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Investigations of botanicals on food intake, satiety, weight loss, and oxidative stress: a study protocol of a double-blind, placebo-controlled, crossover study

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Abstract

Background—Botanicals represent an important and underexplored source of potential new therapies that may facilitate caloric restriction and thereby produce long-term weight loss. In particular, one promising botanical that may reduce food intake and body weight by affecting neuroendocrine pathways related to satiety is *Garcinia cambogia* (*Garcinia cambogia* Desr.)-derived (−)-hydroxycitric acid (HCA).

Methods and Design—The objective of this article is to describe the protocol of a clinical trial designed to directly test the effect that *Garcinia cambogia*-derived HCA has on food intake, satiety, weight loss, and oxidative stress levels, and to serve as a model for similar trials. A total of 48 healthy, overweight and obese individuals (body mass index; BMI range = 25.0 – 39.9) between the ages of 50 to 70 will participate in this double-blind, placebo-controlled, crossover study designed to examine the effects of two doses of *Garcinia cambogia*-derived HCA on food intake, satiety, weight loss, and oxidative stress levels. This trial will take place at the University of Florida (UF)'s Aging and Rehabilitation Research Center (ARRC) and UF Clinical Research Center (CRC). Food intake represents the primary outcome measure and is calculated based on the total calories consumed at breakfast, lunch, and dinner meals during each test meal day at the CRC. This study can be completed with far fewer subjects than a parallel design.

Discussion—Of the numerous botanical compounds, the compound *Garcinia cambogia*-derived HCA was selected for testing in the present study because of its potential to safely reduce food intake, body weight, and oxidative stress levels. We will review potential mechanisms of action and safety parameters throughout this clinical trial, which is registered at ClinicalTrials.gov under NCT01238887.

Trial registration—ClinicalTrials.gov (Identifier: NCT01238887).

Disclosure
The authors have no conflicts of interest to disclose.

Ethical standards
This study was approved by the appropriate ethics committees and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All persons gave their informed consent prior to their inclusion in the study.

Author contributions
Study concept and design: Anton, Leeuwenburgh
Statistical Analysis and interpretation of data: Shuster, Anton
Drafting of the manuscript: Anton, Leeuwenburgh, & Shuster

Keywords

Obesity; Botanicals; Weight Loss; Garcinia Cambogia; hydroxycitric acid; dietary supplement

The epidemic of obesity continues to increase in industrialized nations. Over half of adult Americans are currently overweight or obese and at increased risk for a number of deleterious health conditions, such as cancer, diabetes, and heart disease [1–3]. Moreover, recent evidence suggests adiposity is associated with accelerated aging [4, 5]. Against the backdrop of the increasing obesity epidemic, research over the past sixty years has documented the benefits of caloric restriction (CR), the practice of restricting food (calorie) intake to create a negative energy balance, for increasing longevity and delaying the onset of age-related diseases in numerous species [6]. Preliminary reports suggested that CR may also have beneficial effects in primates [7, 8] and humans [9].

Although it is currently unknown if all humans benefit from adopting a CR lifestyle, extensive research has shown that overweight and obese individuals receive numerous health benefits following weight loss achieved through CR [10]. Long-term compliance to conventional weight loss programs, however, is notoriously poor [11], possibly due to internal feedback systems that defend against body weight loss by signaling the body to increase food intake and decrease energy expenditure in response to caloric restriction. The vast majority of overweight individuals have difficulty sustaining a 20% to 40% reduction in calorie intake [12], which is what appears to be necessary for the long-term maintenance of weight loss [13].

Botanicals represent important sources of potential new adjunctive or alternative therapies for obese and insulin resistant individuals and may enhance the effects of weight loss interventions in reducing systemic oxidative stress levels. Based on recent estimates, Americans spend over 12 billion dollars per year on nutritional supplements [14], and sales of these products are projected to continue to increase by approximately 10% per year [15]. A large number of dietary supplement products are currently promoted for weight loss purposes [16]. Unfortunately, the vast majority of these products have little supportive data from well-designed randomized trials [17]. Given the increasing popularity of these products among overweight and obese individuals, studies are needed to examine their safety and efficacy.

A promising botanical compound that may have beneficial effects on food intake, weight management, oxidative stress, and metabolic disease is *Garcinia cambogia* (*Garcinia cambogia* Desr.)-derived (−)-hydroxycitric acid (HCA). For example, previous animal studies have demonstrated that this compound affects insulin metabolism, leptin, glucocorticoid metabolism, and inflammation [18, 19]. The majority of research on this botanical, however, has been conducted on rodents and short-term human trials without adequate control conditions [20–28]. Moreover, few studies have examined the effect this botanical has on food intake through direct measurement of caloric consumption at breakfast, lunch, and dinner meals in a laboratory setting. Thus, well-controlled, short and longer term human trials are needed to further explore the potential role that *Garcinia cambogia*-derived HCA and other botanical compounds have in food intake regulation and weight management, as well as neuroendocrine functioning and oxidative stress.

The immediate aim of the present study is to investigate the effects of two doses of the *Garcinia cambogia*-derived HCA on food intake, satiety, body weight, and oxidative stress levels. Compared to placebo, it is hypothesized that both doses of *Garcinia cambogia*-derived HCA will reduce food intake, increase satiety, decrease body weight, and reduce

oxidative stress levels. The National Center for Complementary and Alternative Medicine (NCCAM) strongly encourages investigation of at least two dosages of herbal products for many of their supported trials. Hence, as a secondary goal, it is important to document a model design which other investigators may find useful.

1 Methods and design

1.1 Participants

1.1.1 Sample size—Based on a chromium pilot study [29], the repeated measures correlation for food intake 7 weeks apart was 0.60. Thus, conservatively assuming the within subject correlation in food intake was 0.50 or higher. With N=48 matched subjects for a particular contrast, there is at least 80% to detect a difference of 0.47 standard deviations (SD) in caloric intake per kg at P=0.0167 two-sided for each contrast. In terms of calories per day, participants in the chromium pilot study averaged about 6399 kJ/d (SD=400). With a sample size of 48 participants, the present study will have an adequate sensitivity to detect a difference of 798 calories, which represents a mean difference of about 13%. To achieve our study goal of 48 completers, we plan to enroll 60 participants.

1.1.2 Entry procedures—Participants will be recruited through media advertising, direct mail, health promotion events, databases, and referral sources. An effort will be made to recruit an ethnically diverse group of participants (namely, 20% African American). Volunteers who meet the inclusion criteria listed below will be scheduled for a screening visit. During this visit, participants will first sign an informed consent. They will then receive a brief medical evaluation in which their height and weight will be taken, and an interview will be conducted regarding medical history and potential obstacles for completing the study. Participants will also provide a blood sample to ensure they are healthy and suitable to participate in this study, as well as complete questionnaires to assess their mood, eating habits, food cravings, and preference for foods that will be served in this study.

1.1.3 Inclusion criteria—Participants may be included in this study if they meet the following criteria: (1) healthy individual with normal blood chemistries and platelet counts; (2) 50 to 70 years of age; (3) body mass index (BMI) between 25 and 39.9 kg/m^2; (4) for females, post-menopausal (i.e., no menstrual cycle for more than one year).

1.1.4 Exclusion criteria—Volunteers will be ineligible if they have significant medical conditions (for example, history or clinical manifestation of cardiovascular disease, diabetes, cholelithiasis, liver or renal disease, or cancer); abnormal laboratory markers (for example, renal or liver abnormalities, elevated potassium levels or hemoglobin and hematocrit below the lower limit of normal); psychiatric or behavioral problems (for example, eating disorders or a history of drug and alcohol abuse); concomitant medications (for example, steroids). Volunteers will be screened out if they are unwilling or unable to adhere to different supplement regimens over a ten-month period. Other exclusion criteria include the following: (1) current smoker, due to effects of nicotine upon taste and appetite; (2) diagnosable eating disorder, since intentional restriction of eating and binge eating/overeating could increase the variability of the data; (3) use of anti-depressant medications, anti-psychotic medications, or any medications, botanicals, or other products that may potentially influence appetite, hunger, and/or satiety; (4) report of alcohol or substance abuse within 6 months or consumption of more than 14 alcohol drinks/week; (5) report of any allergies to the foods used in the study. The following measures will be used as screening measures: Beck Depression Inventory-II (BDI-2)[30], Eating Inventory (EI) [31], and Eating Disorder Diagnostic Scale [32, 33]. In line with previous studies, potential participants will also be excluded if their scores on the dietary restraint, disinhibition, and

perceived hunger scales of the Eating Inventory are high (>14). This exclusion criteria is based upon the finding that people who have very high scores (>14) on the Dietary Restraint scale tend to restrict eating in the lab and people with very high scores on the Disinhibition (>14) and Perceived Hunger (>14) scales tend to eat more than normal amounts of food.

1.2 Randomization and design

A three-period within subject's crossover design with two doses of *Garcinia cambogia*-derived HCA versus placebo will be used. The order the participants receive each dose of *Garcinia cambogia*-derived HCA or placebo will be counterbalanced and determined through randomization. Participants will be blinded to their assigned condition throughout the study. Thus, participants will complete a total of three 6-week conditions and two 6-week washout periods following a baseline food test day.

1.3 Intervention

Participants will initially complete a baseline food test meal day at the UF CRC, during which they will consume breakfast, lunch, and dinner, as well as complete satiety assessments before and after meals. Following the baseline food test meal day, participants will be randomly assigned to receive one of two doses of *Garcinia cambogia*-derived HCA or placebo (palm oil) in capsule form for a six (6) week duration. In each condition, participants will be instructed to take four capsules three times per day, 30 min before breakfast, lunch, and dinner. After six (6) weeks, participants will be asked to complete a second food test meal day (procedures described above) at the UF CRC. Following this food test day, participants will complete a six (6) week washout period and will then return to the CRC to complete a third food test meal day, which will serve as a baseline for their next assigned condition. Participants will then be asked to complete the next condition, in which they will receive one of two doses of *Garcinia cambogia*-derived HCA or placebo (palm oil) in capsule form for a six (6) week duration. After completing the next assigned condition, participants will again return to the UF CRC to complete a fourth food test meal day. Participants will then complete a second six (6) week washout period and will then return to the UF CRC to complete a fifth test meal day, which will serve as a baseline for their next condition. Participants will then be asked to complete the third and final condition in which they will receive one of two doses of *Garcinia cambogia*-derived HCA or placebo (palm oil) in capsule form, for six (6) week before returning to the UF CRC to complete their final food intake test day.

To enhance retention, participants will receive biweekly phone calls throughout both the treatment and washout periods to remind them of their importance to the study, as well as to ensure no adverse events have occurred. Following the completion of this trial, phone assessments will be conducted at one week and one month to ensure no adverse events have occurred.

Dosage: Two doses of *Garcinia cambogia*-derived HCA will be tested in this trial: (1) 2800 mg/day, and (2) 5600 mg/day. For all three conditions, participants will be instructed to consume 12 capsules per day. In order for participants to be blinded to their assigned condition, participants assigned to the 2800 mg/day condition will receive 6 capsules of the active compounds (a total of 2800 mg/day of HCA) and 6 capsules of the placebo (a total of 2040 mg/day of palm oil). In the proposed double-blind placebo controlled trial, the effects of each of these two doses will be compared to each other as well as to placebo (12 capsules containing a total of 4080 mg/day of palm oil). Compliance with the dosing regimen will be monitored both through interview and by counting capsules left at the end of each treatment period. The company Glykon Technologies Group (Seattle, WA) is providing the *Garcinia cambogia* derived HCA (product lot number – 60024351) and the placebo in the form of

palm oil (product lot number - 60023451) for this study, which are then put into capsule form by the company Capsugel.

1.4 Outcome Measures

1.4.1 Primary

Food Intake: Food intake will be directly measured based on the gram weight of foods served pre- and post- meals to determine each participant's caloric intake. The Clinical Research Center (CRC) of the University of Florida has extensive experience studying the effect of pharmacological compounds, behavioral tools, and food products on eating behavior and satiety. Eating behavior in the laboratory is consistent with eating behavior in the natural environment [34] and food intake measured in the laboratory is highly reliable [35].

1.4.2 Secondary

Body Weight: Weight will be measured at screening, baseline, and every test meal day for the duration of this 36-week trial.

Visual Analog Scales: Visual Analogue Scales (VAS) will be used to quantify subjective ratings of hunger, satiety, fullness, and carbohydrate cravings. When completing the VAS, participants rate the intensity of these subjective states on a 100-millimeter line from "not at all" to "extremely." Flint and colleagues found support for the reliability and validity of VAS in measuring subjective states related to food intake [36]. The VAS ratings will be taken during the afternoon between the lunch and dinner meals.

Eating Inventory: The Eating Inventory (EI) [31] will also be used to assess changes in dietary restraint, disinhibition, and hunger throughout the study.

Food Craving Inventory: The Food Craving Inventory (FCI) [37] was selected for use to provide a reliable and valid assessment of cravings for different types of foods during this study. It has four conceptual factors or subscales: High Fats, Sweets, Carbohydrates/ Starches, and Fast Food Fats that comprise the higher-order construct of "food craving," represented by the FCI's total score.

DNA/RNA Oxidation: Blood will be collected in 10 ml EDTA-Vacutainer® tubes from Becton Dickinson (Franklin Lakes, NJ), and white blood cell (WBC) 'buffy coats' will be collected following centrifugation (800 × g, 20 min, 4ºC) using a large orifice pipette tip and placed into 1.5 ml eppendorf tubes and immediately frozen at −80°C. Buffy coats of WBC will be thawed from −80°C and placed on ice. Working on slush ice (0°C) during all steps, cells will be lysed in 4.5 ml of 3 M GTC buffer (0.2 wt.% N-L-Sarcosine, 20 mM tris, pH 7.5) containing 10 mM of the freshly dissolved metal chelator deferoxamine meylate (DFOM) during homogenization using a Potter-Elvehjem homogenizer. All chemicals and supplies used to extract and analyze nucleic acids have been previously described [38]. After transferring the homogenates to 15 ml Phase-Lock Gel (PLG) tubes, an equal amount of a phenol:chloroform mixture (pH 6.7) will be added, and proteins and lipids will be extracted into the phenol phase. After vortexing in intervals during 10 min keeping the tubes on ice, samples will be centrifuged (2,000 × g, 30 min, 0°C), and the upper aqueous phase containing nucleic acids will be transferred into a new PLG tube. The procedure will be repeated once. After transferring into a new PLG tube, an equal amount of chloroform:isoamylalcohol (24:1) will be added to remove any remaining phenol by hand mixing followed by centrifugation. The procedure will be repeated once, and the upper aqueous phase will be collected and nucleic acids precipitated by adding 1:1 isopropanol,

mixing and incubating at −80°C over night. Total nucleic acids will be collected by centrifugation at 10,000 × g, 0°C for 10 min. Nucleic acids will be washed in 70% ethanol, spun down at 3,000 × g (10 min, 0°C) and air-dried at room-temp for 10 min. RNA/DNA hydrolysis will be performed using Nuclease P_1 and alkaline phosphatase, and 8-oxoGuo/guanosine (Guo) and 8-oxodGuo/2′-deoxyguanosine (dGuo) ratios will be determined using HPLC-ECD with a Coulochem III electrochemical detector (ESA Inc., Chelmsford, MA) as described previously [38].

1.5 Statistical Analyses

The hypotheses comparing the treatments will be tested by the two sample method for crossover studies per Shuster [39]. To study the impact of the duration of washout, we shall compare calorie intake per day for each dose following nothing (infinite washout) vs. placebo (18 weeks) vs. following the other dose (6 weeks). These data will be useful in planning future studies to help determine washout duration. Analysis of variance will be used. Note: Intent-to-treat will be followed as closely as possible. Subjects who complete two periods will contribute to the contrast involved in the two treatments they completed. Every attempt will be made to get calorie intake data on subjects who refuse to comply with the treatments.

For the primary hypothesis, food intake (kcal/day) will be directly measured based on the gram weight of foods served pre and post meals to determine each participant's caloric intake. Intent-to-treat will be followed as closely as possible. Each pairwise contrast will be made stratifying for matched versus unmatched. For example if 48 subjects completed conditions A and B, three subjects completed A only, and two subjects completed B only, the matched vs. unmatched contributions of each participant will be weighted in proportion to the inverse of the estimated variance of the two estimated differences. A significant effect will be determined if the two-sided P-value is below 0.05/3=0.0167 (two-sided). This strict requirement for significance assures that the probability of falsely declaring any pair of treatments as significantly different when there is no difference in the target population is less than 5%. The secondary outcome variables (i.e., satiety and body weight) will be analyzed in a similar manner, with any significant findings needing independent verification in a future study. Note that if there are no unpaired data for a contrast, the method will be equivalent to that given in Shuster [39]. To accomplish the same power objective, a three arm parallel trial would require 291 completers (97 per arm) to achieve the same operating characteristics. Thus, for Phase II type research such as this, three period crossover trials can be very cost effective.

2 Discussion

The primary objective of the present study is to investigate whether the botanical compound *Garcinia cambogia*-derived HCA affects food intake, satiety, body weight, and oxidative stress levels in overweight and obese individuals. Since there is limited information on the dose-response effects of HCA, as well as the effects of doses higher than 2800 mg/day on food intake, body weight, and other metabolic parameters, a dosing study on HCA is likely to yield highly novel information. In the present study, two doses of HCA are being tested. A previous short-term clinical trial found the no observed adverse effect level (NOAEL) in humans is greater than 4000 mg/day [40]. The highest dosage level being tested in the present study is 5600 mg/day; thus, the current study will evaluate whether a higher dose of this compound than has been previously tested may meet the NOAEL criteria.

Despite substantial efforts, rates of obesity continue to increase in America and other industrialized nations. A multitude of factors appear to increase the difficulty of achieving and sustaining weight loss over the long-term. The increasing prevalence and incidence of

obesity and its associated negative health impact has increased the need for effective treatments. Overweight and obese individuals receive numerous health benefits following weight loss achieved through CR, which may be related to reductions in systemic levels of oxidative stress. Long-term weight loss, however, has proven to be very difficult for most individuals to achieve, possibly due to internal feedback systems that signal the body to increase food intake and decrease energy expenditure in response to reductions in caloric intake.

Unfortunately, these strong neuroendocrine signals to increase food intake and decrease energy expenditure following weight reduction appear to remain present until the defended weight is reached [13]. For example, nutrient stimulated glucagon-like peptide-1 (*GLP-1)* release is reduced in obese individuals after six weeks of caloric restricted weight loss [41]. Additionally, leptin is decreased in response to hypocaloric diets prior to changes in adiposity [42], and both leptin and insulin appear to underestimate peripheral adiposity following weight reduction [43]. These findings suggest that individuals would have to consume fewer calories than the signals they receive from the brain and periphery to maintain a reduced body weight [44].

There is evidence, however, that the defended body weight can be manipulated by factors that influence hormonal, metabolic, and neural functions [45], which suggests that treatments that impact these signals may facilitate long-term weight loss. An increased understanding of the pathophysiology of obesity, as well as the biological changes associated with caloric restriction and exercise, may reveal how natural and/or pharmacological compounds may potentiate the effects of behavioral interventions. As noted above, botanicals represent an important source of potential new therapies for overweight and obese individuals.

The present study has a number of strengths. First, the proposed trial will be the first study to directly measure changes in food intake following use of a selected botanical using laboratory based methodology. Second, this trial will incorporate the strengths of two institutes within the University of Florida: *Institute of Food and Agricultural Sciences and Institute on Aging.* Third, the investigative team has conducted a number of clinical trials, particularly in the areas of nutrition, obesity, and aging.

The present study also has a few potential limitations. First, the proposed study does not fully evaluate the effects of multiple dosage levels of the selected botanical compound. Full evaluation would require testing more than three doses in multiple study samples. Additionally, we are only examining the effects of this botanical on systemic markers of oxidative stress and are not collecting biopsies to examine changes in markers of oxidative stress at the cellular level. Another potential limitation is the evaluation of only one of a number of potential botanicals that may influence appetite and food intake. It is possible that other popular botanical compounds may also have significant effects on food intake. Based on a review of the literature, however, the botanical compound *Garcinia cambogia*-derived HCA was selected because of its potential to safely reduce food intake, body weight, and oxidative stress levels, potentially by affecting neuroendocrine pathways related to satiety.

Future research is needed to explore the effects that promising botanical compounds may have on food intake and satiety levels. Additionally, studies are needed to explore the role that botanical compounds alone and in conjunction with weight loss interventions may have in affecting plasma biomarkers of oxidative stress. Moreover, the influence that changes in these biomarkers have on disease processes is an important but understudied area.

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