Relationship between the absorption of 5-hydroxytryptophan from an integrated diet, by means of Griffonia simplicifolia extract, and the effect on satiety in overweight females after oral spray administration.

Running head: Satiety and tryptophan supplementation

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Key words: overweight, dietary supplement, tryptophan, serotonin, 5-hydroxyindoleacetic acid, 5-hydroxytryptophan, body weight

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ABSTRACT

The management of overweight may include the use of dietary supplements targeted to counter the feeling of hunger. A randomized, double-blind, placebo-controlled trial has been performed in 20 overweight females. These subjects were randomly assigned to supplement their diet with either an extract from *Griffonia Simplicifolia* (10 subjects) or a placebo (10 matched subjects) for 4-weeks, in conjunction with a personalised reduced calorie diet. The main aim of this study was to evaluate the efficacy, by the assessment of 24-h urinary 5-hydroxyindoleacetic acid levels (5-HIAA), of 1-month administration of a dietary supplement containing 5-hydroxytryptophan from botanical extracts in healthy, overweight females. Secondary endpoints were the assessment of sensation of appetite (by Haber score), body composition, and severity of binge eating. The supplemented group had a significant increase of 24-h urinary 5-HIAA levels (p<0.001), and a decrease in Haber score (p<0.001) while the placebo group did not show significant changes. With regard to changes in body composition, statistically significant differences between the treatment groups were found for the mean change in BMI, suprailiac skinfold thicknesses, arm circumference and hip circumference. Other parameters were found to be similar in the treated and in the placebo groups.

In conclusion, this study shows that the 5-hydroxytryptophan present in the Griffonia extract, administered via spray to the oral cavity, is adequately absorbed, as confirmed by the increase in 24-h urinary 5-HIAA and that the supplementation of the diet of overweight women with 5-hydroxytryptophan increases the feeling of satiety associated with a decrease in BMI.
INTRODUCTION

Excess body weight is one of the most important risk factors for all-cause of morbidity and mortality. The likelihood of developing such conditions as type-2 diabetes, heart disease, cancer, and osteoarthritis of weight-bearing joints increases as the body weight increases\(^{(1)}\), and these conditions lead to substantial economic costs for the overall health care budget. The short and long-term effects of conventional weight-management programs have been unsatisfactory and thus obese people, and society as a whole, repeatedly call for alternative therapies, including dietary supplements. Currently an important field of research deals with dietary supplements that contain molecules which act by controlling feelings of appetite (2). These appetite modulators include tryptophan, since it is a precursor of the neurotransmitter serotonin (3-5). Tryptophan consumed as part of the diet is transported within the cells, where it undergoes enzymatic hydroxylation with the formation of 5-hydroxytryptophan. Subsequently, a decarboxylation process with production of 5-hydroxytryptamine (5-HT) takes place; 5-HT is a monoaminic neurotransmitter synthesized within the serotoninergic neurons of the central nervous system, as well as within the enterochromaffin cells of the gastrointestinal system. Serotonin is considered to be a modulatory neurotransmitter within the central nervous system, with inhibitory effects on sexual behaviour, sensitivity to pain and appetite (6,7). The urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin, reflects the content and turnover of gastrointestinal (GI) serotonin (8). Plasma tryptophan concentrations are reportedly low in obese subjects, both when measured at single time points (9,10) although not always (11) and throughout a 24-hr period (12). Moreover, plasma tryptophan remains low after weight reduction (12) and decreases with dieting, (13-16), an effect that may be partly responsible for the high relapse rate after diet-related weight loss (17). The role 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 (18). Previous observations have shown that oral administration of 5-hydroxytryptophan (5-HTP) is useful for losing weight (19-22).
Given this background, the aim of the study was designed to determine the efficacy of a dietary supplement with natural plant extracts rich in 5-hydroxytryptophan, administered via the oral cavity for one month in conjunction with a personalized low-calorie diet in overweight females. The efficacy was evaluated 1) by monitoring the body’s absorption of its active principles, assessed by comparing 24-hr urinary excretion of 5-hydroxy-3-indoleacetic acid (5-HIAA) determined at baseline and after 2 months and 2) by assessing the effect on the feeling of appetite.

MATERIALS AND METHODS

Subjects

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. The subjects were recruited from a Outpatient Unit for the Treatment of Obesity, Azienda di Servizi alla Persona di Pavia. The subjects were all females, ageing between 18 and 50 years and were required to be premenopausal, not currently pregnant, normally menstruating, with body mass indexes (BMI; in Kg/m$^2$) ranging between 25 and 35. To be included in the study, the subjects could not present significant alterations in lipid and carbohydrate metabolism (glucose <6.11 mmol/L, total cholesterol <6.20 mmol/L, triacylglycerol <2.28 mmol/L) or be affected by any acute or disabling conditions or by endocrinological, neoplastic, heart disease and autoimmune diseases. Patients were excluded from the study if they met the Diagnostic and Statistical Manual-IV (DSM-IV) (24) criteria for a current diagnosis of major depressive disorder as determined by the Structured Clinical Interview for DSM-IV Axis 1 Disorders (SCID-1). Patients were also excluded if they had a history or current diagnosis of bulimia, panic disorder, obsessive compulsive disorder, post-traumatic stress disorder, bipolar I or II disorder, or schizophrenia. No psychoactive drugs, including anti-obesity agents, were permitted throughout the study. All subjects had to give complete medical histories, and underwent physical examination, anthropometric assessment and routine laboratory tests. Clinical data, alcohol intake, smoking habits and physical activity were recorded. Number of
previous diets and weight history variables were also taken from a diet/weight history questionnaire developed specifically for this study.

Assessment of satiating effect

The satiating capacity was assessed numerically, using a scoring system graded from minus 10, to represent extreme hunger, to plus 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 (24).

Assessment of Binge Eating Severity and of depressive symptoms

The severity of binge eating was assessed using the Gormally Binge Eating Scale (BES) (25). A Beck Depression Inventory (BDI-II) was taken to assess depressive symptoms: a score of 10 to 30 was indicative of depressive symptoms (26). The tests were performed by all the participants at the start of the study and after 30 days of intervention. The psychodynamic tests were conducted under standardized conditions of comfort and silence, with a study technician always in attendance.

Weight-loss program

Subjects were trained to restrict their daily energy intake by a moderate amount, 3344 kJ/d less than daily requirements based on World Health Organization criteria (27) 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 counselling.

Assessment of body composition

Nutritional status was assessed using anthropometric measurements. Body weight and height were measured and the Body Mass Index (BMI) was calculated (Kg/m²). Skinfold thicknesses (biceps, triceps, suprailiac, subscapular) were measured twice using a harpender skinfold caliper at 5 minutes intervals in each site following a standardized technique (28). Sagittal abdominal diameter
was measured at the L4–5 level in the supine position and waist girth was also measured. Anthropometric variables were measured by a single investigator.

Dietary supplement

The subjects received a dietary supplement containing 5-hydroxytryptophan from botanical extracts supplied as spray formulation (5HTP-Nat Exts) via oral cavity or an identical placebo (formulated just with the same product’s excipients: water, fructose, alcohol, potassium sorbate, citric acid, aroma, colour). Subjects were randomized to receive 3 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 4 weeks. The dose of formulation solution was administered to the oral cavity using a spray bottle. The product is a solution composed of different plant extracts; three oral sprays contain 10.24 mg of *Griffonia simplicifolia*, 11.7 mg of *Centella asiatica* L., 11.7 mg of *Taraxacum officinale*, 9.75 mg of *Cynara scolymus*, 4.55 mg of *Paulina cupana* L. Mart, 39 µg of *Alga klamath*. In the previous study (22) the product contained a major quantity of Griffonia extract, but that extract was titrated 25% in 5HTP. The Griffonia extract present in the formulation used in the present study was titrated 95%; therefore the quantity of the extract contained in the formulation was proportionally reduced to keep constant the concentration of 5-HTP in the finished product. All the other extracts were present in the formulation at the same concentration they were in the previous study. The product was manufactured by Medestea Biotech S.p.a., (Torino) - Italy.

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 blinded 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.

Biochemical analyses
Twenty-four hour urinary excretion of 5-hydroxy-3-indoleacetic acid was also determined at baseline and after 8 weeks by the chromatographic-colorimetric method described by Udenfriend et al. (29).

As regard dietary supplement, in the finished product the 5-HTP concentration was evaluated by using a reversed-phase HPLC with a mobile phase consisting of methanol-water (70:30, v/v); wavelength: 278 nm.

Statistical analysis

A between and within group analysis have been made by mean of Wilcoxon and Mann-Whitney test. Mann-Whitney test has been performed on the difference between basal and final time value. All statistical testing was 2-sided. The Kolmogorov-Smirnov (K–S test) test was used to compare the Haber test data between the two groups. It used two-sample K–S test that quantifies a distance between the empirical distribution functions of two samples. The significance level was set at a P value of less than 0.05. The analysis was obtained with Statistical Software XL-STAT (2008).

RESULTS

All the twenty subjects completed the 30 days intervention trial and their characteristics at the commencement of the study are shown in table 1; the Placebo and Supplemented groups were homogeneous for all parameters evaluated.

A significant difference was found between the treatment groups for the mean change in 5-hydroxy-3-indoleacetic acid (Table 1). This significantly increased in the treated group but not in the control group (Table 1). Moreover, a significant difference between treatments was found for BMI (p<0.02), suprailiac skinfold thickness (p<0.005), arm circumference (p<0.001), arm muscle area (p<0.006) and hip circumference (p<0.0001).

The feeling of satiety score was significantly higher in the supplemented group than in the placebo group, as shown in figure 2. It represents the cumulative relative frequencies of both placebo and
food supplement Haber test values (x-axis). The two curves show how many times we observed a relative frequency till reaching the total. Y-axis could be intended as a time profile where 1 means 30 days. In particular the food supplement curve shows relative frequencies of Haber value greater than the control group. The distance between the curves means that the food supplement Haber values are shifted toward a more satiety score than the placebo one. The correlation between the two curves is statistically significant (*p<0.001).

With regard to changes in body composition, statistically significant differences between the treatment groups were found for the mean change in BMI, suprailiac skinfold thicknesses, arm circumference and hip circumference (Table 1). The other parameters were found to be similar in the treated group and in the placebo group (Table 1).

As regards the psychodynamic tests BES and BDI, the scores of the two groups did not show any statistically significant changes from baseline to end of treatment. No statistically significant differences between groups have been detected.

No adverse events were reported during the study in either group.

DISCUSSION

The most important finding of this study concerns the bioavailability of 5-HTP from Griffonia Simplicifolia, over 4-weeks of administration. The comparison of 24-h urinary excretion of 5-HIAA, the major metabolite of serotonin (8), determined at baseline and after 1 months, demonstrated that administration of the formulation containing natural plant extracts rich in 5-HTP, delivered as spray in oral cavity, significantly increases the 24-urinary excretion of 5-hydroxyindole acetic acid.

The administration of the formulation containing natural plant extracts rich in 5-HTP, delivered as spray in oral cavity, significantly increases the 24-urinary excretion of 5-hydroxyindole acetic acid. This is the first study in humans, to our knowledge, that demonstrates the absorption of 5-Hydroxytryptophan (5-HTP) present in the extract of Griffonia semplicifolia, an herb rich in 5-HTP (30), after having sprayed it in small quantity directly into the oral cavity.
Literature refers to the efficacy in controlling appetite of oral solid preparations containing 500-900 mg per day of 5-HTP (19-21). We previously demonstrated that a much lower quantity of 5-HTP (9.7 mg in three sprays), administrated as sublingual spray 5 times a day, is effective in reducing the hunger feeling (22). In the present study we demonstrate that this low quantity of 5-HTP is effective also when just sprayed into the oral cavity. As a matter of fact, it is well known that the great problems of traditional herbal treatments are 1) the lack of well standardized products 2) the lack of precise knowledge concerning the absorption of the product and 3) the lack of scientific evidence of efficacy obtained by means of controlled clinical trials. To overcome these limitations, this study has been conducted 1) with an highly standardized Griffonia Semplicifolia extract rich in 5-hydroxytryptophan, 2) by monitoring the absorption of 5-hydroxytryptophan, as assessed by the 24-urinary excretion of 5-hydroxyindole acetic acid and 3) with a double-blind, placebo-controlled, randomized clinical trial. The issue of standardization, characterization, preparation and absorption of an herbal extract is most important (31).

In addition, it is important to highlight the significant increase in the Haber test score, obtained only in the treated group and not in the placebo group. This significant increase in the feeling of satiety would be the indirect cause that led to follow more closely the prescribed restricted diet and consequently to decrease in BMI in the treated groups, statistically different from placebo. These observations are in agreement with previous studies (19-22). Moreover, the effect on BMI could be linked to the presence of other active components in dietary supplement. The regulation of appetite and body weight involves multiple parallel neuronal and bodily mechanisms. Not surprisingly, it was shown that a medication that targets these mechanisms result in weight loss of 5%-10% in a period of 60-90 days. Although weight loss of this magnitude may produce significant reductions in risk factors associated with cardiovascular morbidity and mortality, patients are more focussed on cosmetically meaningful reductions in weight (~20%-25%). Combining 2 medications that work via different mechanisms, that is, "combination pharmacotherapy," is an approach to obtain cosmetically relevant reductions in weight (32). The dietary supplement studied is a combination
therapy since it contains, in addition to the extract of Griffonia Simplicifolia, Guaranà, known for its stimulatory effect on fat metabolism (33); Centella Asiatica, which is useful for its beneficial effects on blood and lymph circulation (34); Taraxacum, which has an anti-oxidative and diuretic effect (35); artichoke, with its detoxifying action (36); and klamath algae, which has a high content of nutritive substances including phenylethylamine, which improves mood (37). Regarding the mood status and the severity of binge eating, there were no significant differences identified between groups. A significant change in the score of the psychodynamic tests was not expected; it was included in this study to rule out any adverse effects on general health functioning in either group.

Further studies, involving a larger number of patients and also including men, may offer a potential field for the treatment of overweight. The observed safety profile was consistent with the known effects of 5–HTP supplementation from previous studies (19-22).

In conclusion, the present study demonstrated that the 5–HTP present in the extract of Griffonia, administered via spray to the oral cavity, is adequately absorbed, as confirmed by the increase in 24-h urinary 5-HIAA. In addition supplementation of overweight women with 5-hydroxytryptophan increases feelings of satiety associated with a decrease in BMI.
REFERENCES


<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline Mean (SD)</th>
<th>After 1 month Mean (SD)</th>
<th>Change from baseline Mean (SD)</th>
<th>Delta (change ctrl-treated)(SD)</th>
<th>Difference between treatments P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-hydroxy-3-indoleacetic acid (mg/24h)</td>
<td>3.64 (1.38)</td>
<td>3.56 (1.36)</td>
<td>-0.08 (0.431)</td>
<td>5.09 (4.59)</td>
<td>5.17 (4.51)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.00 (5.94)</td>
<td>69.20 (5.63)</td>
<td>-8.8 (8.1)*</td>
<td>-0.8 (-1.8)</td>
<td>1.13 (1.33)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.61 (2.15)</td>
<td>27.31 (2.29)</td>
<td>-0.30 (0.43)</td>
<td>-0.30 (-0.43)</td>
<td>-0.87 (0.58)</td>
</tr>
<tr>
<td>Triceps Skinfold Thickness (mm)</td>
<td>34.00 (5.54)</td>
<td>32.35 (8.07)</td>
<td>-1.65 (3.50)</td>
<td>-0.6 (1.07)</td>
<td>-3.8 (4.10)</td>
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<td>Biceps Skinfold Thickness (mm)</td>
<td>26.05 (9.47)</td>
<td>25.45 (10.18)</td>
<td>-0.5 (4.93)</td>
<td>-0.6 (1.07)</td>
<td>-0.25 (0.79)</td>
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<td>Subscapular Skinfold Thickness (mm)</td>
<td>31.35 (6.81)</td>
<td>30.40 (6.77)</td>
<td>-0.95 (1.92)</td>
<td>-0.95 (1.92)</td>
<td>-3.25 (5.00)</td>
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<tr>
<td>Suprailiac Skinfold Thickness (mm)</td>
<td>32.10 (5.95)</td>
<td>31.75 (6.18)</td>
<td>-0.35 (0.82)</td>
<td>-0.35 (0.82)</td>
<td>-5.4 (5.66)</td>
</tr>
<tr>
<td>Arm Circumference (cm)</td>
<td>31.30 (2.54)</td>
<td>31.15 (2.55)</td>
<td>-0.15 (0.34)</td>
<td>-0.15 (0.34)</td>
<td>-1.85 (1.13)</td>
</tr>
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<td>Arm Muscle Area (AMA) (cm³)</td>
<td>27.72 (7.52)</td>
<td>28.98 (8.31)</td>
<td>1.263 (2.69)</td>
<td>1.263 (2.69)</td>
<td>-3.65 (5.67)</td>
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<tr>
<td>Arm Fat Area (AFA) (cm³)</td>
<td>4.09 (0.91)</td>
<td>4.05 (0.82)</td>
<td>-0.04 (0.16)</td>
<td>-0.04 (0.16)</td>
<td>-0.34 (0.62)</td>
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<td>Muscle Arm Circumference (MAC) (cm)</td>
<td>20.62 (2.21)</td>
<td>20.99 (2.35)</td>
<td>0.37 (0.81)</td>
<td>0.37 (0.81)</td>
<td>-0.79 (1.65)</td>
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<td>Waist circumference (cm)</td>
<td>92 (6.72)</td>
<td>91.35 (6.6)</td>
<td>-0.65 (1.60)</td>
<td>-0.65 (1.60)</td>
<td>-2.4 (3.45)</td>
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<td>Hip circumference (cm)</td>
<td>103.6 (5.57)</td>
<td>103.15 (5.49)</td>
<td>-0.45 (0.76)</td>
<td>-0.45 (0.76)</td>
<td>-5.9 (5.40)</td>
</tr>
<tr>
<td>Waist Hip Ratio (WHR)</td>
<td>0.89 (0.08)</td>
<td>0.89 (0.08)</td>
<td>-0.01 (0.01)</td>
<td>-0.01 (0.01)</td>
<td>0.01 (0.06)</td>
</tr>
<tr>
<td>Beck Depression Inventory</td>
<td>11.3 (9.20)</td>
<td>8.7 (7.56)</td>
<td>-2.6 (5.15)</td>
<td>-2.6 (5.15)</td>
<td>-0.4 (1.96)</td>
</tr>
<tr>
<td>Binge-eating Scale</td>
<td>11.8 (5.55)</td>
<td>10 (5.14)</td>
<td>-1.8 (3.19)</td>
<td>-1.8 (3.19)</td>
<td>-0.2 (3.68)</td>
</tr>
</tbody>
</table>

*P<0.001
26 subjects assessed for eligibility

20 eligible: fulfilled inclusion criteria

6 excluded: 1 refused to participate 5 with laboratory abnormalities

20 randomized

10 allocated to the supplementation
10 received allocated intervention

Followed up at week 4: n = 10
10 analysed

10 allocated to placebo supplementation
10 received allocated intervention

Followed up at week 4: n = 10
10 analysed

Figure 1. Flow diagram of a trial of supplementation versus placebo in the treatment of healthy overweight females.
Figure 2. Evolution profile of the mean Haber score in the treated (continuous line) and control groups (dotted line). * p<0.001