International Journal of Obesity (2009) 33, 1174–1182

0307-0565/09 $32.00
www.nature.com/ijo

ORIGINAL ARTICLE

Satiety and amino-acid profile in overweight women after a new treatment using a natural plant extract sublingual spray formulation

M Rondanelli[1], C Klersy[2], P Iadarola[3], F Monteferrario[1] and A Opizzi[1]

[1]*Section of Human Nutrition and Dietetics, Faculty of Medicine, Endocrinology and Nutrition Unit, Department of Applied Health Sciences, Azienda di Servizi alla Persona, University of Pavia, Pavia, Italy;* [2]*Service of Biometry and Clinical Epidemiology, Fondazione IRCCS 'Policlinico San Matteo', Pavia, Italy and* [3]*Department of Biochemistry 'A Castellani', University of Pavia, Pavia, Italy*

Objective: To test the effect on satiety of a formulation comprising plant extracts naturally containing 5-hydroxytryptophan, delivered as sublingual spray (5HTP-Nat Exts), administered five times a day for 2 months.
Design: Two-month, randomized, double-blind, placebo-controlled trial.
Subjects: A total of 27 healthy, adult overweight women were randomly assigned to the treatment (14) or the placebo group (13).
Measurements: Visual analog scales were used to assess appetite sensations every day. Moreover, the study evaluated the bioavailability of 5-hydroxytryptophan following sublingual delivery over 8 weeks, by comparing 24-h urinary excretion of 5-hydroxy-3-indoleacetic acid (5-HIAA), determined at baseline and after 2 months. Other secondary end points of the study were to compare body composition, depressive symptoms, severity of binge eating and quality of life. Finally, the study tested whether a single administration of 5HTP-Nat Exts in fasting state has an effect on amino-acid profile and on appetite ratings and whether 5HTP-Nat Exts administered before a fixed test meal has any effect on satiety.
Results: The group using the 5HTP-Nat Exts experienced a significantly greater increase in their sensation of satiety over an 8-week timeframe and in fasting state following administration of 5HTP-Nat Exts than the placebo group did (AUC = 305.2 (52.8) vs 236.6 (59.4), mean difference −68.7 (95% confidence interval (CI) −116.2 to −21.2), $P = 0.007$; mean difference in Haber score change 2.5 (95% CI 0.62–3.12, $P = 0.007$)). A difference was observed between the groups for the mean change in 5-HIAA. All the amino acids evaluated after a single administration of 5HTP-Nat Exts were found to be similar. Differences were found for the mean change in body mass index, skinfold thicknesses and hip circumference. The other parameters were found to be similar.
Conclusion: All these findings suggest that 5HTP-Nat Exts may be safely used to treat the problem of appetite control in overweight women during a weight loss program.
International Journal of Obesity (2009) **33,** 1174–1182; doi:10.1038/ijo.2009.155; published online 15 September 2009

Keywords: 5-hydroxytryptophan; sublingual drug delivery spray formulation; weight loss; satiety; appetite

Introduction

Pharmacological, biochemical and behavioral evidence have demonstrated that brain serotonin (5-hydroxytryptamine) is an important regulator of appetite, macronutrient preference and mood, in both animals and humans.[1–7] The observation has repeatedly been made that drugs that enhance transmission across serotonin synapses generally suppress appetite, whereas those that diminish serotonin transmission stimulate food intake.[8–10] Evidence suggesting that defects in the serotonin system may be etiologic of obesity has been also accumulated.[11,12] The synthesis of serotonin in the brain is controlled in part by the availability of its amino-acid precursor, L-tryptophan. Tryptophan pools in the brain are, in turn, influenced by the uptake of tryptophan from the circulation. This uptake process depends on a competitive, saturable transport carrier shared by tryptophan and several

Correspondence: Professor M Rondanelli, Section of Human Nutrition and Dietetics, Faculty of Medicine, Endocrinology and Nutrition Unit, Department of Applied Health Sciences, Azienda di Servizi alla Persona, University of Pavia, Via Emilia 12, 27100 Pavia, Italy.
E-mail: serv.nutrizione@asppavia.it
Received 8 April 2009; revised 24 May 2009; accepted 22 June 2009; published online 15 September 2009

other large neutral amino acids (LNAAs), and the branched-chain amino acids.[13,14] Because of competitive transport, brain tryptophan uptake and, ultimately, serotonin synthesis are influenced by plasma concentrations not only of tryptophan but also of the other LNAAs. The plasma ratio of the concentration of tryptophan to the sum of the concentrations of the other LNAAs (the plasma tryptophan ratio), which summarizes this competitive relation, has been proven to be a useful and reliable predictor of brain tryptophan uptake and central serotonin synthesis.[15,16] Plasma tryptophan concentrations and the ratio of tryptophan to other LNAAs (plasma tryptophan ratio) are reportedly low in obese subjects, both when measured at single time points[17,18] (although not always)[6] and throughout a 24-h period.[19] Moreover, the plasma tryptophan ratio remains low after weight reduction[20] and decreases with dieting,[21–24] an effect that may be partly responsible for the high relapse rate after diet-related weight loss.[25] The function of amino acids in the regulation of food intake has been supported by experimental data suggesting that changes in plasma amino-acid concentrations may modify food intake by affecting the brain availability of neurotransmitter amino-acid precursors.[26] Previous observations have shown that oral administration of 5-hydroxytryptophan (5-HTP) is useful for losing weight.[27–29] Moreover, it has been shown that 5-HTP increases plasma concentration of leptin, the hormone synthesized from fat that inhibits the feeling of hunger in the hypothalamus.[30–33] Currently, 5-HTP is available exclusively in oral or parenteral formulations. In some cases, however, the oral route might not be the best choice for the patient, because of difficulties with swallowing, or when a faster onset of action is required. Furthermore, parenteral administration may not offer a suitable alternative. In recent years, a growing interest in alternative dosage forms for drug administration has emerged. Drug delivery through the oral mucous membranes is considered to be a promising alternative to the oral route. In terms of permeability, the sublingual area of the oral cavity (that is, the floor of the mouth) is more permeable than the buccal (cheek) area, which in turn is more permeable than the palatal (roof of the mouth) area.[34] Moreover, sublingual drug administration is simple and relatively cost effective. Given this background, this study tested whether a composition of natural plant extracts rich in 5-HTP supplied as a sublingual spray (5HTP-Nat Exts) consumed five times per day for 2 months has an effect on satiety. Moreover, another aim of the study was to determine the bioavailability of 5-HTP following sublingual delivery over 8 weeks, by comparing 24-h urinary excretion of 5-hydroxy-3-indoleacetic acid (5-HIAA), the major metabolite of serotonin,[35] determined at baseline and after 2 months. Finally, the study tested whether a single administration of 5HTP-Nat Exts in fasting state has any effect on the plasma amino-acid profile and on appetite ratings and whether 5HTP-Nat Exts consumed before a fixed test meal at lunchtime has any effect on satiety.

The formulation contains extract of *Griffonia simplicifolia*, which is rich in 5-HTP;[36] Guaranà, known for its stimulatory effect on fat metabolism;[37] *Centella asiatica*, which is useful for its beneficial effects on blood and lymph circulation;[38] *Taraxacum*, which has an antioxidative and diuretic effect;[39] artichoke, with its detoxifying action[40] and klamath algae, which has a high content of nutritive substances including phenylethylamine, which improves mood.[41]

Materials and methods

Participants

The study was performed under the approval of the ethics committee of the Department of Internal Medicine and Medical Therapy, University of Pavia. Subjects gave their written consent to the study. Healthy women aged 25–45 years, with a body mass index (BMI) greater than 25 kg m^{-2} and less than 35 kg m^{-2}, were eligible for the study. All subjects had to give complete medical histories, and underwent physical examination, anthropometric assessment and routine laboratory tests. Individuals who had any hepatic or renal disease, diabetes, unstable cardiovascular disease, uncontrolled hypertension, an eating disorder (diagnosed bulimia), active cancer or surgery for weight loss were excluded from the study. Moreover, patients were excluded from the study if they met the Diagnostic and Statistical Manual IV (DSM-IV)[42] criteria for a current diagnosis of major depressive disorder as determined by the Structured Clinical Interview for DSM-IV Axis 1 Disorders. Subjects also were excluded if they were using medications for weight loss or were pregnant or lactating or if they had entered menopause. All participants agreed to refrain from participating in any other weight loss program. Alcohol intake, smoking habits and physical activity were recorded. The subjects were randomly assigned to one of the two groups in a double-blind parallel study.

Food supplement

The subjects received a food supplement composed of natural plant extracts rich in 5-HTP supplied as sublingual spray formulation (5HTP-Nat Exts) or a placebo.

Subjects were randomized to receive three sprays of 5HTP-Nat Exts five times per day—fasting in the morning, at mid-morning, before lunch, fasting in the afternoon and before dinner—or an identical placebo for 8 weeks. The dose of formulation solution was administered to the sublingual mucosa using a spray bottle. The product is a solution composed of different plant extracts. The composition of the product administered in the three sublingual sprays is 39 mg *G. simplicifolia*, 11.7 mg *C. asiatica* L., 11.7 mg *Taraxacum officinale*, 9.75 mg *Cynara scolymus*, 4.55 mg *Paullina sorbilis* L. Mart, 39 μg *Alga klamath*. The product was manufactured by Medestea Research and Production S.p.A. (Torino, Italy).

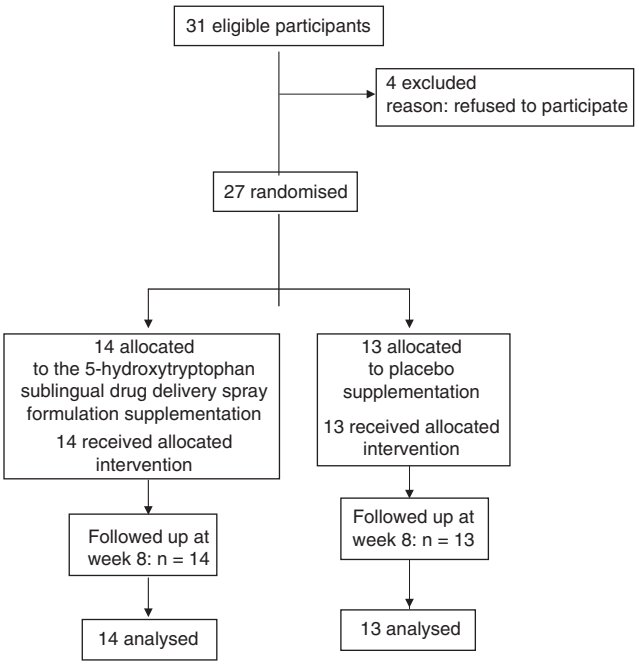

Figure 1 Subject randomization and dropout throughout the study.

The bottles for each treatment group were assigned a subject number according to a coded (AB) block randomization table prepared by an independent statistician. Investigators were masked to the randomization table, the code assignments and the procedure. As the subjects were enrolled, they were sequentially assigned a subject number. Subject randomization and dropout throughout the study are shown in Figure 1.

Procedures

Visual analog scales (VAS) were used to assess appetite sensations in patients for 1 week before the start of the study. Following this week, after 12 h of fasting and abstinence from water since midnight, the subjects arrived, using motorized transportation, at the Endocrinology and Nutrition Unit of Azienda di Servizi alla Persona di Pavia, University of Pavia (Italy) at around 0800 hours. Blood samples were taken in the fasting state and 20 and 40 min after administration of three sprays of 5HTP-Nat Exts or placebo. VAS were used to assess appetite sensations in the fasting state, 20 and 40 min after administration of three sprays of 5HTP-Nat Exts or placebo. Then, between 0845 and 0900 hours a standardized breakfast (four biscuits, one yogurt from skimmed milk and one cup of espresso coffee with one teaspoonful of sugar) was served. Subjects were instructed to eat everything within a 30-min period.

At 1230 hours, three sprays of 5HTP-Nat Exts or placebo were administered to the patients and then, after 10 min, a standardized, solid test-meal, consisting of 80 g rice, 80 g ham, 50 g white bread, 100 g lettuce, 150 g apple, 15 g olive oil with 500 ml tap water, was served. The participants were allowed to eat until satisfied. The lunch was served in a separate room. The subjects were separated by screens so that they could not see each other. They were instructed not to speak to each other. Lunch was consumed and VAS measurements were performed in the same room, where the participants were kept quiet and away from any visual, hearing and olfactory stimuli that might have influenced the measurements.

The meal consisted of 3169 kJ with 15.5 E% (percentage energy) protein, 58.5 E% carbohydrate and 26 E% fat. The total amount of food at the test meal was measured by weighing food items separately before and after eating to the nearest 0.1 g. To avoid reported interference due to premenstrual depression, food intake measurements were not recorded during this time of the month.[43]

Visual analog scales were used to assess appetite sensations before 5HTP-Nat Exts or placebo administration and then every 20 min for 4 h after the standardized lunch.

The following day, the patients started with the weight loss program and began to take three sprays of 5HTP-Nat Exts or an identical placebo, five times per day for 8 weeks. Subjects were asked to compile the VAS every day before the allocated dinner time (between 1930 and 2000 hours).

Twenty-four-hour urinary excretion of 5-HIAA and binge eating severity, depressive symptoms and health-related quality of life were assessed in both groups, both before and after the 8-week treatment period.

Rating of appetite

Visual analog scales were used to assess appetite sensations. Satiety was assessed numerically, using a scoring system graded from −10 to represent extreme hunger, to 10 to represent extreme satiety. Subjects were shown a scale with 20 graduations and asked to indicate how they felt in respect to hunger or satiety by pointing to an appropriate place along the scale. The scale was dotted with phrases describing the various degrees of hunger or satiety, but subjects were free to choose any point along the scale; the point chosen was defined as the Haber score.[44]

To calculate the values of the area under the curve (AUC) in response to the treatment, the VAS measurements for each day were considered in this calculation by using the trapezoid method.

Biochemical analyses

Venous blood samples, taken at 0800 hours, were drawn from the antecubital vein at three time points (before three sprays of 5HTP-Nat Exts or placebo, and 20 and 40 min thereafter) in fasting patients to determine plasma amino-acid concentrations. The concentration of free amino acids in plasma was determined by means of the AminoQuant II amino-acid analyser based on the HP 1090 HPLC system with fully automated precolumn derivatization using both

ortho-phthalaldehyde and 9-fluorenylmethyl chloroformate reaction chemistries according to the manufacturer's protocol. Detection was performed measuring UV absorbance at 338 and 262 nm, respectively. The procedure used was as follows: 2 ml samples of plasma were deproteinized by adding 500 μl of 0.5 N HCL and, after centrifugation at 5000 *g* for 10 min at 5 °C, the supernatant was concentrated up to 200 μl under a nitrogen stream and further filtered on a 45 μm Millipore filter (Millipore Corporation, Bedford, MA, USA). Aliquots (1 μl each) were automatically transferred to the reaction coil and derivatized with the reagents mentioned above. The remaining deproteinized serum was stored at 20 °C. Analyses were performed in duplicate, and the value reported for each amino acid was the mean of two independent determinations. The average minimum detectable level of amino acids was 3–5 pmol for each microliter of material injected. Amino-acid concentration was expressed as pmol per liter.

Twenty-four-hour urinary excretion of 5-HIAA was also determined at baseline and after 8 weeks by the chromatographic-colorimetric method described by Udenfriend *et al*.[45]

Anthropometry, weight loss program and food intake

Nutritional status was assessed using anthropometric measurements at baseline and after 2 months in both groups. Body weight and height were measured and the BMI was calculated (kg m^{-2}). Skinfold thicknesses (biceps, triceps, suprailiac, subscapular) were measured twice using a Harpenden skinfold caliper at 5 min intervals at each site following a standardized technique.[46]

Sagittal abdominal diameter was measured at the L_{4-5} level in the supine position and waist girth was also measured. Anthropometric variables were measured by a single investigator.

As to the weight loss program, subjects were instructed to restrict their daily energy intake by a moderate amount, 2508 kJ per day less than daily requirements based on World Health Organization criteria[47] with a regimen that maintained a prudent balance of macronutrients: 25% of energy from fat, 60% of energy from carbohydrates and 15% of energy from protein. A registered dietician performed initial dietary counseling.

To assess compliance to the weight reduction program a 24-h dietary recall was assessed by the nutritionist.

Assessment of binge eating severity, depressive symptoms and health-related quality of life

Binge eating severity, depressive symptoms and health-related quality of life were each evaluated at baseline and after 2 months in both groups.

The severity of binge eating was assessed using the Gormally Binge Eating Scale (BES)[48]and a Beck Depression Inventory (BDI)[49] was taken to assess depressive symptoms; the tests were conducted under standardized conditions of comfort and silence, in the presence of a study technician.

The subjects studied were tested with the Short-Form 36-Item Health Survey (SF-36),[50] to evaluate their health-related quality of life. The SF-36 questionnaire is a valid generic measure for rating health-related quality of life in several research fields, on the basis of its validity, high internal consistency and high test–retest reliability.

Statistics

Continuous data were described as mean and standard deviation or median and quartiles if skewed; categorical data as counts and percent. To compute AUCs, we rescaled the Haber score from a range of −10 to +10 to a range of 0–10. For the analysis of the primary end point, the AUCs computed from the Haber scores measured over time were compared by means of a Student's *t*-test. Mean differences between groups and their 95% confidence intervals (95% CI) were calculated. Moreover, in a secondary analysis of the primary end point (and for some of the secondary end points), the median change (95% CI) in each group was computed and compared with the Mann–Whitney *U*-test. The difference in median changes (95% CI) was also calculated. The time profile for the other secondary end points was compared by means of general linear regression models, with calculation of Huber–White robust standard errors to account for intrasubject correlation over time. Time profiles were considered significantly different in the presence of a significant interaction term (while adjusting for baseline values).

Stata 10.1 (Stata Corporation, College Station, TX, USA) was used for computation. All tests were two sided. A P-value <0.05 was considered statistically significant. The secondary analysis of the primary end point was tested at the 0.025 level (Bonferroni correction); given the exploratory nature of the secondary end points, no correction for multiple tests was applied. All analyses were performed according to the intention-to-treat principle on the population reported in Figure 1.

Results

A total of 77 women were enrolled, out of 31 eligible participants (four cases were excluded because they refused to participate) (Figure 1). Following the randomization model, 14 patients were included in the intervention group, and 13 in the placebo group. The groups were closely matched in all parameters evaluated in the study (Table 1).

Intergroup differences in the primary outcome variables

The group using the 5HTP-Nat Exts product experienced a significantly larger sensation of satiety overall, evaluated by the AUC, than the placebo group over the 8-week period of the study (AUC = 305.2 (52.8) vs 236.6 (59.4), mean difference −68.7 (95% CI −116.2 to −21.2), $P = 0.007$; Figure 2);

Table 1 Characteristics of the subjects studied at baseline and after 2 months

Variable	*Baseline mean (s.d.)*		*After 2 months mean (s.d.)*	
	Control	*Treated*	*Control*	*Treated*
5-Hydroxy-3-indoleacetic acid (mg/24 h)	1.77 (1.15)	1.70 (1.09)	1.97 (1.28)	4.08 (2.88)
BMI (kg m^{-2})	29.86 (3.47)	29.36 (2.35)	29.65 (3.34)	28.34 (2.01)
Arm circumference (cm)	33.18 (2.82)	32.88 (1.61)	33.09 (2.98)	31.77 (1.36)
AMA (cm^3)	33.48 (6.19)	35.00 (9.11)	33.29 (6.54)	33.80 (7.53)
AFA (cm^3)	4.72 (0.79)	4.78 (0.77)	4.70 (0.83)	4.70 (0.70)
MAC (cm)	22.35 (1.72)	22.72 (2.37)	22.29 (1.83)	22.41 (2.08)
Waist circumference (cm)	98.18 (11.92)	96.15 (5.96)	97.82 (11.75)	94.54 (6.36)
Hip circumference (cm)	113.68 (8.38)	108.31 (4.23)	113.05 (8.27)	105.62 (4.50)
Waist/Hip ratio (WHR)	0.86 (0.06)	0.89 (0.04)	0.86 (0.06)	0.89 (0.05)
Calf circumference (cm)	40.32 (3.03)	38.19 (2.07)	40.14 (2.94)	37.88 (2.13)
Beck Depression Inventory	9.36 (8.19)	11.69 (7.75)	8.36 (8.07)	8.54 (8.35)
SF-36 General Health	62.36 (21.71)	58.46 (19.69)	70.64 (16.52)	58.46 (16.99)
SF-36 Mental Health	65.82 (20.50)	58.77 (17.23)	70.18 (18.54)	59.69 (23.24)
Binge Eating Scale	13.27 (±8.45)	11.92 (6.29)	11.55 (9.84)	8.00 (6.07)
Recall 24 h				
Energy (kJ)	7776.34 (1664.74)	5666.96 (1133.50)	6865.61 (2859.12)	4830.62 (942.73)
Protein (%)	15.92 (3.10)	16.75 (7.27)	14.65 (3.51)	17.71 (3.81)
Lipids (%)	34.85 (6.52)	27.41 (5.82)	33.62 (11.01)	27.24 (12.65)
Carbohydrates (%)	46.63 (11.42)	54.24 (9.95)	50.47 (10.86)	53.57 (12.53)
Simple carbohydrates (%)	16.94 (7.68)	17.44 (9.79)	16.34 (7.39)	20.48 (6.85)
Alcohol (g)	1.97 (4.35)	1.46 (2.71)	0.56 (1.40)	0.98 (3.26)
Fiber (g)	18.87 (12.96)	16.03 (6.66)	19.93 (11.26)	14.30 (4.96)

Abbreviations: AFA, arm fat area; AMA, arm muscle area; BMI, body mass index; MAC, muscle arm circumference.

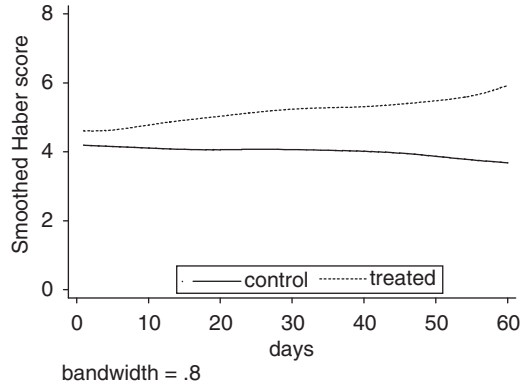

Figure 2 Time profile of the mean Haber score (lowess smoothed estimate) in the treated (dotted line) and control groups (continuous line).

also, the change in Haber score at the end of the study was significantly higher in the treated group than in the control group (mean difference of 2.5, 95% CI 0.62–3.12, $P=0.007$). A significant change with respect to baseline of the Haber score was observed in the treated group (mean change 1.25 (95% CI 0.49–2.25, $P=0.002$), but not in the control group (mean change −1.25 (−1.61 to 1.25), $P=0.32$).

Intergroup differences in secondary outcomes

Appetite ratings. The measurements taken during the first day showed that the administration of 5HTP-Nat Exts (three sprays) in fasting state reduced the appetite rating both after 20 and 40 min, significantly more than the placebo. In particular, as shown in Table 2, the Haber score was significantly reduced in the treatment but not in the placebo group (Table 2). Although the Haber score significantly decreased from baseline to 40 and 240 min in both groups after the standardized lunch, as shown in Table 2, no significant difference was elicited between the treated and the placebo group.

Biochemical analysis. A significant difference ($P=0.005$) was found between the treatment groups for the mean change in 5-HIAA (Table 3). This significantly increased in the treated group but not in the control group. All the amino-acid concentrations evaluated after one administration of 5HTP-Nat Exts or placebo were found to be similar in the treated and in the placebo group (data not shown).

Body composition and food intake. As to changes in body composition, statistically significant differences between the treatment groups were found for the mean change in BMI, skinfold thicknesses (biceps, triceps, suprailiac, subscapular) and hip circumference (Table 3). In all cases, a significant change from baseline was present in the treated but not in the placebo group. The other parameters were found to be similar in the treated group and in the placebo group.

Regarding the evaluation of food intake during the standardized test meal, no specific behavior was observed in macronutrient selections in either of the two groups;

Table 2 Secondary end points: short-term comparisons between treatment and placebo groups (regression model for repeated measures) of appetite ratings (Haber score)

Rating of appetite (Haber score)	*Time (min)*	*Control mean (s.d.)*	*Treated mean (s.d.)*	*Treatment effect (difference in time profile)* P-*value*	*Change over time in control* P-*value*	*Change over time in treated* P-*value*
Before/After 5 jets of 5 HTP-Nat Exts	0	4.56 (0.93)	3.85 (1.19)	0.045		
	20	4.69 (1.19)	4.90 (0.35)		0.55[a]	0.006[a]
	40	4.81 (1.38)	4.90 (0.95)		0.42[b]	0.020[b]
Before/After standardized meal	0	4.65 (1.69)	5.19 (1.12)	0.551		
	40	6.32 (1.61)	7.33 (1.08)		0.035[a]	0.006[a]
	240	4.51 (0.87)	4.23 (1.20)		0.001[b]	0.000[b]

[a]Test comparing time 20 vs time 0. [b]Time 40 vs time 20.

Table 3 Secondary end points: changes over time of 5-HIAA, body composition and food intake

Variable	*Median (95% CI)*		*Δ median (95% CI)*	*Difference between treatments* P-*value*	*Change in control* P-*value*	*Change in treated* P-*value*
	Change from baseline in control	*Change from baseline in treated*	*Δ (change control—change treated)*			
5-Hydroxy-3-indoleacetic acid (mg/24 h)	0.2 (−0.24 to 0.63)	2.05 (0.81–3.85)	−1.8 (−3.4 to 0.7)	0.005	0.266	0.015
BMI (kg/m^2)	0 (−0.53 to 0)	−1.04 (−1.59 to 0.12)	0.85 (0–1.31)	0.008	0.047	0.003
Triceps skinfold thickness (mm)	0 (0–0)	−2 (−4.51 to 0)	2 (0–3)	0.006	0.317	0.006
Biceps skinfold thickness (mm)	0 (−0.29 to 0)	−1.5 (−4.61 to 0)	1.5 (0–4)	0.006	0.526	0.004
Subscapular skinfold thickness (mm)	0 (0–0)	0 (−5 to 0)	1 (0–5)	0.023	1.000	0.016
Suprailiac skinfold thickness (mm)	0 (0–0)	−2.5 (−3 to 0)	2.5 (0–3)	0.004	0.946	0.006
Hip circumference (cm)	0 (−1.43 to 0)	−3 (−4.61 to 0)	2 (0–4)	0.033	0.027	0.004
Beck Depression Inventory	−3 (−4.29 to 1)	−3 (−7.21 to –1)	1 (−2 to 5)	0.344	0.14	0.017
SF-36 General Health	4 (0–20)	0 (−7.63 to 7.42)	9 (−4 to 20)	0.121	0.043	0.806
SF-36 Mental Health	0 (−1.15 to 18.3)	4 (−4 to 10.42)	0 (−8 to 20)	0.791	0.396	0.525
Binge Eating Scale	−3 (−5.57 to 0)	−4 (−4 to –0.39)	1 (−2 to 4)	0.519	0.086	0.004
Recall 24 h						
Energy (kJ)	−1865.28 (−4226.3 to 934.77)	−861.27 (−4374.54 to –82.37)	−606.38 (−2574.26 to 1844.83)	0.457	0.012	0.026
Protein (%)	−0.76 (−6.16 to 9.78)	3.34 (−2.89 to 8.67)	−3.05 (−9.73 to 4.89)	0.409	0.779	0.13
Lipids (%)	−8.05 (−12.41 to –1.97)	−8.3 (−16.46 to 2.8)	−0.56 (−10.17 to 9.09)	0.741	0.012	0.109
Carbohydrates (%)	6.66 (−5.33 to 20.92)	10.24 (−10.28 to 13.78)	4.32 (−8.23 to 18.47)	0.62	0.093	0.286
Simple carbohydrates (%)	2.15 (−10.6 to 9.44)	6.57 (−4.17 to 9.28)	−3.04 (−9.5 to 6.18)	0.457	0.575	0.109
Alcohol (g)	0 (−4.13 to 2.42)	0 (−0.65 to 0)	0 (0–2.25)	0.548	0.682	0.645
Fiber (g)	−1.15 (−15.93 to 12.25)	−4.01 (−20.21 to 3.83)	4.12 (−5.48 to 17.6)	0.409	0.779	0.155

Abbreviation: BMI, body mass index. Comparison of placebo and treatment groups.

differences between the treatment groups were found only for the mean change in lipid percent consumption (Table 4).

Regarding the assessment of 24-h dietary recall, no differences between the treatment groups were found (Table 4).

Binge eating severity, depressive symptoms and health-related quality of life

The mean changes in the scores of the BES and of the BDI were similar in the treated group and in the placebo group (Table 3). For both scales, however, a significant decrease from baseline was observed in the treated group, only. Regarding the evaluation of health-related quality of life, the results showed that the treated group did not differ from the placebo group about changes in general physical, mental, social or emotional functioning, as evaluated by the SF-36 test, throughout the course of the study (Table 4).

Safety. The 5HTP-Nat Exts product was well tolerated, and there were no serious adverse events over the 8 weeks of the study. This fact supports and is consistent with the safety of the use of 5-HTP and the other plant extracts comprising in the formulation, as already reported in literature.[51–53]

Table 4 Secondary end points: comparison of the composition of nutrient intake of the standardized test meal between treated and placebo groups

Variable	*Control mean (s.d.)*	*Treated mean (s.d.)*	*Treatment effect mean difference (95% CI)*	P-*value*
Energy (kJ)	2032.15 (475.45)	2394.94 (602.43)	−362.79 (−864.93 to 139.35)	0.147
Protein (%)	11.74 (3.14)	14.41 (4.34)	−2.67 (−6.20 to 0.86)	0.130
Lipids (%)	39.45 (5.78)	31.48 (7.63)	7.97 (1.68 to 14.25)	0.016
Carbohydrates (%)	46.93 (4.81)	52.76 (9.10)	−5.83 (−12.77 to 1.11)	0.095
Simple carbohydrates (%)	6.68 (5.87)	6.68 (3.78)	0.00 (−4.28 to 4.28)	0.999
Fiber (g)	2.76 (1.48)	3.38 (1.20)	−0.61 (−1.81 to 0.58)	0.295

Discussion

In this study, the treated group experienced a significantly greater increase in the sensation of satiety compared to the placebo group, both after a single 5-HTP Nat-Exts administration in fasting state and over an 8-week period of regular treatment.

It has been shown that appetite increases in response to body weight loss,[54] because of variations in hormone and neurotransmitter levels, such as serotonin, which is one of the most important regulators of appetite.[1,4] The synthesis of serotonin in the brain is controlled in part by the availability of its amino-acid precursor, L-tryptophan;[31] plasma tryptophan levels decrease with dieting,[21–24] therefore the possibility of maintaining the correct levels of tryptophan would be an important goal during a weight loss program to avoid any increase in appetite. In this study, administration of 5HTP-Nat Exts was found to decrease appetite ratings significantly in women after weight loss compared to the placebo group, which also showed a decrease in body weight, due to the restricted diet regime. This, therefore, suggests that the 5HTP-Nat Exts supplement may reverse the expected increase in appetite that normally accompanies weight loss and caloric restriction. The study demonstrated that this effect of 5HTP-Nat Exts administration on appetite was present in a fasting state, whereas no effect was found after a standardized test meal. We suppose that this result was due to the administration method of 5-HTP that was used. A sublingual spray solution dosage form may be an alternative route of tryptophan administration to induce a fast-action onset of satiety, enabling appetite management. This is particularly important considering that the product used in this study had never been studied before. Sublingual administration enables the drug to pass quickly into the bloodstream, because the connective tissue beneath the buccal epithelium contains a profusion of capillaries into which substances can diffuse and thus enter the blood circulation.[55] The quick passage of tryptophan into the bloodstream could explain the significant decrease in appetite ratings at fasting state in the treated group and not in the placebo group, and could also explain the unchanged appetite ratings between the groups after the standardized test meal. Moreover, the quick passage of tryptophan into the bloodstream could also explain the fact that all the amino acids evaluated were found to be similar in the treated group and in the placebo group 20 and 40 min after three sprays of 5HTP-Nat Exts or placebo. The time between the recovery of venous blood samples was probably too far apart from the administration of 5HTP-Nat Exts. Further research with this formulation should specifically address the problem of the time lapse between administration of 5HTP-Nat Exts and the recovery of blood samples. Besides, it can be supposed that all the components of the sublingual drug delivery spray formulation, such as the alkaloids present in *Alga Klamath*,[41] or the antioxidizing substances of *C. asiatica* or *Taraxaco*,[38–39] may have been involved in the effects on appetite observed in the present study.

Another important finding of this study concerns the bioavailability of 5-HTP, following sublingual delivery, over 8 weeks of administration. The comparison of 24-h urinary excretion of 5-HIAA, the major metabolite of serotonin,[31] determined at baseline and after 2 months, demonstrated that administration of the formulation containing natural plant extracts rich in 5-HTP delivered as sublingual spray significantly increases the urinary excretion of 5-hydroxyindole acetic acid.

Regarding the mood status and the severity of binge eating, there were no significant differences identified between groups; even if there had been statistically significant differences between baseline and final scores only in the treated group, this may be due to the presence of 5-HTP in *G. simplicifolia*[7] and also phenylethylamine in *Algae Klamath*.[41]

However, there were no significant differences for any of the measured variables derived from the SF-36. The SF-36 is a general health survey measurement instrument, and the results of this study showed that general health parameters in each group did not differ from each other at baseline and did not change appreciably during the course of the study. A significant change in the SF-36 was not expected; it was included in this study to rule out any adverse effects on general health functioning in either group. The results showed that the treated group did not differ from the placebo group about changes in general physical, mental, social or emotional functioning throughout the course of the study. It should also be noted that there were no significant changes in vital signs and in depressive symptoms, or

adverse events between the two groups; indeed, the patients included in the study were not depressed or affected by severe binge eating disorders.

This study demonstrated that sublingual administration of the 5HTP-Nat Exts over an 8-week period is safe and showed a side-effect profile that was no different than that of the placebo in this population. Moreover, sublingual drug administration is simple and relatively cost-effective.

Therefore these findings, together with the good tolerance observed, suggest that a formulation comprising plant extracts naturally containing 5-HTP delivered as sublingual spray may be safely used to treat the problem of appetite control in overweight subjects during a weight loss program.

Conflict of interest

The authors declare no conflict of interest.

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