school using a convenience sampling technique (20-60 years of age; 23 women and 7 men). All subjects provided written informed consent.

Exclusion criteria

Subjects who were pregnant, were nursing, had diabetes, were on medications, or had any other medical condition were excluded from the study. In addition, subjects were screened for high blood pressure at their first laboratory visit. Measurements of sitting blood pressure were from both arms using the standard clinic procedure of mercury sphygmomanometer with the Korotkoff sound technique according to the recommendations for blood pressure measurements in humans.[37] Subjects with high blood pressure were excluded from participation. The criteria used for high blood pressure was a systolic blood pressure of at least 140 mm Hg and/or diastolic blood pressure of at least 90 mm Hg.

Diet intervention

The 28-day diet intervention program consisted of a cleansing day—fasting with a "cleansing" weight loss tea—and 6 restricted diet days per week. Subjects were requested to refrain from alcohol consumption for the duration of the study period.

The cleansing days were days 1, 8, 15, and 22. On the cleansing days, subjects drank 4 oz of cleansing tea 4 times per day (40 cal per servings). Food was restricted to 1 egg or 1/4 cup of almonds and only if necessary to relieve hunger. Subjects were instructed to drink at least 64 oz of water, preferably filtered or spring water.

The restricted diet days were days 2 to 7, 9 to 14, 16 to 21, and 23 to 28. On the restricted diet days, subjects drank 2 high-protein shakes as replacements for 2 meals (200 cal per shake). The third meal was a 400- to 600-cal low–glycemic index meal from a set food list (eg, 4 oz of lean protein, chicken, or fish; 1 to 2 cups of fresh vegetables; no refined carbohydrates such as bread or pasta) with an emphasis on organic foods. Subjects were provided with a list of allowed foods to eat as meals and snacks and menu suggestions, which included only low–glycemic index foods. Subjects were encouraged to drink as much water as possible throughout the 28-day diet intervention.

Both adherence to the diet intervention and adverse events were monitored qualitatively using a weekly diary. Subjects were instructed to record food and beverage consumed during the study period as well as their adherence to product directions, for example, drank all of the required products—either 4 servings of tea or 2 shakes per day. The diaries were reviewed weekly by a trained clinician to address adverse effects and to continually monitor treatment integrity.

Data collection

Anthropometric measurements included body weight, height, body mass index (BMI), and waist and hip circumferences. Anthropometric assessments were conducted with the subjects wearing light clothing (gown and shorts) and no shoes. Weight was measured

using a high-precision digital scale (DI-10; DIGI Matex, Inc, Singapore; 0.1-kg weight gradations, 225-kg capacity). Height was self-reported by the subject. Body mass index was calculated from the body weight and height (kilograms per square meter), with self-reported height as a potential constant error impacting the validity of BMI. A standard cloth tape measure was used to record waist and hip circumference in inches. The clinician measured the circumferences of each site at least 3 times and averaged the 3 most consistent measurements to enhance reliability. Body landmarks of the umbilicus and greater trochanters were used to standardize measurements of pre-post waist and hip circumferences, respectively, to enhance reliability and validity. The waist-to-hip ratio was calculated from measurements of waist and hip circumferences.

A fasting lipid profile was obtained from each subject pre-post diet intervention. Professional services were obtained from a local hospital to perform the blood draws and analyze the venous plasma samples. Venous plasma (lithium heparin) was collected by standard venipuncture technique from the antecubital vein. Venous plasma samples were analyzed using routine clinical chemistry methods to measure total cholesterol, low-density lipoproteins (LDL cholesterol), high-density lipoproteins (HDL cholesterol), triglycerides, and glucose.

Cellular biochemistry, neurochemistry, and microbial metabolism

Biochemical analyses of first morning urine pre-post diet intervention were performed (test name: 0091-Organix Comprehensive Profile via LC/MS-MS, Spectrophotometry; Metametrix, Inc, Duluth, GA). A total of 45 urine markers provided indices of fatty acid metabolism, carbohydrate metabolism, energy production (Krebs cycle intermediates), B-complex vitamin markers, methylation cofactor markers, neurotransmitter metabolism markers, oxidative damage and antioxidant markers, detoxification indictors, bacteria-general, *Lactobacillus acidophilus*/general bacteria, *Clostridial* species, and yeast/fungus, that is, data entered from the report of Organix Comprehensive Profile.

Urine samples were collected in accordance with instructions provided by Metametrix Clinical Laboratory. In brief, subjects were instructed to void (empty bladder) before going to bed and then place a collection basin over their home toilets. Subjects used a pipette to place 12 mL of their first morning sample plus any overnight sample into a test tube. At the laboratory, urine samples were frozen and then shipped to Metametrix Clinical Laboratory for biochemical analyses. Subjects were instructed to restrict their fluid intake to three 8-oz glasses or less for 24 hours before collecting their urine sample to avoid excessive dilution of the urine. Of this total 24 oz, the subjects were instructed to restrict intake to no more than 8 oz consumed after 8:00 PM. Female subjects started the 28-day diet intervention at the midpoint of their menstrual cycle to avoid collecting urine during menstruation.

Statistical analyses

Pre-post intervention data from the anthropometric measurements, lipid profiles, and urine profiles were compared using paired t tests for each dependent variable. There was no correction for multiple dependent variables, experiment-wise error rate, as the primary purpose of this observational study was hypothesis generation for future randomized control trials with blinding to compare the effects of various commercially available meal replacement products on weight loss and lipid profiles. The usefulness of the levels of specific organic acids in urine samples to assess metabolic processes in future studies was exploratory, and no correction for the experiment-wise error was performed. Treatment integrity and adverse events were described qualitatively from the weekly diaries. Intention-to-treat analyses were used to account for subjects that withdrew from participation, that is, dropouts. For the primary outcome measures, all paired t test results were confirmed by the Wilcoxon signed rank test. The level of significance was .05 for each statistical procedure.

Results

Study population

During a period of 1 month, we recruited 34 subjects to participate in the research study. Four of these subjects were excluded for having high blood pressure. The remaining 30 patients were enrolled into the research study and began the study. One of these subjects withdrew from the research study because of an adverse reaction to the first consumption of the cleansing tea, which included hives, nausea, and vomiting. Twenty-nine subjects successfully completed the study protocol. Dependent variables were analyzed using the intention-to-treat procedure, which assumed the same pre-post values for the one subject who withdrew ($n = 30$, $df = 29$).

Allocated treatment

All subjects were assigned to receive the cleansing tea combined with high-protein meal replacement

shakes. Subjects only complied with the study protocol of 2 shakes and 1 small meal on the restricted diet days or only consuming 4 tea drinks on the cleanse days at a frequency of 21%. However, on the majority of restricted diet days and cleanse days at a frequency of 56%, the subjects only deviated from the study protocol by consuming the allowed snacks to alleviate hunger. Five of the subjects reported that they were unable to drink the tea 4 times per day and only drank the tea 3 times per day on the 4 cleansing days. At a frequency of 23%, subjects failed to consume only the low-glycemic foods on the allowed list on the restricted diet days or ate meals on the cleansing days. All subjects reported that they drank plenty of filtered or spring water, at least 64 oz on both the cleansing days and restricted diet days. Overall, the compliance revealed that eating the calorie-restricted diet of low–glycemic index foods was the most difficult aspect of the diet plan because, 21% of the time, subjects followed the study protocol and, another 56% of the time, the subjects only deviated from the study protocol to eat the allowed snacks. However, at a frequency of 23% for both the diet-restricted days and cleanse days, subjects ate nonallowed foods to alleviate hunger.

Anthropometric measurements

Body weight, waist and hip measurements, waist-to-hip ratio, and BMI were significantly reduced pre-post diet intervention (Table 1, $P < .05$). Weight loss averaged 6.4 ± 4.34 lb with the 95% confidence interval ranging from 4.7 to 8.0 lb. In addition, the amount of weight loss was independent of initial body weight ($r = .26$). At baseline, the range for BMI was from 21.4 to 35.9 kg/m^2, with one outlier at 45.8 kg/m^2. The frequency distributions of BMI at baseline were 11 normal weight (18.5-24.9 kg/m^2), 8 overweight (25.0-29.9 kg/m^2), and 11 obese adults (≥30.0 kg/m^2). The significant change in BMI of 1.0 ± 0.68 kg/m^2 with the 95% confidence interval ranging from 0.77 to 1.28 was consistent with the weight loss data ($P < .05$). Waist-to-hip ratio significantly changed from 0.85 ± .093 to 0.83 ± .090 pre-post diet intervention ($P < .05$).

Lipid profiles

The HDL cholesterol and triglycerides were not significantly changed after the 28-day diet intervention, but these values were normative preintervention. However, total cholesterol and LDL cholesterol were significantly reduced by 13.3 and 11.4 mg/dL, respectively, pre-post diet intervention (Table 2, $P < .05$).

Serum glucose levels

There was no significant change in fasting blood glucose pre-post diet intervention (87.6 ± 8.53 to 86.7 ± 8.61 mg/dL, Table 2, $P > .05$). Fasting blood glucose was in the reference range at both time points.

Cellular biochemistry

Cellular biochemistry was evaluated by measuring Krebs cycle intermediates and markers of fatty acid metabolism, carbohydrate metabolism, and amino acid metabolism.

Krebs cycle intermediates

There were significant increases in the Krebs cycle intermediates (Table 3, $P < .05$) except for α-ketoglutarate and hydroxymethylglutarate pre-post diet intervention (Table 3, $P > .05$).

Table 1 Subject characteristics and anthropometric measurements

	Baseline	Postprogram	Difference	t Value ($df = 29$)
Blood pressure (mm Hg)				
Systolic	117.0 ± 11.10	–	N/A	N/A
Diastolic	77.4 ± 8.73	–	N/A	N/A
Body weight (lb)	175.9 ± 36.77 (132.0-266.0)	169.5 ± 35.85 (131.0-263.0)	−6.4	8.01 *
Height (in)	66.2 ± 3.2	66.2 ± 3.2	N/A	N/A
BMI (kg/m^2)	28.2 ± 5.31 (21.4-45.8)	27.2 ± 5.13 (21.0-44.0)	−1.0	8.25 *
Waist circumference (in)	36.3 ± 5.80 (26.0-48.0)	34.4 ± 5.72 (26.0-48.0)	−1.9	7.74 *
Hip circumference (in)	42.7 ± 4.13 (37.0-55.0)	41.6 ± 4.15 (36.5-54.5)	−1.1	5.01 *
Waist-to-hip ratio [a]	0.85 ± 0.093 (0.65-1.11)	0.83 ± 0.090 (0.68-1.08)	−0.02	4.57 *

Ranges in parentheses.
[a] Mean ± SD of 7 men and 23 women.
* $P < .05$.

Table 2 Fasting blood glucose levels and lipid profile data

	Baseline	Postprogram	Difference	*t* Value (*df* = 29)
Glucose (mg/dL)	87.6 ± 8.53 (72.0-115.0)	86.7 ± 8.61 (70.0-114.0)	−0.9	0.72
Total cholesterol (mg/dL)	192.8 ± 35.58 (133.0-278.0)	179.5 ± 36.66 (114.0-278.0)	−13.3	4.11 *
HDL (mg/dL)	59.4 ± 16.05 (35.0-93.0)	58.6 ± 15.58 (38.0-99.0)	−0.8	0.67
TAG (mg/dL)	98.2 ± 54.65 (40.0-229.0)	92.9 ± 43.34 (46.0-212.0)	−5.3	1.03
LDL (mg/dL)	113.8 ± 29.63 (65.0-184.0)	102.4 ± 28.82 (54.0-189.0)	−11.4	3.65 *

Ranges in parentheses. *TAG*, triacylglycerols.
* $P < .05$.

Markers of fatty acid metabolism

On average, all markers of fatty acid metabolism were within normal limits at the onset of the study and did not change following the 28-day diet intervention (Table 4, $P > .05$).

Markers of carbohydrate metabolism

There was a 26% decrease in pyruvate concentration from 2.7 ± 2.13 to 2.0 ± 1.81 μg/mg creatinine pre-post diet intervention (Table 4, $P < .05$). There were no significant differences in the production of lactate or β-hydroxybutyrate pre-post diet intervention (Table 4, $P > .05$).

Markers of amino acid metabolism

Measurements of organic acids produced in pathways of amino acid metabolism included markers of B-complex vitamins and markers of methylation factors (Table 5). There were significant decreases in levels of α-ketoisovalerate and α-ketoisocaproate pre-post diet intervention (Table 5, $P < .05$). Pre-post diet intervention, there were significant decreases in levels of methylmalonate, an intermediate in the metabolism of valine, and significant increases in levels of formiminoglutamate, an intermediate in the metabolism of histidine (Table 5, $P < .05$).

Oxidative damage and antioxidant markers

Pre-post diet intervention, there was a significant increase in 8-hydroxy-2-deoxyguanosine concentration (Table 6, $P < .05$). *p*-Hydroxyphenyllactate was unchanged pre-post diet intervention (Table 6, $P > .05$).

Detoxification indicators

The data on detoxification markers are in Table 6. There were no significant changes in 2-methylhippurate, glucarate, or α-hydroxybutyrate concentrations pre-post diet intervention ($P > .05$). Pre-post diet intervention, orotate significantly increased ($P < .05$), pyroglutamate significantly decreased ($P < .05$), and sulfate did not change ($P > .05$).

Neurochemistry

There were significant increases in levels of vanilmandelate and 5-hydroxyindole acetate and a significant decrease in the level of homovanillate pre-post diet intervention (Table 7, $P < .05$).

Microbial metabolism

On average, all markers of intestinal bacteria and microbes were within normal limits at the onset of the

Table 3 Markers of cellular biochemistry: Krebs cycle intermediates

Krebs cycle intermediates	Baseline	Postprogram	Difference	*t* Value (*df* = 29)
Citrate (μg/mg creatinine)	468.2 ± 181.79	552.3 ± 218.99	84.1	3.51 *
cis-Aconitate (μg/mg creatinine)	50.8 ± 12.36	55.4 ± 11.45	4.6	2.27 *
Isocitrate (μg/mg creatinine)	59.2 ± 14.07	68.8 ± 14.25	9.6	4.44 *
α-Ketoglutarate (μg/mg creatinine)	16.4 ± 8.25	16.0 ± 10.98	−0.4	0.27
Succinate (μg/mg creatinine)	7.1 ± 4.95	9.5 ± 5.09	2.4	2.59 *
Fumarate (μg/mg creatinine)	0.4 ± 0.39	0.6 ± 0.42	0.2	2.67 *
Malate (μg/mg creatinine)	1.1 ± 0.71	1.5 ± 0.91	0.4	3.07 *
Hydroxymethylglutarate (μg/mg creatinine)	4.5 ± 1.61	4.5 ± 1.53	0.0	0.01

* $P < .05$.

Table 4 Markers of cellular biochemistry: fatty acid metabolism and carbohydrate metabolism

	Baseline	Postprogram	Difference	*t* Value (*df* = 29)
Fatty acid metabolism				
Adipate (μg/mg creatinine)	3.6 ± 1.60	3.8 ± 2.50	0.2	.054
Suberate (μg/mg creatinine)	1.3 ± 1.38	1.0 ± 0.62	−0.3	1.06
Ethylmalonate (μg/mg creatinine)	3.9 ± 1.21	4.1 ± 1.38	0.2	1.64
Carbohydrate metabolism				
Pyruvate (μg/mg creatinine)	2.7 ± 2.13	2.0 ± 1.81	−0.7	2.44 *
Lactate (μg/mg creatinine)	8.3 ± 4.98	7.5 ± 4.30	−0.8	0.92
β-Hydroxybutarate (μg/mg creatinine)	5.9 ± 16.99	3.7 ± 11.05	−2.2	1.03

* $P < .05$.

study and did not change after the 28-day diet intervention (Table 8, $P > .05$).

Adverse effects

As described above, one patient withdrew from participation because of an adverse reaction to the cleansing tea. On 36% of the cleanse days, subjects reported headaches, with another 4% reporting headache with lightheadedness and another 4% reporting lightheadedness only. On 56% of the cleansing days, subjects reported no adverse effects. Across the 28 days in the diet plan, subjects reported no adverse effects at a frequency of 80%. Besides headaches on the cleanse days, hunger, fatigue, mood changes, hot flashes, and diarrhea/constipation were listed as adverse effects that occurred at frequency of 20% during the 28-day diet plan. All reported adverse reactions and effects resolved after resuming normal dietary habits without any long-term adverse effects.

Discussion

The data support the concept that a low–glycemic load diet intervention incorporating a cleansing tea and high-protein meal replacement shakes facilitates the body's ability to safely lose weight while promoting improvements in lipid profiles. As discussed below, clinically meaningful differences occurred for weight loss, total cholesterol, and LDL cholesterol, which suggest that these variables should be used as primary outcomes in future studies on weight management programs. Adverse effects were tolerable, and adherence to the 28-day diet intervention was acceptable. However, beneficial physiologic adaptations that may underlie the improvements in weight status with a low–glycemic load diet intervention incorporating a cleansing tea and high-protein meal replacement shakes require additional research. Significant changes in the levels of specific organic acids in urine samples did not reflect meaningful metabolic effects because all values were within normal limits pre-post intervention. Thus, the usefulness of analyzing urine samples as reliable, valid, sensitive, or specific physiologic markers of energy metabolism changes with dietary modifications remains questionable.

The subjects experienced a healthy weight loss of 6.4 lb, on average, over a 28-day period, which was in agreement with the Department of Health and Human Services guidelines of 1 to 2 lb per week.[38] On average, decreases of 2 in around the waist and 1 in around the hips accompanied the weight loss. The maximum weight loss was 18 lb, with the 95% confidence interval between 4.7 and 8.0 lb; and the maximum inches lost around waist was 4.8 in, with the

Table 5 Markers of cellular biochemistry: amino acid metabolism

	Baseline	Postprogram	Difference	*t* Value (*df* = 29)
B-complex vitamins				
α-Ketoisovalerate (μg/mg creatinine)	0.5 ± 0.17	0.4 ± 0.15	−0.1	2.74 *
α-Ketoisocaproate (μg/mg creatinine)	0.4 ± 0.22	0.3 ± 0.09	−0.1	2.54 *
α-Keto-β-methylvalarate (μg/mg creatinine)	1.0 ± 0.53	1.0 ± 0.41	0.0	0.73
Xanthurenate (μg/mg creatinine)	0.5 ± 0.21	0.5 ± 0.22	0.0	0.55
β-Hydroxyisovalerate (μg/mg creatinine)	5.5 ± 1.59	6.2 ± 2.18	0.7	2.48 *
Methylation factors				
Methylmalonate (μg/mg creatinine)	2.0 ± 0.67	1.7 ± 0.72	−0.3	2.55 *
Formiminoglutamate(μg/mg creatinine)	0.6 ± 0.24	1.0 ± 0.56	0.4	3.90 *

* $P < .05$.

Table 6 Markers of cellular biochemistry: oxidative damage and antioxidants and detoxification

	Baseline	Postprogram	Difference	*t* Value (*df* = 29)
Oxidative damage and antioxidants				
p-Hydroxyphenyllactate (μg/mg creatinine)	0.4 ± 0.36	0.4 ± 0.33	0.0	0.00
8-Hydroxy-2-deoxyguanosine (ng/mg creatinine)	3.3 ± 1.31	3.8 ± 1.14	0.5	2.53 *
Detoxification markers				
2-Methylhippurate (μg/mg creatinine)	0.0 ± 0.11	0.0 ± 0.01	0.0	1.07
Orotate (μg/mg creatinine)	0.5 ± 0.20	0.6 ± 0.27	0.1	4.23 *
Glucarate (μg/mg creatinine)	5.2 ± 1.23	5.7 ± 1.21	0.5	1.89
α-Hydroxybutyrate (μg/mg creatinine)	0.8 ± 0.78	0.9 ± 1.03	0.1	0.28
Pyroglutamate (μg/mg creatinine)	61.1 ± 33.06	29.7 ± 10.15	−31.4	5.00 *
Sulfate (μg/mg creatinine)	231.4 ± 72.22	238.0 ± 69.40	6.6	0.47

* $P < .05$.

95% confidence interval between 1.4 and 2.4 in. Decreases in BMI and waist-to-hip ratio were other indices of improvements in weight status with the low–glycemic load diet intervention. Body mass index decreased from 28.2 ± 5.31 at baseline to 27.2 ± 5.13 kg/m^2 after the 28-day diet intervention. Although BMI significantly decreased by 1.0 kg/m^2 after the 28-day diet intervention, the subjects remained in the overweight category pre-post intervention. The recommended waist-to-hip ratios, which are associated with a decreased risk for cardiovascular disease, are less than 0.83 for women and less than 0.90 for men.[39] Similar to BMI, pre-post changes in the waist-to-hip ratios were not clinically meaningful because the women in the study were not at risk for cardiovascular disease (<0.83) and men remained in the at-risk category (>0.90) throughout the study period.

Besides weight status, the observational study included the measurement of lipid profiles as markers of cardiovascular health. Normative values from the National Cholesterol Education Program were used to define clinically meaningful differences in cholesterol levels.[40] Although HDL cholesterol and triglycerides were not impacted by the 28-day diet intervention, total cholesterol and LDL cholesterol were significantly decreased. The average HDL cholesterol of the study sample was approximately 60 mg/dL, which is at the upper limit of normal for this "good" cholesterol. In addition, weight loss achieved through exercise is more effective at raising HDL cholesterol levels than dieting.[41] Similarly, triglycerides were in the midrange of normal (98.2-92.9 mg/dL, pre-post intervention), with exercise, independent of weight loss and dietary changes, being an effective sole intervention for decreasing triglycerides.[42,43] Total cholesterol of 192.8 mg/dL approached borderline high at baseline among our study sample. After the 28-day diet intervention, the 13.3-mg/dL decrease in total cholesterol was statistically and clinically meaningful, as a value of 179.5 mg/dL reflects a more favorable lipid profile. The statistically significant decrease of 11.4 mg/dL in LDL cholesterol from 113.8 mg/dL at baseline to 102.4 mg/dL after the 28-day diet intervention was clinically meaningful, as LDL cholesterol, postintervention, approached the optimal category. Our results substantiate the concept that weight loss from a calorie-restricted, low–glycemic load diet is an effective method for promoting improvements in total cholesterol and LDL cholesterol levels.[44]

Evidence is beginning to emerge that a broad range of healthy diets with different dietary composition, but emphasizing calorie restriction, are successful for promoting improvements in weight status, metabolism, and cardiovascular health.[2-5] Calorie restriction, without malnutrition of essential nutrients, may also improve mitochondria efficiency and reduce oxidative stress in association with decreases in DNA damage and 24-hour energy expenditure.[6,7,12,45] As shown previously for other commercially available meal

Table 7 Markers of neurochemistry

Neurotransmitter metabolism	Baseline	Postprogram	Difference	*t* Value (*df* = 29)
Vanilmandelate (μg/mg creatinine)	2.1 ± 0.55	2.5 ± 0.77	0.4	3.35 *
Homovanillate (μg/mg creatinine)	3.7 ± 1.10	2.9 ± 1.99	−0.8	2.21 *
5-Hydroxyindole acetate (μg/mg creatinine)	2.8 ± 3.49	6.5 ± 10.76	3.7	2.37 *
Kynurenate (μg/mg creatinine)	1.1 ± 0.40	1.1 ± 0.48	0.0	0.72
Quinolinate (μg/mg creatinine)	5.8 ± 1.54	5.9 ± 1.72	0.1	0.07

* $P < .05$.

Table 8 Bacteria and other microbes

	Baseline	Postprogram	Difference	*t* Value (*df* = 29)
Benzoate (μg/mg creatinine)	1.5 ± 1.90	2.2 ± 3.61	0.7	0.97
Hippurate (μg/mg creatinine)	321.0 ± 333.09	250.2 ± 208.81	−70.8	1.20
Phenylacetate (μg/mg creatinine)	0.1 ± 0.27	0.1 ± 0.00	0.0	1.00
Phenylpropionate (μg/mg creatinine)	0.5 ± 0.00	0.5 ± 0.00	0.0	N/A
p-Hydroxybenzoate (μg/mg creatinine)	0.3 ± 0.33	0.4 ± 0.34	0.1	0.58
p-Hydroxyphenylacetate (μg/mg creatinine)	14.7 ± 8.89	11.9 ± 9.41	−2.8	1.32
Indican (μg/mg creatinine)	52.0 ± 35.74	46.6 ± 22.26	−5.4	0.78
Tricarballylate (μg/mg creatinine)	0.9 ± 0.30	0.8 ± 0.54	−0.1	0.70
L acidophilus (μg/mg creatinine)	1.8 ± 1.59	1.2 ± 1.15	−0.6	1.98
Clostridial species (μg/mg creatinine)	0.2 ± 0.01	0.2 ± 0.18	0.0	1.21
Yeast/fungus (μg/mg creatinine)	25.1 ± 11.40	36.1 ± 46.87	11.0	1.47

replacement products, for example, Medifast (Owings Mills, MD),[12] NutriSystem (Fort Washington, PA),[13] Healthy Solutions (Health Management Resources® Corporation (HMR), Boston, MA),[14] Standard Process (Palmyra, WI),[15] and Ultra-Slim Fast (Unilever Bestfoods, Englewood Cliffs, NJ),[46] the Isagenix 28-day program is an effective and safe weight loss program. Increasing the number of evidence-based weight management interventions available to clinicians for disease prevention is important to address the individualized eating habits of patients and the constraints of time, knowledge, and costs of the providing clinicians. Adherence to the diet plan is the critical factor for weight loss, weight maintenance, and health benefits with evidence-based commercially available programs and products requiring minimal professional intervention.

Besides calorie restriction, the 28-day diet intervention included a cleansing tea. All markers of intestinal bacteria and microbes from the urine samples were within normal limits at the onset of the study and did not change after the 28-day diet intervention.[33] As the reliability and validity of organic acid markers in urine samples to predict the presence of dysbiosis are still questionable, microbiological and biochemical analyses of fecal materials are still the only the accepted methodologies to measure the microbial ecology of the gut in clinical trials on diet plans.[21,29-31]

Limitations

As an observational study, the lack of blinding, randomization, and a control group limits the generalizability of the results. Although baseline weight only accounted for approximately 7% of the variance in weight loss, the inclusion of normal-weight individuals may be a potential confounder. Subject selection bias limits the generalizability of the results. Although subjects achieved their adult heights, using self-reported height may be deemed a constant error in the calculation of BMI. Although the subjects indicated that they maintained their normal level of physical activity and exercise training from the beginning of the study to the end of study, not using a physical activity log to monitor adherence to this study protocol criterion may be a confounder.

Conclusion

The findings from this exploratory study support that a low–glycemic load diet intervention incorporating a cleansing tea and high-protein meal replacement shakes may facilitate weight loss while improving lipid profiles. The physiologic changes from the urine samples did not reflect meaningful metabolic effects.

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