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Nutrition Research 24 (2004) 45–58

NUTRITION RESEARCH

www.elsevier.com/locate/nutres

Efficacy of a novel, natural extract of (–)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX, niacin-bound chromium and *Gymnema sylvestre* extract in weight management in human volunteers: A pilot study

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Received 9 June 2003; received in revised form 18 September 2003; accepted 19 September 2003

Abstract

In this pilot study, the efficacy of a novel, natural extract of a highly bioavailable, calcium-potassium salt of (–)-hydroxycitric acid (HCA-SX) alone and in combination with a niacin-bound chromium (NBC) and *Gymnema sylvestra* extract (GSE) was evaluated for weight loss in moderately obese subjects by monitoring changes in body weight, body mass index (BMI), appetite, lipid profiles, serum leptin and serotonin levels, and enhanced excretion of urinary fat metabolites. *Garcinia cambogia*-derived (–)-hydroxycitric acid (HCA) has been shown to reduce appetite, inhibit fat synthesis and decrease body weight without stimulating the central nervous system. NBC has shown the ability to restore insulin function, metabolize fat, turn protein into muscle, and convert sugar into energy, which plays a role in appetite regulation and facilitates weight loss. *Gymnema sylvestre* is a traditional herb that helps to promote weight loss possibly through its ability to reduce cravings for sweets and control blood sugar levels. A randomized, double-blind, placebo-controlled human clinical study was conducted in thirty obese subjects (ages 21-50, BMI$>$26 kg/m^2) for eight weeks in Elluru, India. The subjects were randomly divided into three groups (10 subjects/group) and given HCA-SX 4,667 mg (60% HCA providing 2,800 mg HCA/day) (Group A), a combination of HCA-SX 4,667 mg, NBC 4 mg (providing 400 μg elemental Cr) and GSE 400 mg (providing 100 mg gymnemic acid) (Group B), or placebo (Group C) daily in 3 equally divided doses 30-60 min before each meal. This

0271-5317/04/$ – see front matter
doi:10.1016/j.nutres.2003.09.007

HCA-SX dose was extrapolated from previously conducted *in vitro* and *in vivo* studies. In addition, subjects received 2,000 kcal diet/day and underwent a 30 min/day supervised walking program, 5 days/week. At the end of 8 weeks, body weight and BMI decreased by 6.3%, respectively, in Group A. Food intake was reduced by 4%. Total cholesterol, LDL and triglycerides levels were reduced by 6.3%, 12.3% and 8.6%, respectively, while HDL and serotonin levels increased by 10.7% and 40%, respectively. Serum leptin levels were decreased by 36.6%, and the enhanced excretion of urinary fat metabolites, including malondialdehyde (MDA), acetaldehyde (ACT), formaldehyde (FA) and acetone (ACON), increased by 125-258%. Under these same conditions, Group B reduced body weight and BMI by 7.8% and 7.9%, respectively. Food intake was reduced by 14.1%. Total cholesterol, LDL and triglyceride levels were reduced by 9.1%, 17.9% and 18.1%, respectively, while HDL and serotonin levels increased by 20.7% and 50%, respectively. Serum leptin levels decreased by 40.5% and enhanced excretion of urinary fat metabolites increased by 146-281%. Group C reduced body weight and BMI by only 1.6% and 1.7%, respectively, food intake was increased by 2.8%, and LDL, triglycerides and total cholesterol decreased by 0.8%, 0.2% and 0.8%, respectively. HDL were reduced by 4.1% while serum leptin levels were increased by 0.3%, and excretion of urinary fat metabolites did not change in MDA, ACT and FA, and marginally increased in the case of ACON. No adverse effects were observed. Results demonstrate that HCA-SX and, to a greater degree, the combination of HCA-SX, NBC and GSE can serve as safe weight management supplements.

Keywords: Obesity; Lipids; Urinary metabolites; *Garcinia cambogia*; Niacin-bound chromium; *Gymnema sylvestre*

1. Introduction

Current statistics demonstrate that more than half of U.S. adults are overweight (61%), defined as having a body mass index (BMI) greater than 25 kg/m^{2,} while more than a quarter (26%) of U.S. adults are obese, having a BMI of greater than 30 kg/m^2 [1,2]. Sixty three percent of men and 55% of women are now overweight or obese in this country [3]. Providing an even worse outlook, national data indicate that 10.5-15.5% of children ages 6 through 19 are severely overweight [4]. According to the World Health Organization, there are over 300 million obese adults globally [5]. Low levels of physical activity and sedentary lifestyles have generally been implicated in the worldwide trend of weight gain [6].

Obesity, resulting from an imbalance between energy intake and expenditure, is the second leading cause of premature death in America. Other potential risks of obesity include cardiovascular diseases, diabetes, cancer and hormonal imbalances in women, leading to sterility [7]. Low caloric diets with and without exercise can help with temporary weight loss. Weight loss drugs that suppress appetite, reduce food intake, increase energy expenditure and/or affect nutrient partitioning or metabolism have potential efficacy but are unfortunately frequently accompanied by adverse side effects [8]. Therefore, supplementation with safe and natural products in addition to a healthy diet and exercise may be helpful.

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(–)-Hydroxycitric acid (HCA) has been reported to cause weight loss in humans without stimulating the central nervous system [9]. HCA is derived from the fruit rinds of *Garcinia cambogia*, which exhibits a distinctive sour taste and has been used for culinary purposes in Southern Asia for centuries to make meals more "filling", and has been reported to reduce food intake in experimental animals, suggesting its role in the treatment of obesity [10–14]. HCA is a competitive inhibitor of ATP-citrate lyase, an extra-mitochondrial enzyme involved in the initial steps of *de novo* lipogenesis [7,10–14]. Consequently, HCA reduces the transformation of citrate into acetyl coenzyme A, a step necessary for the formation of fatty acids in the liver. In addition, there is increased production of hepatic glycogen in the presence of HCA, which may activate glucoreceptors leading to a sensation of fullness and reduced appetite [12,15]. Earlier successful animal trials [13,14] suggest that the human dose of HCA typically recommended in dietary supplements and used in previous clinical trials (1,500 mg HCA/day) is sub-optimal. Several publications have reported the efficacy of HCA in weight management [16–20].

Acute oral, acute dermal, primary dermal, and primary eye irritation studies demonstrated the safety of HCA-SX [21]. The LD_{50} in rats was found to be greater than 5 gm/kg. HCA-SX bioavailability was found to be significantly higher in fasting individuals when consumed at least 30-60 min prior to food consumption [22].

Chromium is important for energy production and plays a role in regulating appetite. Administration of 600 μg elemental chromium as NBC (ChromeMate) in two divided doses daily over a period of 2 months to African-American women with a moderate diet and exercise regimen influenced weight and fat loss and sparing of muscle and body composition [23]. Their blood chemistries revealed no significant adverse effects [23]. Another study at the University of Texas found that young obese women consuming 400 μg of elemental chromium as NBC per day with exercise experienced significant weight loss over an eight week period. Insulin response to an oral glucose load was also lowered in the obese subjects, and no adverse effects were observed [24]. Preuss *et al.* [25] conducted a long term study for 12 months in Fischer F344/BN rats using a chronic dose of 400 μg of elemental chromium per day and no adverse effects were observed in body and organ weights, and blood chemistries [25].

Gymnema helps to promote weight control by its ability to reduce the cravings for sweets and control blood sugar levels [26,27]. A peptide isolated from Gymnema, gurmarin, has also been shown to block the sweet taste of glucose and sucrose in animal models [27]. Gurmarin temporarily binds to the sweet and bitter receptors on the tongue, thereby blocking the taste sensation and reducing sweet cravings [27]. Preuss *et al.* [28] showed a significant lowering of cholesterol with *Gymnema sylvestre* ingestion in hypertensive rats fed a high sucrose diet, whereas the placebo group showed a significant increase in cholesterol levels. *Gymnema* is regarded as very safe and has been administered (400 mg/day) to patients with insulin-dependent diabetic mellitus (IDDM) for 10-12 months with no adverse side effects [29].

The effective dose of HCA-SX was determined by a previous *ex vivo* study on serotonin release from isolated rat brain cortex [21,30] and in *in vivo* studies [13,14]. Based on these studies the human equivalency dose of HCA-SX used in the present study was calculated to be 2,800 mg/day, which is significantly greater than the 1,500 mg/day that is normally recommended in dietary supplements [16].

The present study was designed to examine the efficacy of optimal doses of HCA-SX alone and in combination with NBC and GSE given on an empty stomach in thirty human volunteers. Effects of these supplements were investigated on body weight, BMI (an indicator of obesity health risk), appetite (as determined by weighing the remaining food), lipid profiles, serum leptin levels (a biomarker of obesity regulatory gene), serotonin levels, and excretion of urinary fat metabolites (a biomarker of fat oxidation).

2. Subjects and methods

2.1. Subjects

In this study conducted in Elluru, India, each subject was obese, ages 21-50 years, with a body mass index (BMI) ranging from 30.0 to 50.8 kg/m^2 (BMI requirement was greater than $26 > kg/m^2$). Additional inclusion criteria consisted of having a negative pregnancy test, possessing the ability to understand the risks/benefits of the protocol, willingness to participate in a 30 min supervised walking-exercise program (5 days a week), eat the vegetarian or non-vegetarian prescribed diets of approximately 2,000 kcal/day (17% protein, 25% fat, and 58% carbohydrate) divided into three meals, sign an informed consent form, complete a standard health questionnaire, and participate in 3 clinic visits at 0, 4, and 8 weeks. Subjects were excluded if they were pregnant or nursing, presently taking other weight loss medications, had a history of thyroid disease, cardiovascular disease, or diabetes, suffered from intractable obesity, had defined weight limits or had experienced any recent, unexplained weight loss or gain. Subjects were required to fast overnight, and blood and urine samples were obtained at each clinic visit in the early morning to avoid diurinal variation. An individual diary was maintained for each subject.

Advertisements were placed in local newspapers and overweight subjects who responded and met the inclusion criteria during a screening were scheduled for a baseline visit. The evaluation included a questionnaire, physical examination, electrocardiogram, and screening blood studies. Subjects were then randomized into three groups (10 subjects/group) with equal probability through a random number generator. An Institutional Review Board approval IRB #01-001 was obtained from ASR Academy of Medicinal Sciences for this study. All subjects gave written consent prior to participation.

2.2. Weight reduction protocol

A detailed evaluation was performed at the beginning, week four, and week eight of treatment. Bodyweight, BMI, appetite, lipid profile, serum leptin and serotonin levels and excretion of urinary fat metabolites were evaluated. The patients' diaries were checked on a daily basis.

Body weights of the subjects were measured using an Essae Digi (Model DS-410) digital weighing scale (Essae-Teraoka Pvt. Ltd., Bangalore, India). Height was measured using a Benson Track and Field height scale. BMI was calculated by body weight in kilograms divided by square of height in meters. Appetite reduction was estimated by weighing the

remaining food after each meal. Lipid profile, including high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL)W.S.William Seroy*via* HPLC in conjunction with GCMS using a selective ion monitoring technique as described by Shara et al. [31].

The following six key parameters were monitored in a randomized, double-blind, placebo-controlled study over a period of eight weeks: [1] To assess whether optimal doses of HCA-SX, and the combination of HCA-SX, NBC plus GSE (HCA-SX Formula) produces a greater reduction in body weight than placebo, [2] To evaluate whether HCA-SX and HCA-SX Formula produces a greater reduction in BMI than placebo, [3] To assess whether HCA-SX and HCA-SX Formula has an inhibitory effect on appetite as compared to placebo, [4] To assess whether HCA-SX and HCA-SX Formula produces a beneficial effect on lipid profile, including LDL, HDL, triglycerides, VLDL, and total cholesterol, as compared to placebo, [5] To assess whether HCA-SX and HCA-SX Formula has an inhibitory effect on serum leptin and serotonin levels compared to placebo, and [6] To evaluate whether HCA-SX and HCA-SX Formula causes fat oxidation as estimated by enhanced excretion of urinary fat metabolites, including malondialdehyde, acetaldehyde, formaldehyde, and acetone as compared to placebo.

Subjects were divided into three groups. Group A was given a daily dose of HCA-SX 4,667 mg (60% HCA providing 2,800 mg HCA per day), Group B was given a daily dose of a combination of HCA 4,667 mg, NBC 4 mg (400 μg elemental chromium) plus GSE 400 mg (100 mg gymnemic acid), and Group C was given a placebo (microcrystalline cellulose) in three equally divided doses 30-60 min before breakfast, lunch and dinner for eight weeks.

2.3. Study materials

A natural, highly bioavailable, water-soluble, tasteless and odorless calcium-potassium salt of 60% HCA extract from *Garcinia cambogia* commercially known as Super CitriMax (HCA-SX), niacin-bound chromium supplement commercially known as ChromeMate (containing 10% elemental chromium) and a standardized extract of *Gymnema sylvestre* extract commercially known as Gymnema (GSE, GYM-250) (containing 25% gymnemic acid) were obtained from InterHealth Nutraceuticals, Inc., (Benicia, CA). Unless stated otherwise, all other chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO) and were of analytical grade or the highest grade available.

2.4. Dose determination

Earlier animal studies by Sullivan et al [13,14] indicate that higher levels of HCA than those typically recommended in dietary supplements for human beings are required to produce consistent and reliable results. Levels of HCA-SX used in our study were determined by extrapolation of optional micromolar concentrations of HCA required to evoke peak levels of serotonin release in rat brain cortex study [21,30]. Considering a 5-fold faster metabolism in rats compared to humans, the *ex viso* dose extrapolates to a human dose of 2,800 mg of HCA per day, which approximates the human equivalent does used in the earlier animal studies conducted by Sullivan et al. [13,14].

Table 1
Effects of placebo (Group C), HCA-SX alone (Group A) and HCA-SX formula (Group B) on body weight, BMI, and serum leptin levels in human subjects.

Group	Time Point	Body Weight (kg)	BMI (kg/m^2)	Serum Leptin (ng/ml)	Serotonin (ng/ml)
Placebo (Group C)	I	87.39 ± 5.04^a	34.0 ± 1.42^a	34.60 ± 2.58^a	220.0 ± 17.09^a
	M	86.56 ± 5.00^a	33.7 ± 1.40^a	34.60 ± 2.58^a	239.0 ± 14.16^a
	F	86.00 ± 4.95^a	33.5 ± 1.34^a	34.70 ± 2.34^a	266.3 ± 15.31^b
HCA-SX (Group A)	I	88.50 ± 6.89^a	33.6 ± 1.97^a	45.40 ± 3.54^a	216.0 ± 23.55^b
	M	88.70 ± 6.70^b	32.6 ± 1.94^a	37.30 ± 3.90^b	265.0 ± 24.85^b
	F	83.00 ± 6.80^b	31.5 ± 1.99^b	28.80 ± 3.44^c	302.0 ± 26.74^c
HCA-SX Formula (Group B)	I	87.60 ± 5.12^a	34.1 ± 1.42^a	33.80 ± 3.39^a	243.0 ± 20.92^a
	M	84.00 ± 5.03^b	32.7 ± 1.46^b	27.20 ± 3.05^b	298.0 ± 21.05^b
	F	80.80 ± 4.93^c	31.4 ± 1.39^c	20.10 ± 2.50^c	365.0 ± 22.75^c

Data are presented as group mean ± SEM. Subjects were given placebo (Group C), HCA-SX alone (Group A) or HCA-SX formula (Group B) for 8 weeks. See Subjects and methods section for details. Values with non-identical superscripts are significantly different ($p < 0.05$).

2.5. Data analysis

Two-tailed Student's t test, with a level of 5% significance, was performed on all three groups for each variable to detect any significant changes. The data set that was analyzed had eleven variables of interest, which are body weight, body mass index (BMI), low density lipoproteins (LDL), high density lipoproteins (HDL), triglycerides, very low density lipoproteins (VLDL), total cholesterol, serum leptin, serotonin, enhanced excretion of urinary fat metabolites, and remaining food. [32–34].

In each group, longitudinal data was collected for 3 time points denoted by Initial (I), Middle (M) and Final (F) for the first 10 variables, and at 8 time points for the last variable, which is "remaining food".

To compare the differences at a 5% level of significance, we have differences for "I & M", "M & F" and "I & F" for the first 10 variables. For remaining food, since data was collected at 8 time points, there are 28 possible paired differences. Basic summary statistics and test for differences with respect to least square means, among the time points was conducted for each of the variables for each group at each respective timepoint. $P<0.05$ was considered statistically significant.

3. Results

The present clinical study on HCA reported that subjects taking higher, more optimal doses of a highly bioavailable form of HCA (HCA-SX) not only had a significant weight loss, but reduced food intake, increased fat oxidation, decreased LDL, triglycerides and total cholesterol, increased HDL levels, and decreased BMI compared to placebo. The study also demonstrated some surprising new results: high doses of HCA-SX significantly lowered serum leptin levels and increased serotonin levels as determined by our previous *in vitro* and

Table 2
Effects of placebo (Group C), HCA-SX alone (Group A) and HCA-SX formula (Group B) on appetite in human subjects. Remaining food in grams on the plate as an index of appetite suppression.

Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Placebo (Group C)	82.22 ± 35.22[a]	78.33 ± 27.91[a]	101.11 ± 35.92[a]	122.22 ± 57.80[a]	80.00 ± 32.49[a]	61.11 ± 31.20[a]	22.22 ± 16.90[b]	38.89 ± 23.24[a]
HCA-SX (Group A)	236.50 ± 51.87[a]	258.00 ± 80.85[a]	237.50 ± 40.46[a]	185.50 ± 40.56[a]	279.00 ± 84.61[a]	226.50 ± 56.05[a]	342.00 ± 60.34[b]	317.50 ± 24.42[b]
HCA-SX Formula (Group B)	218.50 ± 26.44[a]	216.00 ± 44.36[a]	252.50 ± 38.69[a]	372.50 ± 57.46[b]	344.00 ± 79.20[b]	418.00 ± 81.37[b]	388.00 ± 49.12[b]	505.00 ± 45.89[b]

Data are presented as group mean ± SEM. Subjects were given placebo (Group C), HCA-SX alone (Group A) or HCA-SX formula (Group B) for 8 weeks. See Subjects and methods section for details. Values with non-identical superscripts in each row are significantly different ($p < 0.05$).

Table 3
Effects of placebo (Group C), HCA-SX alone (Group A) and HCA-SX formula (Group B) on lipid profile in human subjects.

Group	Time Point	LDL (mg/dL)	HDL (mg/dL)	Triglycerides (mg/dL)	VLDL (mg/dL)	Total Cholesterol (mg/dL)
Placebo	I	126.00 ± 8.70[a]	30.00 ± 2.22[a]	140.00 ± 27.99[a]	31.00 ± 5.05[a]	186.60 ± 10.98[a]
(Group C)	M	124.00 ± 7.99[a]	30.00 ± 1.62[a]	138.00 ± 27.54[a]	29.00 ± 5.88[a]	182.90 ± 10.52[b]
	F	125.00 ± 8.00[a]	29.00 ± 1.63[a]	140.00 ± 26.46[a]	31.00 ± 5.86[a]	185.10 ± 10.07[a]
HCA-SX	I	106.00 ± 5.75[a]	30.70 ± 2.48[a]	153.50 ± 31.90[a]	33.00 ± 6.60[a]	169.50 ± 7.77[a]
(Group A)	M	102.00 ± 4.67[a]	30.80 ± 2.09[a]	147.20 ± 31.42[a]	32.00 ± 6.37[a]	164.80 ± 7.21[a]
	F	93.00 ± 5.15[b]	34.00 ± 2.23[b]	140.30 ± 31.32[b]	32.00 ± 5.91[a]	158.80 ± 6.17[b]
HCA-SX	F	112.00 ± 8.12[a]	29.00 ± 1.57[a]	98.00 ± 11.27[a]	26.00 ± 2.63[a]	166.30 ± 8.56[a]
Formula	M	103.00 ± 7.54[b]	31.00 ± 1.54[b]	84.00 ± 8.43[b]	26.00 ± 2.52[a]	160.20 ± 8.62[a]
(Group B)	F	92.00 ± 7.13[c]	34.00 ± 1.75[c]	81.00 ± 9.12[c]	25.00 ± 2.83[a]	151.10 ± 7.73[b]

Data presented as group mean ± SEM. Subjects were given placebo (Group C). HCA-SX alone (Group A) or HCA-SX formula (Group B) for 8 weeks. See Subjects and methods section for details. Values with non-identical superscripts are significantly different ($p < 0.05$).

in vivo animal studies [20,29,12,13]. The addition of NBC and GSE generally caused greater significant changes in all parameters measured.

Table 1 demonstrates the changes in body weight, BMI, serum leptin and serotonin following supplementation of placebo (Group C), HCA-SX (Group A) and HCA-SX Formula (Group B) over the period of eight weeks. There was a distinct change observed at the end of four weeks and eight weeks in both Group A and Group B. In Group C, approximately 0.83 and 1.39 kg reduction in body weights were observed at the end of four and eight weeks, respectively. Under the same conditions, approximately 2.8 and 5.5 kg reduction in body weights were observed in Group A, and 3.6 and 6.8 kg reduction in body weights were observed in the Group B, respectively, at the end of four and eight weeks. Thus, at the end of eight weeks, Group C only showed a reduction of 1.7% BMI while there were a 6.3% and 7.9% reduction in BMI observed in Group A and Group B, respectively.

Group C showed no change in serum leptin levels at the end of four weeks and a 0.3% increase in serum leptin levels at the end of eight weeks, while both Group A and Group B exhibited a significant reduction. Approximately 17.8% and 36.6% reduction in serum leptin levels were observed in Group A and 19.5% and 40.5% reduction in serum leptin levels were observed in Group B, respectively, at the end of four and eight weeks. In Group C, an increase of approximately 8.6% and 21% was observed in serotonin levels at the end of four and eight weeks, respectively. However, Group A demonstrated a 23% and 40% increase and Group B demonstrated a 23% and 50% increase at the end of four and eight weeks, respectively.

Table 2 demonstrates the amount of remaining food over the period of eight weeks for each group, which reflects a trend of appetite suppression in both Group A and Group B. Group C exhibited a slight increase in food consumption of 2.8%. Approximately a 4% and 14.1% reduction in appetite was observed in Group A and Group B at the end of eight weeks, respectively.

Table 3 demonstrates the changes in lipid profiles, including LDL, HDL, triglycerides,

Table 4
Effects of placebo (Group C), HCA-SX alone (Group A) and HCA-SX formula (Group B) on enhanced excretion of urinary fat metabolites (nmoles/ml of urine)

Group	Time Point	MDA	ACT	FA	ACON
Placebo	I	0.168 ± 0.042^{a}	1.527 ± 0.345^{a}	5.034 ± 0.970^{a}	16.36 ± 1.391^{a}
(Group C)	M	0.121 ± 0.020^{a}	1.056 ± 0.178^{a}	5.423 ± 1.093^{a}	13.65 ± 2.556^{a}
	F	0.133 ± 0.026^{a}	1.338 ± 0.092^{a}	4.670 ± 0.572^{a}	18.46 ± 2.462^{a}
HCA-SX	I	0.110 ± 0.029^{a}	1.151 ± 0.131^{a}	4.380 ± 1.595^{a}	20.03 ± 1.281^{a}
(Group A)	M	0.176 ± 0.062^{a}	1.262 ± 0.159^{b}	5.981 ± 0.344^{a}	21.55 ± 1.784^{a}
	F	0.284 ± 0.077^{b}	1.672 ± 0.129^{c}	7.042 ± 0.878^{b}	25.05 ± 1.934^{b}
HCA-SX	I	0.109 ± 0.012^{a}	1.167 ± 0.088^{a}	4.011 ± 0.0375^{a}	18.65 ± 1.526^{a}
Formula	M	0.211 ± 0.072^{b}	1.387 ± 0.11^{b}	5.756 ± 0.519^{b}	24.37 ± 2.292^{b}
(Group B)	F	0.306 ± 0.081^{c}	1.720 ± 0.105^{c}	7.737 ± 0.464^{c}	27.14 ± 2.525^{c}

Data are presented as group mean ± SEM. Subjects were given placebo (Group C), HCA-SX alone (Group A) or HCA-SX formula (Group B) for 8 weeks. See Subjects and methods section for details. Values with non-identical superscripts are significantly different ($p < 0.05$).

VLDL and total cholesterol in Groups A, B and C. There was some reduction in LDL and triglycerides in Group A, however, the changes that were observed in Group B were even more pronounced. Group B demonstrated a boost in HDL levels, while little effect was observed in Group A. The overall total cholesterol level decreased significantly in both Groups A and B. Approximately 3.8% and 12.3% reduction in LDL levels were observed in Group A, while under these same conditions approximately 8% and 17.9% reduction in LDL were observed in Group B at the end of four and eight weeks, respectively. However, Group C only showed a 1.6% and 0.8% decrease in LDL levels at the end of four and eight weeks, respectively. In Group A, approximately 0.3% and 10.7% increases in HDL levels were observed and 6.9% and 20.7% increases in HDL levels were observed in Group B, respectively, at the end of four and eight weeks. No significant changes were observed in Group C. Approximately 4.1% and 8.6% reduction in triglyceride levels were observed in Group A and 15.1% and 18.1% reduction in triglyceride levels were observed in Group B, respectively, at the end of four and eight weeks, respectively. No significant changes were observed in Group C. No significant changes were observed in the VLDL levels in any of the groups. Approximately, 2.8% and 6.3% reduction in total cholesterol levels were observed in Group A and 3.7% and 9.1% reduction in total cholesterol were observed in Group B at the end of four and eight weeks, respectively. No significant changes were observed in Group C.

Table 4 demonstrates the enhanced excretion of urinary fat metabolites, including MDA, ACT, FA and ACON, which were quantified as biomarkers of fat oxidation. Approximately 125-258% increases in total urinary fat metabolites in Group A and 146-281% increases in total urinary fat metabolites in Group B were observed at the end of eight weeks, respectively, as compared to the control samples. In Group C, excretion of urinary fat metabolites did not change in MDA, ACT and FA, and marginally increased in the case of ACON at the end of eight weeks.

In Group A, MDA, ACT, FA and ACON increased by 160%, 110%, 140% and 110% at the end of four weeks, respectively, and 260%, 150%, 160% and 130% at the end of 8 weeks,

respectively. In Group B, MDA, ACT, FA and ACON increased by 190%, 120%, 140% and 130% at the end of four weeks, respectively, and 280%, 150%, 190% and 150% at the end of eight weeks, respectively. In Group C, excretion of MDA, ACT and FA decreased at the end of four eight weeks of treatment, while excretion of ACON decreased at the end of four weeks and increased at the end of eight weeks (Table 4).

Thus, both HCA-SX alone and in combination with NBC and GSE demonstrated significant reduction in body weight, BMI, LDL, triglycerides, total cholesterol and serum leptin as well as significant increases in HDL, serotonin levels and excretion of urinary fat metabolites.

3.1. Adverse events and drop outs

Twenty-nine of the initial thirty subjects completed the study. During the eight week study, adverse events were noted on a daily basis on each subject. There were no serious adverse events noted in any of the groups in this study. It is important to emphasis that the number of patients reporting adverse events in the supplemented groups was not significantly different from Group C.

One subject dropped out of this study on the 21st day for personal reasons, which was not a result of an adverse event caused by the treatment. No patient was removed or dropped out of the study as a result of an adverse event caused by the treatment.

4. Discussion

Obesity, a chronic disequilibrium between food consumption and energy expenditure, continues to be a major health problem in developed and developing countries. Obesity and its related metabolic and cardiovascular complications continue to present an escalating challenge to contemporary medicine [1–3].

HCA, found in citrus fruits such as oranges and lemons, is an organic acid similar to citric acid but its properties are remarkably different from citric acid. HCA has been shown to reduce appetite, inhibit fat synthesis, and decrease body weight without stimulating the central nervous system [11,15,21,30]. Furthermore, HCA does not cause nervousness, rapid heart rate, high blood pressure, or insomnia, symptoms that are often associated with dietary stimulants such as ephedra (Ma-Huang), caffeine or phenylpropanolamine [35]. Thus, HCA may prove to be a safe alternative to these popular diet aids [35].

In our previous studies [20,29] and in the present study, we have demonstrated other important mechanisms including the appetite suppression by HCA and serotonin release by rat brain cortex *in vitro*, regulatory roles on the lowering of lipid profiles, and increased fat oxidation as demonstrated by enhanced excretion of urinary fat metabolites.

Studies by Ramos et al. (1995) demonstrated that 500 mg HCA (CitriMax) taken three times per day before meals for 8 weeks resulted in 215% greater weight loss in twenty overweight adults than those taking a placebo. No adverse events were reported [16]. Of added importance, a significant reduction was also observed in cholesterol and triglyceride levels. Mattes and Borman (2000) demonstrated that a daily dose of 1.2 gm HCA along with

a daily diet of 5,020 kJ (1,200 kcal) for 12 weeks resulted in a significant difference in weight loss (3.7 ±3.1 vs 2.4 ±2.9 kg) compared to placebo [17]. A 6-week randomized, placebo-controlled, single-blind, cross-over study was conducted by Westerterp-Plantenga and Kovacs (2002) in twelve males and twelve females using a daily dose of 900 mg HCA for two weeks. Results demonstrated that HCA supplementation reduced 24 hr energy intake in humans, while satiety was sustained [19].

In examining the "negative studies", Heymsfeld, et al. (1998) provided what became an accepted daily dose of 1.5 gm HCA along with a diet of 5,020 kJ/day or 1,200 kCal/day diet for 12 weeks, and reported that no significant difference in weight loss was observed between the placebo and treatment groups [36]. However, several problems with the study may be responsible for the negative results. Heymsfeld, et al. (1998) quantified the HCA content, but did not assess the bioavailability of the HCA sample used in their study [36]. Many HCA products are less than 50% soluble in water and poorly absorbed. Also, the low-calorie diet (5,020 kJ/day or 1,200 kcal/day) may have accounted for the substantial decreases in body weight of both treatment and placebo groups blunting the ability of HCA to show curbed appetite and reduced food intake.

Our present study used a highly bioavailable calcium-potassium salt of HCA and the optimal dose was determined based on the mechanistic observation of serotonin release in rat brain cortex. Supplements were given on an empty stomach, since we had previously demonstrated that HCA-SX should be consumed at least 30-60 min before meals to enhance bioavailability. This more efficacious dose was extrapolated from *ex vivo* and *in vivo* studies.

Furthermore, our study was designed to better determine the effects of HCA on satiety. A 2,000 kcal/day or 8,372 kj/day was administered, and all unconsumed food was weighed as an approximation of appetite reduction. Supplementation with HCA-SX, and to a greater degree HCA-SX combined with NBC and GSE, significantly reduced appetite as determined by increased amounts of remaining food.

Perceived small weight loss in both the HCA-SX (Group A) and HCA-SX formula (Group B) group is supported by the improvement in BMI which means sparing lean muscle and burning fat, as demonstrated by enhanced excretion of urinary fat metabolites.

Another possible mechanism of action may be HCA's ability to down-regulate the gene that influences obesity and body weight. Leptin is a 167 amino acid protein hormone encoded by the obesity regulatory gene. Synthesized and secreted by adipocytes (fat cells), leptin is present in the bloodstream in amounts related to the amount of fat in the body. Leptin acts primarily on the brain, where it binds to receptors and activates signals that inhibit food intake and increase energy expenditure [37,38]. When receptor-binding activity is diminished, "leptin resistance" develops, i.e., plasma leptin levels increase and lose their ability to inhibit food intake and increase energy expenditure. Studies show that plasma leptin levels are higher in overweight than in non-overweight individuals, and higher in women than in men. Leptin has been shown to be able to modulate insulin secretion and action through these receptors [38,39]. Our findings in conjunction with earlier studies demonstrate that intracellular energy production is important for acute leptin secretion and that potassium and calcium flux may play roles in coupling intracellular energy production to leptin secretion [39,40]. We hypothesize that the calcium-potassium salt of HCA (HCA-SX) may play a role in down-regulating leptin, the obesity regulatory gene.

Serotonin (5-HT) has been implicated in the control of eating behavior and body weight [41,42]. Our previous study demonstrated HCA-SX's ability to increase the availability of serotonin in isolated rat brain cortex and serve as a mild serotonin receptor reuptake inhibitor [20,29]. It is important to note that obese subjects generally show a low level of circulating serotonin level, Our present study, demonstrated increased levels of circulating serotonin in both supplemented groups as compared to the control subjects.

The current study also suggests that HCA has the ability to augment fat breakdown. This is based upon the enhanced excretion of urinary fat metabolites following usage, including malondialdehyde (MDA), acetaldehyde (ACT), formaldehyde (FA), and acetone (ACON), markers of oxidative fat degradation. The sources of these four lipid metabolites are not entirely clear, but the increases in these products are probably due to enhanced β-oxidation of fat. Dhanakoti and Draper [43] demonstrated that urinary MDA excretion was enhanced following enhanced oxidative stress. Furthermore, the fate of radiolabeled MDA administered to rats was found to be extensively metabolized to acetate and carbon dioxide. Based on these observations, the urinary ACT identified in this study may arise from the breakdown of MDA, which is formed as a result of fat oxidation/lipid peroxidation. The enhanced formation of ACON in response to a consequence of enhanced β-oxidation is also well known [44]. Winters et al. [45,46] reported that rat liver microsomes metabolized glycerol to FA. Glycerol is a product of the metabolism of triglycerides by adipose tissue and other tissues that possess the enzyme that activates glycerol, namely, glycerol kinase. Liver and brown tissues are known to have high glycerol kinase levels [45,46]. Other possible sources of FA might include the breakdown of MDA to acetate or ACT and a one carbon fragment [43], and/or the cleavage of a one carbon fragment from acetoacetic acid with the formation of ACON. Under this situation, it should be pointed out that the triglyceride levels were significantly reduced in both HCA-SX and HCA-SX formula groups, accompanied by significant increases in urinary excretion of FA. These results suggest that these supplements may induce enhanced production of glycerol kinase in the biological system, however, this has yet to be proven.

High levels of total cholesterol, LDL cholesterol and triglycerides, as well as low levels of HDL cholesterol, are all risk factors for cardiovascular diseases, diabetes and stroke. The current study shows that supplementation with HCA-SX, and to a greater degree HCA-SX, NBC plus GSE, significantly improves blood lipid profiles.

Taken together, these studies demonstrate that optimal doses of HCA-SX alone and in combination with NBC plus GSE given on an empty stomach are safe, bioavailable, and highly efficacious diet aids that can help reduce excess, or maintain healthy body weight and BMI, and promote healthy blood lipid levels. The reduced serum leptin levels, decreased appetite, reduced food intake, and increased fat oxidation may be, at least in part, responsible for this positive outcome and decrease the risk factors for obesity related degenerative diseases and mortality.

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